COLD SPRING HARBOR LABORATORY

ANNUAL REPORT 1995

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Cold Spring Harbor Laboratory
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Back cover: Poster session at a Cold Spring Harbor scientific meeting in Vannevar Bush Auditorium. (Photographed by Marléna Emmons.)

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Lita Annenberg Hazen (1909-1995)

Lita liked me for my brains and also because as a successful scientist I was optimistic and never worried her about what I should do next. But she did not like to see me becoming almost fat after I took on leading the Human Genome Project and had one job more than I could handle. I then would sense her displeasure when I sat down opposite her at her Pierre Hotel apartment and she would look at my belt not adequate for its job. So when I again became trim, like a long thin DNA molecule, we would not have that awkward start to our conversations where she expressed sharp, clear thoughts and never exuded unreasonable goodliness when the facts were awful.

We had first met when I was badly in need of big money to move the Cold Spring Harbor Laboratory beyond the gene and onto the ultimate challenge of biology, the human mind. I had long known of her philanthropy to biology and medicine, but despite urgings from a colleague who said that our souls were made for each other, I was initially scared to approach her. At a gala several years earlier, I had already gazed upon the firm regality that had emerged out of her Annenberg intellect and money. Yet she could be the friend and patroness who would let me have aspirations beyond common sense.

So I got up my courage and began to let her know me, often in the California beauty of her Hornby Hills home overlooking the Los Angeles Country Club. At first I assumed that her penchant toward biology brains arose out of the tragedy of her eldest daughter Gwynne's premature death from leukemia and her subsequent endowing of a cancer research laboratory at the nearby UCLA Medical Center. In fact, she had to live with the knowledge that it had been her early interest in medical research that had led her to a specialist who used X-rays to treat her daughter's intractable eczema in the days before the cancer-causing attributes of ionizing radiation were firmly established.

Turning away from rationality was not, however, Lita's thing and much happiness in her later life came from bringing together the scientific elite in groups small enough to let her know whom she was helping. For several years, she also provided a major prize in clinical research, but felt almost betrayed when the recipients of her generosity seemed to vanish from her life almost as quickly as they entered her world at the prize ceremony dinners. The receipt of even a holiday card would have made matters different, but I remember that when I was still hot on the trail of scientific discovery, I was so obsessed with the future that the time never came to think backward toward those who had helped propel me forward.

Lita's heart thus increasingly focused on her prestigious several-day-long gatherings of the best minds in biology and medicine whom, with Martin Meyerson's help, she brought together, in charismatic locations like Aspen, Stockholm, Venice, and San Francisco. There one could linger and talk free from the small details of our usual lives that never seemed to transform into any form of the big picture. Of course, Lita could not understand the fast-flowing scientific ideas that came whizzing past her head during formal discussion, but at meal times, she loved replying to the unexpected words and ideas that intellectuals must feed upon to stay alive.

Lita's first visit to the Cold Spring Harbor Laboratory came about in a most unexpected way. Knowing that her friend David Eisenberg was out on Long Island at one of our big symposia, she arrived on an unbearably hot June day at our kitchen door telling my wife Liz that she was a philanthropist in need of cab fare. Liz rose to the financial emergency and soon she and Lita found David below our house in the picnic line for hot dogs. Famished without any lunch, Lita uncharacteristically ate a hot dog and then to everyone's horror collapsed onto the ground. In panic we feared that our philanthropist might not be long for this world. Quickly, however, Lita recovered sufficiently to drink some cold water and within a few minutes Lita and Liz were in the shade of our front porch getting to know each other. Early the next week, the cab fare came in the form of a $25,000 check.

The following year, we began preparing plans for a Neuroscience Center that could enable the Cold Spring Harbor Laboratory someday to become a major force in brain research. In building the complex, we wanted to emphasize our evolution from our past role as a high-powered summer camp for scientists into a major year-round academic center. The architectural trick used to make this point was to be a powerful bell tower that would let us know when the next hour had arrived. Although the tower was bound initially to annoy those who would like every last dollar spent to have a scientific mission, I felt that no one who saw it could ever more doubt that we had a future. And they would see us moving ahead with the architectural flair that most leading academic institutions like to display.

To make this possible, we asked Lita to join our board of trustees. Knowing exactly what I meant Lita accepted, and the next week the big check arrived that let us go forward. Today, the now four-year-old Hazen Tower projects majestically upward outside my office, and when its bell tolls the hour, we remember Lita's great generosity and faith that through scientific research the human condition will improve.

In her last several years, age began to be cruel to Lita's mind, and Liz and I increasingly felt dreadful when her memories of the immediate past no longer held. But almost to the very end, she was still a force to be reckoned with. On her last visit to Lita's 29th floor Pierre apartment, Liz made reference to an acquaintance as one of Lita's close friends. Immediately Lita replied that this individual was a good friend and not a close friend. After coming back to Cold Spring Harbor and relating this incident, Liz burst into tears. We believe that Lita saw us as her close friends.

October 5, 1995

James D. Watson

PRESIDENT'S REPORT

Ten years have now passed since serious consideration was first given to starting the Human Genome Project, the task of determining the order (sequence) of the four base pairs (AT, TA, GC, and CG) along the 24 very long DNA molecules that collectively comprise the human genome. Contained within these DNA sequences are the genetic instructions of our 24 different human chromosomes (22 autosomes and two sex chromosomes X, Y), each of which contains a single DNA molecule. These DNA molecules are the most important molecules underlying human life since each contains on the average some 4000–5000 of our human genes, each gene representing a long linear collection of base pairs positioned at a precise site along one of our chromosomes. In proposing to sequence the some 3×10^9 base pairs (bp) of the human genome, our primary aim was to identify the location and structure of all our genes along their respective chromosomes. With this information in hand, the genetic code lets us automatically know the precise amino acid sequences of the protein products of each gene.

Knowing the location and structure of each of our human genes would provide an enormous resource to the worlds of biology and medicine. To start with, the amino acid sequences of many human proteins by themselves would give us strong clues as to their primary functions. So through the possession of the human genome, we would have our first overview of the totality of the molecular functions underlying the development of a fertilized human egg into a functioning adult human being. Equally important, the establishment of the location and structure of all the human genes would immeasurably speed up the identification of those normal human genes whose mutant forms are the cause of the countless genetic diseases that diminish the lives of so many human beings and their respective families.

When first proposed, however, the Human Genome Project generated deep controversy within the community of molecular biologists, the very scientists whose expertise in manipulating and analyzing DNA would lie at the heart of its successful completion. Among many younger scientists, deeper anxieties developed that first were publicly expressed at our June 1986 Symposium on the Molecular Biology of *Homo sapiens*. Toward its conclusion, a late-afternoon discussion on the pros and cons was chaired by Wally Gilbert and Paul Berg, whose past research on DNA sequencing and recombinant DNA technologies had helped make the Human Genome Project an attainable goal. They both wanted the project to start soon, but a number of younger scientists in the audience worried that its costs might be so high that it would be completed only by containing the momentum of their own research projects. Here in the United States, many of us were initially troubled that the American component of the project might be largely directed by administrators in the Department of Energy (DOE) who oversee the biology programs of the national laboratories at Los Alamos and Livermore. They were far from the heart of the recombinant DNA world, yet they were saying, starting in 1986, that they should lead this effort since it would require the technological and logistical bases that only the super-sized national laboratories possessed.

Much of the scientific opposition to the Human Genome Project thus evaporated when the National Institutes of Health (NIH) came into the act, early in 1988, proposing that the project be scientist-led as opposed to bureaucrat-led. Moreover, unlike the DOE administrators, who obstinately insisted that they would only support human DNA sequencing, the scientist-led NIH endeavor, of which I became the first director, proposed that the much smaller genomes of biologically well-studied model organisms, such as the bacterium *Escherichia coli* (4.5×10^6 bp), the budding yeast *Saccharomyces cerevisiae* (1.5×10^7 bp), the worm *Caenorhabditis elegans* (10^8 bp), and the fly *Drosophila melanogaster* (1.5×10^8 bp), should be sequenced prior to the large-scale sequencing of human DNA. There has been much conservation of gene structure during the evolution of life on the earth, and the amino acid sequences of, say, many bacterial proteins show strong similarities (homology) to those human proteins that carry out similar functions. From the start of the Human Genome Project, we perceived that the establishment of most human gene structures would proceed much faster if the structures of their less complex bacterial, yeast, worm, and fly equivalents had already been established.

Not only do these model organisms have smaller genomes, they also have much higher gene densities along their respective chromosomes. In humans, probably less than 5% of the base pairs code for amino acid sequences, whereas in bacteria and yeast, virtually all of their base pairs specify amino acid sequences. As a result, identifying the genes of bacteria and yeast is almost a routine endeavor. In contrast, identifying the human genes from human DNA sequences is much trickier since their coding (exon) sequences are interrupted by much larger amounts of noncoding (intronic) sequences. Initially unclear was whether spotting the genes within *C. elegans* and *Drosophila* would be a noticeably simpler task than for their human equivalents. Although their introns would be much smaller, their numbers might be almost as large.

Several other factors led to the NIH proposal that genomic sequencing should start with model organisms. To begin with, the effective cost of DNA sequencing in 1988 was at least five dollars per base pair. Before extensive human DNA sequencing commenced, the cost per base pair had to be reduced by a factor of ten to fifty cents or less per base pair. This would allow the Human Genome Project to be completed at a cost of no more than three billion dollars. Given that this project was likely to require 15 years from inception to completion, its annual cost when in full gear need be no more than 200 million dollars a year, a figure that amounted to less than 2% of the total worldwide project for nonclinically directed biomedical research.

Happily from the start of serious discussion, much lower sequencing costs seemed a realistically attainable objective since the first DNA sequencing machines were just becoming commercially available. Although they initially had teething difficulties and even the first labs possessing them were more often using manual sequencing, the best guess was that machine-based production-line procedures would become increasingly more effective over the next several years.

Cost was not the only reason why human DNA genome sequencing was initially put on the back burner. Unlike with the smaller model organisms where the ordered cloned DNA segments were available for sequencing, only very restricted parts of human DNA had been cloned and sited relative to known markers on the human genetic map. In fact, the human genetic map of 1988 was

of much too low a resolution to specify closely the relative chromosomal location of most genetic disease genes. So the first major objective of the Human Genome Project had to be the mapping of a very large number of new genetic markers. Here the correct decision was made to abandon the use of polymorphic markings that reflected differences in restriction enzyme cutting sites. In their place were to be generated markers based on frequent polymorphisms in the length of the CACA-repetitive segments present in very large numbers throughout the human genome. Such segments are easily displayable on gels using polymerase chain reaction (PCR) amplification and are suitable for analysis on the same machines used for DNA sequence analysis. Already this first goal of the Human Genome Project is essentially complete largely due to the very effective generation of very large numbers of CA repeat markers by Jean Weissenbach at Genethon, the large genome center established near Paris in 1990 by the AFM (Association Française contre les Myopathies). By late 1995, they had created polymorphic CA repeat markers on the average for every 500,000 bp.

The existence of these ordered genetic markers greatly simplified the second main goal of the Human Genome Project—the making of a physical map of cloned segments of human DNA running from one end to the other of each of the 24 human chromosomes. Key to the success of making such physical maps has been the development of methods for the cloning of DNA fragments large enough so that their ordering into increasingly larger sets of overlapping fragments (contids) was a practical objective. Here a major breakthrough occurred in 1987 at Washington University, where in Maynard Olson's laboratory, procedures were developed to clone 3×10^5 to 5×10^5-bp-sized fragments of human DNA in yeast artificial chromosomes (YACs). Such inserts were far larger than the biggest human DNA segments previously cloned. Later at Genethon, Daniel Cohen's use of megabase-sized (10^6) fragments allowed the 1993 construction of the first physical maps, albeit with many gaps, for all of the human chromosomes. This task has now been brought close to completion through the development of highly automated production-line mapping procedures at the Genome Center directed by Eric Lander at the Massachusetts Institute of Technology. The physical mapping phase of the human genome is thus almost over, leaving now the more mundane task of generating the sets of overlapping smaller DNA segments now used to generate actual DNA sequence data.

Completion of the genetic and physical maps should not be seen as merely the prelude for the final goals—sequencing and gene identification—of the Human Genome Project. These maps are now very useful in their own right as today's tools for speeding up the discovery of more human disease genes. Ten years ago, with the resources then available, the cloning of many important human disease genes was at best very difficult and more often effectively an unattainable objective. Not enough genetic markers then existed to accurately pinpoint the cloned chromosomal regions containing their disease genes. For example, even though the gene for Huntington's disease had been located in 1984 to near the end of the small arm of chromosome 4, its eventual cloning took ten years, well into the period when the Human Genome Project began to speed up the pace of finding disease genes. And although it took four years from the 1990 mapping of the breast cancer gene BRCA1 to the long arm of chromosome 17 to its eventual cloning in 1994, the ever-increasing resources provided by the Human Genome Project led to cloning of a second breast cancer gene, BRCA2, in only a little more than a year after its mapping to chromosome 13. Likewise greatly speeded

up by the new genetic resources has been the identification of all the major genes responsible for predisposition to Alzheimer's disease. As with Huntington's disease and breast cancer, only through the finding of genes that lead to its predisposition have we been able to begin to think rationally at the molecular level about how the generation of Alzheimer's disease is initiated. The monies thus spent so far on the Human Genome Project already may have been returned to the public through better ways to approach the eventual control of these horrific diseases.

Clearly, disease gene hunting will proceed even faster once all of the human genome has been sequenced and all of the human genes have been identified. Then, once we suspect that a disease gene resides along a specific section of a human chromosome, we can look to see whether any genes in that location with already known functions might be good candidates for the respective disease. We know such an approach can greatly shorten the time associated with disease gene hunting. For example, when there was suggestive evidence that a gene leading to late-onset Alzheimer's disease mapped to a specific region of chromosome 19, the known genes within this region were examined, particularly the one coding for apoliprotein E, a protein associated with the amyloid-containing plaques characteristic of diseased brain portions of Alzheimer victims. Quickly it was found that Alzheimer patients preferentially carried the D polymorphism that only rarely is found in unaffected individuals. In this way, the important role of apoliprotein E in the etiology of Alzheimer's disease became established. Strong medical reasons thus dictate the complete sequencing of the human genome as soon as possible.

The third major goal of the Human Genome Project, using the sequencing of model organisms to develop production-line procedures for low-cost sequencing, is also close to completion. The first available DNA sequencing machine, jointly developed between 1982 and 1986 in Lee Hood's Caltech lab by Lloyd Smith and at Applied Biosystems (ABI) by Mike Hunkapiller, has lived up to the task. That the ABI sequences could be the workhorse of the Human Genome Project has been convincingly demonstrated through the project to sequence the 100-Mb genome of *C. elegans* through the collaborative efforts led by John Sulston at the Sanger Centre just outside Cambridge, England, and by Robert Waterston at Washington University. Funded by both the National Center for Human Genome Research and the British Medical Research Council, the *C. elegans* genome project started in 1990. By 1995, both labs were up to sequencing some 10 million *C. elegans* base pairs per year with the cost falling to fifty cents per base pair, the figure that had been set for the start of large-scale human DNA sequencing.

Initially unclear, however, was whether human genome sequencing (because of its much larger repetitive DNA component) would prove more expensive. Fortunately, this is not the case since both the Sanger Centre and the Washington University groups have found that it is at least as easy to sequence human DNA as worm DNA. Although it was initially feared that the computerized DNA assembly programs might not improve at the same rate as the sequencing machine technologies, this has not been the case. Particularly invaluable have been the new sequence assembly programs created by Phil Green, first at Washington University and more recently in Seattle at the University of Washington. Thus, there is a realistic chance for the cost of sequencing to fall by another factor of two over the next year or two. If so, only 750 million dollars need be spent to total-

ly sequence the Human Genome by 2005, the target date set in 1990 when the Human Genome Project funds first began really to flow. The Human Genome Project may thus be completed not only on time, but also below its original target price of three billion 1988 dollars.

Initially we believed that most human genes would not be revealed until the last several years of the Human Genome Project when the production-line sequencing was in full swing. Today, however, the partial structures of possibly more than one half of all human genes have already been found through the large-scale examination of their expressed gene products, the messenger RNA (mRNA) molecules, which function as the actual templates for protein synthesis. Through knowing their structures, we automatically know the sequences of the genes on which they are made. More importantly, the molecular processes that produce functional mRNA molecules splice (cut) out the intron sequences, effectively leaving only base pairs that code for the amino acid of their gene protein products. To learn the sequence of these mRNA products, which often are tissue-specific, the enzyme reverse transcriptase is used to make complementary DNA (cDNA) copies of the mRNA molecules. It is these cDNA polynucleotide chains that are then sequenced.

This expressed sequence tag (EST) approach was first effectively explored in the laboratory of Craig Venter when he was at NIH. By examining ESTs from many different cell types, Venter's lab was able quickly to catalog the sequence of several tens of thousands of different ESTs. How many different genes gave rise to the ESTs, however, was unclear since most EST sequences are only several hundred bases long. Moreover, through differential splicing, single genes are capable of generating several distinct EST products. To help promote his EST approach, Venter created a privately funded institute, The Institute for Genome Research (TIGR), located near NIH in Bethesda. Within a year, sufficient additional ESTs had been sequenced to generate overlaps between many of the ESTs and so generate more complete versions of the functional mRNA transcripts revealed by EST analysis. Venter currently believes that his some 175,000 different ESTs arise from at least 30,000 (and possibly as many as 60,000) different genes, possibly more than half of the total human gene number, which has been estimated to lie between 60,000 and 120,000.

The EST methodology, however, will never totally characterize the human complement. It will always be strongly biased against rarely expressed genes. Moreover, it can never expose the nature of the upstream and downstream regulatory regions that are seldom transcribed into mRNA products. It has, however, already revealed the existence of many human genes that otherwise we would only have later known when their respective DNA genomic fragments had been sequenced. It was the thought that many such EST sequences might be commercially valuable that, in fact, led to the multimillion dollar funding of TIGR by its parent biotechnology company, Human Genome Sciences. But this TIGR funding came with the price that the ESTs so obtained would remain secret until Human Genome Sciences had been given not only a first look, but also control over their future commercial exploitation. All of the sequenced TIGR human ESTs are still kept out of public databases, although in September 1995, the functions of many ESTs that are similar to already known genes (proteins) were released. But before a given laboratory can obtain real sequence information about these ESTs, it must sign an agreement limiting their commercial use, a requirement that many scientists and companies find difficult to accept. To circumvent the restric-

tion governing the use of Venter's EST database, a second massive EST sequencing effort began some 18 months ago at Washington University, with the funds provided by the pharmaceutical giant Merck. As soon as these Merck-funded ESTs are sequenced, they are placed in the Genbank public database and analyzed for sequence overlaps to identify those coming from the same gene transcripts. By now the number of ESTs in the public database is beginning to approximate those held privately. More data as to the tissues where these ESTs are expressed remain privately held.

The value of many EST sequences will improve when the chromosomal DNA segments from which they are expressed become known. Unless, however, there is reason to believe that an EST comes from a gene important for either biological or commercial reasons, the cost of finding its location on the human physical map will keep most such sequences unassigned until their respective genomic segments are sequenced as part of the Human Genome Project. Over the near-term, most subsequent EST studies will likely be driven by commercial considerations.

The EST approach to gene finding never made sense for the smaller model organisms such as bacteria and fungi since the total sequencing of their genomes always seemed to be realistic objectives once the cost of sequencing became no more than fifty cents per base pair. The first bacterial genome so sequenced has been that of *Hemophilus influenzae* (2×10^6 bp) done at TIGR using DNA provided by Hamilton Smith of Johns Hopkins Medical School. Revealing the genome underlying the existence of a single cell was clearly a milestone in the history of biology. Most important, more than two thirds of its 1800 genes were assigned precise metabolic functions. Equally exciting was the subsequent sequencing at TIGR of a much smaller *Mycoplasma genitalium*, a tiny bacterial type cell that exists as a parasite inside its much larger vertebrate host cell. Because this tiny cell obtains so many of its essential building blocks from its host cell, it does not need the proteins (genes) involved in their respective synthesis. As a result, its functioning and replication require only some 450 different genes. Here again, precise functions have been assigned to some 60% of their genes. Genome analysis gives us the reassuring conclusion that we already have identified more than half of the molecular components essential for the function and multiplication of single-celled organisms.

The same message now comes from the virtually complete sequencing of the budding yeast *Saccharomyces cerevisiae*. This success results from a very large international collaborative effort involving laboratories in Europe, the United States, Canada, and Japan. Already the public databases contain the sequences of virtually all of its some 6000 genes. Happily we already know the function of about half, with more having homology with genes in other organisms. Most exciting to cell biologists is the fact that some 30% of the genes code for proteins whose structures suggest that they are membrane components, reinforcing the importance that membranes play in intracellular metabolism of even the smallest eukaryotic cells.

Although more than half of the yeast genes were sequenced through a cottage industry approach involving about 100 different laboratories, costs dictate that most future genome sequencing successes be the products of highly automated large laboratories such as the Sanger Centre, the Washington University Genome Center, and TIGR. Only they will prove capable of reducing sequencing costs much below the fifty cents per base pair milestone. In such large centers, a

single ABI sequencing machine can generate over a year 10^6 bp of highly accurate final sequence. Only through the creation of several more such large-production sequencing facilities will the human genome be sequenced by the year 2005. The Sanger Centre and the Washington University facility have already reached sizes where we can be confident that the 10^8-bp *C. elegans* genome will be finished at high accuracy by the turn of this century. They have tackled first the more gene-rich internal chromosomal regions, leaving the repetitive sequence-rich chromosomal ends to be sequenced as the project draws to its conclusion. Here the decision must be made whether the same accuracy should accompany the sequencing of uninformative DNA segments that goes into sequencing genetically important regions. Clearly, no one wants to miss the presence of real genes interspersed within much larger lengths of biologically irrelevant DNA. The job will not be well done if say 5% of the some 16,000 *C. elegans* genes are missed. On the other hand, if only 16 out of 16,000 were to be missed, the study of *C. elegans* development and functioning might proceed effectively unimpeded.

The question of how accurate we want the final sequence to be may prove far more difficult to settle for the human genome. We must remember that the true objective of the Human Genome Project is gene identification and mapping, not the accurate sequencing of a particular human genome. Many polymorphic differences exist within most repetitive DNA segments as well as within the unique segments coding for intron DNA sequences. Once we have identified a particular DNA signal as repetitive or intronic, should we sequence it to the same accuracy as those segments coding for obvious biological functions? Common sense tells us we should not, and already there are proposals to first do a relatively low-resolution genome sequencing effort over the entire human genome. Although such a scan would miss a few percent of the human genes, it would nonetheless largely establish the general nature of the human genome. How this dilemma is finally answered is likely to depend on whether the current cost of sequencing can be reduced over the next several years to say ten cents per base pair. If so, there is no reason not to go for the same accuracy that we want to achieve from the larger-model organisms such as *C. elegans* or *D. melanogaster*. But if further cost reductions prove hard to achieve, the argument to concentrate our accurate sequencing efforts on biologically relevant sequences may come to the fore. In any case, there is enough money in current fiscally strained genome budgets to give confidence that at least 95% of our genes will be identified by 2005.

Our job will then be to much more deeply understand how the human body develops and functions, using the information provided within the human genome. Effectively, this century will be perceived biologically as that of the gene. In this coming century, we will have the opportunity to see in detail how the controlled expression in space and time of our some 100,000 genes gives rise to our human bodies with brains so powerful that we finally have the means to define the molecular essence of what it means to be human.

At the same time, the sequencing momentum generated by the Human Genome objective will positively spill over into virtually all aspects of biological research. Most pleasing will be the ever-growing ability to see how genomes have evolved and expanded during the three billion years of biological evolution on earth. As the cost of sequencing moves down to the level of cents per base pair, the genomes of a large variety of evolutionarily diverse species will be open to analysis. Here at Cold Spring Harbor, we are involved in the international effort

to sequence the smallest known plant genome, that of *Arabidopsis thaliana* (around 10^8 bp). Just as the complete genome sequence for a yeast has transformed how we comprehend the nature of the yeast cell, the possession of the complete genome of this small member of the mustard family will radically push forward research on all forms of higher plants, including trees.

We need not fear that biology will slow down in the foreseeable future.

April 25, 1996 **James D. Watson**

DIRECTOR'S REPORT

History will almost certainly judge the current era of biology as one of the most productive periods of scientific endeavor, comparable to the dramatic discoveries in quantum physics in the early half of this century. The discoveries in physics led to a fundamental understanding about the nature of the atom and the beginnings of time. The understanding was so deep that it drove some to philosophy to "explain" the unexplainable, whereas others pursued more practical endeavors that prepared the way for a major technological revolution. On the other hand, the dramatic increase in our ability to study life means that experimental biologists will be kept busy for at least the next 100 years and thus may not have time for more philosophical pursuits. Moreover, because advances in biology are so intimately linked to human health, the biologists will always have important practical problems to unravel. How this might be done best should be a topic of constant conversation.

Advances in the basic biomedical sciences are coming at a very rapid pace, and from all directions. The current efforts to sequence the genomes of many organisms, the daily identification of interesting new proteins and discovery of their functions, and the remarkable ability to investigate gene function using powerful genetic manipulations offer unparalleled opportunities for medicine. The amount of biological information that is emerging from this revolution is staggering, and there is an urgent need for more efficient ways to keep track.

Luckily, within the last year or so, there has surfaced a potential solution to the information storage and retrieval problem. A number of journals are now completely available in electronic form on the Internet (for example, the *Journal of Biological Chemistry* is a particularly useful online resource). In the best cases, the full text of the journal can be rapidly searched for information. Key words and references in the research articles are electronically linked to information databases, such as databases containing genome and protein sequences, genetic and disease databases, and even other scientific literature. This makes it relatively easy to seek out relevant information that is connected to the original research. I suspect that in the very near future, a substantial amount of biology can be "done" by computer informatics experts who seek connections between published experimental results. It may well be that a new field of biology, "virtual physiology," will emerge as an essential contributor to progress in the biological sciences. Ideally, these virtual physiologists will link up with the experimental scientists so that biology does not become completely theoretical. For example, even today there are those who make claims about the function of proteins based on DNA sequence similarities when they have no intention of following up on these claims.

At the same time, scientific meetings such as those held here at the Laboratory will be ever more important as the amount of information increases. Such conferences provide to a scientist an overview of a field in a matter of a few days which probably would take weeks or even months to comprehend by reading the literature.

As exciting as the advances in modern biology are, there is a pressing need for institutions such as our own to better facilitate connections in biology, particu-

larly the transfer of the exciting advances in basic science to clinical research. Interaction between basic and clinical research groups is now cast by the National Institutes of Health (NIH) as "translational" research, but more often this term is used by scientists to secure grant funds than it is for advancing real transfer of information between the sciences.

There are often long lag times between basic research discoveries and clinical applications. It is common for the brightest minds that contribute to the spectacular advances in basic research to be unaware of the day-to-day problems faced by the clinician. It is equally common for the clinician to be unapprised about new discoveries in basic research. As the advances in basic science become more extensive and complicated, it will be increasingly necessary to bring those interested in transferring basic discoveries to the clinic together with the clinical researchers. This will be particularly necessary for the critical design of patient-based research because of the complexities in planning good clinical studies and the enormous costs that are often associated with this type of inquiry. How clinical research might be best done and how basic scientists might contribute are problems that need attention.

With these thoughts in mind, a meeting was organized at the Banbury Conference Center in October, 1995, that focused on neurofibromatosis type 1 (von Recklinghausen disease, NF1), a devastating disease that affects about 1 in 4000 people throughout the world. This dominantly inherited trait causes learning disabilities in young children, and children and adults are affected with a variety of deformities, such as café-au-lait spots, neurofibromas, optical and bone problems, and malignancies. The NF1 mutations were mapped to chromosome 17 in 1987, and the altered gene that causes these severe abnormalities was cloned in 1990 through a cooperative research effort, spearheaded by Francis Collins, now head of the National Center for Human Genome Research at the NIH.

The protein product from this gene is large and its full functions are not known, but the protein displays similarities to a known regulator of the human RAS protein. As shown by Michael Wigler and his colleagues at Cold Spring Harbor, as well as others, when mutated, the human *RAS* oncogene contributes to cancer progression in a large fraction of human tumors. This provides an interesting, but still speculative, link between tumor formation and the NF1 protein. Because of the links with human cancer, a great deal has been done on the biochemistry of the RAS protein. This information has come from basic research on the RAS protein in species as diverse as yeasts, the fruit fly *Drosophila*, and mammals such as mice, and there are very interesting potential anti-cancer drugs that have been developed by the pharmaceutical industry based on this basic research.

There is thus an enormous amount of information known about the biochemistry and genetics related to NF1 disease. The gene that causes the primary problems is in hand and a great deal of information is known about the possible biochemical pathways the NF1 protein controls. Yet there are significant problems that clinicians have in diagnosing and treating the disease. Despite the apparent genetic simplicity, there is extreme clinical variability in the outcome of the disease; diagnosis, particularly of the cognitive deficits, is a major problem for the clinician and a detailed description of the nature of some of the clinical defects is lacking. Because these clinical problems still exist, but also because there is a strong interest by scientists in the biology of the NF1 protein, it seemed that a meeting that brought together clinicians, scientists interested in clinical and basic research, and investigators from the pharmaceutical industry to dis-

cuss the disease would be valuable. Although there have been other meetings on NF1, the charge at the Banbury meeting was to discuss how clinical science and medicine would be best advanced by the better design of clinical research and better coordinated basic research. Another goal was to explore progress in both clinical research and therapeutic strategies targeted at all aspects of the disease. Because so much is known about the underlying mechanisms that lead to NF1, our agenda was to assess critically how this understanding was being exploited for further treatment and what might be done to speed clinical progress. Our intention was to use NF1 as a model disease to determine some of the problems that arise in moving basic research into the clinic and how best to facilitate good clinical research. In this respect, the meeting was an outstanding success.

It was apparent during the meeting that while basic research on this disease was progressing well, clinical research lagged sadly behind and many translational opportunities were missed. There appeared to be too many cases where there was insufficient clinical information available to enable therapeutic strategies to be assessed. For example, it was clear that there was a dearth of systematic, longitudinal studies on the development and growth of neurofibromas. Even the origin of the cells in the neurofibromas remains an enigma. In another case, it was very clear that although the vast majority of human cancers containing mutations in the RAS protein affected one of the three RAS proteins in humans (K-RAS), the best animal model available for assessing drugs that target the RAS pathway is a transgenic mouse expressing an activated form of a different human RAS protein (H-RAS). Thus, the relevance of this animal model to human disease and for testing existing drugs that target the RAS pathway is questionable. The latter deficiency has implications far beyond NF1 research.

As a result of lengthy discussions at the meeting and conversations since then with many investigators, a series of recommendations emerged that I believe may greatly enhance clinical studies on NF1. More importantly, however, this type of approach could become a paradigm for facilitation of needed clinical research generally.

The NF1 community of basic scientists and clinicians present at the meeting, including representatives from large pharmaceutical houses, decided to establish small working groups whose charge is to solicit consensus opinions on the deficiencies in translational and clinical research. The groups chosen for NF1 covered five areas: orthopedics, cognition, neurofibromas, malignancies, and optic glioma. Each of these areas was selected because they represent distinct clinical problems. Each group has a Chair to coordinate the agenda, which is to identify deficiencies in the clinical knowledge base, initiate new ideas for clinical research, coordinate multicenter clinical proposals, and maintain limited databases of information. These groups in turn will report to a parent steering committee (headed by Dr. Bruce Korf at the Children's Hospital in Boston) that will oversee the progress of the individual groups, coordinate research between the groups, and set any recommendations that might be forwarded to the NIH. These recommendations could be then sent to the office of the Director at an appropriate Institute within the NIH where they could decide, in consultation with the working group Chair, a suitable method of approach. In many situations, this could be a very effective mechanism for the NIH to identify high priority areas for program announcements for future peer-reviewed research. This mechanism would then ensure that the research was investigator-initiated, was deemed by

the scientific community to be of high value, and that any research funded did not bypass the normal stringent NIH standards for reviewing and funding science.

As a result of the Cold Spring Harbor meeting on NF1, the mechanism has been established, and already the groups are working to discuss how clinical research in this area might be enhanced. There is every reason to believe that this experience with NF1 could be copied for many other areas of clinical research that are tied to specific diseases or groups of disease. The NF1 meeting held here, and importantly, the mechanism for generating subsequent recommendations, could become a model for future meetings on other equally pressing medical research. It should be the responsibility of research institutions to facilitate such meetings, to host the initial meetings where the problems that exist are discussed, and then to facilitate the establishment of working groups. Ideally, institutions should coordinate these meetings with the NIH by inviting Institute Directors or appropriate program staff, as well as with research foundations and academic or research societies. Certainly, the ability to access and exchange information on the Internet will also help in such endeavors and may ultimately provide a vehicle for providing information about the disease to the public. In many ways, the search for the affected gene and a possible cure for Huntington's disease that was coordinated by Nancy Wexler from Columbia University is a shining example of how clinically relevant research might be advanced by discussion groups. Unfortunately, not every important problem in medicine has someone like Nancy Wexler to keep the momentum going. Perhaps the example set at the Banbury Center will become a valuable precedent, and it is hoped that this mechanism for facilitating clinical research will spread throughout the biomedical research community.

The NIH would need to be receptive to such proposals, but clearly if highly relevant and important science, particularly in the clinical arena, were to derive from such meetings, then I believe they would be more than welcome. At present, in many Institutes at the NIH, it has become the role of program staff to initiate and solicit new research via the "Request for Application" (RFA) mechanism. It is my experience that many of these RFAs happen to be in obvious well-funded areas and that they shy away from the "not-so-obvious" basic research and clinical research. The NF1 model will allow clinicians, scientists, and the pharmaceutical experts to provide important advice and suggestions to the Institute Directors and program staff at the NIH prior to decisions they make on the general directions for extramural research. I believe the mechanisms discussed above will not only further progress in clinical research, but also provide a valuable mechanism to collect scientist- and clinician-driven rationales for convincing the public and Congress to continue to support biomedical research.

April 26, 1996 **Bruce Stillman**

HIGHLIGHTS OF THE YEAR

Research Highlights

Cold Spring Harbor Laboratory research focuses on three major areas: cancer, neurobiology, and plant genetics. In 1995, all three areas saw exciting progress. Our neurobiology program had a banner year with tremendous breakthroughs in understanding the genetic basis of learning and memory. Senior Staff Scientist Tim Tully came to Cold Spring Harbor in 1991 concurrent with the dedication of the Beckman neuroscience building. He brought with him fruit flies and the training apparatus he had developed for them.

Tim Tully

Tully is a behavioral geneticist and performs behavioral experiments on the fruit fly *Drosophila*. Using a Pavlovian approach, he and his colleagues train the fruit flies to avoid a particular odor by associating it with an electrical shock. With the help of an outstanding research technician, Maria Del Vecchio, Tully found that normal wild-type fruit flies require ten training sessions with rest intervals in order to learn an associative learning task and to produce maximum long-term memory. Additional training sessions provide no additional benefit to long-term memory. In contrast, repeated sessions without rest intervals (massed training) produced no long-term memory. Thus, like humans, *Drosophila* remembers best when a task is learned over a period of time as opposed to learning a task crammed into one intense training session, which produces little, if any, long-term memory. By comparing the results of experiments using genetically altered fruit flies with those done using normal wild-type flies, Tully and his colleagues have associated differences in learning patterns with genetic mutations, tracking down the genes and proteins critical to the learning process.

Alcino Silva

While Tully is training fruit flies, Senior Staff Investigator Alcino Silva is one floor below in Beckman doing similar studies with mice. Silva began by studying the effects of mutations in mice on learning and memory. His work focuses on the function of the hippocampus, a region of the brain that is critical to the formation of new memories in mice and humans. He first studied how removal of the gene that produces an enzyme called CaMKII affects learning and memory in mice. Alcino uses a standardized test known as the Morris Water Maze to test learning in mice.

In the Morris Water Maze, the mice are placed in a tank of cloudy water, where a platform is hidden somewhere just below the surface. Initially, the mice do not know where the platform is, but eventually they learn with the help of a flag on the platform and large geometric shapes on the walls that serve as orientation markers, or landmarks. Both normal mice and mice lacking the CaMKII gene soon learn to find the platform easily by swimming to the flag. When the flag is removed, however, the normal mice continue to swim directly to the platform following clues from the orientation markers, whereas the mice with the CaMKII gene deleted swim aimlessly about, having failed to retain the spatial orientation necessary to remember where the platform is located. Subsequent experiments to test visual impairment, decreased motivation, and other possible effects of the CaMKII deletion proved that in every other way these mutant mice are normal—only their ability to learn how to follow the orientation markers is apparently severely impaired.

These parallel studies on flies and mice have converged on the importance of

Jerry Lin

David Beach

Carol Greider

a protein called CREB, which regulates gene transcription. Tully, his associate Jerry Yin, and Silva have discovered that the CREB gene has a critical role in the formation of long-term memory in both flies and mice.

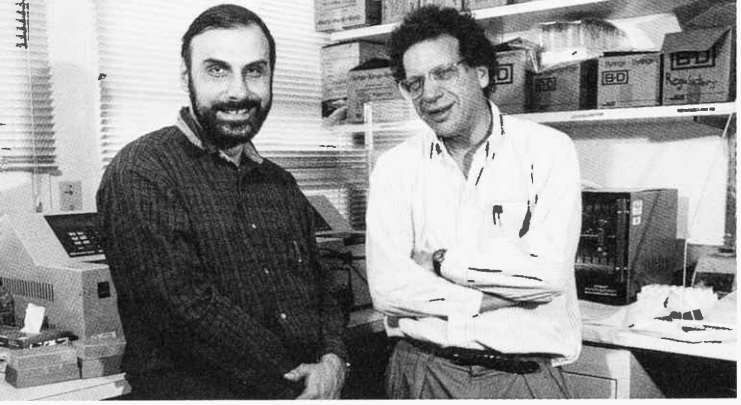
Jerry Yin, a Senior Staff Investigator, studies the genetic components of learning and memory. In collaboration with Jonathan Wallach, a graduate student in the lab of Chip Quinn at the Massachusetts Institute of Technology, Jerry discovered that the CREB gene produces two proteins, an activator and repressor. Tully and Yin blocked the activity of the CREB protein in the fruit flies by stimulating the repressor form using a genetic tool known as a heat shock promoter. By attaching the heat shock promoter to the CREB repressor, and then raising the flies' body temperature by warming the chamber in which they are trained, they were able to block the activity of CREB in the fly. To study CREB in mice, Silva obtained mice from Günther Schutz in Heidelberg that lacked the CREB gene. Experiments by both Cold Spring Harbor groups—using flies and mice—yielded the same results: CREB-impaired organisms have short-term memory intact, whereas the formation of long-term memory is impeded.

In 1995, a series of experiments by Tully and Yin produced an unprecedented result. Although in the earlier experiments they along with Alcino Silva were able to prevent learning by inhibiting the activity of the CREB protein, last year they asked whether increasing the activity of CREB would improve memory, a much more difficult experimental result to achieve. Again, using the heat shock promoter, Tully and Yin hyperstimulated the activator form of CREB. In doing so, they were able to produce *instant* long-term memory in fruit flies after only one exposure to a given task!

The generation of such "photographic" memory has not yet been accomplished in mammals—Silva does not have the luxury of using a heat shock promoter in his mice. Mice do have such a genetic switch, as do humans, but as warm-blooded creatures, they are only mildly susceptible to changes in body temperature based on changes in the atmosphere. However, as the research progresses and scientists look for other genetic tricks that might be utilized in mammals, we look forward to achieving even greater understanding of learning and memory—knowledge that may lay the groundwork for treatment of learning disabilities, Alzheimer's disease, trauma, and other disorders.

As exciting as the Cold Spring Harbor neurobiology program is, there remains an unfaltering dedication here to basic cancer research. David Beach's lab continues to make progress in studies of cell cycle control. Recent work in his lab further elucidated the role of three genes that encode related regulatory proteins that are similar to the yeast CDC25 protein, two of which—CDC25A and CDC25B—have been implicated in breast cancer. Overexpression of one or both is seen in 70% of breast cancer tumors. Work on this very promising area of cancer research is ongoing and promises to bring new light to the search for effective breast cancer treatment.

Carol Greider studies telomerase, an enzyme that she discovered 10 years ago, while a graduate student with Elizabeth Blackburn at the University of California, Berkeley. Since that time, continued research has shown that telomeres, the ends of chromosomes, are normally shortened with each replication cycle in cells that ultimately die. Telomerase maintains the length of the chromosome's ends and when absent in unicellular organisms causes them to senesce. Interestingly, telomerase is found in approximately 90% of tumor cells but not in most normal cells. 1995 was a year of extraordinary progress in the search for greater understanding of this immortalizing and potentially oncogenic enzyme.



N. Lisitsyn, M. Wigler

V. Sundaresan, U. Grossniklaus, R. Martienssen

Early in the year, Greider along with Kathleen Collins and Ryuji Kobayashi purified essential protein components of the telomerase enzyme and cloned their corresponding genes. Months later, Greider, in collaboration with researchers at Geron Corporation in Menlo Park, California, cloned the RNA component of human telomerase, termed hTR. Maria Blasco of Greider's lab cloned the telomerase RNA component found in mice, an accomplishment that will allow direct experimental testing of the role of telomerase in normal mammalian development and cancer. Greider and associates also succeeded in expressing a complementary RNA strand, which latches on to the active telomerase RNA, thereby inhibiting the telomerase activity and allowing the shortening of telomeres and ultimate cell death. We hope to know soon whether telomerase might represent an ideal target for anti-cancer drugs.

Michael Wigler and Nikolai Lisitsyn recently developed a technique for gene-finding that has led not only to significant discoveries in their own lab, but also to discoveries nationwide by assorted researchers in various fields. Representational Difference Analysis, or RDA, is a high-tech method of comparing the DNA of healthy tissue with that of diseased tissue. RDA has been used in the Wigler lab and elsewhere to locate mutations in cancer cells from the breast, kidney, bladder, lung, colon, skin (melanomas), and brain (neuroblastomas). RDA has also enabled researchers at Columbia University to identify a virus suspected of causing Kaposi's sarcoma in AIDS patients, scientists at Abbott Laboratories to identify three previously unknown hepatitis viruses, and researchers at Pathogenesis to identify a form of human herpesvirus that may be associated with multiple sclerosis. RDA was also successfully employed by researchers at Johns Hopkins to screen for mutations in pancreatic cancer. The technique was granted a patent by year end and is considered to be a most promising tool in the fight against cancer and infectious disease.

Our plant genetics program, which carries on the Nobel prize-winning work of the late Barbara McClintock, made great strides as well in 1995. Plant geneticists Rob Martienssen and Venkatesan Sundaresan and their colleagues developed a powerful new system for identifying genes involved in plant growth and development. The system utilizes McClintock's maize transposable elements and is being used to generate a large collection of *Arabidopsis* plants, each carrying a transposon tag integrated into or near a different plant gene. This system facilitated identification of the *prolifera* gene, a homolog of yeast and mammalian genes that regulate DNA replication.

With strong progress in neurobiology, cancer, and plant genetics, we now look forward to an equally exciting 1996.

Günter Blobel

Symposium LX: Protein Kinesis

This year's annual CSH Symposium on Quantitative Biology marked the event's 60th anniversary. The topic of discussion for 7 days in early June was Protein Kinesis: The Dynamics of Protein Trafficking and Stability. Günter Blobel, of the Howard Hughes Medical Institute and Rockefeller University and a CSHL trustee, delivered the annual Dorcas Cummings lecture entitled "How Proteins Find Their Addresses in Cells." Dr. Blobel presented a clear overview of the complex system of traffic and transit of proteins within cells, drawing analogies including molecules as cars and microtubules as tracks and describing the types of information encoded in the amino acid sequence of each protein. Dr. Blobel's talk was followed by the traditional Symposium dinner parties in the homes of local Laboratory supporters. At the close of the Symposium, George E. Palade, Nobel prize winner in 1974 for his work on the structural organization of the cell, delivered the summary.

At this year's Symposium, we inaugurated the Reginald G. Harris Lecture in honor of the former director of the Laboratory who initiated the Cold Spring Harbor Symposium in 1933. Harris recognized the need to bring biologists together to discuss their science and to provide a forum for making biology more rigorous; hence, the name "Cold Spring Harbor Symposia on Quantitative Biology." We were pleased to have Dr. Randy Schekman, University of California at Berkeley, present the first Reginald Harris Lecture.

Banbury Conference Center

The J.P. Morgan Executives' Meeting. It is hard to believe that 1995 marked the tenth year of our program designed for the senior executives of pharmaceutical and biotechnology companies. Initiated in 1986, these meetings have achieved a remarkable reputation for combining the best in modern research with the best from the business world. That this has been so is due in large part to our succession of sponsors—first Shearson-Lehman Brothers, then Baring Brothers, and now J.P. Morgan Co. This year's meeting was entitled Infectious Diseases: Ancient Plagues, New Epidemics and covered topics ranging from a history of epidemics to the ways in which X-ray crystallography is being used to understand immunological responses to viruses. A highlight of the meeting was the presentation by Ham Smith on the rapid sequencing of the complete genomes of *Hemophilus influenzae* (1.6 million base pairs) and *Mycoplasma genitalium* (0.6 million bases pairs). This served to show how far we have come since the first meeting on "The Genetic Knowledge of Man" in 1986. At that meeting, Lee Hood talked of his work on sequencing the whole of the HLA region, a project that was at the outer limits of what was then possible. Since we have come so far in ten years, who can tell what might be the subject of our twentieth meeting?

Meetings on Breast Cancer. Two meetings at Banbury Center exemplified our commitment to understanding and using our knowledge of the genetic causes of breast cancer. The first meeting was a joint venture of the Banbury and DNA Learning Centers to provide a forum for members of Long Island's 1 in 9 Breast Cancer Action Coalition to learn in more depth about molecular genetics and cancer. In addition to talks on the fundamentals of genetics from Mark Bloom, Dave Micklos, and Jan Witkowski, there were talks by invited speakers such as Elizabeth Claus of Yale University School of Medicine and experiments at the DNA Learning Center. The second meeting, Molecular Diagnostics of Breast

Cancer, brought together the world's leading breast cancer epidemiologists and molecular geneticists to examine the implications of the cloning of the BRCA1 breast cancer gene (an event that was followed, some two months after the meeting, by the isolation of the BRCA2 gene).

Duchenne Muscular Dystrophy. In 1980, we held a meeting at Banbury Center to examine how the then new advances in molecular human genetics could be applied to what was perceived at that time as the most intractable of the human inherited disorders. Six years later, the gene was cloned, and 15 years later, we were able to hold an open meeting in Grace Auditorium to discuss the various approaches to therapy.

The meeting was intended to provide an opportunity for parents of affected children to hear from key researchers, including Gordon Foulkes, Chief Scientific Officer of Oncogene Science, Inc. The Laboratory has been working with Oncogene Science in utilizing a new strategy in the search for compounds that will turn on a gene that may replace the one damaged in Duchenne muscular dystrophy. We cannot tell how likely success will be, but all avenues must be explored to find a treatment for this terrible disease.

50 Years of Phage

This year marked the fiftieth anniversary of phage research at Cold Spring Harbor. It was in the summer of 1945 that the first course on bacteriophage research was introduced here by Max Delbrück. An annual phage meeting ensued, commencing in 1947 in Nashville, Tennessee, and moving to Cold Spring Harbor in 1950, where it was wisely scheduled to dovetail with the phage course, encouraging course participants to stay on and attend the meeting. The course ran through 1970, after which it evolved into what is today called the ABG course, for advanced bacterial genetics, and the meeting has been at the Laboratory every year since 1950, with the exception of one. The meeting in August was both a celebration of the rich history of phage research and a forum for the presentation of new advances building upon the foundation of knowledge laid here so many years ago. Participants shared memories and data and many younger attendees

François Jacob, Gunther Stent, Marilyn Zinder

were excited to become acquainted with people whose names they knew from the classic papers.

Notable participants included Waclaw Szybalski, Evelyn Witkin, Gunther Stent, François Jacob, Franklin Stahl, Takashi Yura, Rollin Hotchkiss, Norton Zinder, Charles Yanofsky, Dale Kaiser, Boris Magasanik, and Gisela Mosig; it was especially delightful to welcome back the witty and charming Manny Delbrück. The meeting was enriched by historic displays of abstracts dating back to the 1950s, photographs, and memorabilia. As the days passed, a sign-in book for reminiscences was filled with insights from phage participants old and new, creating a wonderful new artifact for the Cold Spring Harbor phage collection. By all accounts, the event was a great scientific, historic, and social success.

Fifth Anniversary of the Human Genome Project

In November, 1995, the Human Genome Project celebrated its fifth anniversary. In honor of this milestone, a group known as The Genome Action Coalition (TGAC) sponsored the First Annual James Watson Lecture and Awards Ceremony at the National Academy of Sciences in Washington D.C.

TGAC is a voluntary association of more than 70 patient advocacy groups, professional organizations, and biotechnology and pharmaceutical companies who have pledged their support to this important project through its completion. In recognition of my role (J.D.W.) as the first director of the National Center for Human Genome Research at the National Institutes of Health, I delivered the introduction and opening remarks. Dr. Francis Collins, its current director, then gave an update on the status of the progress of genome mapping and sequencing. The featured speaker for the afternoon was Senator Mark Hatfield of Oregon, the Chairman of the Senate Appropriations Committee and one of the leading congressional advocates of biomedical research. Senator Hatfield spoke about the relationship between groundbreaking genetics research and public policy.

The event was also honored by the attendance of Secretary Donna Shalala of the Department of Health and Human Services, who was awarded the TGAC National Policy Leadership Award, and Senator Pete Domenici of New Mexico, Senator Tom Harkin of Iowa, and Congressman John Edward Porter of Illinois, who received TGAC Congressional Awards for their support of basic research.

Thomas Jessell

Board of Trustees

In 1995 the Board of Trustees nominated and elected three new members: William E. Murray, Whitney D. Pidot, and Lola N. Grace.

Mr. Murray is an attorney in private practice specializing in estates and trust. Bill has been a friend to the Laboratory for many years—he assisted in establishing the Samuel Freeman Computer Laboratory (1985), the Neuroscience Center (1988), and the Samuel Freeman Laboratory of Cancer Cell Biology (1989).

Mr. Pidot is an attorney in corporate law, currently a partner at the firm Shearman & Sterling in New York City. Mr. Pidot is a long-time neighbor of the Laboratory residing in nearby Locust Valley.

Mrs. Grace is a managing director of Sterling Grace Capital Management in New York and is a director of the Society of Memorial Sloan-Kettering. Lola and her husband John are part of an outstanding family of Laboratory supporters. The late Oliver Grace, John's father, and his wife Lorraine have been true friends of

the Laboratory for many years; in 1984, they gave funds toward the construction of Grace Auditorium, allowing the Laboratory to create an exceptional forum for world-renowned scientific meetings, and in 1987, they established the Oliver Grace Chair for Cancer Research which I (J.D.W.) am privileged to hold.

Thomas Jessell completed his six-year term on the Board this year. Dr. Jessell, a former instructor of Cold Spring Harbor neuroscience courses, continues his studies on developmental neurobiology at Columbia University and was recently elected a Fellow of the Royal Society. We wish him the best in his future endeavors.

Townsend J. Knight, Esq., completed his most recent term on the Board and has been named an Honorary Trustee. Townie is a descendant of John D. Jones, founder of the Biological Laboratory at Cold Spring Harbor in 1890 and of the Wawepex Society, an organization established to administer the family's philanthropic interests. Among the family's charitable ventures were the donation of land and monies that were used to establish the first research laboratory at Cold Spring Harbor (known now as Jones Laboratory) built in 1893 and the establishment of the Knight Trust. Townie has served on the Board for many years (1971–1975, 1982–1995), and his continuing presence as an honorary trustee will provide us with invaluable advice and counseling.

Townsend J. Knight, Esq.

Trustee Receives Lasker Award

Scientific trustee Don Wiley, Ph.D., of Harvard University, received the 1995 Albert Lasker Medical Research Award for distinguished contributions to biomedical research. Dr. Wiley, professor of biochemistry and biophysics at Harvard University, was recognized for his revolutionizing work in immunology.

Robertson Research Fund

A tradition of giving was established by the Robertson family of Lloyd Harbor back in 1973, when Charles Robertson gifted nearly $8 million to the Laboratory to create the Robertson Research Fund. At that time, he also initiated the transfer of the Robertson property on Banbury Lane, now the site of our Banbury Conference Center, and a fund for its maintenance. Then, two years later in 1975, the Banbury Fund—the Robertson family's private foundation—established the Marie H. Robertson Memorial Fund to support neuroscience research at Cold Spring Harbor. Its availability soon after allowed the establishment of year-round research in leech neurobiology by Birgit Zipser in the newly renovated Jones Laboratory.

In 1995, the Robertson Research Fund—its value now up to $56 million—distributed $1.6 million to basic research at Cold Spring Harbor, supporting 16 staff scientists; CSH fellow Scott Lowe, who works in James Laboratory on apoptosis and cancer therapy; postdoctoral fellow Chantal Autexier, who studies telomeres (the ends of chromosomes) in Carol Greider's lab; and graduate student Shirley Pinto, who is studying the *latheo* gene, implicated in learning and memory in *Drosophila*, in Tim Tully's lab. The Marie Robertson Memorial Fund also supported research into the genetic basis of learning and memory in *Drosophila* in the Tully lab, including support for postdoctoral researcher Michael Regulski, and in mice in Alcino Silva's lab, including support for postdoctoral researcher Yoon H. Cho.

Major Gifts

We are again grateful for the strong support of our Trustees, interested individuals, foundations, corporations, and other charitable organizations. A major priority this year was the proposed acquisition of the beautiful Friends World College property in Lloyd Harbor. Through the generosity of our trustees, the Dolan Family Foundation, Thomas Saunders, David Luke, Edwin Marks, John Reese, an anonymous donor, and the William Stamps Farish Foundation, we received pledges in excess of $3 million toward the purchase. In consideration of concerns voiced by a group of community members, we have withdrawn our application for the use of the Friends World College campus from the Village of Lloyd Harbor and, with our Board of Trustees, are discussing alternative projects to meet the needs for administrative and editorial space and additional scientist housing. We deeply appreciate the support of our friends at a time when we hoped to preserve the lovely 30-acre estate while fulfilling pressing needs of the Laboratory.

Our Undergraduate Research Program (URP) received important additions to its endowment with gifts of $100,000 from The Garfield Foundation; $100,000 from the estate of Joan Redmond Read; $60,000 from our friend and colleague Bentley Glass; $40,620 from the Burroughs Wellcome Fund, proceeds of $51,000 from the wonderfully warm and well-received concert given by Flicka von Stade and Friends in the spring, and $7,500 from Jephson Educational Trust.

The Laboratory science programs were well supported during the year with a gift of $290,000 from Westvaco for the plant genetics program; $150,000 from the McKnight Endowment for Science and one of $120,000 from the John Merck Fund, both for Dr. Alcino Silva's lab; $150,000 from the Prichard Trust for scientific equipment; $100,000 from the Glaxo Research Institute and $75,000 from Alan and Edith Seligson, each toward postdoctoral research fellowships; $45,000 from 1 in 9: Long Island Breast Cancer Coalition for Dr. Mike Wigler's lab for cancer research, especially breast cancer; $30,000 from the Goldring Family Foundation for postdoctoral support in Dr. Bruce Stillman's lab; $20,000 from the Irving A. Hansen Memorial Fund; $20,000 from Arrow Electronics for equipment; $15,000 from the Lauri Strauss Leukemia Foundation for Dr. Nick Tonks; $15,000 from Mrs. Oliver R. Grace for equipment; $10,000 from the Dextra Baldwin McGonagle Foundation and $10,000 from the Edward S. Moore Foundation for scientific equipment.

A most gracious gift was made by the Olin family in the form of three magnificant sculptures. Gerry and Sue Lin have been generous supporters of the Laboratory and recently, after the death of Sue's father, William Cohen, the Olins had to handle the distribution of his estate. Located on Mr. Cohen's property in Sands Point were original works of art ranging in height from 6 feet to 12 feet tall. The Olins were interested in placing these large sculptures in an appropriate environment and chose to offer them to the Laboratory to be placed on our beautiful grounds.

A cor-ten steel sculpture called *Profile/Canto #1*, by Ernest Trova—an imposing 8 feet high and 12 feet across—was relocated to the field on Bungtown Road across from the Yellow House. *Draco*, a gray Vermont marble piece by Kenneth Campbell now stands on the lawn of Ballybung while a second Campbell piece, twice the height at 12 feet and crafted of white marble, named *Pyramus and Thisbe*, stands just outside the north door of the Beckman Neuroscience Center. The campus has been both enriched and beautified with the addition of these one-of-a-kind heirlooms.

James D. Watson, Sue Olin, and Gerald Olin

Our endowment funds fared well, led by a gift of $150,000 from Bill and Marjorie Matheson for the Matheson Fund; $50,000 from the Banbury Fund for the Robertson Chair; and an addition of $74,000 to the Harrison Chair.

Support for research into the causes of Duchenne muscular dystrophy continued, with a contribution of $52,000 from John Cleary. The DNA Learning Center received a grant of $50,000 from Genentech to help in the construction of the new Cellarium exhibit and the Barker Welfare Foundation provided $15,000 for program support.

The companies contributing to the Cold Spring Harbor Corporate Sponsor Program continue to provide a very substantial underpinning of the meetings program in Grace Auditorium and at the Banbury Center. Full acknowledgment of their generosity can be found in the Finance Section.

DNA Learning Center

Story of a Gene opened in May. The first exhibition to be developed in-house at the DNA Learning Center (DNALC), it traces the story of human growth hormone (HGH), an ideal case study in modern human genetics. HGH affects an obvious physical trait (height), its physiology and cell biology are well understood, and it was one of the first human therapeutics produced by recombinant DNA. The backdrop for the exhibit is the remarkable Cellarium, a room-size mural depicting the expression of the hormone within a single pituitary cell. Interactive exhibits challenge visitors to consider the parameters of human growth and the physical effects of growth hormone, as well as the controversial use of growth hormone to improve lifestyle for humans and milk production in dairy cows. November saw the publication of *Laboratory DNA Science*, a college-level course in recombinant DNA techniques developed by Learning Center staff members Mark Bloom and Dave Micklos, in collaboration with Cold Spring Harbor alumnus Greg Freyer of Columbia University.

The DNALC continues to play a unique part in promoting genetics education. In 1995, no fewer than 8,000 students and teachers came to the DNALC for hands-on DNA workshops and an additional 10,000 visitors saw the exhibits Story of a Gene and Long Island Discovery.

Scientists Recognized

Our reputation as an institution staffed by the best and the brightest is frequently reinforced by recognition in the form of awards to our scientists. This year, two Cold Spring Harbor scientists received major prizes: Michael Hengartner received the first annual Pharmacia Biotech & Science Prize for Young Scientists. Sponsored by *Science*, the journal of the American Association for the Advancement of Science, the award recognizes outstanding molecular biologists early in their careers. The grant of $20,000 was formally presented at a ceremony in Sweden and recognized the excellence of Michael's Ph.D. thesis on apoptosis, or programmed cell death, which was completed while he was a graduate student at the Massachusetts Institute of Technology.

Carol Greider was honored by the American Society for Cell Biology, with the presentation of the first annual Glenn Foundation Award at the ASCB's 35th annual Symposium in December. The $5,000 grant is in recognition of Greider's work on telomerase, a protein implicated in extending the lives of cancer cells indefinitely.

Michael Hengartner

CSHL Press

Twenty new books were published, plus a new videotape set and an audio tape/CD. The total titles in print now number more than 220. The most successful new title was *PCR Primer*, a laboratory manual on amplification techniques, which sold more than 2,500 copies when released in the last quarter of the year. Also notable were four other lab manuals developed from practical courses taught at the Laboratory and two scholarly monographs edited with colleagues by two of the Laboratory's senior scientists: *Telomeres*, assembled by Carol Greider and Elizabeth Blackburn, and *Translational Control*, edited by Michael Mathews with John Hershey and Nahum Sonnenberg. A special reprint of François Jacob's autobiography *The Statue Within* was published to coincide with the fiftieth anniversary of the Laboratory's bacteriophage course.

The journal program continued to expand in scope and frequency. The successful five-year old bimonthly methods journal *PCR Methods and Applications* evolved into the monthly research journal *Genome Research*. The print issues were supplemented with information such as video clips made available through the journal's World Wide Web site, the first use of the Internet in this way by a science journal. *Genes & Development* consolidated its position in the top ten of the world's biology journals with another successful year editorially, increased circulation, and an increasingly important contribution to the financial performance of the Press.

The first issue of the annual directory, *The Lab Manual Source Book*, contained information from more than 600 suppliers about 15,000 products used in laboratory work with genes, cells, and proteins. It was distributed to 40,000 scientists worldwide. In addition, an interactive database, BioSupplyNet, was created on the World Wide Web to allow scientists rapid access to more detailed information about available products and their sources of supply.

CSHL Association

The annual meeting of the CSHL Association began with lunch at Ballybung on February 5 and was followed by a special lecture by Rich Roberts, the Laboratory's most recent Nobel Laureate. Roberts, who won the highest award of science in 1993, spoke to the audience of directors and friends of the Laboratory about his work on RNA splicing and about the extraordinary festivities associated with the Nobel prize ceremony in Stockholm, Sweden.

Joan Pesek, Associate Director for the annual fund, left the Association after nearly nine years; we will miss her enthusiastic participation. Jean Schwind, former grants assistant here at the Laboratory, has stepped into the position and is effectively handling the many facets of Association fund-raising.

One of the goals of the Association is to reach out to a new generation of supporters for basic research. Toward this end, the Association has initiated the Next Generation Outreach Program, a lecture series designed to stimulate the interest of a generation of 30–40-year-old community members and to bring the importance of basic research home to the audience. In the first lecture, Tim Tully discussed breakthrough discoveries in learning and memory and their implications for humans. The second lecture included Carol Greider, who discussed the importance of fellowships on the path to independent research, and Winship Herr, who discussed educational programs at the Laboratory. In the third lecture, Michael Wigler addressed what is probably the most prevalent question about basic research today: What kind of progress are we making in cancer research?

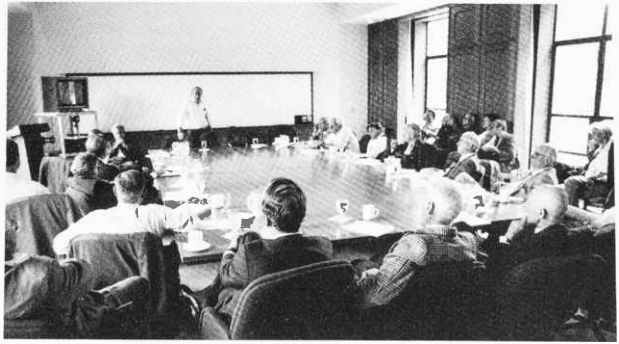
The final lecture featured David Micklos of the DNA Learning Center, who discussed personal and social implications of reading DNA.

The Association has an outstanding history of support of science and education here at the Laboratory, support that has played a crucial part in fostering our research, especially that of our young scientists. We are sure that through this outreach we will inspire participation by the next generation.

President's Council

The President's Council was formed in an effort to bring together a small group of individuals with a keen interest in science and the work of Cold Spring Harbor Laboratory. Through their annual commitment of $25,000, the members provide support for the Cold Spring Harbor Fellows program. The funding is critical in attracting top young scientists fresh from their Ph.D. studies. It allows them the opportunity to pursue their own research, rather than assisting in the laboratory of an established scientist.

A major feature of the Council is an annual meeting to bring together this select group of leaders from business, finance, and science to discuss the latest developments in genetic research and biotechnology. The Council's first meeting, held May 12–13, began with lunch at Ballybung for Council members and their guests. Senior Scientist Adrian Krainer and CSH fellow Ueli Grossniklaus spoke to the group about the importance of the Cold Spring Harbor Fellows program in allowing scientists to do unrestricted independent research. The discussion at the meeting focused on the Societal Implications of Modern Human Genetics. The mix of minds of leaders in the business world and scientific community evoked provocative discussion as to the implications and issues that are coming to the forefront as our knowledge of the genetics of human behavior increases. The keynote speaker, Dr. Nancy Wexler, Columbia University College of Physicians & Surgeons, opened the meeting on Friday evening with her talk on The Promise and Perils of Human Genetics. A panel discussion was held on Saturday morning to discuss the ethical, legal, and social issues arising from our increasing genetic knowledge. Leading the discussion were Dr. Tom Bouchard, University of Minnesota; Dr. Tom Caskey, Merck & Co. Inc.; Dr. Tom Murray, Case Western Reserve University School of Medicine; and Dr. Norton Zinder,



President's Council

Rockefeller University. The day and the meeting ended with the guests gathering once again at Ballybung for a parting luncheon.

Founding members of the President's Council include Abraham Appel, Appel Consultants; Michel David-Weill, Lazard Freres & Co.; Frederick Frank, Lehman Brothers, Inc.; Leo A. Guthart, ADEMCO; Charles E. Harris, Harris & Harris Group, Inc.; Walter B. Kissenger, Long Island Research Institute; David Mahoney, The Charles A. Dana Foundation; Donald A. Pels, Pelsco, Inc.; George B. Rathmann, ICOS Corporation; Frank E. Richardson, Wesray Capital Corporation; Hubert J.P. Schoemaker, Centocor, Inc.; James H. Simons, Renaissance Technologies Corp.; George Soros, The Soros Foundations; Margo Walker, Citivilla Properties; and Sigi Ziering, Diagnostic Products Corporation.

Special Events/Fundraisers

On March 25, Flicka von Stade and Friends charmed and entertained a packed house in Grace Auditorium for a second time. The very talented and equally beautiful diva strayed from her operatic style, performing a spectacular blend of ethnic folk songs, bluesy jazz, a little ragtime, and several rearrangements of classics by Bach and Shubert. Accompanied by Bill Crofut on banjo and vocals, Chris Brubeck on bass and trombone, and Joel Brown on guitar, Flicka raised additional funds for the Frederica von Stade Endowment, a fund that will provide an annual fellowship for our Undergraduate Research Program.

In June, 1 in 9: The Long Island Breast Cancer Action Coalition held their second annual Michael Scott Barish Sand Soccer Tournament in Long Beach. In October, several Lab members were invited to participate in 1 in 9's first annual Governor's Dinner Dance at the Seawane Club in Hewlett Harbor. Proceeds of the outdoor sporting event and the black-tie gala attended by Governor George and Libby Pataki were donated to the Laboratory in support of Michael Wigler's lab and the Human Cancer Fund. In a public ceremony in November, 1 in 9 presented the Laboratory with a check for $45,000, more than doubling their initial grant to us in 1994 in support of Dr. Wigler's cancer research.

Carol Large

In September, CSHL Association director Carol Large coordinated a new event at Cold Spring Harbor: the Old Westbury Gardens Tree Symposium. Sponsored by the Westvaco Corporation, the event was conceived by Carol and organized in cooperation with Old Westbury Gardens and the Cornell Cooperative Extension of Nassau County. Horticulturists, gardeners, and other lovers of things green were drawn to Grace Auditorium to hear a day-long forum where horticulturists, landscape designers, and plantsmen presented, discussed, and debated their views on the present and future use of trees in the landscape. Participants included noted author Professor Michael Dirr of the University of Georgia and Robert Halpern of the Wildlife Conservation Society. The benefit raised nearly $8,000 for the CSHL Association which supports scientific research by supporting research fellows.

A most rewarding cultural event is our Young Artist Concert series. Young classical musicians, many of whom have played to audiences around the world, perform in Grace Auditorium during our scientific meetings. Melvin Chen and Alexis Pia Gerlach performed in April at the Cytoskeleton meeting, on piano and cello, respectively. In May, Todd Palmer played clarinet and Margaret Kampmeier played piano before an audience of scientists interested in RNA processing. Respite from transcription factor studies in September was provided by Catherine Cho and Benjamin Loeb on violin and piano. Violinist Dmitri Berlinsky

and pianist Elena Baksht, both natives of Russia, provided melodic entertainment for the DNA replication crowd gathered at the Laboratory later in September with pieces from Ravel, Tchaikovsky, and Franck.

Some of the Young Artists performances are organized through Young Concert Artists, Inc. (YCA), a management company; others are privately sponsored. All are conducted free of charge for the scientists working at and visiting Cold Spring Harbor Laboratory. The Laboratory's long-time friend Roger H. Samet graciously sponsored the Chen/Gerlach performance, and Dr. Mark Ptashne, moved by an earlier YCA performance, donated $5,000 through the D'Egville Foundation to provide support for the promotion of young artists and was the sponsor for the Cho/Loeb concert.

Family Spirit

The events that contribute to the Laboratory's family atmosphere spanned the year. The Easter Egg Hunt returned to Airslie lawn this past spring, sending children with baskets scampering about in search of brightly colored eggs. The summer brought the annual July Staff Picnic, which took place on one of the notoriously hot, over-100° days. Well-attended by staff, family, and friends, the harbor beach was the most popular place to be, second only to the shade beneath the trees along the shore.

Laurie Landeau, our long-time friend and former trustee, held her annual August beach party for the scientific staff at her still wonderfully undeveloped Eaton Neck property. In the cold of winter, children of Laboratory staff attended the annual Christmas party replete with gifts for every child, and food and drink for everyone. Afterward, the crowd gathered around the Christmas tree on Bungtown Road for caroling and the tree lighting. Carolers were distracted from the cold by the smooth sounds of the American Concert Band, a five-piece brass ensemble well-versed in the traditional songs of the season.

The Lab Makes History

In a satisfying culmination of 25 years of historic preservation here at Cold Spring Harbor, the Laboratory was honored in June with a certificate proclaiming its historic significance at both the state and national levels. Thanks to the tireless efforts of Liz Watson and her dedication to the architectural and historical integrity of each building on the grounds of the Laboratory, we have succeeded in earning a place on the New York State and National Registers of Historic Places (NRHP).

The nomination to the Register of Historic Places was initially inspired by the magnificent and historically true restoration in 1980 of Davenport House—the pumpkin-colored Victorian house built in 1884 that faces Route 25A at the entrance to the Laboratory. As Liz assembled the NRHP proposal, it became clear that virtually all of our buildings were historically significant: Airslie House, built in 1806 for Major William Jones; Davenport Laboratory (now Delbrück Laboratory), built in 1926 and subsequently named for Nobel prize winner Max Delbrück who established the famous "phage course" at Cold Spring Harbor; our grants and development building, Wawepex, built in ca. 1825 during the whaling era and named for the Indian word for "at the good little watering place." Indeed, the land itself, including the field on which Barbara McClintock grew the corn

Liz Watson, Bernadette Castro

used in her Nobel prize-winning work, earned a place on the National Register, as did the Cold Spring Harbor Fish Hatchery, established in 1887.

The designation celebrates not only the historic scientific and cultural significance of the Laboratory, its grounds, and the Fish Hatchery, but also the dedicated efforts of Liz and of Jack Richards of our Buildings & Grounds Department, who supervised much of the restorative construction. Bernadette Castro, Commissioner of New York State Department of Parks, Recreation, and Historic Preservation, presented a beautifully framed certificate to Liz at a breakfast ceremony in Blackford Hall on June 2. Among those in attendance from the Laboratory were Dr. Bruce Stillman, Director; Morgan Browne, Administrative Director; Dr. Winship Herr, Assistant Director; Arthur Brings, Director of Facilities; Susan Cooper, Director of Public Affairs and Development; Nathaniel Comfort, Science Writer who supplied the written documentation necessary for the nomination; and Jack Richards. Representing the CSH Fish Hatchery were Norman Soule, Director, and his wife Mary Ann and board members Mary Jo Hossfeld, Richard Cohen, George Dennis, Carol Dubois, Chris Nuccio, Muffy Osterhus, Mark Trotter, and Charles Holcomb. In addition, Allison Hain of School District 2 and Laurie Hempton, Historic Preservation Field Representative for the New York State Office of Parks, Recreation, and Historic Preservation (who worked so diligently with Liz to bring the project to fruition), were on hand to celebrate the designation.

Elizabeth Borden

Gavin Borden Lecture

Our graduate students are a vital part of Cold Spring Harbor Laboratory, both for their research and for the vitality they bring. This year, the Laboratory instituted a new event to honor a friend to science and the Laboratory and to acknowledge the role of the graduate students. The Gavin Borden graduate student seminar and lecture was named for the energetic and charismatic publisher of *The Molecular Biology of the Cell* (MBC) who died in 1994 of cancer.

Bruce Stillman (*Left*) and Bruce Alberts (*center*) at the Gavin Borden Lecture

The program opened on March 9 with a lecture by one of the six authors of *Molecular Biology*, Bruce Alberts, President of the National Academy of Sciences and Professor of Biochemistry and Biophysics at the University of California. The lecture, geared toward graduate students—Borden's target audience as a writer—was entitled "Avoiding a Train Wreck: What Happens When RNA Polymerase and DNA Polymerase Collide." After the lecture, there was a reception for Dr. Alberts, and then he and the graduate students had dinner together.

The following day, Dr. Alberts met individually with me (B.S.) and Assistant Director Winship Herr, and senior scientists Carol Greider, Michael Wigler, Nick Tonks, and Tim Tully. After a lunch with members of the scientific staff, Dr. Alberts met with all of the graduate students for an informal discussion. Topics discussed included the varied careers that young scientists can consider. Dr. Alberts' visit ended with supper at Ballybung with the Watsons. The education of graduate students was a mission dear to Gavin Borden's heart and the Laboratory proudly and enthusiastically supports that important charge.

Funding for the creation of the Gavin Borden Lecture series was provided by Gavin's widow Elizabeth Borden, myself (J.D.W.) and Liz, Dr. Bruce Alberts, and Robert Winthrop.

Undergraduate Research Program (URP)

The Undergraduate Research Program at Cold Spring Harbor Laboratory provides an opportunity for college undergraduate students to participate in active research projects under the supervision of Laboratory staff scientists. Since the inception of the URP program in 1959, 440 students have participated in the course and many have gone on to productive careers in biological science.

The URP seeks to provide not only a greater understanding of the fundamental principles of biology, but also an increased awareness of experimental approaches to science and a deeper understanding of the major issues in the fields of genetics and molecular and cell biology. The participants also garner a personal acquaintance with research, research workers, and centers for study.

This year, 22 students were selected from more than 280 applicants. Support for the URP was provided by Bio-Rad Laboratories, Burroughs-Wellcome Fund, C. Bliss Memorial Fund, The Garfield Internship, Hanson Industries, Jephson Educational Trust, Libby Internship, National Science Foundation, Phillips Petroleum Foundation., Inc., Powers Foundation, William Shakespeare Internship, and the Frederica von Stade Internship. (For a list of these students by name and university, see the URP Section in this Annual Report.)

Partners for the Future

In a mission to open Laboratory doors to an ever younger audience, the Laboratory has solicited nominations from all Long Island high schools each year since 1990. The Partners for the Future program is now in its sixth year, and once again, we have five talented high school students doing original research in the laboratories of staff scientists. The five students chosen to participate in the program come to the Laboratory each day after school, October through March, and do original experiments under the guidance of a scientist mentor. The culmination of the program is an oral presentation by the students in which they present the results of their months of study to an audience of proud parents, teachers, and mentors.

The 1995–1996 Partners, their schools, and mentors are Hayley Solomon of Plainedge High School with Dr. James Dezazzo; Danielle Cain of Sachem High School in Lake Ronkonkoma with Dr. Michael Regulski; Ilana Kurshan of Huntington High School with Dr. Venkatesan Sundaresan; Dominik Rosa of Commack High School with Dr. Hong Ma; and Elizabeth Tegins of St. Anthony's High School in South Huntington with Dr. Kim Arndt.

Project WISE

In an effort to encourage bright young women to enter into the fascinating world of science and technology, the Laboratory has entered into a program with the State University of New York, Stony Brook, called Project WISE (*W*omen *i*n *S*cience and *E*ngineering). Stony Brook recruited our participation, as well as that of Brookhaven National Laboratory and the American Association of University Women, in this program designed to expose high school girls to the sciences. Mary Horton of our Grants Department orchestrated our involvement, which included sponsoring two trips to the DNA Learning Center. On the first trip, the students used restriction enzymes to cut DNA from bacteriophage λ and then analyzed the fragments, all under the guidance of Laboratory scientist Diane Esposito.

On the second trip to the Learning Center, each young woman isolated a sample of her own DNA and fingerprinted it using the automated polymerase chain reaction (PCR). In addition to the DNA Learning Center workshops, the participants had dinner with and visited the laboratories of six Laboratory scientist mentors: Hollis Cline, Carol Greider, Ann Sutton, Roberto Malinow, Luis Peña, and John Horton. Each student participates for four years—9th through 12th grade. The Laboratory will, each year, instruct the 10th graders in molecular biology and genetics, offering a foundation upon which they can build a future in technology and the sciences.

Top row: James D. Watson, Mike Riggs, Bruce Stillman
Middle row: Madeline Wisnewski, Linda Rodgers, Annette Kirk
Front row: Marlene Rubino, Andrea Stephenson, Georgia Binns

Long-term Service

Nine Laboratory employees celebrated long-term service anniversaries this year. Madeline Wisnewski, scientific secretary, celebrated her 25th year with us. Madeline joined us in 1970 and has worked in various offices throughout the Laboratory, including as secretary to Dr. Watson, before landing in Demerec Laboratory where she works with Drs. Helfman, Greider, and Spector. Vincent Carey, grounds foreman, and Robert McGuirk, senior laboratory technician, both celebrated their 20-year milestones. Fifteen-year honorees included Michael Riggs, laboratory technician, and Linda Rodgers, research associate, members of Michael Wigler's lab; Bruce Fahlbusch, buyer for the Buildings & Grounds Department; Georgia Binns, research associate in Ryuji Kobayashi's lab; Marlene Rubino, administrative assistant in Environmental Health & Safety; and Andrea Stephenson, now meetings administrator.

Changes in Administrative Staff

This was a year of transition for the Laboratory's computer department. Fred Stellabotte, former Computer Systems Manager, left us to take a position with the Avis Corporation. Jerry Latter, former manager of the Quest Protein Database, was appointed Director of the Information Services Department (ISD) and charged with improving and restructuring the Laboratory's use and support of computers. Latter is orchestrating improvements in the staffing, networking, and approach to troubleshooting for the extensive network of hardware and software of some 560 employees who use a variety of computing systems.

The Buildings & Grounds (B&G) Department has undergone administrative changes as well. Jack Richards retired from his position as Director of B&G, but he remains active in architectural and construction projects. Jack was convinced to join the Laboratory in 1969 after bidding on the renovation of the James Laboratory Annex. Over the years, he has played a valuable part in consulting

with architects and supervising construction and renovation; his attention to detail and quality workmanship during the preservation of many Laboratory buildings was without question a factor in the Laboratory's successful nomination to the State and National Registers of Historic Places. As Jack would tell it, however, it was the construction of the wastewater treatment plant in the 1970s that presented the greatest challenge. That facility, planned and constructed under his direction, maintained a perfect record for meeting health department standards during its years of operation and is among the most attractive settings on the harbor. It is with great pleasure that we acknowledge Jack's extraordinary contributions by naming the future B&G building in his honor.

Art Brings has stepped into the Director's position, managing the staff of 80 who comprise B&G, Environmental Health and Safety, Security, and the Harris Animal Facility. It is a credit to Jack and Art that we have maintained and beautified the grounds and buildings, continually striving to provide not only a pleasant, but also a healthy and safe working environment for all members of the Laboratory staff.

Changes in Scientific Staff

Each year, the Laboratory is infused with fresh new intellect ranging from bright and enthusiastic graduate students and postdoctoral researchers to experienced senior staff scientists. At the same time, we inevitably see the departure of others.

Dan Marshak, a protein chemist who was a part of the Laboratory for 10 years, went on to a position with Osiris Therapeutics, Inc. in Baltimore, Maryland, where we wish him all the best. Nikolai Lisitsyn, Senior Staff Investigator and codeveloper of a powerful new technique for genetic research known as Representation Difference Analysis (RDA) with Michael Wigler, has accepted a position as assistant professor at the University of Pennsylvania, School of Medicine in Philadelphia and Hiroyuki Nawa, Senior Staff Investigator interested in mechanisms of neuronal communication, accepted a professorship at Niigata University in Japan. Harriet Feilotter, Staff Investigator with us since 1990, was an integral part of the Dana Consortium—the Laboratory's collaboration with researchers at Johns Hopkins and Stanford University established to identify genetic components of manic depressive disorder. Harriet has moved to Queen's University in Ontario as a research associate.

Several staff associates have accepted positions elsewhere and have departed Cold Spring Harbor: William I. Chang left the Marr lab to accept a position as Senior Software Engineer at Infoseek Corporation in Mountainview, California; Mi Sha left the Roberts lab to a position as staff scientist at the Genetic Institute in Cambridge, Massachusetts; Ann Sutton departed from the Arndt lab to accept a visiting professorship at SUNY, Stony Brook; Xu Duffy from the Wigler lab went to a research scientist position at North Shore University Hospital here on Long Island; and Michael White moved to the University of Texas Southwestern Medical Center in Dallas, as an assistant professor. Computer scientist Marty Hiller left the Marr lab this year to fill the same position with Millenium Pharmaceuticals in Cambridge, Massachusetts, and Howard Hughes Medical Institute associate Hui Zhang left the Beach lab to become a lecturer at Yale University School of Medicine.

Five visiting scientists completed their stays at the Laboratory in 1995. Young-Seuk Bae returned to Kyungpook National University in South Korea from the

Marshak lab; Doug Demetrick returned to Foothills Medical Center at University of Calgary in Canada from the Beach lab; Carina Dennis left the Helfman lab to return to the Ph.D. program at Oxford University in the United Kingdom; Boris Kuzin returned to the Russian Academy of Science in Moscow from the lab of Grigori Enikolopov; and Peter Barker departed from the Wigler lab.

Two special visitors to the Laboratory, Jonathan Montague and Greg Jeffries, have returned to the United Kingdom. Both graduates of Eton College in Windsor came to Cold Spring Harbor to study with our scientists for one year before proceeding on to university life. Jonathan studied plant genetics in Rob Martienssen's lab and has gone on to begin his studies at Oxford University. Greg worked with Michael Hengartner on studies of programmed cell death in *C. elegans* and is now attending Cambridge University. It was our pleasure to host Jonathan and Greg, and we wish them well in their studies.

New Staff Members

More than 30 new postdoctoral researchers and 20 graduate students joined the Laboratory this year. Tatsuya Hirano joined us as a Senior Staff Investigator, after 6 years at the University of California, San Francisco, studying chromosomal dynamics. Leemor Joshua-Tor also signed on as a Senior Staff Investigator; she arrived from Caltech in Pasadena to join our Keck Structural Biology Laboratory. Peter Nestler made the short trip from Columbia University in New York to join us as a Staff Investigator. Peter will be working on combinatorial chemistry.

We have several new visiting scientists this year: Benjamin Horwitz came from Israel to work on plant genetics in the lab of Hong Ma; Isabelle Jupin arrived from the Institut Jacques Monod University in France to work with Linda Van Aelst on viral/host interactions in viral RNA replication; Leslie Kerrigan came up from Osiris Therapeutics in Baltimore to work in Dan Marshak's lab with Timothy Connolly, who was here on sabbatical; Boris Kuzin from the Russian Academy of Science in Moscow is studying the role of nitric oxide in *Drosophila* development in Grigori Enikolopov's lab; Roberta Maestro has come to the lab of David Beach from CRO Avino in Italy to study the p53 gene and apoptosis; R. Sanders Williams came on sabbatical leave from University of Texas, Southwestern Medical Center to work in the Stillman lab on regulation of DNA replication; and De Ye from the Biotechnology Institute in University Park, Pennsylvania, joined Venkatesan Sundaresan's lab and is working on embryogenesis in *Arabidopsis*.

Promotions

Several staff members were recognized this year with promotions. Two scientists, Tim Tully, Cold Spring Harbor Laboratory's *Drosophila* learning and memory expert, and plant geneticist Rob Martienssen were promoted from Senior Staff Investigator to Senior Scientist. Staff Investigators Michael Hengartner and Yi Zhong—both having arrived fresh out of graduate school—were promoted to Senior Staff Investigator positions. Michael will continue to study apoptosis, and Yi will focus on neural development and learning and memory. Joseph Colasanti, a postdoctoral researcher in Venkatesan Sundaresan's lab, and Michael White, a postdoc in the Wigler lab, were both appointed Staff Associates. Shou Waga, a postdoc in the Stillman lab who studies mechanisms and regulation of eukaryotic DNA replication, was promoted to Staff Investigator.

Postdoctoral Departures

Masahiro Akiyama left Bruce Stillman's lab for an associate professor position at the Nara Institute of Science and Technology in Japan; Zoltan Asztalos went from Tim Tully's lab to a scientist position with ERATO, the Yamamoto Behavioral Genes Project in Tokyo; Wei Guo left David Helfman's lab to continue postdoctoral research at the Scripps Research Institute in San Diego; Chang-Deok Han went from a postdoctoral position with Rob Martienssen to a position as a research scientist at the Gysong-Song National University in Korea; Keiko Mizuno left Hiruyuki Nawa's lab; Piruz Nahreini went from Mike Mathews' lab to a Scientist II position at Ribozyme Pharmaceuticals Inc., in Colorado; and Catherine Weiss left Hong Ma's lab to be a research biologist at American Cyanamid, Agriculture Research Division. David Beach's lab saw the departure of three postdoctoral researchers: Scott Davey to an assistant professorship at Queen's University, Cancer Treatment and Research Foundation in Ontario; Taekook Kim to a research associate position at Harvard University, Molecular and Cellular Biology Department; and Brad Nefsky to continue his postdoctoral research elsewhere. Ariel Avilion and Kathleen Collins both left Carol Greider's lab—Avilion to do her postdoctoral research in Robin Lovell-Badge's laboratory at the MRC in London and Collins to an assistant professorship at the University of California, Berkeley, Department of Molecular and Cell Biology. Nick Tonks said good-bye to Susann Brady-Kalnay, who left to become an assistant professor at Case Western Reserve University in Cleveland, and to Hong Sun, who is now assistant professor at Yale University, Department of Genetics. Yoon Cho left Alcino Silva's lab to do additional postdoctoral research at the Center for Behavioral Neuroscience at SUNY Stony Brook and Bruno Frenguelli left the Malinow lab to continue his postdoctoral research at the University of Bristol, Anatomy Department in the United Kingdom. Kim Arndt bade farewell to Cecilia Devlin, who is continuing her postdoctoral research in Akron, Ohio, and to Fong C. Lin, who is doing the same at New England Biolabs in Beverly, Massachusetts. Doris Germain from Bruce Futcher's lab is continuing her postdoctoral research at the Peter MacCallum Cancer Institute in Melbourne, Australia. Dorre Grueneberg followed Michael Gilman to Ariad Pharmaceuticals in Cambridge, Massachusetts, and Henry Sadowski accepted a position as assistant professor at Mt. Sinai School of Medicine, Biochemistry Department, in New York. Louis A. Peña and Christian van den Bos left Dan Marshak's lab—Peña to become a research associate professor at Memorial Sloan-Kettering Cancer Center, Rockefeller Research Laboratory in New York, and van den Bos to accept a staff scientist position at Osiris Therapeutics, Inc. in Baltimore.

Graduate Students

Ann Ryan and Ken Simon left the Gilman lab to continue their graduate studies at Ariad Pharmaceuticals, and Terrance Vale left the Beach lab and Yaolin Wang left the Zhong lab each to do the same—Vale at the University of Texas Southwest Medical Center and Wang at University of Kansas.

Many of our researchers obtained their degrees and made the transition from graduate student to postdoc. David Casso, of David Beach's lab, went on to do postdoctoral research at the University of California, San Francisco, and Sonja Witte went from the Cline lab to do the same at Massachusetts College of Pharmacology at Quincy College. Michele Cleary, of Winship Herr's lab, will be

going on to postdoctoral work at Shirley Tilghman's lab at Princeton University, but for the time being has remained at Cold Spring Harbor, working for the journal *Genes & Development* in an editorial capacity, assisting Dr. Watson with revisions of his book *Molecular Biology of the Gene*, and continuing to do research in the Herr lab. Cynthia Sadowski of Nouria Hernandez's lab earned her degree and will be leaving to do postdoctoral research at New York University in February 1996. Nick Chester, from the Marshak lab will be doing his postdoctoral research at the Howard Hughes Medical Research Institute, Department of Genetics at Harvard University; Yuliang Ma from Mike Mathews' lab went on to the University of California at San Diego; Robert Mihalek from Tim Tully's lab is doing postdoctoral research at University of Pittsburgh; and Tao Zhong of Kim Arndt's lab went on to Massachusetts General Hospital. Maureen Barr left the Wigler lab to do her postdoctoral research at the California Institute of Technology and George Tokiwa left Bruce Futcher's lab to do postdoctoral research at New York University Medical Center. Patricia (Beth) Elliott has entered into the plant breeding business here on Long Island.

Planning our Entry into the 21st Century

In this report, we see the research made possible by the great burst of building activities that were planned in the last decade (1980–1989) of the Laboratory's first century. It was the creation of the Walter Page Laboratory (1987) and the acquisition of our Uplands Farm Experimental Station (1984) that allow us an international role in plant genomics and developmental biology. And only through the 1991 completion of our Beckman Laboratory did we have the capability of being a world leader in how genes control the acquisition of memory. By our imaginative expansion (1993) of McClintock Laboratory, we have remained at the center of cell cycle research with its vast potential for the eventual control of cancers. The new space so provided let us also create the Lita Annenberg Hazen Genome Center.

Now we must initiate a new set of planning initiatives to ensure that we enter the 21st century with the facilities needed for continued innovation. At this year's annual November meeting of our Board of Trustees, we set up four new committees to make recommendations during 1996 as to how best to ensure our continued leadership roles. One, headed by me (B.S.), is looking into the future of neurobiology, in particular, how we should exploit further our research on learning and memory in flies and mice to encompass the problem of human memory and learning. The second committee, headed by Winship Herr, will look into the Laboratory's future role in graduate education. We now have 51 graduate students doing their Ph.D. thesis requirements here, almost all enrolled in graduate programs at SUNY, Stony Brook. The question we need to ask is whether the ever-increasing size of our graduate training program might demand our becoming a degree-granting institute. Our third committee is looking into the question of whether the Laboratory should be more pro-active in the commercial exploitation of research done here at Cold Spring Harbor. During the past decade, some ten biotechnology companies have been developed using ideas and technologies generated here. Only two, however, Protein Databases and Oncogene Science, are located here on Long Island. Now a committee, headed by John Maroney, is asking whether future laboratory inventions would more likely stay on Long Island if we help bring into existence a nearby biotechnology-oriented industrial park.

Lastly, we have set up a committee to look into how we should house our growing numbers of graduate students and postdoctoral fellows. Neither of these categories of younger scientists receives salaries commensurate with the relatively high cost of housing on the north shore of Long Island. To plan how to respond, Morgan Browne heads a committee that is looking into ways to generate nearby housing units. All of these groups are to report to the Board of Trustees at our 1996 annual November meeting.

During 1996, we will thus be very future-oriented. In this way, we will continue to promote the world of biology that still has so much to offer to human society.

April 30, 1996

Bruce Stillman, *Director*

James D. Watson, *President*

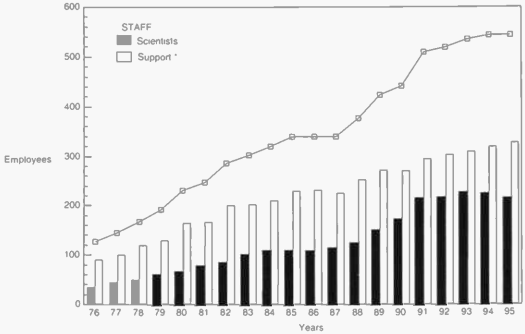
ADMINISTRATION

The past year was a very satisfactory one here at Cold Spring Harbor Laboratory. The scientific staff continued to make important and surprising discoveries about cancer, neuroscience, and plants, which are the Laboratory's major areas of research, and there was much well-deserved recognition in the national media. Equally pleasing was the growing impact of the Laboratory's broad range of education programs, which sometimes are overshadowed by the greater visibility of research.

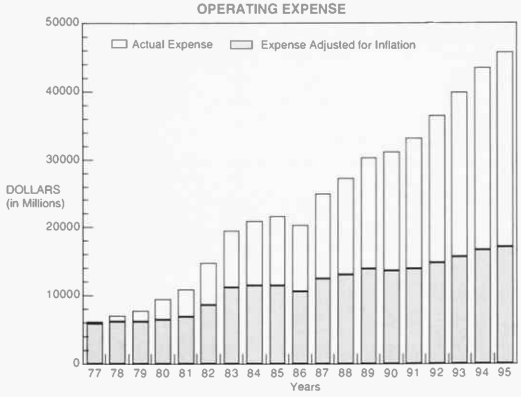
During 1995, approximately 6000 scientists from all over the world came here to present and discuss their latest results at Grace Auditorium symposia, to define and focus on new issues in science at Banbury Center seminars, and to learn new scientific techniques at the unique postgraduate level courses held in our teaching labs in Beckman and Delbrück, as well as at Banbury. In addition, there were more than 100 postdoctoral fellows in training on our campus and more than 50 graduate students fulfilling thesis requirements for their Ph.Ds. Twenty-two university students gained admission to our highly competitive Undergraduate Research Program and worked side by side for the summer with senior scientists at the laboratory bench. At our DNA Learning Center, the excitement and implications of molecular biology and genetics were conveyed both to science teachers and to the next generation of young scientists through hands-on laboratories, lectures, exhibits, and multimedia curricula. Our academic press published 3 science journals and 20 new books.

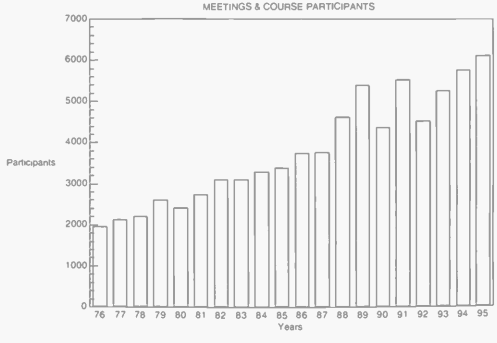
Financial results for 1995 were very much better than expected at the beginning of the year. Revenues reached a new high of $43.8 million, and there was a surplus from operations of $92,000 after provision of $2.8 million for depreciation. There was no need to utilize reserves of $1.15 million established in prior years to cover start-up expenses of the neuroscience program or shortfalls in research funding. Contributing to the good results were the success of Laboratory scientists in competing for Federal grants, strong meetings attendance, increasing royalties from technology transfer, and higher investment income. The Laboratory's budget has been balanced in each of the past seven years, and operations over that period have produced $17.2 million of positive cash flow that has been invested in maintaining and renewing infrastructure, building new facilities, and replacing and acquiring scientific equipment.

A lean and unbureaucratic administrative structure at the Laboratory has an important role in keeping costs in check and greatly facilitates rapid decision making. Government pressure to reduce indirect costs continues and now seems focused on separating such costs into administrative and facility categories and most likely imposing a cap on one or both. Recent initiatives at the Laboratory to address indirect costs have included reduction of staff through attrition and lowering overtime costs through improved scheduling and outsourcing of services such as housekeeping for off-site facilities. Waste disposal costs have been reduced through improved management of hazardous materials and a recycling program for paper and bulk products. This July we will switch over from secondary to primary metering of electricity, permitting the purchase of power at wholesale rates. We have plans for more efficient lighting systems and a major capital investment to replace the HVAC system of Demerec Laboratory—all



* 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





designed to reduce substantially our staggering energy bill of over $1.7 million per year.

Very important to the Laboratory's good financial results have been the additional revenues generated by the Meetings and Courses Department, headed by David Stewart, and Commercial Relations, led by John Maroney. The traditional science meetings held in Grace Auditorium have for many years been characterized by well chosen subjects, a focus toward younger scientists who are given ample opportunity to present their work, and an ambiance unique to Cold Spring Harbor Laboratory which keeps many of the scientific groups returning year after year. New in recent years are additional meetings, apart from the traditional scientific symposia but still scientifically interesting and appropriate to the Laboratory, that enable corporations and other organizations to make use of our extensive facilities during the quiet portions of the year.

The success of technology transfer under John Maroney's leadership has been remarkable. Royalty income continues to expand and the Laboratory has become an equity participant in seven new biotechnology companies that have been founded, to a greater or lesser extent, on technology developed here. The Laboratory has led the way in committing the proceeds from technology transfer exclusively to the support of future science. This was done by establishing the Science Fund, a special category of endowment, where are placed the royalty and equity proceeds from all such activities. In recent months, several of the Laboratory holdings have become publicly traded at substantial valuations, lending credence to our dream that one day the Science Fund may represent a major new endowment for science.

The Laboratory's current endowment, which collectively includes the Robertson Funds and the Cold Spring Harbor Fund, reached an all time high of nearly $109 million at year end, ahead 20% from the previous year. The investments include a balanced mix of domestic and foreign equity, fixed income securities, and short-term liquid instruments. During the year, the funds benefited from outstanding market returns in both equity and fixed-income markets, an addition of $500,000 from Laboratory operations, and a $50,000 gift from the Banbury Fund directed toward further building the Charles S. Robertson Neuroscience Chair. Over the years, the Laboratory has positioned the annual drawdown from endowment at a very conservative average of less than 3.5% of market value, and this has been a major factor in the growth of the Funds. Endowment is our primary source of internal funding for science and provides important support for $30 million of tax-exempt bonds issued in recent years to finance construction of science and infrastructure facilities. Almost since inception, the firm of Miller Anderson & Sherrerd has provided outstanding investment management for the equity and fixed-income portions of the endowment. During the past two years, U.S. Trust has managed short-term liquid investments. Now, due to the very substantial growth of the funds, our Finance and Investment Committee has initiated a search to add one or more managers to provide further diversity and assist in continuing the high level of past market performance.

Very disappointing during the year was the unexpected and unfortunate outcome of the Laboratory's effort to acquire the campus of Friends World College in Lloyd Harbor. In 1992, the College, for financial reasons, had transferred its programs to Long Island University and offered the property for sale. The Village then approached the Laboratory to find a compatible use that would preserve public access and the historic buildings, the activities of the Sagamore Rowing Club, and the remarkably beautiful open land and waterfront views of the 28-acre

site. After extended fund-raising and negotiations, the Laboratory proposed to the Village a plan to use the campus for the editorial and administrative activities of our academic press and for housing needs of our meetings visitors and scientific staff.

Much misinformation and exaggeration about the proposed use of the property were circulated around the Village, and there was vehement opposition from some residents. There was concern over traffic, misunderstanding that our academic press was a commercial venture, and fear of a precedent set for large-scale multifamily housing. There was mistrust of the Laboratory's intentions despite our more than 15-year exemplary management of the much larger Robertson (Banbury Center) property, where many Village residents enjoy the freedom to walk its woodland and open areas.

The Laboratory tried to address concerns through discussion with Village officials and small groups of residents. We proposed a new access route to resolve traffic problems permanently. We dropped the proposed use by our academic press. We offered to purchase the site as is, using existing buildings for existing uses with only the future right to build a small amount of additional housing. We also proposed protective covenants for the open land.

Although the Laboratory received broad support from a large segment of the Village residents, there remained a well-organized group of residents adamantly opposed to the project. Village officials instructed the College to evict all current residents from the site and then notified the Laboratory that the educational use permit was canceled and would not be renewed.

Having hoped to go forward in partnership with the Village, we had no wish to polarize the residents. The College was under intense financial pressure and time was of the essence. Therefore, with much regret, we decided not to pursue the matter further.

We especially thank those individuals and Foundations who pledged financial support for the project. We have offered each the return of all funding with interest, and we are much encouraged by their expressed intent to participate instead in other high-priority projects in science and infrastructure.

The Buildings & Grounds Department, under the very capable leadership of Art Brings, had a busy year in 1995 despite the village-wide moratorium which stopped all nonresidential construction throughout the year. Most obvious from an appearance standpoint, Bungtown Road (from Route 25A to Nichols), which is a Village street, and the south section of the Grace parking lot were repaved at our expense. New curbing was installed in front of Blackford Hall and on portions of Bungtown Road. A new berm was constructed and landscaped at the Waterside parking lot to screen cars from across the harbor. The Bonn House, Fire House, Nichols, Williams, Osterhaut, Hooper, and the Banbury Conference Center were all painted, and Richards, Wawepex, the Library, and Hershey received new roofs. Further landscaping and a lawn sprinkler system were installed at Ballybung and Jones.

Less visible but no less important, the HVAC system of Sambrook Laboratory was replaced to improve temperature and humidity control. The Hooper Dormitory was completely renovated with new carpet, furniture, ceiling fans, lighting, photographs, and paint, which were warmly welcomed at the fall meetings by many of our visiting women scientists. At Uplands Farm, two new state-of-the-art greenhouses were built to replace dilapidated structures. At the DNA Learning Center, a new exhibit, "Story of the Gene," was constructed as was office space for a computer design facility. On October 6, Doubleday House was

severely damaged by fire. Fortunately, no one was injured and the house was back in use by year end.

Under the watchful and seasoned eye of Jack Richards, now Director of Special Projects, planning moved forward on future projects temporarily stymied by the moratorium. These include the Child Care Center, the aptly named Richards Building and Grounds Facility, and additional cabins for our meetings visitors. Earlier in the planning stage are a new imaging building, additional offices for our neuroscientists, new space for software design, and a long awaited athletic facility. There is much to do, and we are counting on Jack not to become too enamored of his new home in Florida.

Public Affairs and Development, ably led by Susan Cooper, portrays the Laboratory to the outside world, schedules and manages the many community and other events held at the Laboratory, and is responsible for the fund-raising activities that have grown enormously in number and diversity in recent years.

During 1995, much attention was directed toward relations with our local villages. Communication with residents on Long Island's North Shore is difficult in the absence of frequently published local newspapers. It depends primarily on personal contact achieved at small group meetings and mailings to individuals of fact sheets, newsletters, and personal correspondence. Much was accomplished by these in countering misinformation and portraying accurately the Laboratory's activities and intentions. In addition, the Laboratory's program of lectures, seminars, and special events for the community was continued and expanded.

Fund-raising focused on a number of priorities, foremost of which were Friends World College, the Child Care Center, and program and equipment support for science. Much effort was also directed toward supporting the very important annual funds of the CSHL Association and of the DNA Learning Center Corporate Advisory Board. The Association Fund, under the leadership of John Cleary, supported by Jean Schwind, raised a record $650,000. The Corporate Advisory Board Fund, led by Rick Clark, supported by Laura Hundt, raised $130,000. Laura deserves special recognition for organizing and managing the 2nd Annual CSHL Golf Tournament which raised nearly half of this latter total.

Probably the high point of the year was the gala "Flicka von Stade and Friends" concert held on March 25 which raised $55,000 for the Undergraduate Research Program's von Stade Endowment.

In December, much to our regret, Jill Clark departed for Boulder, Colorado, where her husband, Rick became chief operating officer of Insession Inc., a promising young computer software company. Jill had proved herself an invaluable member of the staff who always could be counted on to convey with great style the essence of a project to a foundation or major donor. In April of this year, the Burroughs Wellcome Foundation announced a $1,000,000 grant, initiated and shepherded by Jill, for our Undergraduate Research Program. We miss her and she will be difficult to replace.

In the Library, Margaret Henderson and her staff continued to provide us with invaluable resources despite a creeping encroachment upon available space by other departments. After much analysis, the water leakage problem in the basement was finally resolved, but already these offices are being spoken for by more voices than can possibly reside there. During the year, much use was made of an additional online reference service: Medline Grateful Med, Current Contents Life Sciences for Mac. Thanks to funding from the previous year by the Gladys Brooks Foundation, a microfilm reader/printer was purchased and back volumes of *Biochemica et Biophysica Acta* were made available. A new Library com-

mittee, consisting of Yuri Lazebnik (Chair), Tracy Calhoun, Grisha Enikolopov, Ueli Grossniklaus, and Margaret Henderson, was formed to make helpful suggestions.

Other very important administrative departments such as Grants, headed by Susan Schultz; Human Resources, led by Cheryl Sinclair; and Purchasing, headed by Phil Lembo are indispensable to the smooth functioning of the Laboratory. In particular, the very sizable cost savings uncovered through the quiet and extremely thorough professionalism of Phil and his staff have been eye openers to us all. Phil's first year at the Laboratory clearly demonstrates that he was the perfect choice to head the Purchasing Department.

Bill Keen, our Controller, and Barbara Wang, Assistant Controller, as always have succeeded in managing our finances and budget to ensure that any surprises turn out to be good ones. In 1995, in close conjunction with Jerry Latter and his newly organized and smoothly functioning Information Service Department, Bill and Barbara successfully met the very major challenge of overseeing the conversion to updated software and new computer hardware in all administrative departments. By year end, the task was nearly complete and improved efficiency for all was becoming obvious.

Sadly it must be reported that on October 21st, Roberta Salant lost her beloved and devoted husband, Jack, to a sudden and completely unexpected death. Her courage in carrying on so valiantly through the months since her terrible loss has impressed upon all of us the remarkable quality of this grand lady, who each year takes such good care of the needs of myself, John Maroney, and the Laboratory's Board of Trustees.

Now half-way into 1996, it is pleasing to report further improvement in the Laboratory's relations with the Village of Laurel Hollow. The effort to establish better communication with residents has made many aware that the Laboratory voluntarily provides substantial financial support to the Village for use of services and also assists the school district with both cash payments and extensive in-kind services for the education received by the Laboratory children living in tax-exempt housing. Last fall, the Village approved the environmental impact statement prepared by the Laboratory for the Village Board of Zoning Appeals, which concludes that there would be no significant environmental impact from the Child Care Center or from any of the other seven projects covered by the study. At year end, the Village provided a draft of its proposed new zoning ordinance and began discussions with Laboratory representatives concerning its provisions. The Laboratory has serious objections and reservations about the ordinance and has registered them clearly for the record. Nevertheless, we are moving forward with the Village in a new sense of cooperation that has characterized recent relations. In April, the Village Board passed the new ordinance and lifted the almost two-year moratorium on nonresidential construction. The Board then approved the use of the de Forest Stables for the Child Care Center and has since also approved its site plan. Shortly, a building permit should be issued that will allow the Child Care Center to proceed in time for a spring 1997 opening.

G. Morgan Browne
Administrative Director

May 16, 1996



RESEARCH

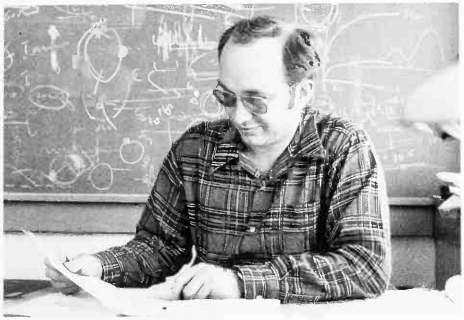


Top: Nancy Kaplan; Lin Ying Xie; Qizhi Wang

Center: Hollis Cline; Ahmed Samatar; Jane Hoffman

Bottom: Salim Mamajiwalla; Benjamin Lee; Grigori Enikolopov





1977

1979

With Joe Sambrook 1981

With Michele Manos and Barbara Ahrens 1981

With Michele Calos 1986

1986

Yakov Gluzman 1948-1996

Cold Spring Harbor Laboratory lost a dear colleague on April 6, 1996, with the untimely death of Yakov Gluzman. Even after he left Cold Spring Harbor in 1987, he remained loyal, coming back just this year to help us prepare our application for continued funding of the Tumor Virus program project, now in its 25th year. This important program project has been indelibly changed with his loss.

Yasha, as he was known, was always different. He was not even born on his official birthday of January 2, 1948. He was born at home on December 22, 1947, but following a practice not uncommon at that time, his parents delayed reporting his birth until after the new year, thereby delaying by a year his induction into the Soviet military.

He was born and grew up in Chernovski, a predominantly Jewish city in Ukraine. In 1965, he was admitted to Moscow State University where he studied biology. Shortly after his marriage in 1969, his wife, along with members of her family, emigrated to Israel, but he was not allowed to leave. To increase his chances of obtaining an exit visa, Yasha left the university just prior to completing his Diploma studies and became a carpenter, because carpentry was viewed by the Soviet regime to be less important to the country's welfare than scientific professions. Despite this move, however, it was only after extensive political appeals, including an appeal by the U.S. Congress, that he was granted an exit visa by the Soviet government in 1971.

When Yasha arrived in Israel, he met his 14-month-old son, Ilan, for the first time. Shortly thereafter, on the basis of an interview, he was admitted to the prestigious Weizmann Institute without the requisite degree. Under the guidance of Ernest Winocour, he performed his doctoral studies on the transformation and replication properties of the simian DNA tumor virus SV40. Although he had devised several ruses to avoid the military while in the Soviet Union, in Israel he served in the army and saw combat in an artillery unit during the 1973 Yom Kippur war. After obtaining his Ph.D. degree in 1977, he came to Cold Spring Harbor Laboratory as a postdoctoral fellow with Joe Sambrook to continue his studies on tumor viruses.

Even as a graduate student, Yasha had had the vision to use viruses as tools to help mankind. At that time, he studied how SV40 can mutate such that its ability to replicate and kill cells is destroyed while its ability to induce cells to proliferate remains intact. At Cold Spring Harbor, he elegantly engineered mutations into SV40 to produce a debilitated virus that, although unable to replicate itself, could allow the replication of other engineered DNA molecules carrying the SV40 origin of replication. He generated cells carrying this disabled virus and called them COS cells. COS cells could be made to produce large amounts of biomedically important proteins. The importance of these cells was recognized immediately, and, to this day, they are used worldwide in biomedical research and in the fight against human disease.

Yasha remained at Cold Spring Harbor until 1987 and made important contributions to the fields of SV40 DNA replication and control of gene expression. In 1987, he decided to become directly involved in the development of pharmaceuticals and moved to Lederle Laboratories in Pearl River, New York. This move afforded him the opportunity to apply his efforts directly to the discovery of treatments for human disease. There, he helped develop drugs to treat viral infections, including respiratory syncytial virus infection, the cause of a potentially serious respiratory disease in children.

Yasha was a true virologist, who appreciated that, if one listened carefully, viruses had much to tell about themselves. He was highly regarded for his ability to examine experimental problems with clarity and from unique perspectives; often, he inspired his colleagues to re-evaluate their scientific assumptions and interpretations. As a colleague, Yasha had a special sense of humor. Friends remember him for his famous bear hugs, which once accidentally broke the ribs of a colleague. He was also known for turning upside down and dangling by the ankles a student who was not thinking clearly.

In the recent months preceding his death, he was making plans to return to Israel and start his own biotechnology company, where he could continue to develop anti-viral drugs.

His life was too short. We will sorely miss him, not only for his friendship, but also for the discoveries he never had a chance to make.

TUMOR VIRUSES

The Tumor Virus section continues to use viruses to probe 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. We continue to probe the mechanisms and regulation of DNA replication, transcription, mRNA processing, and translation. Our studies of the cellular responses to viruses are teaching us about the control of signal transduction in human immunodeficiency virus-infected cells and the mechanisms of apoptosis or programmed cell death, a natural cellular defense to virus infection. This year, Scott Lowe, who trained with our former colleague Earl Ruley while at MIT, joined our program as a Cold Spring Harbor Fellow. He and Yuri Lazebnik have joined forces to understand the regulation of apoptosis in cancer cells: Scott Lowe is using a genetic approach and Yuri Lazebnik is dissecting the process biochemically. The combining of such complementary approaches is characteristic of the research process at Cold Spring Harbor and is one of the important elements that keeps the Tumor Virus program vital.

DNA SYNTHESIS

B. Stillman R.S. Williams, S. Waga, M. Akiyama, G. Cullmann, V. Ellison, M. Hidaka, M. Iizuka, P. Kaufman, R. Li, C. Liang, C. Mirzayan, A. Verreault, M. Weinreich, K. Gavin, H. Rao, L. Zou, V. Filadora, J. Mitchell, M. Waga, C. Driessens

During the past 12 months, we have continued to focus our efforts on understanding many aspects of the mechanism and control of DNA replication in eukaryotic cells. As in the past, these studies have relied on a multidisciplinary approach by drawing upon the advantages offered by different experimental systems. Biochemical studies on the small DNA tumor virus, simian virus 40 (SV40), during the past 11 years have led to the identification of the human cell proteins that cooperate with SV40 T antigen to replicate the viral genome. The functions of many of these proteins have been discovered, and similar proteins have been identified in the yeast *Saccharomyces cerevisiae*, where we have been able to demonstrate a role for these proteins in replication of the cell genome. We have also continued to study the mechanism of chromatin assembly during SV40 DNA replication and the function of chromatin assembly factors from human cells. Furthermore, during this year, similar chromatin assembly activities have been identified in *Drosophila* and yeast cells.

Studies on the mechanism and control of the initiation of replication of cell chromosomal DNA have continued by focusing on the origin recognition complex (ORC), a multisubunit protein that binds to cellular origins of DNA replication and determines where replication will begin. These studies include the identification of proteins that interact with ORC either to establish a prereplication complex at origins of replication or to activate these prereplicative complexes following commitment to cell division and entry into S phase. Just as we have studied in parallel the mechanism of DNA replication at the fork in both human and yeast cells, we have now found similarities between the proteins that are required for the initiation of DNA replication in yeast with proteins present in mammalian cells. This important development should eventually enable identification of the DNA elements in mammalian chromosomes that determine the location of origins of DNA replication and how they might be controlled through the cell cycle and during tissue development.

Mechanism and Control of DNA Synthesis at the Replication Fork

G. Cullmann, V. Ellison, R. Li, S. Waga

SV40 LARGE TUMOR ANTIGEN

SV40 encodes a protein, the large tumor (T) antigen, that performs multiple functions during the initiation of DNA replication. These include binding to the specific DNA sequences that determine the location of the origin of DNA replication, a DNA helicase function that facilitates unwinding of the DNA beginning at the origin and an additional function that directs loading of the DNA polymerase α/primase complex on to the template DNA. A continued collaboration with Dr. Daniel Simmons (University of Delaware) has generated data suggesting that T antigen also has an additional function in DNA replication via interaction with topoisomerase I, an enzyme that untwists the double helix as the helicase unwinds DNA at the replication fork.

REPLICATION FACTOR C

As part of a continuing effort to understand the functions of the eukaryotic cell DNA replication proteins, we have systematically isolated the genes encoding the proteins that function at the replication fork. This year, the five genes encoding the replication factor C (RFC) protein subunits have been isolated from the yeast *S. cerevisiae* and they were all shown to be essential. This was somewhat surprising because all of the proteins have extensive sequence similarity, particularly the four small subunits. Also of interest was the sequence comparison of the RFC proteins with the functionally related proteins present in prokaryotes and bacteriophages. Unlike other replication proteins such as the proliferating cell nuclear antigen (PCNA), where there exist functionally related proteins in eukaryotes and prokaryotes that do not have obvious amino acid sequence similarities, the RFC family of proteins are quite conserved in sequence, even in organisms as diverse as bacteriophage T4, *Escherichia coli*, *S. cerevisiae*, and humans (Fig. 1). One of the RFC genes in *S. cerevisiae*, encoding the largest subunit of the five-subunit protein complex, proved to be identical to the *CDC44* gene. Mutations in *cdc44* result in a phenotype consistent with a defect in chromosomal DNA replication, and studies in Dr. Connie Holms' laboratory (University of California, San Diego) have shown that mutations in the gene encoding PCNA suppress *cdc44* mutations. These observations provide strong genetic evidence in support of our previous biochemical evidence for an interaction between RFC and PCNA during SV40 DNA replication in vitro.

INTERACTIONS BETWEEN p21 AND PCNA

The p53 tumor suppressor protein has been shown to function in a cell cycle checkpoint control pathway in mammalian cells. For instance, in response to DNA damage caused by genotoxic stimuli such as UV or γ-irradiation, the level of p53 protein increases and arrests cell cycle progression in the G_1 or G_2 phase so that replication of damaged DNA or segregation of damaged chromosomes can be prevented. p53 is a transcription factor that activates the expression of a set of unrelated genes. Among them, the gene encoding p21 (*Waf1, Cip1, Sdi1*) appears to encode a critical component of p53-mediated G_1 arrest in response to DNA damage. The p21 protein is capable of binding directly to cyclin/CDK complexes, and when present in excess, it inhibits the activity of the kinase necessary for G_1 to S phase progression in the normal cell cycle.

Of particular interest to us was the finding in David Beach's laboratory at Cold Spring Harbor that in normal human diploid cells, but not in many transformed cells or p53-deficient cells, p21 formed a quaternary complex with a cyclin, a CDK, and

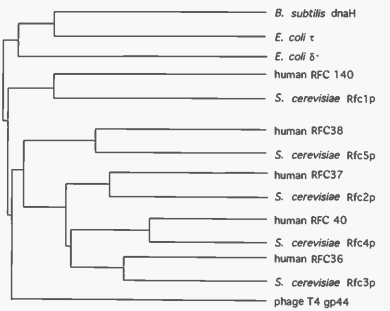


FIGURE 1 RFC and related proteins. A tree comparing the sequences of RFC and related proteins. The horizontal distance is a measure of relatedness.

PCNA. We thought it likely that this quaternary complex functions in normal cells to regulate coordinately cell cycle controls, DNA replication, and DNA repair and therefore collaborated with David Beach and Greg Hannon (CSHL) to test this idea.

Results reported in the previous Annual Report demonstrated that p21 differentially regulated DNA replication and repair in response to DNA damage. p21 inhibited PCNA-dependent DNA replication and did not require the presence of CDK cyclin proteins.

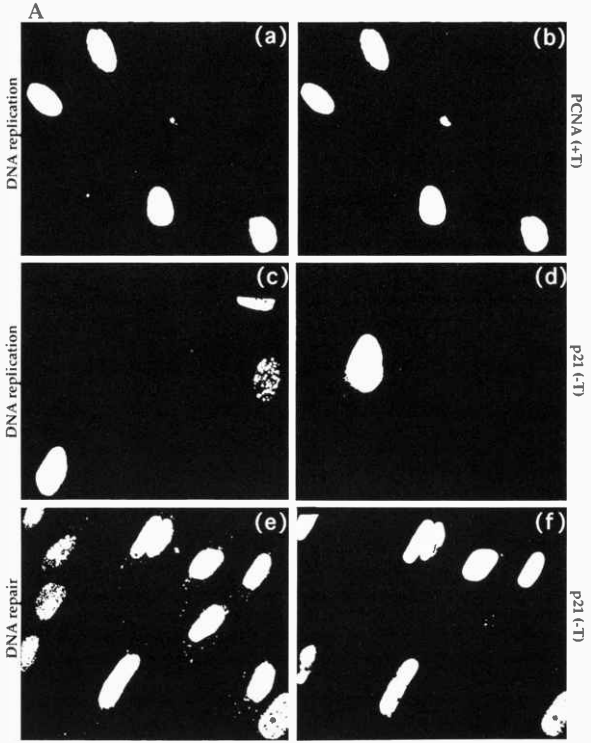


FIGURE 2 Immunofluorescence of the localization of PCNA and p21 in human diploid fibroblasts. (*Left panels*) Incorporation of bromodeoxyuridine (BrdU) into DNA either in S phase DNA replication (*a* and *c*) or DNA repair in G_1 phase (*e*). The BrdU staining was detected by immunofluorescence using anti-BrdU-antibodies. (*Right panels*) Immunofluorescence directed against either PCNA (*b*) or p21 (*d* and *f*). The cells in *a* were extracted with Triton X-100 prior to cell staining to show PCNA associated with DNA replication sites. (*a* and *b*) PCNA is present in a Triton-insoluble form only in cells actively replicating DNA in S phase, but is extracted in cells in G_1 and G_2. (*c* and *d*) p21 is absent in the cells replicating DNA, but is present in the cells that are not replicating DNA. In contrast, *e* and *f* show that p21 is present in all cells that are repairing UV-induced DNA repair.

In contrast, DNA repair was completely refractory to p21, even though this repair was dependent on PCNA. We then proposed that the interaction between p21, PCNA, and the CDK-cyclins served to coordinate DNA replication and repair with cell cycle progression, both in normal cycling cells and in cells that had suffered DNA damage.

We first tested this model by examining the levels of p21 in human diploid fibroblasts during the normal cell cycle and in cells irradiated with UV. During the normal cell cycle, p21 levels were high in G_1 phase, but the protein disappeared as cells entered into S phase (Fig. 2). These observations were consistent with our in vitro data demonstrating that p21 inhibited PCNA-dependent DNA replication.

Next, to determine whether DNA repair in vivo actually takes place in the presence of elevated levels of p21, we examined the cellular distribution of p21 and PCNA following UV irradiation of normal human diploid cells that were in the G_1 phase of the cell cycle. Immunostaining experiments with anti-p21 and anti-PCNA antibodies have shown that the majority of cells actively repairing DNA contained high levels of p21 in the nucleus, indicating that DNA repair can occur in the presence of p21 induced by DNA damage (Fig. 2). The p21 levels in the cells corresponded well with the levels of p53 induced by the DNA damage. Therefore, this in vivo observation is also consistent with the in vitro data described above.

The cellular distributions of p21 and PCNA following UV irradiation were examined further as follows. In this series of experiments, normal human diploid cells synchronized by serum deprivation were irradiated with UV and released into G_1 by the addition of serum. Either 0, 4, or 16 hours later, the cells were fixed and double stained with anti-p21 and anti-PCNA antibodies. Consistent with the previous data showing the p53-independent induction of p21 by serum, a transient induction of p21 was seen upon addition of serum without UV irradiation, but the level of p21 diminished as the cells progressed into S phase. In contrast, in the UV-irradiated G_1 cells, high levels of p21 were induced by DNA damage and were sustained in nuclei for at least 48 hours after release into G_1. The levels of PCNA seen in nuclei appeared to be constant throughout the time course, whether cells were irradiated with UV or not. Interestingly, although both PCNA and p21 in nuclei of the untreated cells could be extracted with Triton X-100 prior to fixation of the cells, in the UV-irradiated cells, a large fraction of p21 as well as PCNA in the nuclei became resistant to extraction with detergent. These results suggest that upon DNA damage, p21 and PCNA redistribute in nuclei and become more tightly associated with the nuclear structure, most probably sites of DNA repair.

To further assess the involvement of p21 and PCNA in DNA repair in vivo, we examined the cellular localization of these proteins following UV irradiation in the cells derived from patients with the cancer-prone disease, xeroderma pigmentosum, either complementation group A (XPA) or group G (XPG). These two cell lines are deficient in global nucleotide-excision repair. Immunostaining revealed a distinct and punctate pattern of p21 that colocalized with PCNA in these cells, whereas in normal cells, staining with anti-p21 or anti-PCNA antibodies was more homogeneous. In contrast, in Cockayne's syndrome and XPC cells that are deficient in transcription-coupled DNA repair, but not in global nucleotide-excision repair, the p21 and PCNA staining patterns were the same as those in the wild-type cells. We argued from these data that p21 and PCNA play a part in the sensing of genome-wide DNA damage following UV irradiation, a hypothesis we propose to pursue in the future.

Taken together, we assume that p21 plays a part in coordinating cell cycle control, DNA replication, and DNA repair to maintain the integrity of the genome. It is possible that in normal cells, a quaternary complex consisting of cyclin, CDK, PCNA, and p21 might be responsible for such a regulatory function. Therefore, the rearrangement of the quaternary complex that is observed in many tumor cells might cause genomic instability due to the uncoupling of cell cycle controls with the replication and repair machineries.

Chromatin Assembly

P. Kaufman, A. Verreault

CAF1

Our previous work on DNA replication-dependent chromatin assembly has identified a multisubunit chromatin assembly factor called CAF1 that promoted the assembly of nucleosomes during DNA replication in vitro. The cloning and characterization of cDNAs from human cells encoding the two largest subunits of CAF1 have been reported previously. In

collaboration with Ryuji Kobayashi here at the Laboratory, the p50 subunit of CAF was sequenced and cDNAs encoding the protein were isolated. The amino acid sequence of this subunit (now called p48) is 95% identical to another protein called p46 that is encoded by a separate gene. There are also two related genes in the yeast *S. cerevisiae*. All of these proteins contain a repeated sequence motif called the WD40 (or β-transducin) repeat, a motif that is also present multiple times in the p60 subunit of CAF1. Notwithstanding the similarity of the p46 and p48 proteins, only the p48 protein appears to be a part of CAF1 in vivo.

Sequence database searches revealed that the p46 and p48 proteins had been identified by Dr. Eva Lee (University of Texas, Austin) as proteins that bind to the retinoblastoma tumor suppressor protein. It is intriguing that these proteins were identified as Rb-binding proteins, although many proteins are known to bind to Rb. We have confirmed that the p46 and p48 proteins bind Rb in vitro, but we as yet do not have evidence that they bind Rb in vivo in a physiologically significant manner.

IDENTIFICATION OF A LARGE CHROMATIN ASSEMBLY COMPLEX

CAF1 appears to function as a molecular chaperone for an acetylated-H3/H4 multiprotein complex to facilitate assembly of chromatin during DNA replication. We have continued to search for other proteins that are required for replication-dependent chromatin assembly by fractionating cell extracts and reconstituting DNA replication and replication-coupled chromatin assembly with purified proteins. To this end, we have reconstituted DNA replication with fractions containing all the known human-cell DNA replication proteins, purified CAF1, purified histones H2A and H2B from chromatin, and other fractions from human-cell extracts. Using this reconstituted system, we have discovered a large, multiprotein complex that has been purified to near homogeneity which will assemble chromatin under conditions where pure CAF1 will not. This chromatin assembly complex (CAC) contains the three CAF1 subunits, acetylated forms of histones H3 and H4 (but not H2A and H2B), and other as yet unidentified proteins. The histone H4 is acetylated on lysine residues 5, 8, and 12 near the amino terminus of the protein, but not on lysine 16, an acetylation commonly associated with gene transcription. Histone acetylation on lysine residues 5 and 12 has long been known to be associated with the assembly of chromatin during the S phase of the cell cycle.

DROSOPHILA CAF1

The analysis of the human-cell CAF1 using the SV40 system has provided great insight into the mechanism of chromatin assembly during DNA replication, but it does not afford the ability to take a genetic approach to determine the function of the proteins in vivo. We have therefore searched for CAF1 activity in *Drosophila* and yeast and have found promising activities in both. The *Drosophila* studies have been done in collaboration with Jim Kadonaga'a laboratory at the University of California, San Diego. The first surprise was that extracts from *Drosophila* early embryos (prior to cellularization of the syncytial nuclei) are able to support SV40 DNA replication in an origin- and T-antigen-dependent manner. Importantly, we found that the *Drosophila* extracts supercoiled the DNA in a replication preferential manner and a CAF activity was purified from the extract. In collaboration with Ryuji Kobayashi, some of the proteins present in the *Drosophila* CAF1 (dCAF1) have been sequenced and were found to be very highly similar to the human CAF1 subunits.

Cell Chromosome Replication

M. Akiyama, K. Gavin, M. Hidaka, M. Iizuka, C. Liang, C. Mirzayan, H. Rao, M. Weinreich, R.S. Williams, L. Zou

INITIATION IN YEAST CELLS

Studies on the replication of cell chromosomes now focus almost entirely on the mechanism and control of the initiation of DNA replication in both human and yeast cells. These studies were greatly advanced a few years ago by the discovery of the origin recognition complex (ORC) in *S. cerevisiae*. This initiator protein functions in the initiation of DNA replication by binding to the replicator sequences in the genome which determine the location of an origin of DNA replication. In the last year, we have reported the isolation of all of the genes encoding the ORC subunits and have shown them to be essential for progression through S phase. Temperature-sensitive mutations in

the genes encoding the ORC subunits affect the frequency of initiation at individual replicators. If the function of ORC is reduced to very low levels in the cell, the frequency along the chromosome of initiation of DNA replication becomes so low that the cells die.

The only sequence similarity to other proteins present in the protein sequence databases was found with the Orc1p. This protein is related as sequence to two proteins: the Sir3p that controls silencing of transcription of the yeast mating-type genes and the Cdc6p, a protein that controls the initiation of DNA replication. We have demonstrated that the Orc1p has a specialized domain that is dispensable for its function in DNA replication but is essential for transcriptional silencing. This further strengthens the link between control of gene expression by silencing and the initiation of DNA replication.

In last year's Annual Report, we reported that the Cdc6 protein (Cdc6p) binds to ORC and cooperates to determine the frequency of initiation of DNA replication at individual origins. This protein is particularly interesting because others have shown that it is expressed in a cell-cycle-dependent manner prior to establishment of a prereplicative complex at each origin and that the protein is very unstable. We became interested in Cdc6p because genetic studies suggested an interaction with ORC. In the past year, other genetic studies have indicated an interaction between the MCM family of proteins and ORC. Interestingly, there was no genetic interaction between CDC6 and any of the MCM genes. The MCM proteins, of which there are five in *S. cerevisiae*, have been shown by others to be required for the initiation of DNA replication in yeast and other organisms. They are part of a regulatory factor called the replication licensing factor that plays some part in limiting DNA replication to once per cell cycle. Physical mapping of the initiation of DNA replication at several origins has shown that mutations in the gene encoding Cdc6p and each of the five genes encoding the MCM family of proteins reduce the frequency of initiation of DNA replication at each origin (Fig. 3). Thus, the ORC proteins, the MCM proteins, and Cdc6p all cooperate to establish prereplicative complexes at origins of DNA replication.

In contrast, temperature-sensitive mutations in genes encoding other proteins that control the initiation of DNA replication, such as Cdc7p and Cdc28p, eliminate initiation at the nonpermissive temperature but show normal initiation at the permissive temperature. Thus, these proteins seem to function as on-off switches for initiation (see Fig. 3). We are now examining the physical interaction between all these proteins.

INITIATION IN HUMAN CELLS

During the last year, we have expanded our studies to include the important problem of the mechanism and control of the initiation of DNA replication in mammalian cells, particularly in human cells. We posited that the initiation of DNA replication in mammalian cells occurs in a sequence-specific manner as it does in yeast. This was not an obvious conclusion because a number of observations suggested that vertebrate DNA replication was different from that of yeast, particularly the studies with *Xenopus* eggs and transfection of plasmids containing human DNA sequences into mammalian cells. We further hypothesized that the process of initiation was conserved and that ORC would have a similar role in yeast and in mammalian cells. It was therefore logical for us to search for the human ORC proteins. This approach has been successful with the demonstration that both Orc1p and Orc2p are found in human cells, as well as in other species such as other yeasts, plants, and invertebrates. Parallel research in Dr. Michael Botchan's laboratory at the University of California,

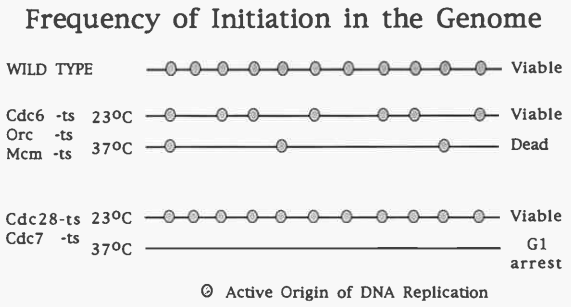


FIGURE 3 Frequency of initiation of DNA replication in the genome for various temperature-sensitive mutants in genes encoding proteins that control progression from G_1 into the S phase during the cell cycle. Shown is a representative chromosome from a single cell in a population of cells. The shaded ellipse represents an active origin at a particular locus. The frequency of active origins along a chromosome is determined by the Cdc6p, ORC, and MCM family of proteins, and this frequency is affected by temperature. In contrast, Cdc28p and Cdc7p act as on-off switches, either fully on at the permissive temperature or off at the nonpermissive temperature.

Berkeley, has identified ORC in *Drosophila*. We are now searching for other ORC proteins from human cells.

The discovery that the ORC complex is conserved strongly suggests that the mechanism of initiation of DNA replication is also similar in yeast and mammalian cells. In particular, we are interested in whether or not the sites for initiation along a chromosome are determined by specific DNA sequences and how these might be linked to other nuclear processes during cell growth and differentiation.

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MOLECULAR BIOLOGY OF PAPILLOMAVIRUSES

A. Stenlund M. Berg, E. Gillitzer, T. Sedman, J. Sedman, G. Chen, 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) give rise to tumors that are prone to progress toward malignancy, especially frequently cervical carcinoma. In total, HPV DNA can now be found in biopsies from approximately 80% of all cervical carcinomas.

A key impediment to the study of papillomaviruses has been the inability to define a simple in vitro cell culture system for HPVs, largely due to the fact that these viruses normally require specialized

differentiating cells that only with great difficulty can be generated in cell culture. Therefore, a bovine papillomavirus (BPV-1) has become the prototype virus for the papillomavirus group largely because a convenient cell culture system exists for this virus. In this cell culture system, viral gene expression, oncogenic transformation, and viral DNA replication can be studied. The DNA replication properties of papillomaviruses show some unique and interesting characteristics. As part of their normal life cycle, these viruses can exist in a state of latency which is characterized by maintenance of the viral DNA as a multicopy plasmid in the infected cells. The copy number of the viral DNA appears to be tightly controlled, and the viral DNA is stably inherited under these conditions. This system therefore provides a unique opportunity to study plasmid replication in mammalian cells.

In previous years, we have reported the development of a short-term replication assay that has enabled us to define the viral components that are required for viral DNA replication. More recently, we have directed our attention toward detailed biochemical analysis of the replication process. We are studying the biochemical properties of the two viral proteins (E1 and E2) that are required for viral DNA replication. We are also studying how these two proteins interact with the ori and with each other to generate initiation complexes.

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 can provide sequence specificity in the formation of the initiation complex. We have also completed some work (in collaboration with T. Melendy in B. 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.

Role of the Transcription Factor E2 in BPV DNA Replication

J. Sedman, 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. 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 E1-E2-ori complex is formed by cooperative binding of E1 and E2 to ori and requires low concentrations of E1. Formation of the E1-ori complex requires higher concentrations of E1 and forms on the same ori sequence but 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. The sum of these results indicates that the ability to form the E1-E2-ori complex is important for replication activity in vivo.

For replication in vitro under standard conditions, E1 alone is sufficient to initiate replication in an ori-specific manner. This result presents 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. A clear difference between in vitro and in vivo conditions for replication is the presence of vast quantities of competing cellular DNA sequences under in vivo conditions. Since E1 appears to have a relatively modest sequence specificity, E2 might function to in-

crease the selectivity of binding. By challenging binding of E1 with nonspecific competitor DNA, in the absence or presence of E2, we have demonstrated that the sequence specificity of E1 is greatly increased in the presence of E2. Consequently, we have modified the conditions used for replication in vitro by including competitor DNA. This modification results in a complete dependence on E2 protein for replication, resulting in a cell-free replication 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.

Binding of E1 and E2 to the BPV ori

J. Sedman, T. Sedman, A. Stenlund

An important factor in elucidating the function of the two different E1-containing complexes that can form on the ori is the specific composition of the complexes and the stoichiometry of binding. This information is also important to determine if a precursor-product relationship exists between the two complexes. We have therefore performed molecular mass determinations of the two different E1-containing ori complexes using a combination of glycerol gradient centrifugation and gel filtration as well as cross-linking studies. The results of these studies show that E1 in the absence of E2 binds to the ori as a trimer. The formation of the trimer is induced by the presence of the specific binding site for E1 and does not occur in the absence of DNA. Interestingly, this trimer of E1 is topologically linked to the DNA, indicating that E1 forms a ring-like structure that encircles the DNA. In the presence of E2, E1 binds as a monomer to the same binding site. High-resolution footprinting as well as interference analysis demonstrates that the monomer of E1 binds in an identical position as one of the E1 molecules in the trimeric complex, indicating that maybe the E1-E2-ori is a precursor for the trimeric E1-ori complex.

Structure-Function Studies of the E1 Initiator Protein

E. Gillitzer, A. Stenlund

Viral initiator proteins are characterized by a number of biochemical activities that are required for initiation of DNA replication. These include sequence-specific DNA-binding activity, DNA helicase activity, ori unwinding activity, and DNA-dependent ATPase activity. In addition, E1 can interact with itself to form multimers and can also interact specifically with the E2 protein. Some of these activities are associated with the monomeric E1 protein, whereas other activities require homo association and formation of multimeric E1 complexes. We have initiated a mutational analysis of the E1 polypeptide to define the domains in E1 that are required for these different activities. We are initially focusing on mapping the domains of the E1 protein that are required for formation of the two different ori complexes (E1-ori and E1-E2-ori), i.e., DNA-binding activity, homo association, and association with E2. By detailed deletion analysis, we have so far identified a 17-kD minimal DNA-binding domain that also contains the sequences required for interactions with E2.

Physical Interactions between the E1 and E2 Proteins

M. Berg, M. DaCosta, A. Stenlund

In most eukaryotic replicons that have been studied so far, binding sites for transcription factors constitute a part of the *cis*-acting sequences required for replication activity. In the majority of cases, including, for example, SV40, polyomavirus, and ARS elements from *Saccharomyces cerevisiae*, this auxiliary activity can be supplied by various transcriptional *trans*-activators with little apparent specificity. A similar requirement exists also for papillomavirus replicons; however, only the virus-encoded transcription factor E2 can serve as an auxiliary factor for 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. Because the E2 proteins are well conserved between different papillomaviruses and have a conserved overall structure, we tested E2 proteins from other papillomaviruses for interaction with BPV E1 and for replication. E2 from HPV-11 failed to interact with BPV E1 in either of these assays. This observation presented us with an

opportunity to map the regions of E2 that were required for this interaction by construction of chimeric BPV/ HPV-11 E2 proteins. This approach has a distinctive advantage over conventional mutagenesis in that the overall structure of the protein can be maintained.

We have used this procedure to generate a large number of chimeric E2 proteins. These chimeras were tested for their ability to interact physically with BPV E1 in a biochemical assay and for the ability to support replication in vivo. The results from these experiments have revealed a more complex situation than we had anticipated. We find that (1) multiple regions from both the amino-terminal *trans*-activation domain of E2 and the carboxy-terminal "hinge" and DNA-binding domain cooperate to generate a strong interaction with E1; (2) the interaction between the E1 and E2 proteins appears to take place in a two-step process where an initial weak physical interaction between E1 and the DNA-binding domain of E2 allows a stronger, productive interaction to take place between E1 and the activation domain of E2; and (3) the specificity of the interaction between E1 and E2 resides in the first step, i.e., in the interaction between E1 and the DNA-binding domain of E2, whereas the activation domains of both BPV E2 and HPV-11 E2 are equally capable of interacting with E1.

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. Replication of SV40 DNA relies on SV40 large-T antigen for ori recognition, ori unwinding, and helicase activity. Using 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 these factors, an involvement in chromosomal DNA replication has been demonstrated through genetic experiments in *S. 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 of 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.

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TRANSCRIPTIONAL REGULATION

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M. Tanaka D. Aufiero R. Freiman V. Meschan W. Tansey
R. Babb C. Hinkley L. Peña A. Wilson
M. Boutros C. Huang

We study the mechanisms of transcriptional regulation in eukaryotes. To probe these mechanisms, our studies focus on the interactions of viral and cellular regulatory factors, particularly between the herpes simplex virus (HSV) protein VP16 and its cellular targets: When HSV infects cells, VP16, a virion protein, is released into the infected cell, whereupon it associates with two cellular proteins—Oct-1, a POU-domain-containing transcription factor, and HCF—on VP16-responsive elements in HSV immediate-early (IE) promoters. Formation of this VP16-induced complex results in activation of transcription of the HSV IE promoters.

Our studies can be divided into three general areas: (1) the study of how activators, such as VP16, and the basal transcriptional machinery interact to stimulate transcription, (2) the mechanisms of formation of the VP16-induced complex, and (3) the cellular functions of the VP16-associated protein HCF. This section also includes independent studies by M. Tanaka, which probe promoter occupancy by transcription factors in vivo.

Enhancer Function

S. Atanasoski, C. Hinkley, W. Tansey

In these studies, we examine how sequence-specific activators that assemble on transcriptional enhancers stimulate transcription through interactions with the basal transcriptional machinery assembled on core promoter elements. We have studied the generation of two neural POU-domain enhancer-binding proteins called N-Oct-3 and N-Oct-5 from a single open reading frame, and the interaction of transcriptional activation domains with their regulatory targets.

N-Oct 3 and N-Oct 5 were first identified in electrophoretic mobility retardation assays through their ability to bind to the octamer sequence ATGCAAAT. These two N-Oct factors are detected in extracts from tumor-derived and normal neural cells. They are present differentially, however, in extracts from melanocytes and melanoma cells: N-Oct 3 is present in extracts from both melanocytes and melanoma cells, whereas N-Oct 5 is more evident in extracts from metastatic melanoma cells. We have found in collaboration with A. Fontana and E. Schreiber (University of Zurich) that a cDNA encoding N-Oct 3 directs synthesis of both the N-Oct 3 and N-Oct 5 proteins and that the N-Oct 5 protein in neural and melanoma cell extracts is also related to N-Oct 3. N-Oct 5, however, is apparently not expressed in vivo: It is not detected if cells are rapidly lysed in SDS or if extracts are prepared with a cocktail of protease inhibitors that includes the serine-protease inhibitor 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF). These data suggest that the enhancer-binding protein N-Oct 5 is a specific in vitro proteolytic cleavage product of N-Oct 3 and is not directly related to melanocyte malignancy. Thus, the cell-specific appearance of N-Oct 5 may relate to the levels of proteases in different cell types.

To study the interaction of the transcriptional activation domains of enhancer-binding proteins with regulatory targets, we are taking two approaches: a global approach in which we probe these interactions in yeast with the 2-hybrid assay and a directed approach in which we probe the interaction of activation domains with specific basal transcription factors. In the latter experiments, we have focused on the interaction of activation domains with the TATA box-binding protein TBP. TBP has been shown to associate in vitro with many viral and cellular transcriptional activation domains and has been frequently suggested to be a direct target of transcriptional activators. We examined the functional relevance of activation domain–TBP association by examining the effects of mutations in TBP on activation domain association. Figure 1 shows the results of such an experiment. Mutations in a specific loop of TBP (S3′/S4′) disrupt association of TBP with activation domains from both VP16 (see lane 5, Fig. 1A) and the cellular tumor suppressor gene product p53 (not shown). This mutation, however, does not affect the response of TBP to these activators in vivo (see lane

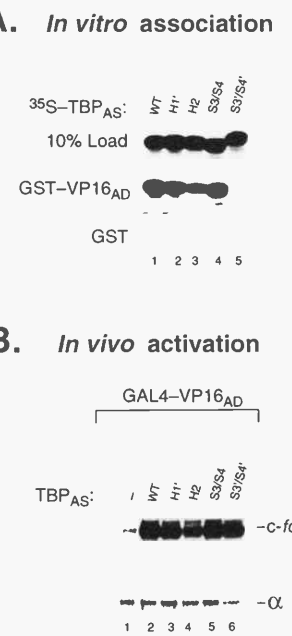

FIGURE 1 A double alanine substitution in human TBP disrupts association with the VP16 activation domain in vitro but does not affect response to VP16 in vivo. (*A*) Radioactively labeled wild-type and mutant TBP molecules were tested for association with agarose beads carrying glutathione-*S*-transferase sequences alone (GST) or GST sequences fused to the VP16 activation domain (GST-$VP16_{AD}$). Wild-type TBP (lane *1*) and three of the four mutant TBPs (lanes *2–4*) bound to GST-$VP16_{AD}$ tightly and specifically; binding of the *S3′/S4′* mutant TBP (lane *5*) to GST-$VP16_{AD}$ was reduced by 25- to 30-fold. (*B*) Wild-type and mutant TBP molecules were analyzed for their ability to support response to the VP16 activation domain in human HeLa cells, using the altered-specificity TBP assay. The figure shows the results of an RNase protection analysis, quantitating correctly initiated transcripts from a c-*fos* reporter (c-*fos*) and from an α-globin internal control plasmid (α). (Lane *1*) Activity of the c-*fos* reporter in the presence of a GAL4-$VP16_{AD}$ fusion protein, but in the absence of altered-specificity TBP; (lane *2*) rescue of transcription by wild-type altered-specificity TBP. Note that all of the mutant TBPs (lanes *3–6*), including the *S3′/S4′* mutant—which does not associate with the VP16 activation domain in vitro—support wild-type levels of response to GAL4-$VP16_{AD}$ in vivo.

6, Fig. 1B). These results suggest that the ability of TBP to associate with activation domains in vitro is not absolutely required for its ability to respond to activators in vivo. One possibility is that association with TBP is only one of multiple redundant pathways for activation of transcription by activators and that disruption of any one of these redundant pathways is insufficient to disrupt transcriptional activation.

Viral *Trans*-activation

R. Babb, M. Cleary, C. Huang

To study viral *trans*-activation, we probe the structure and function of the VP16-induced complex and its individual components. One of those components is Oct-1, which on its own recognizes a variety of *cis*-regulatory sequences, including the 8-bp octamer sequence ATGCAAAT found in many enhancers and promoters and the VP16-responsive TAATGARAT (R = purine) motif found in HSV IE promoters. Oct-1 recognizes these regulatory elements via a bipartite DNA-binding domain, called a POU domain. POU domains contain an amino-terminal POU-specific (POU_S) domain, which in Oct-1 recognizes the ATGC portion of the octamer sequence, tethered by a hypervariable linker to a carboxy-terminal POU-type homeo (POU_H) domain, which recognizes the AAAT portion of the octamer sequence. When bound to the octamer sequence, the Oct-1 POU_S and POU_H domains do not touch one another, suggesting that their interactions with DNA are largely independent of one another. Consistent with this view, in previous years, we have presented genetic studies which indicate that the two POU subdomains can adopt different arrangements on different binding sites.

These results have placed particular interest on the role of the POU domain linker in the binding of the POU domain to DNA. For example, the linker could influence how the POU subdomains can arrange themselves on the DNA. To test this hypothesis, we assayed the effects of progressive shortening of the linker on binding to different sites. We assayed binding to two Oct-1-binding sites that we identified a number of years ago in the SV40 enhancer, one similar to the octamer sequence and one quite different from the octamer sequence. Using these sites, we found that the length of the Oct-1 POU-domain linker could have very different effects on Oct-1 binding to DNA: On the octamer-like site, shortening the linker segment resulted in a progressive loss of binding affinity, whereas on the site that bears little octamer similarity, shortening the linker segment had no deleterious effect until it was only four amino acids long. These results suggest that the requirements of the linker vary depending on the positioning of the two POU subdomains on DNA.

To physically probe the conformation of the Oct-1 POU subdomains on different binding sites, we adopted, in collaboration with Shannon Pendergrast

(CSHL), a protein-DNA cross-linking strategy developed by S. Pendergrast and R. Ebright at the Waksman Institute. For this approach, a photoactivatable cross-linking agent is covalently attached to a unique cysteine in the protein of interest. The modified protein is allowed to bind to DNA and is then subjected to ultraviolet irradiation, which causes the modified cysteine side chain to form a covalent bond with nearby DNA. Protein-DNA complexes are separated from free DNA, and the DNA is cleaved at the site of cross-linking, giving precise information about where a given protein or protein subunit contacts DNA. These studies have shown that the POU subdomains do indeed adopt different arrangements on different binding sites: On a VP16-responsive TAATGARAT site that bears little similarity to an octamer sequence, the POU_S domain is positioned on the side opposite of the POU_H domain from where it is positioned on an octamer site.

To study the role of VP16 in VP16-induced complex formation, we have compared the activities of two VP16 proteins: the HSV-1 VP16 protein and its homolog from bovine herpesvirus 1 (BHV-1). We have shown that these two Oct-1 coregulators possess similar, if not identical, homeodomain recognition properties but possess different virus-specific *cis*-regulatory specificities: The HSV-1 VP16 protein activates transcription from an HSV-1 VP16-response element and the BHV-1 VP16 protein activates transcription from a BHV-1 VP16-response element. A distinct 3-bp segment, the "D" segment, lying 3′ of the canonical TAATGARAT motif, is responsible for the differential *cis*-element recognition and transcriptional activation by these two homeodomain coregulators. These results demonstrate how homologous homeodomain coregulators can direct differential transcriptional regulation by a single homeodomain protein.

Cellular Functions of HCF

M. Boutros, R. Freiman, Y. Liu, A. Wilson

Although the involvement of HCF during HSV infection is understood in some detail, little is known about its role in uninfected cells. In previous years, we have shown that HCF is a family of polypeptides, the large majority of which range in size from 110 kD to 150 kD. These polypeptides are processed from a large precursor protein of approximately 300 kD. The processed polypeptides represent amino- and carboxy-terminal fragments resulting from proteolytic cleavage at a series of centrally located 26-amino-acid repeats called the HCF repeats. Curiously, the amino- and carboxy-terminal HCF fragments resulting from cleavage remain associated with one another after cleavage.

Although HCF is a collection of large polypeptides, only the amino-terminal 380 residues of HCF are required to associate with VP16 and stabilize VP16-induced complex formation. This region consists of a series of six repeats related to the GG motif found in a variety of proteins. In other proteins, the repeated GG motif (or related sequences) directs formation of a "super-barrel" structure of six β-sheets, each made up from four β-strands; each of the six β-sheets forms one blade of a propeller-like structure. Mutational analysis indicates that the six blades of the propeller-like structure in HCF do not all have the same role in stabilizing the VP16-induced complex.

One of the roadblocks in the study of the cellular functions of HCF has been the lack of a known cellular function. Recently, however, a hamster cell line that has a temperature-sensitive defect in cell proliferation caused by a missense mutation in the HCF gene has been discovered (H. Goto and T. Nishimoto, Kyushu University). These cells stop proliferating at 39.5ºC, but they can resume proliferating when restored to the permissive temperature. The missense mutation that causes the HCF defect lies within the amino-terminal VP16-interaction domain (H. Goto and T. Nishimoto, pers. comm.). We therefore asked, in collaboration with Drs. Goto and Nishimoto, whether this same mutation would influence VP16 function.

Indeed, in a transient expression assay, VP16 can activate transcription in the mutant cells at the permissive temperature but not at the nonpermissive temperature. These results argue that HCF is indeed important for VP16 activation of transcription in vivo and that VP16 targets a part of the cell machinery important for cell proliferation. We next tested the effects of the HCF mutation on the ability of VP16 to induce complex formation and found that the mutant HCF, prepared from cells grown at the nonpermissive temperature or synthesized by translation in vitro, is defective for stabilization of the VP16-induced complex. This defect in VP16-induced complex formation correlates with an inability of the mutant HCF

protein to associate with VP16 effectively. Thus, the very same mutation that causes an arrest in cell proliferation causes a defect in VP16 function. These results lead us to hypothesize that VP16 binds to HCF by mimicking an important cellular cofactor for HCF function, a function that is related to cell proliferation.

Enhancement of Activator Binding to a Promoter In Vivo by Cooperativity and Activation Domain Function

M. Tanaka

Binding of transcriptional activators to a promoter is an important process in transcriptional activation. I have continued to investigate the in vivo determinants of activator binding to a promoter in yeast cells. A quantitative in vivo methylation protection assay has previously led to the unexpected observation that activator binding to a promoter is enhanced in vivo by transcriptional activation domains of activators. An extension of the analysis has now revealed another determinant: the number of activator-binding sites present in a promoter. Thus, multiple sites within a promoter can cooperatively recruit cognate factors in vivo, and they do so regardless of whether the factors contain an effective activation domain.

Modulation of activator binding by these two determinants—potency of activation domains of activators and the number of binding sites present in a promoter—plays an important role in determining transcriptional responses of a promoter. For example, potent activators can efficiently stimulate transcription at low concentrations because they readily bind to a promoter. Similarly, a promoter containing many binding sites can direct high levels of transcription because it effectively recruits activators. In contrast to these in vivo results, neither of these determinants showed an effect on activator binding to DNA in a typical in vitro assay. It thus appears that, in vivo, binding of activators to a promoter is determined not only by direct interaction between activators and a specific DNA sequence, but also by other interactions, which may involve factors such as general transcription factors and histones.

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RNA SPLICING

A.R. Krainer J. Cáceres A. Mayeda T.-L. Tseng
A. Hanamura M. Murray I. Watakabe
D. Horowitz M. Pastrnak Q. Wu

MECHANISMS OF CONSTITUTIVE AND ALTERNATIVE PRE-mRNA SPLICING

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 to alter gene expression, either tissue-specifically or in response to a developmental program or to extracellular signals. Both constitutive and alternative splicing mechanisms involve multiple protein components, as well as RNA components that are part of small nuclear ribonucleoprotein (snRNP) particles. Our lab has focused on the identification, purification, and molecular characterization of protein factors that are necessary for the catalysis of splicing and/or for the regulation of alternative splice site selection.

A major portion of our efforts has been devoted to the characterization of three families of RNA-binding proteins: the SR proteins, exemplified by SF2/ASF; the hnRNP A/B proteins, of which the best characterized is hnRNP A1; and the more recently discovered SF7 factors. Individual SR proteins function as essential constitutive splicing factors and are also involved in constitutive or regulated enhancer-dependent splicing. In addition, they modulate alternative splicing in vivo or in vitro in a concentration-dependent manner. This activity is antagonized by hnRNP A/B proteins to modulate alternative 5′-splice site selection and by SF7 proteins to determine alternative 3′-splice site selection.

ROLE OF THE STRUCTURAL DOMAINS OF SR PROTEINS IN SUBNUCLEAR LOCALIZATION AND ALTERNATIVE SPLICING IN VIVO

J. Cáceres used epitope-tagged SR protein cDNAs to analyze the role of different domains of SF2/ASF and other SR proteins in alternative splicing in vivo and in localization to the proper nuclear regions. Using SF2/ASF mutants that he previously analyzed in vitro, he examined their activity in HeLa cells by transient overexpression and cotransfection of an alternative splicing reporter gene. In collaboration with T. Misteli and D. Spector (CSHL), he used indirect immunofluorescence and confocal microscopy to measure the presence of wild-type or mutant epitope-tagged proteins in the nucleoplasmic speckle region. Surprisingly, the RS domain of SF2/ASF was found not to be required for localization in the speckles. In contrast, the RS domains of other SR proteins that, unlike SF2/ASF, contain a single RRM proved to be essential, in agreement with work by other laboratories. In general, an excellent correlation was observed between the ability of mutant proteins to localize to the speckles and their ability to affect alternative splicing in vivo. In addition, SR proteins lacking either RRM1 or RRM2 showed significant alternative splicing activity in vivo. This apparent discrepancy with our earlier in vitro studies could be due either to improper folding of mutant proteins in vitro or to the use of different substrates in the two assays. Additional pre-mRNAs are being assayed to address the issue of specificity and to determine which domains contribute to such substrate specificity.

ISOLATION AND CHARACTERIZATION OF NOVEL SR PROTEINS

To gain insights into the unique properties of individual SR proteins, cDNAs for three new human SR proteins were cloned and characterized, in collaboration with Dr. John Bell (John Radcliffe Hospital, Oxford) and his associate, Dr. Gavin Screaton, who did a sabbatical in my laboratory (Screaton et al. 1995). By use of reverse transcriptase–polymerase chain reaction (RT-PCR) with degenerate primers, Screaton cloned full-length cDNAs corresponding to human SRp55 and SRp40 (for which partial peptide sequence or homologs from other species had been pre-

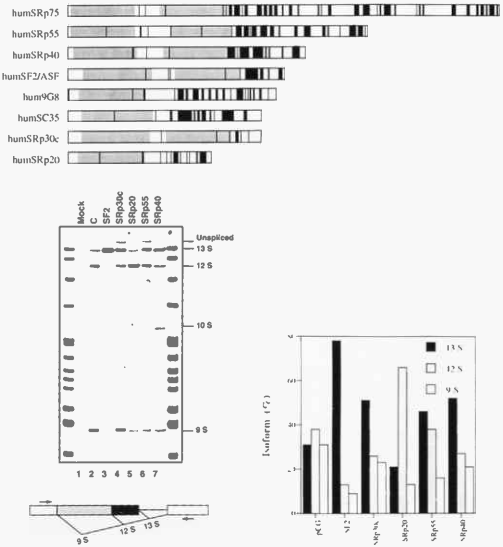

FIGURE 1 Domain structure of human SR proteins and effect of selected SR proteins on alternative splicing of adenovirus E1A in vivo. (*Top*) Schematic representation of domain structures of all eight human SR proteins drawn to scale. Gray shading represents RRMs. Thin dark vertical lines represent individual RS or SR dipeptides. Thicker lines indicate clustering of RS or SR dipeptide repeats, with the thickness being proportional to the number of consecutive repeats. (*Bottom, left*) In vivo analysis of alternative splicing activity of cloned SR proteins. HeLa cells were transiently cotransfected with the adenovirus E1A gene, and one of the indicated SR protein cDNAs, subcloned in the pCG expression plasmid. Total RNA was analyzed by RT-PCR, with 5′-end labeling of one of the primers. (Lane *1*) Mock transfection; (lane *2*) control cotransfection with pCG lacking an insert; (lanes *3–7*) cotransfections with the indicated SR protein cDNAs. The mobilities of unspliced pre-mRNA and of 13S, 12S, 10S, and 9S mRNAs are indicated on the right. The diagram below shows the structure of the E1A gene, the RT-PCR primers, and the major mRNAs generated by alternative 5′-splice site selection. The 10S and 11S mRNAs arise from double splicing events and are not shown in the diagram. The data were quantitated by PhosphorImage analysis, and the amounts of 13S, 12S, and 9S mRNAs are expressed as a percentage of the sum of all three mRNA isoforms. (*Bottom, right*) Quantitation of relative use of alternative E1A 5′-splice sites in response to SR protein overexpression.

viously described) and to a novel SR protein, SRp30c, which has an unusually short RS domain. The structures of known human SR proteins are shown in Figure 1 (top). Additional cDNA clones for SRp40 and SRp55 were also isolated, which reflect extensive alternative splicing of the corresponding pre-mRNAs. The predicted protein isoforms lack the carboxy-terminal RS domain and might be involved in feedback regulatory loops.

A. Mayeda and G. Screaton expressed the three cDNAs encoding the full-length SR proteins in *Escherichia coli*, and the purified recombinant proteins

were shown to be active in general splicing, i.e., they functionally complemented a HeLa cell S100 extract deficient in SR proteins. In addition, J. Cáceres and G. Screaton used a transient cotransfection assay to test the ability of human SRp30c, SRp40, and SRp55 to modulate alternative splicing in vivo. Comparison with known SR proteins in this assay revealed striking differences that appear to reflect unique substrate specificities. For example, SF2/ASF and SRp40 promote use of the adenovirus E1A 13S 5′-splice site, whereas SRp20 favors specifically the 12S 5′-splice site (Fig. 1, bottom). We further showed that expression of the endogenous SR proteins is regulated differentially in stimulated T lymphocytes, concomitantly with changes in the alternative splicing patterns of CD44 and CD45 cell surface molecules. FACS analysis revealed that individual T cells simultaneously express CD44 and CD45 isoforms that represent opposite patterns of exon skipping and inclusion, suggesting that different regulators (e.g., SR proteins or their antagonists) control alternative splicing of these pre-mRNAs or that perhaps the same regulator acts on them with opposite polarity (Screaton et al. 1995).

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 the SF7 factors, respectively) 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 considered, the system gains a complexity perhaps sophisticated enough to control the numerous alternative splicing choices made in living cells. In particular, these proteins may be responsible for global regulatory mechanisms that affect alternative splicing of large sets of pre-mRNAs in different tissues, developmental stages, and/or in response to external signals.

SR PROTEINS FROM PLANTS

In collaboration with A. Barta's group in Vienna, A. Mayeda characterized purified SR proteins isolated from tobacco and carrot cells. In addition to the observed cross-reactivity with antibodies directed to human SF2/ASF or to SR proteins, it was possible to show that purified plant SR proteins are active in constitutive and alternative splicing when assayed in heterologous HeLa cell extracts, with either mammalian or plant pre-mRNAs. Although biochemical studies of plant pre-mRNA splicing have been hampered by the lack of in vitro systems, these studies show that SR proteins are conserved in structure and function between plant and animal kingdoms (Lopato et al. 1996).

SEQUENCE-SPECIFIC RNA-BINDING PROPERTIES OF hnRNP A/B PROTEINS

I. Watakabe is studying the sequence-specific binding properties of hnRNP A/B proteins. SELEX experiments showed that hnRNP A2 and B1 bind with high affinity to short RNAs containing at least one copy of a hexameric sequence related to the hnRNP A1 recognition site identified by G. Dreyfuss and colleagues. Filter-binding experiments showed that the three proteins have similar sequence specificities and selectivities. Mutational analysis of the consensus high-affinity binding site shows that the hexamer is necessary but not sufficient for high-affinity binding. Analysis of hnRNP A1 mutants that are inactive in alternative splicing in vitro, but active in general RNA binding, showed an excellent correlation between ability to switch alternative splice site use and sequence-specific binding to the SELEX winner sequences. These results suggest that sequence-specific RNA binding by hnRNP A/B proteins contributes to their alternative splicing function.

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. D. Horowitz recently identified a 343-amino-acid human protein, hPRP18, which has 30% identity to yeast PRP18. The homology is primarily in the carboxy-terminal third of the protein, which is also conserved in rice and nematode ESTs. He expressed hPRP18 in *E. coli* and raised antibodies to it,

which were used to deplete hPRP18 from HeLa cell nuclear extracts. Immunodepletion specifically and completely blocked the second step of splicing, leading to accumulation of splicing intermediates. Recombinant hPRP18 restored the second step of splicing, demonstrating that hPRP18 is essential for catalytic step II. Recombinant yeast PRP18 also restored activity to the depleted human extract, showing that the yeast and human proteins are functional homologs. However, hPRP18 cannot functionally replace yeast PRP18, either in vivo or in vitro. hPRP18 does not appear to be stably associated with snRNPs. Unexpectedly, anti-hPRP18 antibodies cross-react with a protein that appears to be a novel component of U4/U6 and U4/U6·U5 snRNP particles, which will be purified and characterized.

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 most 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 proteins were purified on the basis of immunoreactivity, and partial amino acid sequence was obtained in collaboration with R. Kobayashi (Protein Chemistry Facility).

Tseng is focusing on a fission yeast protein that, in addition to reacting with the above antibodies, contains an RNA-recognition motif, as determined from partial peptide sequence and nucleotide sequence of RT-PCR fragments. Cosmid genomic and cDNA clones have been obtained and are presently 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.

SPLICING OF PRE-mRNAs WITH NONCONSENSUS SPLICE SITES

Q. Wu has been studying the biochemistry of splicing of a very small class of introns with unique 5′- and 3′-splice sites that do not conform to the consensus sequences. The few known examples share unique 5′, 3′, and putative branch site elements, and the work of R. Padgett and colleagues suggests that these introns require minor snRNAs, such as U12 and perhaps U11, for processing in vivo. Interestingly, pre-mRNAs that contain these nonconsensus introns also contain conventional introns. Examples include the human proliferating cell nucleolar antigen P120, a cartilage matrix protein, an ion channel subunit, and the *Drosophila* Prospero gene. J. Steitz and colleagues have recently described splicing of the P120 unusual intron in vitro and shown dependence on U12 snRNA. We have also recently demonstrated accurate splicing of the unusual intron in a sodium channel subunit pre-mRNA in human extracts and are currently characterizing the splicing pathway and analyzing the requirement for specific snRNPs and protein factors.

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PROTEIN SYNTHESIS

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

Gene expression is controlled at a variety of levels and through many different mechanisms. The research in our laboratory is focused on several systems which illustrate the diversity of regulatory processes in human cells. Some of these systems involve viral genes that interact with and modulate cellular pathways, thereby impinging on processes such as cellular growth control and viral multiplication. The following paragraphs summarize the progress made in 1995 on each topic.

During the year, Mingsong Liu and Qizhi Wang joined the laboratory while Piruz Nahreini left to take up a position in the Biotech industry. Yuliang (Frank) Ma completed his graduate training and took his well-earned Ph.D. degree with him to Jim Feramisco's laboratory at the University of California, San Diego.

Regulation of PCNA

B. Lee, M. Liu, P. Wendel, M.B. Mathews

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

Transactivation of the PCNA promoter by the E1A 243R protein is mediated through a novel *cis*-acting element termed the PERE (the *P*CNA *E*1A-*r*esponsive *e*lement) lying about 50 nucleotides upstream of the PCNA transcriptional start site. The PERE contains a sequence resembling an activating transcription factor (ATF)-binding site, and studies reported last year established that it binds the transcription factor ATF-1. Other PERE sequences may bind an as yet unidentified transcription factor. In addition, an adjacent site located downstream from the PERE was shown to bind the enhancer-associated protein RFX1. Further work, by gel mobility shift analysis, indicates that RFX1 binding is also influenced by a sequence located upstream of the PERE. Furthermore, mutagenesis of this region implies an interrelationship between the ATF and RFX1 sites. For example, the formation of the RFX1-containing complex P1 is dependent on the integrity of both the ATF and RFX1 sites. Experiments with antibodies directed against the two factors have so far failed to detect the simultaneous binding of ATF-1 and RFX1 to the same DNA probe. On the other hand, measurements of the stability of DNA-protein complexes raise the possibility that the complex containing RFX1 may be able to compete with the PERE-binding factor(s) in binding to the same DNA sequence in vivo.

Mutational analysis of the E1A gene had suggested that regions of the E1A 243R protein that bind to cellular factors p300 and p107 are required for optimal PCNA induction by E1A. To address the possible roles of these two proteins in PCNA transactivation directly, we obtained the cDNAs for both p300 and p107 and tested them in HeLa cells using transient expression assays. Our results further implicate p107 in the transactivation of the PCNA promoter by E1A 243R. Transient expression of the wild-type p107 protein reduced transactivation in a dose-dependent manner. p107 mutants deficient in their capacity to bind the E1A protein were equally inhibitory, indicating that the reduced transactivation is not simply due to sequestration of E1A 243R by p107 in an inactive complex. Furthermore, a monoclonal antibody raised against p107 specifically supershifted one of the DNA-protein complexes formed

on the PCNA promoter, providing evidence that p107 associates with the promoter in vitro.

The p300 protein has recently been shown to possess properties of known transcriptional adaptor proteins. Overexpression of wild-type p300 had no effect on transactivation of the PCNA promoter by E1A in transient expression assays. However, transactivation was impaired by p300 mutations that abrogate E1A binding. transactivation by E1A mutants deficient in their ability to bind to wild-type p300 was impaired to a similar degree, suggesting that p300 can mediate E1A transactivation of the PCNA promoter. Taken together, these data support the view that E1A 243R transactivates the PCNA promoter through more than one pathway (Fig. 1).

Regulation of HIV-1 Gene Expression

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

The human immunodeficiency virus, HIV-1, is the causative agent of AIDS. It carries regulatory genes which play important roles in its life cycle and pathogenicity and operate by unprecedented mechanisms. The HIV-1 protein Tat stimulates transcription from the HIV promoter, located in the viral long terminal repeat (LTR). It increases the rate of transcriptional initiation and augments elongation by overcoming premature transcriptional termination. Tat's antitermination function can be reproduced in a cell-free system using purified, bacterially expressed Tat to stimulate HIV transcription. This greatly facilitates biochemical investigations of its mode of action. We reported last year that the ability of Tat to transactivate in vitro depends on preincubation with cell extract or S-100, implying that a cellular factor(s) is required to "potentiate" Tat transactivation. Potentiation could be due to the formation of a complex between Tat and cell protein(s) or to covalent modification of Tat. To understand the nature of this activity, we have embarked on its purification. The activity has been carried through several steps including ammonium sulfate precipitation, ion exchange and gel filtration chromatography, and glycerol gradient centrifugation. Present information suggests that potentiation activity may copurify with a small protein (<30 kD). The purification studies are ongoing.

One attractive hypothesis to explain Tat transactivation is that in vitro it improves the processivity of RNA polymerase complexes, possibly by recruit-

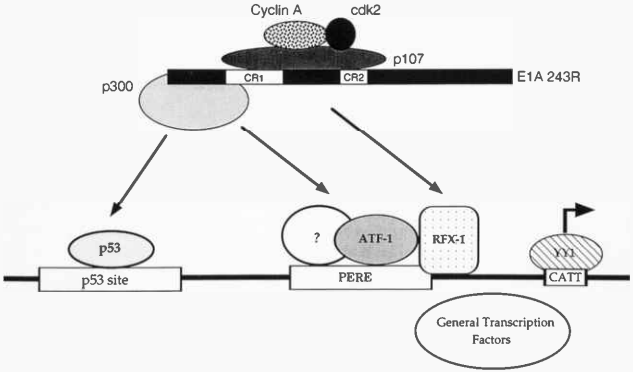

FIGURE 1 Schematic representation of the human PCNA promoter and its interactions with the E1A 243R oncoprotein. E1A 243R transactivates the PCNA promoter via the PERE, an element which binds the ATF-1 transcription factor. Transactivation by E1A 243R is apparently mediated through its interactions with cellular proteins p300 and/or p107. Additionally, there is an upstream p53 binding site through which the p53 transcription factor can transactivate PCNA in a manner that can be repressed by E1A 243R.

ing or modifying cellular elongation factors. Tat is able to bind a cellular kinase, TAK, which phosphorylates 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. We are also attempting to purify this activity. Our initial approach was to try to deplete TAK from HeLa cell extracts by an affinity chromatography method, but TAK could not be removed efficiently from the extract under various experimental conditions. Subsequently, we turned to conventional chromatographic procedures and discovered that TAK separated into more than one fraction, possibly because it is assembled into several different complexes. During the early steps of purification, TAK copurified with TFIIH, which was recently shown to contain CAK (cyclin-dependent activating kinase) as one of its components. CAK has CTD kinase activity which is associated with TFIIH. However, TAK can be distinguished from CAK by several different biochemical procedures, and it appears to be a novel kinase.

Translation of an RNA Polymerase III Transcript

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

We have shown that a transcript generated by RNA polymerase III (pol III) can be translated in vivo to synthesize functional protein. The HIV-1 Tat-coding sequence was placed under the control of the adenovirus VA RNA promoter, a strong pol III promoter. When the pVA-Tat chimera was transfected into human cells, uncapped and nonpolyadenylated VA-Tat RNA was produced, transported to the cytoplasm, recruited by polysomes, and translated. The resultant Tat protein was detected by its ability to transactivate 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 demonstrate that not all of the functional mRNA in a cell must be made by pol II. Nevertheless, no naturally occurring pol III-generated mRNAs have been characterized. Why should this be?

One possible explanation is that RNAs generated by pol III are relatively poor templates for protein synthesis. As noted in last year's Annual Report, the rate of initiation of translation on VA-Tat RNA is low in comparison to mRNA made by pol II, probably because the terminal structures characteristic of normal mRNAs (5′caps and 3′poly[A]) enhance translational efficiency. A second possibility is that intron removal from pol III-generated transcripts may be an inefficient process. To test this hypothesis, we asked whether a pol III transcript can be spliced in vivo. The first intron sequence of the β-globin gene (130 bp long) was inserted into the Tat-coding region of pVA-Tat in such a way that its removal by splicing would yield an uninterrupted Tat-coding sequence. Cells transfected with this construct, pVA-Tat.int, displayed no detectable Tat activity, whereas those transfected with pCMV-Tat.int, an equivalent construct transcribed by pol II, expressed Tat as expected. Examination of the transcripts by RNA protection analysis confirmed that RNA from cells transfected with pVA-Tat.int was not spliced, unlike that from pCMV-Tat.int-transfected cells. These results imply that the pol III transcripts could not be spliced in vivo, suggesting that they are not handled in the nucleus in the same way as conventional mRNAs.

In the course of this work, we noticed that pol III failed to terminate transcription at a site which matches the published criteria defining a pol III termination signal. These features, which were deduced from a study of the *Xenopus* 5S ribosomal RNA gene, include a run of four or more thymidines in a GC-rich context; adenosine residues close to the stretch of Ts were reported to be inhibitory. Since the site had been introduced a short distance (30 nucleotides) downstream from the transcriptional B box, we first considered the possibility that this promoter element might occlude the termination signal or otherwise render it inactive. However, the natural VA RNA termination signal was not bypassed when it was brought as close as 14 nucleotides to the B box. Site-directed mutagenesis of the termination signals showed that As in close proximity to the run of Ts did not influence the efficiency of termination, whereas Gs were very inhibitory. These findings suggest that the mechanisms of termination are different for the 5S rRNA and VA RNA genes; this difference may reflect differences between these two pol III genes in the architecture of their promoters and their mechanism of transcriptional initiation.

Translational Control

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

The protein kinase DAI, the double-stranded RNA (dsRNA)-activated inhibitor of translation, now generally known as PKR, is emerging as a pivotal regulator of cell activity. It has been implicated in the control of cell growth, transformation and differentiation, apoptosis, and signal transduction, as well as translation. PKR is a serine/threonine kinase, and many of its actions are mediated via the phosphorylation of the protein synthesis initiation factor eIF2 (eukaryotic initiation factor 2). Phosphorylation of eIF2 leads to the slowing or cessation of translation. PKR is present in most cells and tissues in a latent (inactive) state, and as its name implies, it is activated by RNA regulators. A current model posits that PKR activation requires dimerization of the enzyme, which allows intermolecular phosphorylation to occur. This autophosphorylation has been correlated with the activation of PKR as an eIF2 kinase.

To understand the activation process in detail, we have set out to characterize the autophosphorylation sites. Seven such sites were identified through peptide mapping and sequencing (conducted with Dan Marshak and Georgia Binns, Protein Chemistry Core Section), and each of them was mutated to alanine (which cannot be phosphorylated) to explore their roles in enzyme function. One site, Thr-258, appears to play a role in the activation of the kinase. Experiments conducted with Sean Lee and Mariano Esteban (SUNY, Brooklyn) and with Patrick Romano and Alan Hinnebusch (NICHD, Bethesda) showed that mutation of this site decreased enzyme activity in simian cells and in yeast. In a kinase assay, conducted in vitro, mutation of the neighboring sites (Ser-242 and Thr-255) had no effect on their own, but these mutations exacerbated the deleterious effect of the Thr-258 mutation. Thus, phosphorylation of the three sites may act synergistically to activate the enzyme, and they may comprise part of an autoregulatory region of the enzyme. Four additional sites lie in the spacer region between the two RNA-binding motifs that constitute the dsRNA-binding domain. Mutation of these sites suggests that they do not play a role in activation of the enzyme. We are also examining the effects of these mutations on cell growth and tumorigenicity in mammalian cells.

eIF2 is not the only substrate for PKR, however. Included in a growing list of proteins that can also be phosphorylated by this kinase is IκB, the inhibitory subunit of transcription factor NF-κB; an unidentified 90-kD protein from rabbit reticulocytes; and the HIV-1 transactivator Tat, mentioned above. We have explored some of the characteristics of the interaction between Tat and PKR, demonstrating that PKR can bind and phosphorylate both the two-exon form of Tat (Tat-86) and the single-exon form (Tat-72). Phosphorylation of Tat by PKR is dependent on the prior activation of the enzyme by dsRNA. Although the amino-terminal region of Tat (residues 2–36) is dispensable for binding, PKR did not bind or phosphorylate Tat mutants containing only the amino-terminal 48 residues, suggesting that the interaction between PKR and Tat may require the RNA-binding region of Tat. Purified Tat-72 competed with eIF2 for phosphorylation by activated PKR, and, reciprocally, Tat was able to inhibit the activation of PKR. Current efforts are aimed at determining the site(s) of Tat phosphorylation and the significance of the phenomenon for the activities of both Tat and PKR.

Virus-Host Interplay

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

PKR is an important component of the host antiviral defenses. Its synthesis is induced by interferon, and it is activated upon virus infection, with the result that generation of viral progeny is curbed. In the face of this threat to their survival, viruses have evolved countermeasures to protect themselves against activation of the kinase. One of the best known of these is the production by adenoviruses of small RNA molecules, the virus-associated (VA) RNAs, some of which serve to prevent PKR activation. In previous years, we described how adenovirus 2 (Ad2) VA RNA_I blocks PKR activation and took a phylogenetic approach to help determine structural features that are critical to its function in vivo. We also explored the structure and function of VA RNA_I in vitro.

The related viral product, Ad2 VA RNA_{II}, is transcribed from a neighboring gene and shares many properties with VA RNA_I, yet it does not effectively interfere with PKR activation. VA RNA_{II} is synthesized by pol III, but much less abundantly than VA RNA_I. Although most human adenoviruses produce two VA RNAs, a minority of them (~20%) do not

possess a VA RNA_{II} gene. Whereas some viruses apparently did not develop a second gene, in others it seems to have been lost or inactivated secondarily. Nevertheless, when the VA RNA_{II} gene is present, its sequence is at least as highly conserved as that of adenovirus VA RNA_I; furthermore, VA RNA_{II} adopts a secondary structure different from that of VA RNA_I. We therefore speculate that it plays a different role in virus infection, possibly one that is less essential in some tissues or circumstances. To address this hypothesis, we have begun to identify cellular proteins (other than PKR) that interact with VA RNA_{II} and might shed light on its role.

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MOLECULAR MECHANISMS OF APOPTOSIS

Y. Lazebnik L. Faleiro J. Rodriguez
H. Fearnhead Y. Xu

Our laboratory focuses on the molecular mechanisms of apoptosis, a process that is critical for normal development and that causes disease including cancer when it malfunctions. During apoptosis, a cell ac-

tivates the machinery responsible for the cells self-destruction, and it appears that a key part of this machinery are ICE-like proteases (ILPs), a family of highly specific and closely related cysteine proteases. ILPs are constitutively expressed in cells as inactive precursors, and their activation appears to coincide with the onset of apoptotic execution. We have developed a cell-free system that reproduces the execution stage of apoptosis in vitro and provides a powerful model for studying the role of ILPs in this process. The goal of our laboratory is to identify and characterize the ILPs that are involved in apoptosis and to study their regulation. These studies will provide the molecular framework with which to manipulate apoptosis in order to selectively kill cancer cells.

During the last year, we were joined by Lavina Faleiro and Joe Rodriguez as graduate students and Howard Fearnhead as a postdoctoral fellow.

Identification of ILPs Involved in Apoptosis

L. Faleiro, Y. Lazebnik

Although it is believed that ILPs are essential for apoptosis in mammals, their identities and functions are not well established. The current strategy to identify ILPs involved in apoptosis is to obtain the ILPs on the basis of their homology with known ILPs and then investigate whether each particular ILP plays a part in apoptosis. Although this approach has been extensively used, it is not known precisely which of the growing number of cloned ILPs are involved in apoptosis. To avoid characterizing ILPs not related to apoptosis, we chose an approach based on biochemical activity. ILPs are expressed as latent precursors, which are processed into active proteases. We decided to use affinity probes to identify ILPs that are activated during apoptosis. The ILP affinity probes are biotin-labeled peptide inhibitors that bind irreversibly to the catalytic site of active ILPs. The ILPs labeled with such inhibitors can be visualized by using electrophoresis followed by immunoblotting and probing with avidin-peroxidase.

Using this technique, we found that multiple ILPs are apparently activated during apoptosis. This confirmed our biochemical data which suggested that multiple ILP activities with distinct specificity are involved in cell destruction during the cell death. In collaboration with Scott Lowe's laboratory (CSHL), we are going to determine whether distinct sets of ILPs were activated in different cells and following different stimuli.

During the last year, we began to identify the ILPs that are involved in apoptosis. In collaboration with the laboratories of Dr. D. Nicholson (Merck Frosst, Canada) and Dr. N. Thornberry (Merck, New Jersey), we expanded upon our observation that an ILP which is activated during apoptosis cleaves the nuclear enzyme poly(ADP-ribose) polymerase (PARP). This ILP was purified by using an affinity probe that mimics the cleavage site in PARP. The purified enzyme was identified by peptide sequencing as cpp32, a previously cloned protease whose function was unknown. We are continuing identification of other ILPs activated during apoptosis.

A Cell-free System That Reproduces Apoptosis in Human Tumor Cells

H. Fearnhead

The cell-free system of apoptosis that we developed initially employed extracts made from a chicken cell line. Despite the progress that we made using this model system, we decided to develop a similar system based on human tumor cells for the following reasons: (1) Our long-term goal is to use apoptosis to kill cancer cells in humans; (2) most of the research on ILPs has been carried out with human or mouse models; and (3) we envision that our research will eventually merge with other fields, such as cell cycle, DNA replication, and signal transduction, which rarely use chicken as a model. Therefore, we reproduced the cell-free system using extracts made from apoptotic Jurkat cells, a well-characterized cell culture model of human T-cell leukemia. All of the changes that we observed in the chicken system, such as changes in nuclear morphology, DNA cleavage, and cleavage of PARP, were reproduced with the extracts from Jurkat cells.

During the last year, our system was also reproduced in other laboratories by using a number of different cell types and ways to induce apoptosis. This indicates that the apoptotic events reproduced by this cell-free system are conserved in different cell types and species.

Activation of ILPs at the Onset of Apoptosis

H. Fearnhead

It appears that neither protein nor mRNA synthesis is required to carry out apoptosis in many cell types. This observation implies that apoptotic ILP precursors are present in viable cells and are activated at the onset of apoptosis. Having identified several active ILP species in apoptotic cells, we wanted to know if these enzymes are present in intact viable cells and whether these enzymes can be activated in a cell-free system.

We found that extracts made from untreated cells contain ILPs required for apoptosis and that these ILPs can be activated by adding exogenous ICE-like enzymes. Intriguingly, activation of ILPs rendered these extracts active in our cell-free assay of apoptosis. This indicated that the apoptotic machinery can be activated directly through activation of ILPs and independent of upstream signaling pathways. Thus, ILPs are an attractive target for drugs designed to induce apoptosis in cells where upstream signaling pathways are blocked, for example, by a tumor virus.

Proteins Interacting with ILPs

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

Active ILPs are required for the onset of apoptosis. Since ILPs are directly implicated in the destruction of cell structures such as the nuclear lamina and since specific inhibitors of ILPs prevent apoptosis, it is likely that ILP activation is the event that occurs at the end of the signaling pathways and is the first irreversible step inevitably leading to cell death. Therefore, understanding the mechanism of ILP activation is of paramount importance, because if this process is blocked in a cancer cell, the cell will become resistant to any cytotoxic drugs that induce apoptosis, for example, the majority of currently used chemotherapeutics. For the same reason, ILP activation is an attractive target for pharmacological intervention to circumvent defects in the apoptotic pathways upstream of the execution machinery, such as mutations in the p53 tumor suppressor. The mechanism and regulation of ILP activation are not known. It is possible that this regulation is carried out by proteins that interact with ILPs. Therefore, we began systematic screening for the interacting proteins by using the yeast two-hybrid system. In our initial screen, we used CED-3, an ILP that is essential for apoptosis in nematodes, and identified several proteins that interact with this protease. We are currently determining whether these proteins play a part in apoptosis.

Substrates and Inhibitors of ILPs

J. Rodriguez

Apoptosis involves multiple processes, including restructuring of the cytoskeleton, disassembly of the nucleus, degradation of DNA, inhibition of protein and RNA synthesis, and expression of antigens facilitating the engulfment of apoptotic cells by other cells. If ILPs are triggering some of these changes, they most likely do so by proteolytic cleavage of specific substrates. Depending on the substrate, such cleavages may have different effects, such as inactivation or activation of the protein, alteration of the protein structure and function, or disassembly of a cellular structure. Only a few substrates for ILPs during apoptosis are known, and they provide insufficient insight into how ILP activity causes cell death. Most of these substrates were found by serendipity. During the last year, we began to develop approaches that would allow us to screen for new ILP substrates.

We also began a search for cellular inhibitors of ILPs. Although such inhibitors have not been identified, viral inhibitors of ILPs have been reported. These proteins are potent inhibitors of apoptosis in several experimental systems and appear to be part of the viral anti-host defense mechanism, suggesting that they mimic cellular proteins. Thus, identification of viral and cellular inhibitors of ILPs should provide new insights into how the activation of ILPs is regulated.

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REGULATION OF CELL SIGNALING IN HIV PATHOGENESIS

J. Skowronski S. Bronson A.J. Iafrate
Y.-N. Chang M. Lock

AIDS is invariably associated with the depletion of the regulatory subset of T cells that express CD4 protein on the cell surface. CD4+ T cells are preferentially infected by the human immunodeficiency virus (HIV), which suggests that viral infection may be lethal for these cells. Our main interest is in the molecular mechanisms underlying the development of AIDS, in particular, in the functional consequences of the interaction between immunodeficiency virus proteins and the cellular regulatory machinery. In the last year, our work continued to focus on two HIV-1 proteins, Nef and Env. Both proteins have profound effects on the normal function and/or survival of CD4+ T cells and these effects are likely to play an important part in AIDS.

Nef is a small cytoplasmic protein found in all immunodeficiency viruses. Nef is important for efficient virus replication in vivo but is dispensable for the viral life cycle under in vitro conditions. The critical in vivo function of Nef is not understood. We have previously shown that Nef can alter the development of CD4+ T cells when expressed in immature T-cell precursors in the thymus. This effect correlated with down-regulation of CD4 molecule expression on the cell surface and disruption of the association of CD4 with the Lck protein tyrosine kinase. The CD4-Lck complex is required for normal signaling by the T-cell antigen receptor. These effects of Nef on CD4 and on the CD4-Lck complex will disrupt the normal function of CD4+ T cells.

In the last year, we performed detailed genetic analysis of the interaction between Nef and the T-cell antigen receptor (TCR) machinery. These studies demonstrated that in addition to disrupting the CD4-Lck complex, Nef also disrupts another aspect of TCR function. This other effect of Nef involves a membrane proximal block in TCR signaling that is independent of Nef's ability to down-regulate CD4 expression. In a second development, we have constructed new lines of transgenic mice that express Nef in peripheral T cells. This is an important model because peripheral CD4+ T cells are the major targets for HIV infection in humans. Finally, we have initiated genetic studies of the cytotoxic effect of HIV Env expression in T cells.

Nef Alters the Normal Function of Peripheral CD4+ T Cells in Transgenic Mice

Y.-N. Chang, S. Bronson, A.J. Iafrate, M. Lock

Little is known about the interactions between the HIV-1 genes and the regulatory machinery in infected cells. To address the consequences of Nef on normal T cells in vivo, we directed expression of the HIV-1 Nef protein to these cells in transgenic mice. A T-cell-specific expression vector containing Nef-coding sequences placed under control of transcription control elements derived from the T-cell-specific CD2 gene was permanently integrated into the mouse germ line. Transgenic animals derived with this construct showed high-level Nef protein expression in the mature cells of the T lymphocyte lineage. This is an important model because mature T cells that express CD4 protein on their surface are the major target of HIV infection in humans.

A compilation of results from a detailed study of T cells from CD2 N1[NL43]#2 transgenic mice is shown in Figure 1. Several lines of evidence from these animals indicated that the Nef protein has a dramatic effect on the development and function of T

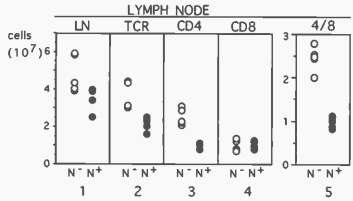

FIGURE 1 Altered T-cell subsets in CD2 N1(NL43)#2 mice. The absolute number of lymph node cells (LN), T cells (TCR), and $CD4^+$ and $CD8^+$ T lymphocytes in mesenteric lymph nodes from transgenic animals (N^+, *closed circles*) and from littermate controls (N^-, *open circles*) is shown. The percent of cells that express the αβ-TCR and CD4 or CD8 molecules was determined by three-color flow cytometry. The absolute number of CD4 and CD8 T cells was calculated from the total number of cells recovered from lymph nodes, as determined by hemocytometer counting. The ratio of CD4 to CD8 T cells is also shown (4/8). Each mouse analyzed is represented by five dots and each dot represents a result of a single determination.

cells that express the CD4 molecule. As shown in Figure 1, the frequency of the mature T cells in the thymus and in the lymph nodes of transgenic animals was abnormally low (panel 1). This decrease was largely due to an abnormally low frequency of $CD4^+$ T cells, and the representation of $CD8^+$ T cells was found to be normal (compare panels 3 and 4). Moreover, expression of the CD4 protein on the majority of transgenic $CD4^+$ lymph node cells was threefold to fourfold lower than that observed on $CD4^+$ lymphocytes from littermate animals. CD4 is a component of TCR, and it is likely that Nef-induced T-cell perturbations reflect altered TCR function in Nef-expressing T cells. Our current studies address the effect of Nef on TCR-regulated processes in T cells such as the induction of proliferation and apoptosis.

Functional Analysis of Nef Interaction with T-cell Receptor Machinery

A.J. Iafrate, S. Bronson

TRANSIENT ASSAY OF THE EFFECT OF NEF ON T-CELL ACTIVATION

To confirm our observations from transgenic mice in human T cells and to address the relationship between the effect of Nef on CD4 expression and on TCR signaling, we developed in vitro assays of Nef's interaction with the TCR machinery using a human $CD4^+$ T-cell line. Because constitutive expression of Nef in T-cell lines may result in a selection for mutants in signal transduction pathways, we developed a transient expression system to study the effect of Nef on TCR function. In this assay, expression vectors encoding the wild-type, or mutant, Nef proteins were introduced into $CD4^+$ Jurkat T cells by transfection. Subsequently, cells were stimulated via TCR by overnight culture in the presence of α-TCR monoclonal antibody (MAb). One of the effects of TCR signaling is the induction of the very early activation antigen CD69 on the surface of stimulated cells. Therefore, induction of CD69 expression provided a convenient indicator of TCR function. Flow cytometric analysis of CD4 and CD69 expression permitted us to quantitate the effect of Nef on CD4 and CD69 expression simultaneously.

NEF BLOCKS A MEMBRANE-PROXIMAL EVENT RELATED TO CALCIUM SIGNALING IN THE TCR PATHWAY

As illustrated in Figure 2, transfection of Jurkat T cells with the CD3 NA7 vector, but not with a control "empty" CD3-β plasmid, resulted in an approximately 50-fold decrease in CD4 expression in a large fraction of electroporated cells (compare panels 1 and 2). The NA7 allele, like the NL43 allele, is a natural and very potent allele of HIV-1 *nef*. We know from control experiments that the $CD4^{low}$ cells reflected the population of successfully transfected cells. α-TCR stimulation of the mock-transfected cells resulted in an approximately six- to tenfold increase in CD69 expression, when compared to that in the unstimulated population (compare panels 1 and 3). In contrast, treatment of cells transfected with the CD3 NA7 vector resulted in induction of CD69 expression only in the $CD4^{high}$ cells. CD69 expression in $CD4^{low}$ cells was not increased (see panel 4). Thus, Nef blocks induction of CD69 expression by TCR stimulation. This effect of Nef was also observed with SIV Nef protein (see Fig. 2B). Therefore, the blocking of TCR signaling by Nef reflects a conserved function of the viral protein.

Nef does not block CD69 induction following stimulation with α-TCR monoclonal antibody in the presence of calcium ionophore, which bypasses the TCR to induce calcium signaling. Thus, Nef appears to suppress a membrane-proximal event that involves calcium signaling. Interestingly, Nef did not interfere

The cleavage products remain noncovalently associated and move to the cell surface where they can bind the CD4 protein expressed on the surface of an opposing cell. Env is toxic to the cells and Env-induced death of cultured T cells appears to result from two distinct effects. One effect involves the formation of large multinucleated syncytia and results from the fusion of cells bearing the gp120/gp41 envelope heterodimer on their surface with cells expressing the CD4 molecule. This process involves adhesion of the two cells mediated by gp120-CD4 interaction and membrane fusion mediated by the gp41 Env subunit. The other effect involves the death of individual cells and occurs in the absence of syncytia formation.

To dissect the molecular basis for Env-induced cell death, we have established an inducible Env expression system in a human T-cell line and developed a simple assay to correlate Env expression with cell death. As expected, induction of wild-type HIV-1 Env protein expression in this system, but not of other control proteins, resulted in massive death of productively transfected cells. The cell death occurred rapidly, within hours, following the onset of Env expression. In our system, Env-induced cell death did not involve cell fusion and therefore was likely to reflect cell-autonomous events that take place in Env-expressing cells. Interestingly, two mutant Env proteins that are deficient in binding to CD4 and in mediating membrane fusion, respectively, failed to induce cell death in our system. Our results suggest a model where intracellular interaction of Env with CD4 leads to membrane fusion events within the cells and mediate the lethal effects of the viral protein. Future work will be directed at understanding downstream events in this pathway.

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BIOCHEMISTRY OF CELLULAR GROWTH AND DIFFERENTIATION

D.R. Marshak E. Araya, N. Carpino, N. Chester, T. Connolly, S. Goren, J. Kahler, C. (Walker) Kelley, M. Pittenger, D. Rizzieri, C. van den Bos

Our goal is to attack biological problems of the control of cell growth in several cell systems that are models of different cancers. The members of this laboratory conduct specific research on signal transduction processes involved in cell growth and differentiation. We use a multidisciplinary approach using advanced methods of cell biology, analytical biochemistry, and molecular biology. The projects in this laboratory have been various, ranging from basic research on protein phosphorylation, to myelogenous leukemia, and to bladder cancer in connection with parasitic infections. Most recently, we have begun work on mesenchymal tissues, involving the mesenchymal stem cells, under a research agreement with Osiris Therapeutics, Inc., in Baltimore. During 1995, Dr. Marshak maintained a transitional status between Cold Spring Harbor Laboratory and Osiris Therapeutics, where he serves as the Senior Vice President, Research and Development. By the end of 1995, the members of this laboratory had left Cold Spring Harbor or moved to other positions. During the past 10 years, Dr. Marshak and his laboratory groups have been successful in the science of growth control, and we all look forward to the new challenges ahead. Cold Spring Harbor has been a fantastic environment in which to grow, discover, and learn. Our thanks to all our friends and colleagues for their support during our transitional year, and we have no doubt that many connections will remain intact.

Molecular Cloning and Expression of Protein Kinase CKII Subunits

N. Chester, D.R. Marshak [in collaboration with J. Horton, Cold Spring Harbor Laboratory]

Protein kinases are enzymes that catalyze the transfer of phosphates from the γ-phosphate position of ATP

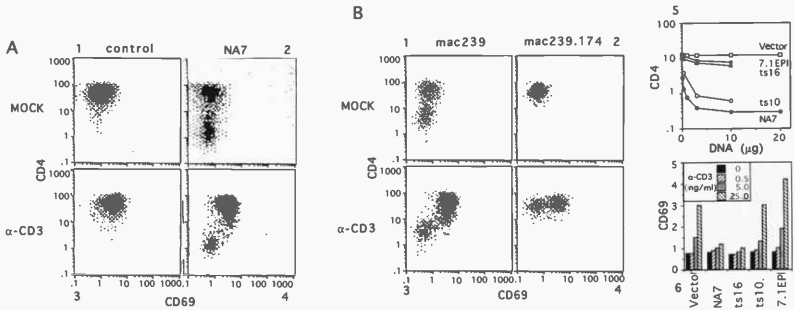

FIGURE 2 HIV-1 Nef interferes with two aspects of T-cell receptor machinery: CD4 expression, and induction of CD69 expression, on the cell surface. (*A*) Two-color FACS analysis of the effect of HIV-1 Nef on CD4 and CD69 expression. CD4 and CD69 expressions on the cell surface are shown on the ordinate and abscissa, respectively, in logarithmic scale. (*B*) Mutations separate the effects of Nef on CD69 and on CD4 expression. (Panels *1–4*) Two-color analysis of CD4 and CD69 expression in cells transfected with plasmids comprising the wild-type SIV mac239 (mac239), or the mutant mac239.174 (mac239.174), alleles and nonstimulated (Mock), or stimulated overnight with α-CD3 MAb (α-CD3). (Panels *5–6*) Dose-response analysis of the effect of mutant HIV-1 Nef proteins (ts16, ts10, 7.1Epi) on CD4 and CD69 expression. The empty CD3-β plasmid (vector) and CD3 NA7 expression vector (NA7) were used as negative and positive controls, respectively. Jurkat T cells were coelectroporated with different amounts of expression plasmids (0 to 15 μg, shown in the abscissa). (*Upper panel*) CD4 expression is shown in the ordinate as peak channel number of CD4 fluorescence; (*lower panel*) CD69 expression on cells stimulated overnight with indicated amounts of α-CD3 MAb is shown in the ordinate as peak channel number of CD69 fluorescence.

with induction of IL-2Rα chain expression by α-TCR stimulation. Thus, Nef blocks only a subset of signals emanating from the TCR. Further experiments to define the Nef-induced biochemical defect in the TCR pathway in Jurkat T cells are under way.

EFFECTS OF NEF ON CD4 AND CD69 EXPRESSION CAN BE SEPARATED BY MUTATION

We identified mutations that disrupt preferentially the effect of Nef on CD4 expression and on the induction of CD69 expression, as shown in Figure 2. For example, a single-amino-acid substitution in the 239.174 SIV Nef protein disrupted the effect of Nef on CD4, but not on CD69 antigen expression (compare panels 1 and 2 and 3 and 4 in Fig. 2A). Results from dose-response analysis of selected mutant HIV-1 Nef proteins are shown in Figure 2B. The ts16 mutation disrupted the effect of Nef on CD4, but not on induction of CD69 expression by α-TCR monoclonal antibody (see ts16 in the upper and lower panels). In contrast, the ts10 mutation had little effect on the ability of Nef to down-regulate CD4 expression, but disrupted the ability of Nef to block induction of CD69. Finally, the 7.1EPI mutation disrupted both the effect of Nef on CD4 and on CD69 expression.

These observations demonstrate that Nef perturbs two separate aspects of TCR machinery in T cells. One effect of Nef is to down-regulate surface expression of CD4. The other effect is to block a membrane-proximal event in the TCR pathway. It appears that the ability of Nef to block TCR signaling is independent from its ability to down-regulate CD4 expression. Current experiments address the molecular mechanisms involved in these two effects of Nef on the TCR machinery. How these two effects of Nef modulate the normal function of CD4+ T cells in vivo is being addressed in experiments with CD2 N1[NL43] mice that express Nef protein in mature T cells.

Mechanism of Cell Death Induced by HIV-1 Env Protein

M. Lock

The envelope protein (Env) is produced as a large precursor (gp160) that is subsequently cleaved in the Golgi apparatus into two subunits, gp120 and gp41.

to a hydroxyl moiety (serine, threonine, or tyrosine) on proteins. The enzyme protein kinase 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, α and β, with molecular masses of 37–44 kD and 24–28 kD, respectively, with an apparent subunit composition of $\alpha_2\beta_2$. The α-subunit is found in two different forms, known as α and α′, that arise from separate genes. To answer questions about the mechanism of enzyme regulation, we have cloned and expressed DNA molecules coding for the full-length forms of the α and β subunits, which has allowed us to insert the coding sequences for the CKII subunits into various expression vectors. 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, β. The catalytic and regulatory subunits combine in a functional complex whether mixed in vitro or co-expressed in the same bacterial cells. We have purified large amounts of the regulatory subunit for structural analysis. In collaboration with J. Horton, we found that a truncation of the protein can occur during expression at a site within the carboxy-terminal domain. Using carboxy-terminal protein sequencing and mass spectrometry, we were able to identify the sites of cleavage and show the basis for production of full-length protein. Dr. Horton has crystallized the β-subunit of CKII in forms that appear to diffract X-rays and has produced larger crystals to solve the three-dimensional structure of this protein. Whereas the catalytic subunits of CKII are homologous to other known protein serine/threonine kinases, the β-subunit is unique. We expect that the structure of this regulatory subunit will be a novel contribution to the field.

Subcellular Pools of Casein Kinase II

N. Chester, D.R. Marshak

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 (non-muscle 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. These antibodies allowed us to continue our studies of the subcellular localization of the subunits of the enzyme during the cell division cycle of HeLa cells. N. Chester has used the antibodies developed in the lab to do detailed immunoprecipitation and immunoblots of the CKII subunits. First, he found that the synthetic rate for the CKII β-subunit is significantly longer than that of the catalytic subunits, whereas the degradation rates for all the subunits appear to be similar. Second, using steady-state metabolic labeling, he identified different pools of CKII that vary in subunit stoichiometry and activity. He has performed experiments in HeLa cells to determine if different pools of CKII are localized to different subcellular compartments. There is evidence that the α′ subunit may have a higher relative concentration in the nucleus. Previous work from Dr. Marshak in collaboration with J. Diaz-Nido in Madrid indicated that this subunit may have unique regulation during neuronal differentiation during embryonic development.

Cell Cycle Regulation of Protein Kinase CKII

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

During the past year, we have continued study of cell cycle regulation of protein kinase CKII and its effect on cyclin-dependent kinases in the control of cell division. The enzyme $p34^{cdc2}$ is a cyclin-dependent kinase that is a key regulator of the G_2-M transition in the cell cycle, and appears to play a part in the G_1-S transition as well. We previously reported that human $p34^{cdc2}$ is phosphorylated on Ser-39 during the G_1 phase of the HeLa cell division cycle. The enzyme apparently responsible for this phosphorylation event is CKII, based on in vitro phosphorylation data, substrate specificity, and correlation of the activities in vivo. To evaluate the functional role of this phosphorylation event in eukaryotic cells, we turned to the budding yeast, *Saccharomyces cerevisiae*, in which we could genetically manipulate the homolog of

mammalian $p34^{cdc2}$, known as CDC28. In collaboration with A. Sutton, we have demonstrated that the homologous serine is phosphorylated on CDC28 in yeast as on $p34^{cdc2}$ in mammalian cells. To test the functional role of this site in yeast, we constructed a yeast carrying a mutation that results in the substitution of an alanine residue for the serine. The mutant plasmid was introduced into a yeast defective for wild-type CDC28. The phenotype of the mutant is a small cell size, indicating a premature entry into S phase. This result suggests that the phosphorylation of the serine on CDC28 in yeast allows the cell to extend G_1. Further genetic and biochemical experiments have indicated that this is not due simply to a lesion in cyclin binding, as first put forward as an explanation. Expression of human cyclin A in yeast containing the serine-alanine mutation in CDC28 does not result in altered amounts of activity or protein precipitating in CDC28/cyclin complexes. Furthermore, CLN mutants in yeast do not have any effect in combination with the serine-alanine CDC28 mutants. Thus, the exact mechanism of action remains somewhat obscure. However, C. van den Bos has succeeded in expressing the human cdc2 Ser-39 mutants in HeLa cells, and his initial results indicate that there is a similar size and cell cycle checkpoint phenotype in this human cell line.

Biochemical Mechanisms in Chronic Myelogenous Leukemia

N. Carpino, S. Goren, J. Kahler, D.R. Marshak
[in collaboration with B. Clarkson, MSKI, New York, and O. Witte, University of California, Los Angeles]

Chronic myelogenous leukemia (CML) is a disease characterized by the presence of a chromosomal translocation in somatic cells of the hematopoietic system. This chromosomal aberration causes the expression of a fusion protein, known as $p210^{bcr:abl}$. The $p210^{bcr:abl}$ fusion protein functions as an unregulated tyrosine kinase, which is associated with proliferation of myeloid progenitor cells. When patients progress to blast crisis, there are massive numbers of myeloid progenitors in the peripheral circulation with a lack of differentiated myeloid cells and their corresponding functions in these hematopoietic lineages. Our studies are aimed at understanding the action of $p210^{bcr:abl}$ in two different ways.

First, S. Goren has taken the approach of trying to characterize sites on the p210 protein that are phosphorylated. She has identified what appear to be sites for the cell-division-cycle-regulated protein kinase, $p34^{cdc2}$. These sites appear to be phosphorylated in vitro and have sequences that match consensus phosphorylation sites for cyclin-dependent kinases. Clones encoding mutant forms of the p210, or its homolog, p185, were developed. The phenotypes of the mutant proteins were characterized by assays with retroviral vectors for transforming ability and subcellular localization. So far, no distinct phenotype of these mutants has been proven.

Second, N. Carpino has developed a purification method for a protein that is hyperphosphorylated on tyrosine residues in cells containing $p210^{bcr:abl}$, and which may be a substrate for the abnormal tyrosine kinase in leukemic cells. This protein, known as p62, has been purified to apparent homogeneity using phosphotyrosine antibodies and classical chromatographic methods. Sequence analysis of the protein was conducted with R. Kobayashi (CSHL), and N. Carpino is cloning a cDNA encoding the protein. This molecule may give us the first clue at the downstream events in myeloid cells transformed with $p210^{bcr:abl}$.

Schistosoma haematobium Soluble Egg Antigens and Bladder Cancer

D.R. Marshak, C. (Walker) Kelley [in collaboration with T. Gaafar, Cairo, Egypt]

This project arose from a collaboration with Dr. T. Gaafar in Cairo entitled "Biochemical, molecular, and immunological characterization of the *Schistosoma haematobium* soluble egg antigens that induce *in vitro* human granuloma formation," which has been part of the Schistosomiasis Research Program. This program is directed toward developing a vaccine for schistosomiasis, a disease that plagues Africa and Asia and is prevalent in Egypt. We chose to work on the soluble egg antigens of the bladder form of the disease, as candidates for synthetic vaccines. In addition, there is a high correlation between chronic *S. haematobium* infection and bladder cancer, suggesting a molecular connection between the immunological response of the patient to chronic infection and the ultimate formation of tumors. Our strategy has been to isolate and characterize the antigens in hopes of identifying peptides for vaccine materials and, potentially, making a connection to bladder cancer.

Specific *S. haematobium* soluble egg antigens

(SEA) were previously identified by electrophoresis, electroelution, and granuloma formation in vitro. Protein bands were identified with relative mobilities at 84, 66, 57, 40, and 30 kD. Proteinase digestion and peptide maps by high-performance liquid chromatography (HPLC) were performed on preparations of each SEA protein. The chromatograph effluent was monitored for ultraviolet absorbance at 214 nm and 280 nm. Peaks of absorbance were collected and analyzed for peptide content and structure. Sequence analysis by repetitive Edman degradation was performed on significant peaks. The primary sequence information was used in two different ways. First, sequences were used to search the existing databases including GENBANK, PIR, and SwissPro for homologies with known proteins or open reading frames in identified genes. Second, in cases where the sequences are novel, synthetic peptides were prepared by solid-phase methods, cleaved to solubilize the products, and purified to apparent homogeneity. The identity of the peptide product was documented and validated by HPLC and plasma desorption mass spectrometry. Some of the peptides have been used as antigens in rabbits to raise antisera that react with the SEA protein. These results were documented by immunoblotting and immunohistochemistry by Dr. Gaafar and her students and colleagues in Cairo. In addition, the synthetic peptides themselves are candidates for use in vaccines directly, and they can be tested in the rodent models of schistosomiasis or in models of granuloma formation in vitro.

The 30-kD SEA protein was obtained in sufficient amounts from preparative electrophoresis experiments. Peptide maps of this protein produced 17 fractions that were potential candidates for sequence analysis, of which 11 were completed. Two of these yielded very revealing amino acid sequence results that show some homology between the 30-kD SEA protein and a family of proteins known as 14-3-3. The 14-3-3 proteins have been implicated in the regulation of protein kinases involved in signal transduction at the plasma membrane surface. Specifically, 14-3-3 may play a part in the interactions between the tyrosine kinase receptors and the Ras protein-stimulated mitogenic pathway that involves the mitogen-activated protein kinases. It is known that abnormally functioning Ras (such as arise from point mutations) leads to cancer, and Ras was one of the first cellular proto-oncogenes identified. In patients that have chronic infection and immunological reaction to the SEA, there are likely antibodies formed against the 30-kD SEA. Because of the homology with 14-3-3, these antibodies could contribute to dysfunction of the Ras signaling system, giving rise to cellular transformation. One would suppose that this effect is most intense near the site of the bladder granulomas. These conjectures, based on the remarkable homology data between the 30-kD SEA and 14-3-3, may provide a clue as to the link between *S. haematobium* infection and the high incidence of bladder cancer.

Mesenchymal Stem Cells

D.R. Marshak, T. Connolly, M. Pittenger [in collaboration with Osiris Therapeutics, Baltimore, Maryland]

Under a research agreement with Osiris Therapeutics, we began the study of mesenchymal stem cells. These cells are adherent, marrow-derived, fibroblastic cells that appear to give rise to various lineages of the mesenchymal tissues, including bone, cartilage, muscle, tendon/ligament, fat marrow stroma, and other connective tissues. The cells are difficult to isolate and to grow. During the first half of the year, we initiated studies to examine the growth and differentiation of the cells under various conditions. The cells can differentiate down several mesenchymal lineages in vitro, and they have been shown to regenerate mesenchymal tissues in vivo. Cultures of these cells are being developed for autologous cell-based implants for regenerative tissue therapy.

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MOLECULAR GENETICS OF EUKARYOTIC CELLS

Members of this section study mainly the pathways of signal transduction that regulate growth, differentiation, and gene expression. Nick Tonks and co-workers have determined the X-ray crystal structure of the PTP1B phosphate bound to its substrate and developed mutants that bind and trap substrates. They have studied the relation of phosphatases to the state of phosphorylation of the catenins, intracellular proteins that may mediate signaling due to cell-cell contact. Linda Van Aelst's group has identified new candidate targets of mammalian Ras and Rac proteins and has begun the study of their involvement in malignant transformation, metastasis, and cellular morphology. Michael Hengartner's laboratory has studied three aspects of programmed cell death (apoptosis) in the nematode *C. elegans*, identifying potentially new suppressors of previously characterized mutants in the central apoptotic pathways and identifying two groups of mutations in genes required for the host to recognize the apoptotic cell. Michael Wigler's laboratory has identified the function of new proteins in the Ras signaling pathways of yeasts and demonstrated the multifunctionality of Ras in mammalian cells. Nikolai Lisitsyn's group, in collaboration with Dr. Wigler's lab, has identified numerous new loci that are frequent sites of genetic alteration in human gastrointestinal and breast cancers. David Helfman's group has continued to explore the physiological role of the various tropomyosin isoforms and to define the components of the splicing complexes that determine the tissue-specific splicing patterns. David Spector's group has succeeded in visualizing the dynamic recruitment of factors to subnuclear compartments in the splicing and transcription of RNA in vivo and developed cell-free systems for the manipulation and assay of these events.

MAMMALIAN CELL GENETICS

M. Wigler L. Van Aelst, E. Chang, M. Hamaguchi, M. White, C. Yen, P. Barker, D. Dong, D. Esposito, V. Jung, R. Lucito, M. Nakamura, C. Nicolette, W. Wei, M. Barr, K. Chang, K. O'Neill, M. McDonough, J. Stolarov, H. Tu, G. Asouline, X. Duan, B. O'Connor, M. Riggs, L. Rodgers, J. Troge

Work in our group is divided into three parts. The first is the study of the signal transduction pathways that relate to the Ras proteins, small GTP/GDP-binding regulatory molecules. Ras proteins are frequently activated by mutation in human cancers. The second is the application of representational difference analysis (RDA) to the definition of the acquired genetic lesions of breast and gastrointestinal cancers. RDA is a method, developed in collaboration with Nikolai Lisitsyn, for the comparison of similar genomes (Lisitsyn et al., *Science 259:* 946 [1993]). The third is the development of combinatorial techniques for the discovery of small molecules capable of binding to and altering the biological activity of signal transduction proteins. These studies, emerging out of earlier work with chemists at Columbia University, are conducted in collaboration with Peter Nestler (Ohlmeyer et al., *Proc. Natl. Acad. Sci. 90:* 10922 [1993]).

Ras Signaling Pathways

L. Van Aelst, E. Chang, M. White, V. Jung, C. Nicolette, M. Barr, K. O'Neill, J. Stolarov, H. Tu, K. Chang, M. McDonough

Previous work from our group defined the effector targets of the Ras proteins in the budding yeast *Saccharomyces cerevisiae* (Toda et al., *Cell 40:* 27 [1985]), the fission yeast *Schizosaccharomyces pombe* (Wang et al., *Mol. Cell. Biol. 11:* 3554 [1991]; Van Aelst et al., *Proc. Natl. Acad. Sci 90:* 6213 [1993]; Chang et al., *Cell 79:* 131 [1994]), and mammalian cells (Van aelst et al., *Proc. Natl. Acad. Sci. 90:* 6213 [1993]). Most recently, we provided the most rigorous genetic evidence that one target for Ras in mammalian cells is the Raf protein (White et al. 1995). Our studies also provide clear evidence for other targets important to the ability of Ras to induce malignant transformation, morphological change,

mitosis, and gene expression (White et al. 1995; Joneson et al. 1996). We have therefore directed considerable effort to the discovery of, and analysis of, other potential targets for Ras (Van Aelst et al. 1995). The candidate targets are found by two hybrid interactions (Fields et al., *Nature 340:* 245 [1989]). The threshing of candidates proceeds by mutational analysis, coupled with gene transfer and tests for genetic interactions.

In the paper by White et al. (1995), we demonstrate a general genetic approach to testing the importance of particular protein/protein interactions. Using the two-hybrid system, we generate allele-specific mutations between genes encoding interacting proteins. We are applying this approach to a number of signal transduction problems, including an analysis of the interactions between Raf and 14.3.3, a highly conserved protein with which Raf interacts (Freed et al., *Science 265:* 1713 [1994]; Irie et al., *Science 265:* 1716 [1994]) and an analysis of other candidate Ras targets.

Concomitant with these mammalian studies, we have continued our studies of the Ras pathway in yeasts. In particular, we have identified a new gene product that appears to facilitate the arrival of Ras at its proper site within *S. cerevisiae* (Jung et al. 1995). We continue to explore the function of Cap, an adenylyl-cyclase-associated *S. cerevisiae* protein that is required, in vivo, for Ras activation of adenylyl cyclase (Field et al., *Cell 61:* 319 [1990]). Two-hybrid and biochemical studies indicate that Cap can bridge adenylyl cyclase to Sla2, a yeast homolog of mammalian talin. Mutational studies indicate that this bridging function is required for the sensitivity of adenylyl cyclase to Ras in vivo.

Our earlier studies in *S. pombe* revealed two distinct Ras-dependent pathways, one leading to activation of a MAP kinase module, through the direct activation of the Byr2 protein kinase (Wang et al., *Mol. Cell. Biol. 11:* 3554 [1991]; Van Aelst et al., *Proc. Natl. Acad. Sci. 90:* 6213 [1993]), and one leading to the activation of a Rho-like protein, Cdc42, through a protein complex involving the Scd1 and Scd2 proteins (Chang et al., *Cell 79:* 131 [1994]). Both pathways are required for sexual conjugation. We have identified a second component that interacts with Byr2, namely Ste4, a leucine zipper protein, and have shown that Ste4 and Ras1 both bind the regulatory domain of Byr2, but at separable sites. The Ste4 and Ras1 proteins evidently act together, although they can act partially independently to activate Byr2. Ste4 shows weak homology with Ste50, an *S. cerevisiae* protein involved in sexual differentiation in that organism. Ste50 binds Ste11, the *S. cerevisiae* homolog of Byr2, and Ste50, like Ste4, is capable of homotypic interaction. We have hypothesized that Ste4 (and perhaps Ste50) acts by causing the dimerization of its target protein kinase.

A potential target for *S. pombe* Cdc42 has been identified, the Shk1 protein kinase (Marcus et al. 1995). It is the homolog of mammalian $p65^{PAK}$ and *S. cerevisiae* Ste20, and genetic evidence suggests it may mediate some (if not all) of the effects of Cdc42 on cell shape and conjugation. Thus, this kinase may also be indirectly under the control of Ras1 in *S. pombe*, through the latter's effects on Scd1 and Scd2.

Genomic Difference Analysis of Human Cancer

M. Hamaguchi, R. Lucito, W. Wei,
C. Yen, P. Barker, D. Broek

In 1993, we published a powerful method for genomic difference analysis called RDA (Lisitsyn et al., *Science 259:* 946 [1993]). In collaboration with N. Lisitsyn, we have utilized RDA to identify probes for loci that undergo amplification, homozygous loss, or loss of heterozygosity in tumor cell lines and in tumor cells (Lisitsyn et al. 1995a,b). Several of the loci we have identified are frequently the sites of genetic alteration in cancers of gastrointestinal origin. We are searching for transcripts from these loci. Using RDA, we have also discovered several loci in humans that appear to contain deletions (or insertions) within the normal population.

Most of our effort now focuses on breast cancer, using material sent to us from collaborators at Sloan-Kettering Memorial Cancer Center (Dr. Larry Norton) and North Shore University Medical Center (Dr. Margaret Kemeny). From these sources, we have identified greater than a half dozen loci, including several previously unidentified, that become amplified in breast cancer, and greater than a half dozen loci that appear to suffer repeated homozygous loss in breast cancers. Together, these probes detect lesions in nearly 90% of aneuploid tumors. Our future work will include continuing to identify loci that are common sites of genetic alteration in breast cancer, correlating these alterations with disease outcome and response to therapy, and isolating the genes and gene products that are the targets of these alterations.

Combinatorial Chemistry

D. Dong

In 1993, together with the Still lab at Columbia University, we published a method for the efficient generation of libraries of organic molecules indexed with molecular tags (Ohlmeyer et al., *Proc. Natl. Acad. Sci. 90:* 10922 [1993]). Recently, Peter Nestler from the Still lab was appointed to the staff at Cold Spring Harbor Laboratory. In collaboration with him, we have planned to generate libraries of branched peptide-like molecules in search of small molecules that can behave locally like antibodies, i.e., that can bind to directed protein targets with high specificity. We are especially interested in targeting domains of the Ras protein, in the hope of interfering with its function. In preparation for this, we have generated reagents and designed systems to detect the binding between Ras and members of our libraries.

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SMALL G-PROTEINS AND SIGNAL TRANSDUCTION

L. Van Aelst M. McDonough
R. Packer

The Ras superfamily of small GTPases comprises a group of molecular switches that regulate an astonishing diversity of biological processes. In particular, members of the Ras and Rho subfamilies are involved in regulating signal transduction pathways mediating processes such as transformation, differentiation, metastasis, and cytoskeletal organization. The molecular basis of most of these pathways remains to be elucidated. We are interested in the mechanisms by which Ras and Rac exert their wide variety of activities.

The most extensively studied GTPases so far are the Ras proteins. Cellular *ras* genes were found to be frequently activated by mutation in a wide variety of human cancers. One major breakthrough was the finding that the serine/threonine kinase Raf is a critical downstream target of Ras, required for Ras-mediated transformation (Van Aelst et al., *Proc. Natl. Acad. Sci. 90:* 6213 [1993]; White et al. 1995). However, it is now abundantly clear that additional functions of Ras contribute to mammalian cell transformation and that Raf activation is not required for the Ras-mediated effects on morphology. One part of our research is focused on the identification of other Ras targets important for the Ras-mediated effects on transformation and morphology.

The Rac1 proteins have moved to center stage with recent discoveries of their involvement in morphogenesis, neutrophil activation, invasiveness, DNA synthesis and transformation, transcriptional regulation, and developmental processes. However, little is known about the biochemical nature of the pathways mediating these events and whether these pathways are interconnected. So far, only a few Rac-interacting proteins isolated have been shown to play a part in Rac-mediated cellular processes. The Tiam1 protein, involved in T-cell invasion and the induction of membrane ruffles, has recently been shown to be an exchange factor for Rac (Michiels et al., *Nature 375:* 338 [1995]), and the serine/threonine kinase PAK, which is stimulated by Rac in a GTP-dependent manner, has recently been reported to be required for JNK and p38 mitogen-activated protein kinase activation (Bagrodia et al., *J. Biol. Chem. 270:* 1 [1995]; Zhang et al., *J. Biol. Chem. 270:* 23934 [1995]). To obtain a better understanding of Rac function, a detailed identification and characterization of Rac-interacting proteins will be required. We have isolated several Rac interacting proteins and are now in the process of further characterizing their function.

Multiple Downstream Effectors of Ras

L. Van Aelst

Despite the evidence that Raf is a critical downstream effector of Ras function, we recently obtained evidence that cellular transformation by Ras can be mediated through both Raf-dependent and -independent pathways (in collaboration with M. Wigler and C. Der, University of North Carolina at Chapel Hill). Two Ras effector domain mutants, Ras (12V,37G) and Ras (12V,40C), which were defective for Raf binding and activation, induced potent tumorigenic transformation of some strains of NIH-3T3. These Ras mutants induced a transformed morphology that was similar to that induced by activated Rac proteins (Khosravi-Far et al., *Mol. Cell. Biol.*, in press [1996]). The identity of these Raf-independent pathways has not been established. Recently, evidence has been provided that Raf activation is not required for the Ras-mediated effects on morphology (Joneson et al., *Science 271:* 810 [1996]). This Raf-independent pathway remains to be defined as well. Several potential Ras effectors have been identified by us using the two-hybrid system (Van Aelst et al. 1995). Among these were RalGDS, a global homolog of RalGDS, and a protein called AF6. AF6 has been previously described in a single example as a fusion partner for ALL1 in acute lymphoblastic leukemias (Prasad et al., *Cancer Res. 53:* 5624 [1993]). The carboxyl terminus of AF6 shows homology with the tail domain of myosin-1 isoform from yeast and myosin heavy chain from *D. discoideum*. AF6 shows homology with the GLGF repeat within this region. Interestingly, AF6 is so far the only identified Ras-binding protein able to interact with the Ras (12V,40C) mutant. We are currently testing whether AF6 has a role in Ras-mediated effects on transformation or morphology using transfection and microinjection assays.

Regulators and Effectors of Rac Function

M. McDonough, R. Packer, L. Van Aelst

We employed the yeast two-hybrid system to identify Rac-binding proteins and to analyze interactions between Rac and previous identified and novel Rac-binding proteins. To date, three classes of specific, positive clones have been obtained. One class was composed of a number of related library plasmids, all of which encoded fusions between the GAL4 activation domain and a previously identified protein, D4. This protein is 67% identical to the bovine rhoGDI (GDP-dissociation inhibitor) protein and has been shown to have GDI activity against Cdc42Hs and Rac proteins in vitro (Adra et al., *Genes Chromosomes Cancer 8:* 253 [1993]). A second class of positive clones consisted of multiple isolates of plasmids encoding fusions between the GAL4 activation domain and a previously unknown protein. We called this protein POR1 for *p*artner *o*f *R*ac1 (see below). In collaboration with T. Joneson and D. Bar-Sagi (SUNY, Stony Brook), we further analyzed the function of this protein (Van Aelst et al., *EMBO J.* in press [1996]). We demonstrated that POR1 binds directly to Rac1 in a GTP-dependent manner and that a mutation in the Rac1 effector binding loop shown to abolish membrane ruffling also abolishes interaction with POR1.

Truncated versions of POR1 inhibit the induction of membrane ruffling by an activated mutant of Rac1, V12Rac1, in quiescent rat embryonic fibroblast REF52 cells. Furthermore, POR1 synergizes with an activated mutant of Ras, V12Ras, in their induction of membrane ruffling. These results suggest a potential role for POR1 in signaling events that lead to membrane ruffling. The third class of positive clones was represented by a single isolate. This library plasmid contained a cDNA insert of 500 bp, and sequence analysis also showed it to be a previously unidentified gene. We recently obtained the full-length gene and are presently further investigating this clone.

To dissect further the physiological roles of Rac1 and its interacting proteins, we are applying the two-hybrid system to identify mutant Rac1 proteins that show differential impairment of their ability to bind to distinct Rac1-interacting proteins, and we are testing for the altered activity of these Rac1 mutants. To date, four groups of Rac mutants have been obtained that possess different binding profiles toward the following Rac1-interacting proteins tested: PAK3, D4, POR1, and POR2. We are currently testing the activity profiles of these Rac1 mutants. These studies will be very useful in defining which pathways contribute to certain processes such as transformation and invasiveness.

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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 in understanding the biological function of these genes. Our approach to detecting DNA changes in tumors is based on the application of a new powerful technology for finding the differences between genomes, developed in M. Wigler's lab here at the laboratory (Lisitsyn et al., *Science 259:* 946 [1993]). This year, our efforts have been focused on finding and physical mapping of homozygous deletions in DNA of colon and kidney tumors. We have sought to identify new tumor suppressor genes encoded in deleted regions by positional cloning and mutational analysis of candidate genes.

Identification and Physical Mapping of Homozygous Deletions in Colon and Kidney Tumors

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

We have continued our extensive search for homozygous DNA losses in kidney and colon cancer cell lines and biopsies, using a new powerful technology for finding the differences between the genomes, called RDA (representational difference analysis). This year, one new probe detecting homozygous loss in a kidney cancer cell line has been discovered and mapped to the 9q34 locus by fluorescence in situ

hybridization (in collaboration with D. Esposito, CSHL).

Construction of cosmid/PAC contigs spanning the regions of homozygous loss (200 kb long each) has been completed for two previously identified homozygous deletions located on chromosomes 3p14 and 20p11 and is under way for two deletions located on chromosomes 9q34 and 11p15. Rapid construction of contigs has been achieved by subtraction of yeast DNA from YAC clones, selected with original RDA probes, and cloning of sequence-tagged sites (STSs) from difference products. These STSs have been sequenced and used for polymerase chain reaction (PCR) search of overlapping P1 and PAC clones.

We have tested for the presence of several STSs from each deleted region in a collection of DNAs isolated from more than 100 cancer cell lines. Although all four regions are known to suffer frequent losses of heterozygosity in many types of tumors, frequent homozygous losses have been detected only in the chromosome 3p14 and chromosome 20p11 regions, mainly in cell lines established from cancers of the digestive tract. These studies may lead us to the discovery of new genes and genetic pathways that have an important role in regulation of growth, differentiation, genomic stability, and other cellular processes. Analysis of mutations inactivating these genes may provide useful information for the development of new efficient diagnostic and prognostic markers of cancer.

Positional Cloning of a Potential Tumor Suppressor Gene from the 3p14 Region

N. Lisitsina, N. Lisitsyn [in collaboration with S. Thiagalingam 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 digestive tract, and constructed a cosmid/PAC contig spanning the region of common loss. Based on the absence of the microsatellite instability phenotype in most colon cancer cell lines with homozygous losses in the region, a potential tumor suppressor gene has been proven 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).

We have made an attempt to clone candidate genes from the region by cDNA subtraction approach (RNA RDA). cDNAs prepared from five tumor cell lines with homozygous deletions of the 3p14 locus have been pooled and taken in large excess for subtraction of cDNAs isolated from two cell lines that have at least one genomic copy of the locus. Obtained difference products have been found to be derived from irrelevant tumor-specific messages located outside of the locus.

Overlapping cosmid and PAC clones, spanning the region of homozygous loss, have been used for the search of transcribed sequences by hybrid selection and exon trapping methodologies. The sequence of the central cosmid from the region of most frequent losses has been determined and searched for the presence of potential exons using the GRAIL program. Expression of four potential exons has been detected in colon and stomach cells, and sequences of these exons have been used for cloning of full-length cDNAs by library screening and RACE (rapid amplification of cDNA ends). Full-length cDNA of one candidate gene has been constructed, but further analysis has ruled out this gene from the list of candidates. Cloning and mutational analysis of three other cDNAs are under way. We hope that these studies will lead us to the cloning and characterization of one of the most frequently mutated genes in human cancer.

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PROTEIN TYROSINE PHOSPHATASES AND THE CONTROL OF CELLULAR SIGNALING RESPONSES

N.K. Tonks A.M. Bennett, 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, T. Tiganis, S.H. Zhang

The phosphorylation of tyrosyl residues in proteins is a key component of the regulation of signaling pathways that control many fundamental physiological processes. 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) which, like the kinases, comprise both transmembrane, receptor-linked forms and nontransmembrane, cytosolic species and represent a major family of signaling enzymes. The structures of the PTPs indicate important roles in the control of key cellular functions. We are utilizing a variety of strategies to characterize the normal physiological function of several members of the PTP family.

During the last year, Sue Brady-Kalnay and Hong Sun completed their postdoctoral studies and took up faculty positions at Case Western and Yale, respectively. We were joined by Anton Bennett and Tony Tiganis as postdoctoral fellows.

Identification of PTP Substrates

A.J. Flint, A.J. Garton, K.R. LaMontagne
[in collaboration with David Barford, Oxford University, United Kingdom]

Crystal Structure of PTP1B: In collaboration with David Barford, we determined the crystal structure of the catalytic domain of PTP1B. In 1995, we extended this analysis by determining the structure of PTP1B complexed with a high-affinity peptide substrate representing an autophosphorylation site of the epidermal growth factor receptor. 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"—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 approximately 9 Å exactly matches the length of a pTyr residue, is a major determinant of specificity for phosphotyrosine. Another determinant of specificity 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.

From this structure, we were able to define a number of residues in the enzyme that were essential for substrate recognition and catalysis. We generated a series of point mutants in each of these residues, expressed and purified the mutant proteins, and examined the consequence of the mutation on catalytic function. Through this approach, we have generated a form of PTP1B that maintains a high affinity for substrate but does not catalyze dephosphorylation effectively. This has afforded us a unique approach to identification of physiological substrates. Following expression, the mutant PTP binds to its physiological substrates in the cell but, because it is unable to dephosphorylate the target, the mutant and substrate become locked in a stable, "dead-end" complex. This

complex can be isolated by immunoprecipitation and associated proteins identified by immunoblotting with antibodies to phosphotyrosine (pTyr). In addition, immunoblotting lysates of cells expressing the mutant PTP with antibodies to pTyr can be used to identify proteins whose phosphorylation state is altered as a consequence of expression of the mutant. We have now initiated such an approach to substrate isolation with intriguing results.

Identification of Substrates for PTP1B: Chronic myelogenous leukemia (CML) is a clonal disorder of the hematopoietic stem cell characterized by the Philadelphia chromosome (Ph) 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. Expression of p210 bcr:abl produces the abnormal patterns of tyrosine phosphorylation that result in the dysfunctional maturation of the hematopoietic stem cell that is the characteristic of CML. Therefore, CML represents one of the best defined examples in which aberrant tyrosine phosphorylation is the underlying cause of a human disease. We have observed that in cells expressing p210 bcr:abl, the expression of PTP1B is enhanced specifically severalfold. The intrinsic PTK activity of p210 bcr:abl is essential for this effect. The changes are specific for PTP1B and are not observed in the closely related homolog TCPTP, which displays 74% identity to PTP1B in its catalytic domain. Furthermore, the bcr:abl, leukemia-inducing form of the PTK is required; v-Abl which shares the same PTK domain does not alter PTP1B levels, neither do the other oncogenic PTKs, such as Src, nor non-PTK oncoproteins such as Myc that have been tested. Perhaps most interestingly, this enhanced expression of PTP1B has also been observed in Ph^+ B lymphoid cells derived from a CML patient, relative to Ph^- cells from the same patient. We have now been able to show that, upon expression of the substrate-trapping mutant form of PTP1B, we can isolate a complex between p210 bcr:abl and the mutant PTP1B, whereas the mutant does not trap v-Abl. Our data suggest that PTP1B may be a specific cellular antagonist of p210 bcr:abl function, and we are currently following this up by testing the effects of this PTP on signal transduction and transformation events triggered by the PTK.

We have also extended this analysis to PTP1B in other cell systems. In COS cells, the substrate-trapping mutant of PTP1B forms a complex with four proteins from the spectrum of phosphotyrosyl proteins in the cell. One of these is the receptor for epidermal growth factor, which is one of the family of ligand-activated receptor PTKs. One of the others, p70, appears to be a substrate for the Src PTK, since its phosphorylation state is enhanced in cells expressing the v-Src oncogenic PTK. We believe that these phosphotyrosyl proteins are bona fide substrates of PTP1B because other phosphotyrosyl proteins in the cell do not form stable complexes with the mutant and because the association is disrupted by the active site-directed inhibitor, vanadate. We are currently examining which of the sites of tyrosine phosphorylation in the EGF receptor are recognized by PTP1B in order to understand further the part played by PTP1B in controlling cellular responses to EGF.

Identification of Substrates for PTP-PEST: Initial studies with the cytoplasmic enzyme PTP-PEST also appear exciting. Following pervanadate treatment of HeLa cells, to inhibit PTP activity and thereby enhance phosphotyrosine levels in intracellular proteins, 50–100 pTyr proteins, i.e., potential PTP substrates, can be recovered. Upon treatment of the resultant lysate with PTP-PEST in vitro, we observed selective dephosphorylation of a 130-kD protein, p130. Furthermore, the substrate-trapping mutant of PTP-PEST was found to be capable of forming a stable complex with p130 exclusively. We have now identified this protein as p130Cas, which has an integral role in signaling events initiated by the v-Src and v-Crk oncoproteins. We are now investigating links between the dephosphorylation of p130Cas by PTP-PEST and the control of cell growth.

These examples illustrate the power of this unique approach to identification of physiological substrates and thus to providing insights into the physiological function of members of the PTP family. We are now expanding this technology to identify physiological substrates for a variety of receptor and cytoplasmic PTPs currently being studied in the lab.

Identification of Regulators of PTP Function

A.M. Bennett, R.L. Del Vecchio, Y.F. Hu, S.N. Mamajiwalla, A.A. Samatar, T. Tiganis, S.H. Zhang

In general, the structure of PTPs can be described in terms of a conserved catalytic domain to which is

fused on either the amino- or carboxy-terminal side a noncatalytic segment that serves a regulatory function and can be used to distinguish individual PTPs. It is now apparent that PTPs are regulated at multiple levels.

Receptor PTPs and Cell:Cell Contact: For the receptor PTPs, the noncatalytic, extracellular segments present the potential for regulation of activity by ligand binding. Although we and others have begun to define potential ligands for these enzymes, there are currently no examples in which engagement of the extracellular segment of a receptor PTP alters the activity of the intracellular catalytic segment. Instead, control of activity through control of subcellular distribution is an important theme that appears to underlie regulation of PTP function. We have characterized a receptor PTP, PTPμ, which possesses an extracellular segment that has homology with the immunoglobulin superfamily of cell adhesion molecules, and have shown that it takes part in homophilic binding interactions, i.e., the extracellular segment of PTPμ on one cell binds to the extracellular segment of PTPμ on the surface of an adjacent cell. In addition, through a portion of its intracellular segment, it interacts with a distinct family of cell adhesion molecules, the cadherin/catenin complex. This association targets PTPμ to specialized points of cell:cell contact termed adherens junctions at which it appears to regulate the adhesive and signaling function of the cadherins. This observation is provocative because tyrosine phosphorylation of the cadherin/catenin complex has been observed under a variety of physiological conditions potentially contributing to transformation and metastasis. It appears that PTPμ may provide the regulatory balance to this phosphorylation event, thus maintaining the cadherin/catenin complex in its functional, dephosphorylated state (Fig. 1).

A second mechanism of control that we have observed involving another broadly expressed receptor PTP, DEP-1, is modulation of the level of PTP protein. The most striking property of this enzyme is that its expression is increased dramatically in dense

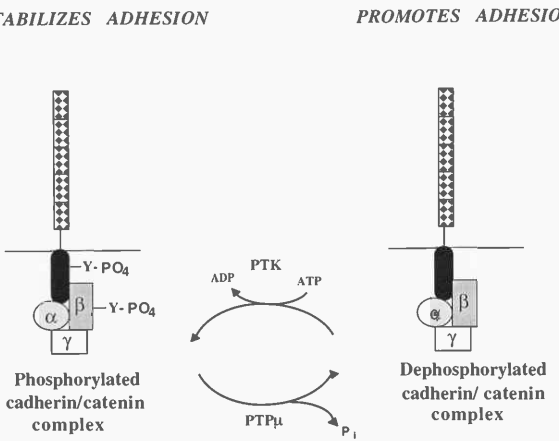

FIGURE 1 Schematic representation of the effects of tyrosine phosphorylation on the cadherins and the role of PTPμ in regulation of adhesion. Tyrosine phosphorylation of cadherins and catenins (mediated by PTKs, such as possibly the EGF receptor) reduces adhesion. This event is opposed by the activity of PTPs, such as PTPμ, which would promote adhesiveness by maintaining cadherins in a functional dephosphorylated state. α, β, γ represent α, β, γ catenin. (Reproduced from Brady-Kalnay and Tonks, *Curr. Opin. Cell Biol.* **7**: 650–657 [1995].)

relative to sparse cell cultures, hence the name *D*ensity *E*nhanced *P*TP-1. Thus, as cells approach confluence and adjacent cells begin to touch each other, surface expression of DEP-1 is enhanced. These observations suggest that DEP-1, and possibly PTPμ, may be important contributors to the mechanism of contact inhibition of cell growth. As normal cells reach confluence and make contact, they stop growing; however, in cancer cells this growth inhibition is disrupted. Since tyrosine phosphorylation has been implicated in promoting cell growth, PTPs as the natural antagonists of PTK function may exert a negative effect on growth-promoting signals by triggering net dephosphorylation of proteins in the membrane. Thus, receptor PTPs may be able to sense directly cell:cell contact. Engagement of their extracellular segments may either modulate activity directly or target the enzyme to specific junctional complexes so as to trigger dephosphorylation of a defined subset of pTyr proteins and initiate the growth inhibitory response. We are currently trying to define the important substrates of the receptor PTPs in this process and characterize how complex formation between PTPμ and the cadherins is regulated.

Regulation of Cytoplasmic PTPs: This concept of control of activity through control of subcellular location also applies to the cytoplasmic PTPs. For example, we have identified two *Xenopus* cytoplasmic enzymes, PTPX1 and PTPX10, which are characterized by amino-terminal segments that have homology with lipid-binding proteins and which are found associated with intracellular membranes. In brain, these PTPs associate with synaptic vesicles, suggesting a role in control of synaptic function. The enzymes SHPTP-1 and SHPTP-2 are characterized by the presence of SH2 domains in their amino-terminal segments. These are domains of 100 residues that bind to phosphotyrosine residues in defined primary sequence contexts and direct the association of these PTPs with specific sites of tyrosine phosphorylation in growth factor and cytokine receptors. Thus, SHPTP-1 and SHPTP-2 are targeted to signaling complexes in the plasma membrane. Although there are some examples, such as these, where the structure of the enzyme immediately suggests where it is targeted and the other proteins with which it interacts, in most cases, the binding partners have to be identified in order to characterize this regulatory feature. We are taking an approach involving a combination of classic protein purification techniques and molecular biology, in particular the 2 hybrid screen in yeast. Interesting observations are now beginning to emerge.

The enzyme TCPTP, which is closely related to PTP1B, occurs in two forms generated by alternative splicing. In one form, p48TC, the noncatalytic segment terminates in a hydrophobic patch that targets the enzyme to ER membranes. We have identified a 120-kD protein that binds to this form of TCPTP specifically and thus may control that particular targeting function. We are in the process of characterizing this protein. The second form lacks the hydrophobic patch and is targeted to nuclei. We have identified a complex between this form of the PTP, p45TC, and the nuclear import factor p97. In addition, 2 hybrid screens have detected an interaction between p45TC and the nuclear import factor karyopherin α. We have also defined the sequences within the noncatalytic segment of p45TC that are essential for binding. This may allow us to interfere specifically with the ability of this enzyme to get to the nucleus. We can examine the consequences of disrupting translocation of p45TC to the nucleus on pTyr levels in nuclear proteins and try to correlate such changes with effects on cell function. In conjunction with the substrate trapping technology, this should provide important information on the cellular targets of TCPTP and thus its physiological function. A number of potential targets spring to mind, in particular the STAT family of transcription factors that are crucial mediators of the cellular response to cytokines and growth factors.

A second example is an enzyme, PTPH1, first characterized in my lab, that features a long noncatalytic amino-terminal segment that displays features of proteins that associate with the cytoskeleton. We have shown that this segment associates in vivo with a protein termed 14-3-3, which has been demonstrated to play a crucial part as a scaffold upon which signal transduction complexes are formed. In addition, enzymatic characterization of PTPH1 in vitro reveals that this noncatalytic segment acts as an autoinhibitory domain and directly modulates catalytic activity. We are currently looking for binding proteins that may relieve this autoinhibition. We hope that these approaches will provide insights into potential roles of PTPH1 in controlling signaling events triggered by changes in the cytosketetal architecture.

Once again, we are expanding the scope of such analyses to encompass other members of the PTP family. As the examples illustrate, by gaining an understanding of the regulatory proteins with which a PTP interacts and its location within the cell, we can accrue important insights into physiological function.

Dual Specificity Phosphatases and Growth Control: We are focusing on MKP-1 which is the product of an immediate early gene, the expression of which is induced rapidly following a variety of cell stimuli. We have defined its point of action in vivo as being the dephosporylation and inactivation of MAP kinases. The MAP kinases have been implicated as common and essential components of signaling pathways induced by diverse stimuli, suggesting that MKP-1 will be a crucial, central player in the control of cell function. As well as dephosphorylating MAP kinase, it appears that MKP-1 may be a substrate for MAP kinase.

We are currently characterizing the potential regulatory significance of this dynamic interplay between the kinase and the phosphatase. In addition, we are using MKP-1 as a tool to study the role of MAP kinases in model systems for cell growth and differentiation.

Characterization of Function through Analysis of Gene Disruption

M.J. Gutch

A variety of model systems can be used for a genetic approach to analysis of PTP function. The model system that we are using is the nematode *Caenorhabditis elegans*, and the experiments are being performed in collaboration with Michael Hengartner's lab here at Cold Spring Harbor. An attractive feature of *C. elegans* is that many of the properties of its signaling pathways are conserved with those found in mammals. Thus, the lessons learned in *C. elegans* should be applicable to humans.

The strategy for this approach is first to perform gene knockouts of PTPs in the nematode. Should the PTP be an important regulator of a signal transduction pathway, then its elimination would be likely to result in a mutant phenotype. This alone may provide information with respect to the specific signaling pathway that the PTP is regulating. Moreover, the analysis can be refined by chemical mutagenesis of the PTP mutant nematodes to identify enhancers or suppressors of the knockout phenotype. Mapping of these enhancers or suppressors may also help to identify potential substrates of PTP. Our efforts are currently focused on two PTPs that have human homologs; one contains SH2 domains and appears to be a homolog of SHPTP-2 and the other, similar to PTPH1, contains a band-4.1 domain found in cytoskeleton-associated proteins. Currently, we have created knockout strains for both PTPs and are characterizing the mutant phenotypes.

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MOLECULAR CELL BIOLOGY

D.M. Helfman C. Chen, M. Gimona, J. Grossman, W. Guo, J.P. Liu, M. Meyer, A. Rai, M. Selvakumar, C. Temm-Grove, J. Wang, A. Watakabe

Our laboratory is interested in studying the regulation and function of genes encoding the cytoskeletal and contractile components of eukaryotic cells. These components have important roles in cell movements, muscle contraction, cell division, intracellular transport, and regulation of cell shape. Eukaryotic cells contain three major filamentous systems: actin filaments, intermediate filaments, and microtubules. Each of these filamentous systems contains a number of different protein components, although different cell types and tissues express specific protein isoforms which comprise these structures. In particular, we have been interested in understanding the regulation and function of tropomyosin (TM) gene expression in muscle and nonmuscle cells. TMs are a diverse group of actin-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 TM isoforms are expressed from four separate genes in vertebrates by a combination of alternative RNA splicing and alternative promoter usage. The research in our laboratory is focused on two related problems in molecular and cell biology: (1) the mechanisms responsible for the generation of tissue-specific and developmentally regulated patterns of alternative RNA splicing and (2) the functions that the different isoforms of TM have in various cell types.

During the year, two members of our group moved on to new positions. Wei Guo has taken a postdoctoral position at The Scripps Research Institute, La Jolla, California, and Constance Temm-Grove has taken a faculty position at the University of Arizona, Tucson. Below is a summary of our studies during the past year.

Regulation of a Muscle-specific Exon β-TM in Pre-mRNA

Y.-C. Wang, J.P. Liu

We have been using the rat β-tropomyosin (β-TM) gene as a model system to study the mechanism of alternative splicing. The rat β-TM gene spans 10 kb with 11 exons and encodes two isoforms, namely, skeletal muscle β-TM and fibroblast TM-1. Exons 1–5, 8, and 9 are common to both mRNAs expressed from this gene. Exons 6 and 11 are used in fibroblasts, as well as in smooth muscle cells, whereas exons 7 and 10 are used exclusively in skeletal muscle cells. Previous studies suggested that the splicing of exons 5–7 (skeletal muscle-type splice) is suppressed in HeLa (nonmuscle) cells by a negative factor. We have been developing an in vitro splicing system from the mouse BC3H1 myogenic cell line in order to understand the regulation of the splicing of β-TM pre-mRNA in skeletal muscle cells. Using a complementation assay of the BC3H1 nuclear extract or its fractions added to the human 293 nonmuscle cell nuclear extract, we have identified an activity in the BC3H1 nuclear extracts that promotes the splicing of exons 5–7. Efforts are under way to purify the factor(s) responsible for this activity.

Previous studies in vitro and in vivo suggest that the critical *cis*-acting elements involved in the suppression of the 3′ splice site of exon 7 in nonmuscle cells appear to be located in the 3′ end of intron 6 and the 5′ end of exon 7. Transient expression in HeLa cells of a chimeric minigene containing exon 7 and its flanking intron sequences of β-TM inserted between two adenovirus exons resulted in an RNA splice product excluding exon 7. When the critical *cis*-acting elements in intron 6 mentioned above were mutated, exon 7 was then included in the splice product, suggesting that the sequences in intron 6 through exon 7 and part of intron 7 contain sufficient information for the suppression of exon 7 in nonmuscle cells (Guo and Helfman, *Nucleic Acids Res. 21*: 4792 [1993]). Similar results were obtained when the same constructs were transfected into mouse NIH-3T3 fibroblasts. When the chimeric minigene was transfected into myogenic cells, exon 7 was also excluded from the spliced RNA product. These results suggest that additional sequences are required for muscle-specific splicing. We are now carrying out more in vivo studies to identify the *cis*-acting elements required for use of exon 7 in muscle cells.

A Multiprotein Complex Assembles on the *Cis*-acting Sequences Involved in Alternative Splicing of β-TM Pre-mRNA

J. Grossman, M. Meyer

In previous studies, we identified one protein, the polypyrimidine-tract-binding protein (PTB), that specifically interacted with sequences upstream of exon 7 that are involved in alternative splicing of β-TM pre-mRNA. To identify additional proteins that interact with sequences within the pre-mRNA, [^{35}S]methionine-labeled nuclear extracts from HeLa cells were mixed with nonbiotinylated RNAs, and the RNA-protein complexes were recovered by immunoprecipitation using monoclonal antibodies to PTB. The same protein patterns were observed using biotinylated-RNA containing intron 6 and recovering the RNA-protein complexes using streptavidin-agarose beads. In addition to PTB, a novel set of proteins were found to assemble and coprecipitate on intron 6. 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 heterogeneous nuclear RNP (hnRNP) proteins are bound to different regions of the pre-mRNA. Comparison of the proteins that assemble on introns from the α- and β-TM genes which utilize distant branch points revealed a common set of proteins that bound to these introns. These studies identified a set of proteins, in addition to PTB, that are likely involved in the use of distant branch sites. In addition, 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.

Role of Exon Sequences in Splice Site Selection

M. Selvakumar

In vitro studies using the β-TM gene to study the alternative splicing of exon 6 (nonmuscle/smooth muscle-type splice) revealed that exons 5 and 6 can be spliced together only if (1) a previous splicing event joins exon 6 to 8 or (2) there is a poly(U) substitution in the intron upstream of the 3′ splice site of exon 6. Thus, both intron and exon sequences can contribute important *cis*-acting elements. Studies in other systems have identified purine-rich motifs as important sequence elements in exons. Such so-called exonic enhancers are believed to act as binding sites for cellular factors such as SR proteins. Examination of sequences within exons 6 and 8 of the β-TM gene revealed the presence of several such motifs. Mutational analyses indicate that some of these motifs are essential for pre-mRNA splicing

When pre-mRNAs containing multiple exons and introns (exons 5 through 8) are used, the major splice product detected in vitro is exon skipping (splicing of exon 5 to exon 8). However, exon 6 is included if a poly(U) tract is included upstream of exon 6. Interestingly, mutating the two purine-rich motifs in exon 6 resulted in exon skipping with this pre-mRNA. Thus, sequences within exon 6 are required for cooperation of the 3′ and 5′ splice sites flanking exon 6. RNA-binding assays are under way to identify factors that interact with these sequence elements. To further characterize these mutations in an in vivo context, experiments are under way using transient transfection assays of minigenes in HeLa cells. Preliminary data indicate that these purine-rich elements are essential in vivo.

Cis-elements That Block the Use of a Skeletal Muscle-specific Exon 7 in Nonmuscle Cells

C. Chen

In previous studies, we localized the critical elements for preventing the use of exon 7 in nonmuscle cells to sequences within exon 7 and the adjacent upstream intron. We have constructed a number of pre-mRNAs to better understand how mutations within these elements contribute to activation of the muscle-specific exon in a nonmuscle environment. Two questions were addressed: (1) How do mutations in exon 7 activate the use of this exon in nonmuscle cells and (2) can the 3′ splice site of exon 7 be used when other exons are substituted? To address the first question, we took advantage of our observation that exon 5 cannot be spliced to exon 6 unless it is first spliced to exon 8 (see above section). Pre-mRNAs containing exon 5, intron 5, and exon 7 were also unable to be spliced in vitro. In contrast, introduction of a mutated exon 7 (Ex-1 mutation), which we previously demon-

strated leads to activation of the skeletal muscle exon in nonmuscle cells in vivo, was now efficiently spliced. Work is in progress to determine if the exon mutation functions by alterations in RNA secondary structure or by providing a binding site for a splicing factor. In a second series of experiments, we studied the 3′ splice of exon 7 in its normal context. We have shown that a pre-mRNA containing exon 5/intron 6/exon 6/intron 6/exon 7 + exon 8 is spliced inefficiently in vitro. Interestingly, substitution of exons 7+8 with sequences containing exons 6+8 leads to strong activation of this 3′ splice site. Thus, even in the presence of the upstream elements previously shown to inhibit the use of exon 7 in nonmuscle cells, the presence of exon sequences containing a strong exonic enhancer (see previous section) was able to lead to efficient use of this 3′ splice site. These results further demonstrate that alternative splicing of exon 7 (skeletal muscle-specific splice) is regulated, in part, by sequences within exon 7.

Stable Expression of Epitope-tagged TMs in REF52 Fibroblasts

M. Gimona

Rat fibroblasts express at least seven different isoforms of tropomyosin. To investigate the in vivo utilization of the individual TM subunits, we epitope-tagged a variety of rat muscle and nonmuscle TM isoforms at their amino-terminal ends, cloned them into mammalian expression vectors, and used these constructs to transfect REF52 fibroblasts. The isoforms containing a tag appear to be fully functional in in vitro actin-binding experiments, retain their ability to form dimers in vitro and in vivo, and are able to incorporate in actin filaments in a fashion indistinguishable from endogenous TM (Gimona et al. 1995). We have now established stable cell lines expressing various TM isoforms including fibroblast

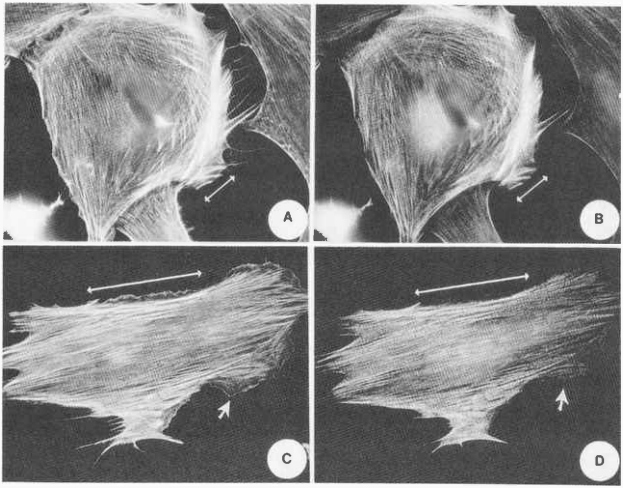

FIGURE 1 Stable expression of epitope-tagged rat fibroblast TM-5 and skeletal muscle β-TM in REF52 cells. Cells expressing TM-5 (panels *A* and *B*) and skeletal muscle β-TM (panels *C* and *D*) were visualized using double-label immunofluorescence. TM-5 is found localized along actin stress fibers as well as at the cell periphery (double arrows in panels *A* and *B*). In contrast, the skeletal muscle β-TM is absent from membrane protrusions like lamellipodia (arrow in panels *C* and *D*). The cells were double stained with anti-Ha antibody to visualize the epitope-tagged TM using GaM-FITC (panels *B* and *D*) or with phalloidin-rhodamine to demonstrate the localization of F-actin (panels *A* and *C*).

TM-1, -2, -4, -5, -5a, or -5b; smooth muscle α-TM; and skeletal muscle α- or β-TM. The epitope tag residing on each exogenous TM isoform allows us to determine the cellular localization of specific TM isoforms in the context of the entire complement of the endogenously expressed subunits in REF52 fibroblasts (Fig. 1). Both muscle and nonmuscle TMs were incorporated into actin stress fiber bundles. We are using these cells to determine the distribution of TM isoforms in dynamic states such as cell movement and cell division. We have analyzed the localization of individual TM isoforms in membrane protrusions and leading lamellae. We show that only the low-molecular-weight TMs, with the exception of TM-4, are being incorporated into the terminal actin structures and that micro spikes (short, thin arrays of tightly bundled actin filaments within the leading edge of migrating cells) are devoid of TM. We further observed that in all cases, the association of TMs with the actin filaments gave rise to a discontinuous staining pattern, indicating zones ranging from 0.1 to 0.4 μm on actin bundles that are devoid of TM. The observed pattern appears to complement that recently shown for nonmuscle myosin which is likely to be organized in these "bare zones," suggesting a "pseudo-sarcomeric" organization of contractile elements in fibroblasts. The understanding of the spatial organization of actin and myosin and the definition of the force-producing units in nonmuscle cells are essential for the understanding of cytoskeletal modulations occurring during cell motility in nonmuscle cells.

Ca^{++}-dependent Interactions of S100A2 with Muscle and Nonmuscle TMs

M. Gimona [in collaboration with Ryuji Kobayashi, Cold Spring Harbor Laboratory, Zeev Lando, Sigma, Israel, Joel Vandekerckhove, University of Ghent Belgium, and Apolinary Sobieszek, Institute of Molecular Biology, Austria]

We have purified the 10-kD Ca^{++}-binding protein S100A2 from porcine stomach smooth muscle tissue. Zero-length chemical cross-linking using EDC revealed the association with muscle and nonmuscle TMs in vitro. The cross-linked product indicated the formation of a 1:1 complex between S100A2 and TM and the interaction was shown to be Ca^{++}-dependent. Monoclonal antibodies raised against S100A2 were used to determine its cellular localization in the porcine epithelial cell line LLC PK1. The localization of S100A2 was observed to change with the differentiation state of these cells. Whereas S100A2 was found to be absent from actin stress fibers, it became colocalized with the actin containing microvilli upon differentiation. Immunoprecipitation of [^{35}S]methionine-labeled extracts failed to coprecipitate TM and S100A2, indicating a transient association between these two molecules. Affinity chromatography of cell extracts on immobilized recombinant TMs, however, supported the Ca^{++}-dependent in vitro interaction between S100A2 and both muscle and nonmuscle TMs, suggesting that the binding site resides in one of the regions conserved among various TM isoforms encoded by exons 3–5 or 7–8. Our data demonstrate the possible interaction of S100A2 and TM in the free form, but not with the microfilament-associated portion, and indicate a differentiation-related function for S100A2.

Low-molecular-weight Fibroblast TM-5: Localization and Coiled-coil Interactions

C. Temm-Grove, W. Guo

Previous studies have shown that three distinct genes encode six isoforms of tropomyosin in rat fibroblasts: The α gene encodes TM-2, TM-3, TM-5a, and TM-5b; the β gene encodes TM-1; and the TM-4 gene encodes TM-4. We report here the characterization of a cDNA clone encoding the most recent rat fibroblast TM to be identified, herein referred to as TM-5, which is the product of a fourth gene that is homologous to the human hTMnm gene, herein referred to as the rat slow-twitch α-TM gene. When fluorescently labeled TM-5 was microinjected into living rat fibroblasts, it localized to the stress fibers and ruffles of the leading lamella. The coiled-coil interactions of TM-5 with other low-molecular-weight (LMW) and high-molecular-weight (HMW) TM isoforms were studied. TM-5 and TM-4 were capable of dimerizing with each other as well as with other LMW isoforms (TM-5a and TM-5b), but not with the HMW isoforms (TM-1, TM-2, and TM-3). In addition, TM-5a and TM-5b were unable to heterodimerize with each other. Since the ratio of the various TM isoforms is not stoichiometric in a particular tissue or cell type, it is likely that different ratios of homodimers and heterodimers occur. How the different combinations of subunits will affect the rela-

tive affinity of TM for actin, head-to-tail overlap, and interaction with other proteins remains to be determined.

Epithelial Cells Express a Complex Pattern of TM Isoforms That Exhibit Distinct Localization

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

We have used antibody studies to characterize the expression and localization of TM isoforms in various kinds of epithelia, including brush border (BB) type (e.g., LLC-PK1, Caco-2, and primary intestinal epithelium) and non-BB (e.g., PtK_2 and NRK-52E). Analyses using a panel of antibodies directed against HMW and LMW TMs revealed that HMW isoforms (TM-1, TM-2, TM-3, TM-6) were localized to stress fibers, but not to adhesion belts, whereas the LMW isoforms (TM5b and TM5) were found in adhesion belts in primary BB cells and LLC-PK1 cells. These results were confirmed using epitope-tagged constructs for transient transfection followed by fluorescence microscopy. These results demonstrate that epithelial cells express a complex pattern of TM isoforms, which exhibit differential localizations within the cells and different patterns or expression depending on the origin and stage of differentiation of the epithelia.

Characterization of TM-binding Proteins in Brain

A. Watakabe [in collaboration with Ryuji Kobayashi, Cold Spring Harbor Laboratory]

We have identified and characterized two proteins that bind to the neuron-specific tropomyosin isoform, TMBr-3. Western blotting using antibodies against known TM-binding proteins showed that one of them is tropomodulin, a TM-binding protein originally identified in erythrocytes. When we tested the extracts derived from various tissues, tropomodulin was present in other tissues, but the other TMBr-3-binding protein was detected only in brain. To further characterize this protein, we have purified it using a TMBr-3 affinity column. By microsequencing, we obtained several peptide sequences of this protein and we cloned its cDNA from a rat brain cDNA library. Nucleotide sequencing revealed that this is a novel protein. Interestingly, this protein had 60% amino acid sequence identity to tropomodulin. Considering its sequence identity, similar molecular weight, and ability to bind to tropomyosin, this protein is a novel isoform of tropomodulin. We designated this isoform as N-tropomodulin (N-Tmod). To distinguish the two isoforms of tropomodulin, we termed the original isoform a E-tropomodulin (E-Tmod). We have produced recombinant N- and E-Tmods and tested their binding to TMBr-3. As expected, both proteins bound to TMBr-3. In addition, both Tmods bound to two other TM isoforms expressed in brain. Thus, there are two highly related TM-binding proteins in brain, N-Tmods and E-Tmods.

The pattern of E-Tmod expression is quite different from that of N-Tmod. E-Tmod is expressed mainly in skeletal and cardiac muscles, and also in brain, lens, and erythrocytes. Moreover, E-Tmod expression in brain starts after birth, unlike N-Tmod. Recent reports demonstrated that E-Tmod is a pointed-end capping protein of F-actin and that it is necessary for the stabilization of the sarcomeric structure of cardiac muscle. Considering the primary sequence homology, it is likely that N-Tmod is also an F-actin capping protein. In developing neurons, the formation and reorganization of cytoskeletal structures, especially actin filaments, are in a dynamic state. N-Tmod likely plays an important part in such processes. Experiments are currently in progress to study its function.

Identification of TM-binding Proteins

A.J. Rai

We are focusing our efforts on the detection of proteins that bind to tropomyosins in an attempt to delineate isoform-specific functions. Toward this end, we are using several approaches to identify and characterize proteins that interact with nonmuscle TM isoforms. One approach is a gel overlay assay. Briefly, proteins are resolved by SDS-PAGE and transferred to a nitrocellulose membrane. The membrane is probed with purified TM and then subsequently incubated with antibodies that recognize the specific isoform of interest. In this manner, proteins that bind to TM can be detected. We are cur-

rently using this approach to compare TM-binding protein in normal and transformed cells. By identifying such proteins, we hope to better understand the transformed state.

Using the gel overlay assay, we have begun to characterize a TM-binding protein present in transformed fibroblasts. These cells exhibit an altered cytoskeleton and a rounded morphology. Previous studies have demonstrated that one of the HMW TM isoforms (TM-1) is absent and two LMW isoforms (TM-4 and TM-5b) are highly elevated. Preliminary experiments from our lab showed that forced expression of TM-1 alone was not sufficient to restore normal cytoskeletal structure and cell shape, suggesting that other factors are involved in these aspects of the transformed phenotype. Therefore, we have begun to determine what other factors are responsible for alterations in actin structure. Preliminary results suggest the presence of a protein that interacts with both HMW (284 amino acids) and LMW (248 amino acids) TMs, which is detected only in the transformed cells. Experiments are currently in progress to fully characterize this protein and determine its role in the transformed phenotype.

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CELL BIOLOGY OF THE NUCLEUS

D.L. Spector S. Huang P. Mintz
P. Lorenz T. Howard
T. Misteli J. McCann

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

Visualization of the Dynamic Nature of Pre-mRNA Splicing Factors in the Mammalian Cell Nucleus

T. Misteli, D.L. Spector

Pre-mRNA splicing factors are distributed non-homogeneously in 20–40 speckles of irregular shape in the mammalian cell nucleus. The morphological appearance of the speckles is highly dependent on the transcriptional activity of the nucleus, which suggests that the speckles are dynamic nuclear structures. To observe directly the dynamic movements of splicing factors in the mammalian cell nucleus, we have taken advantage of the recently described green fluorescent protein (GFP). GFP is an auto-fluorescent protein from the jellyfish *Aequorea victoria* which when irradiated with blue light emits green light without the requirement for any cofactors. We have generated fusion proteins between the essential pre-mRNA splicing factors SC35 and SF2/ASF and the GFP to study the dynamic behavior of functional compartments within the mammalian cell nucleus during interphase by time-lapse fluorescence microscopy. This allows for the first time the direct visualization of movements of splicing factors within the cell nucleus. GFP-SF2/ASF fusion proteins localize to endogenous speckles and are phosphorylated in HeLa and BHK

cells. In actively transcribing interphase nuclei, the speckles enriched in pre-mRNA splicing factors are relatively stationary with respect to their position in the nucleus. In contrast, dynamic reorganizations can be detected at the periphery of speckles. Most prominently, branches of pre-mRNA splicing factors can be seen extending into the nuclear space. These extensions may represent splicing factors which are recruited from their storage sites in the larger speckles (interchromatin granule clusters) to the site of transcription of active genes (perichromatin fibrils). We are currently testing this hypothesis by using inducible gene expression systems, and we are further investigating the nature and significance of the observed movements.

Semi-Intact Cells to Study Nuclear Organization: A Role for Ser/Thr Phosphatase 1 in the Distribution of Pre-mRNA Splicing Factors

T. Misteli, D.L. Spector

The organization of pre-mRNA splicing factors in nuclear speckles has been well characterized by fluorescence and electron microscopy. However, relatively little is known about the molecular mechanisms involved in the organization of pre-mRNA splicing factors in nuclear speckles. One reason for this has been the inability to manipulate freely the experimental conditions in the nucleus. In an attempt to overcome this problem, we have developed a semi-intact cell system to study the organization of pre-mRNA splicing factors in the nucleus. HeLa cells are permeabilized with detergent, and the soluble nucleoplasmic factors are washed away. The nuclear environment is then reconstituted by addition of a nuclear extract from HeLa cells (Fig. 1A). Both transcription and DNA replication occur in the semi-intact cells. In addition, the morphological rearrangements observed in living cells upon inhibition of transcription or in the presence of kinase inhibitors could be reproduced in semi-intact cells. We found that the nuclear fraction in the absence of soluble nuclear factors did not support any change in the organization of splicing factors but that soluble factors were required for this process (Fig. 1B). One of the required soluble factors was identified as a member of the serine/threonine protein phosphatase 1 family (PP1) of phosphatases by inhibitor titration experiments and sensitivity to inhibitor-2 (Fig. 1C). This implies an important role for phosphorylation/dephosphorylation in the organization of pre-mRNA splicing factors and is consistent with the identification of two kinases which specifically phosphorylate the SR-family of essential pre-mRNA splicing factors (for review, see Fu, *RNA 1:* 663 [1995]). Using the semi-intact cell system and intact cells, it should be possible to characterize further

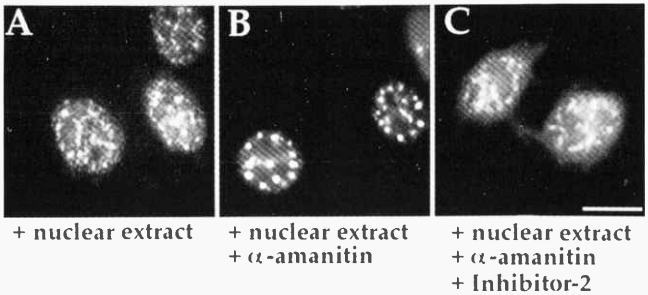

FIGURE 1 Semi-intact cells show a typical speckled distribution pattern of splicing factors in the presence of nuclear extract (*A*). Nuclear extract is required for the observed rounding up of nuclear speckles after α-amanitin treatment (*B*). Inhibitor-2 prevents the rounding up of speckles, demonstrating a role for PP1 in the distribution of splicing factors (*C*). Bar, 10 μm.

the role of phosphatases and kinases in the organization of pre-mRNA splicing factors and to identify other factors that have a role in the organization of splicing factors in nuclear speckles.

Differential Localization of Polypyrimidine Track-binding Protein

S. Huang, D.L. Spector [in collaboration with M. Ellisman and T. Deerinck, UCSD Microscopy and Imaging Resource]

Polypyrimidine track-binding protein (PTB), also known as the hnRNP I protein, has been characterized as an RNA-binding protein that forms a complex with nascent RNA transcripts and is involved in alternative splice site selection. Using a monoclonal antibody that specifically recognizes PTB, we have found that PTB shows a differential localization pattern among different cell types. In primary human fibroblast cell lines, such as Detroit 551 and WI-38, PTB is localized in a diffuse nuclear pattern. However, when several transformed cell lines were examined, such as HeLa and 293 cells, PTB was found to be localized in a half-moon-shaped cap on the surface of the nucleoli in addition to being diffusely distributed throughout the nucleoplasm. Occasionally, a portion of the cap appeared to extend into the nucleoli. The percentage of transformed cells exhibiting such a phenotype varied among different cell lines. The number of caps within each nucleus ranged from one to three. The presence of these structures appears to be inherited as these structures are always present in both daughter cells after cell division and in small clonal populations of HeLa cells. In addition to PTB, RNase P RNA, a small RNA involved in the processing of tRNA, was also found in these cap structures (Matera et al., *J. Cell Biol. 129:* 1181 [1995]). The presence of these structures in transformed cells may be related to the altered physiology of the cancer cell. However, their absence in primary cells suggests that they are not required for fundamental cellular functions.

To understand further the structural characteristics of this nuclear component, we have examined the PTB-containing nucleolar cap at the electron microscopic level using a recently developed photooxidation method. This method allows for a direct correlation of the fluorescent and electron microscopic images and results in excellent cellular preservation. We have found that the PTB protein cap is closely associated with the surface of the nucleolus. The surface of the cap structure appears irregular in shape, and a clearly defined boundary around the cap structure was not observed. More interestingly, a plate-like structure was observed between the nucleolus and the PTB protein cap. This structure appears to contain an array of short fibrils connecting the surface of the nucleolus and the PTB protein cap. Studies are currently under way to decipher the three-dimensional organization of this site of interaction between the nucleolus and the cap structure.

Visualization of Subdomains within Nuclear Speckles Enriched in Pre-mRNA Splicing Factors

P. Mintz, D.L. Spector

We are interested in the functional and structural organization of pre-mRNA splicing factors in the mammalian nucleus. Immunofluorescence studies using antibodies against pre-mRNA splicing factors have revealed a speckled pattern and diffuse nucleoplasmic labeling in the mammalian nucleus. In addition, nonsplicing factors such as poly(A)-binding protein II (Krause et al., *Exp. Cell Res. 214:* 75 [1994]) and a hyperphosphorylated form of RNA polymerase II (Bregman et al., *J. Cell Biol. 129:* 287 [1995]) have also been localized in a speckled pattern in the mammalian cell nucleus. Although the speckled regions appear to contain many different factors when examined by standard immunofluorescence microscopy, each speckle appears to be homogeneously labeled for a particular factor. No information is available with regard to the sublocalization of specific splicing factors within speckles. Since it is possible that splicing factors are further compartmentalized in these regions, we have re-examined their distribution in speckles by confocal laser scanning microscopy using serial dilutions of antibodies against specific factors. We have found that splicing factors are further compartmentalized into numerous subunits within a speckle that are heterogeneous in size and average between 0.2 μm and 0.4 μm in diameter (Fig. 2). We have termed these components subspeckles. Sub-

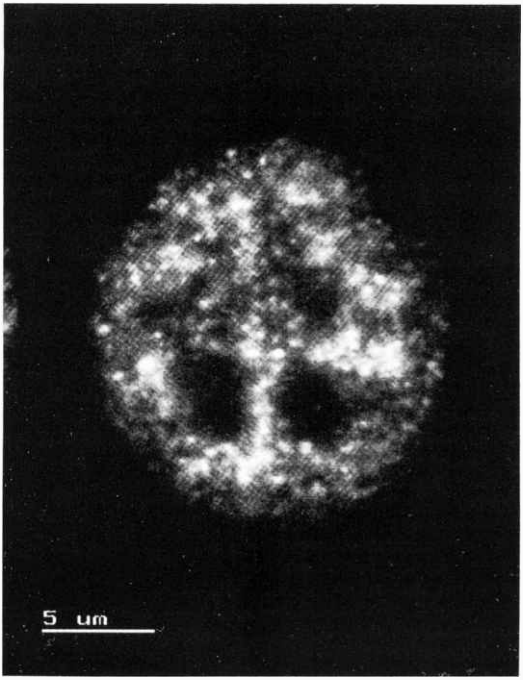

FIGURE 2 Optical section showing the distribution of the U2 snRNP B′′ protein in subspeckles. Note that in places loops and strings of subspeckles can be observed.

speckles were also observed to be diffusely distributed throughout the nucleoplasm. In some cases, strings and loops of subspeckles were observed to extend from the highly dense speckled regions. Upon inhibition of RNA polymerase II transcription, the strings and loops of subspeckles were no longer observed. Subspeckles were also not observed in coiled bodies.

To examine the functionality of these nuclear substructures, we are currently evaluating their association with sites of transcription of transiently expressed intron-containing transcripts.

Cell Biology of Antisense Oligonucleotides

P. Lorenz, D.L. Spector [in collaboration with C.F. Bennett, ISIS Pharmaceuticals]

Antisense oligonucleotides can be valuable tools to inhibit specifically the expression of a target gene. Their specific action is based on their ability to hybridize to complementary regions in the target RNA(s). Several mechanisms of action for antisense molecules have been proposed, including interfering with RNA processing, transport and/or translation,

and degradation of the local hybrid by RNase H activity. However, in vivo merely circumstantial evidence of specific antisense activity has been provided. In particular, the interactions of antisense molecules with their targets within the cell are barely characterized in terms of stoichiometry and structure of the complexes, where the interactions take place, and what their fates are.

We have set out to characterize the intracellular behavior and exact localizations of antisense oligonucleotides and to relate the localizations to their exerted antisense activity. Thus far, we have concentrated on phosphorothioate oligonucleotides (PS-ONs) because of their stability to nucleases. We have used PS-ONs directed against regions of an inducible endogenous RNA, intercellular adhesion molecule 1 (ICAM-1), in HeLa cells. A fluorescent tag at the 5′ or 3′ end of the antisense molecule (20 mer) allowed us to follow the distribution of the PS-ONs and compare it on the single cell level to the exerted inhibition of ICAM-1 protein expression measured by indirect immunofluorescence. Addition of the antisense oligonucleotides to the culture medium resulted in the PS-ONs ending up in vesicular structures in the cytoplasm. These structures probably belong to the endosomal/lysosomal compartment of the cell. No decrease in ICAM-1 protein staining could be detected under these conditions. In contrast, administration of the antisense molecules by addition to the culture medium in the presence of cationic lipids (lipofectin) or by direct microinjection into the cells efficiently targeted the PS-ONs to the nuclei. Concomitantly pronounced decreases of ICAM-1 protein could be observed in these cells. These results suggested that the antisense activity of the ICAM-1 oligonucleotides was executed within the nucleus. The nuclear signals of the PS-ONs consisted of an overall diffuse nucleoplasmic staining and numerous bright spherical structures. In situ fractionation of cells revealed that most PS-ONs were bound to the nuclear matrix. The spherical structures have been found to be unrelated to the antisense activity as control PS-ONs, despite their lack of such activity, also displayed the same localization in the nucleus. In addition, these spherical structures could not be assigned to known nuclear structures such as speckles enriched in splicing factors, nuclear bodies, or centromeres. Interestingly, when two different PS-ONs were introduced independently of each other into cells, even by two different means, they could be found to colocalize at the same spherical structures. We are currently trying to visualize both the antisense oligonucleotides and their specific target RNAs simultaneously in cells in order to identify the specific sites of action of the antisense molecules.

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GENETIC ANALYSIS IN *CAENORHABDITIS ELEGANS*

M. Hengartner T. Casci (URP) D. Hoeppner Q. Liu
S. Desnoyers G. Jefferis M. Spector
T. Gumienny J. Keller S. Tharin

Our laboratory uses the small nematode *Caenorhabditis elegans* as a model organism for the study of interesting biological problems. The two main areas that we are currently investigating are (1) programmed cell death (apoptosis) and (2) nervous system function and development. Because important biological processes are evolutionarily conserved, we believe that the general rules that we observe in *C. elegans* will also prove to apply to humans.

Programmed Cell Death

Programmed cell death (PCD, also known as apoptosis) is a mechanism that multicellular organisms have developed to eliminate cells that are not needed or are potentially dangerous. PCD has important roles in animal development and homeostasis and occurs in a wide variety of tissues in both vertebrates and invertebrates. Proper control of PCD is crucial:

Breakdown in the regulation of this process contributes to the pathogenesis of a number of diseases, including cancer, auto-immunity, neurodegenerative diseases, ischemic stroke, and myocardial heart infarct.

The worm *C. elegans* provides an attractive system for the study of PCD, as its development has been extensively characterized and the animal is readily amenable to genetic and molecular manipulations. So far, we and others have identified 17 genes that affect PCD in *C. elegans* (Fig. 1), and we have molecularly characterized a number of these genes. For example, we found that the gene *ced-9*, which is required to prevent the death of cells that should survive, is a homolog of the mammalian proto-oncogene *bcl-2*. Interestingly, *bcl-2* is thought to have a similar function in mammals as *ced-9* has in *C. elegans*. Similarly, CED-3, required for PCD in worms, is homologous to a family of cysteine proteases required for apoptosis in mammals. The conservation in sequence and function of these cell death genes strongly suggests that the molecular mechanism for PCD has remained conserved through evolution and that nematodes and mammals share a common molecular pathway for cellular suicide. Thus, the knowledge gained about PCD in *C. elegans* promises to be relevant to our understanding of apoptosis in human development and disease. 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.

Identification of Proteins That Interact with CED-9

M. Spector, G. Jefferis, T. Casci

The CED-9/Bcl-2 family members possess no known enzymatic activity, suggesting that these proteins might inhibit PCD 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 LexA DNA-binding domain as a bait. We have identified 11 independent clones that specifically interact with the LexA-CED-9 bait. We are currently confirming these interactions in vitro and testing the interacting proteins for effects on *C. elegans* cell death. To complement and assist the two-hybrid studies, we have generated antibodies against CED-9 that will be used to identify proteins that coimmunoprecipitate with CED-9.

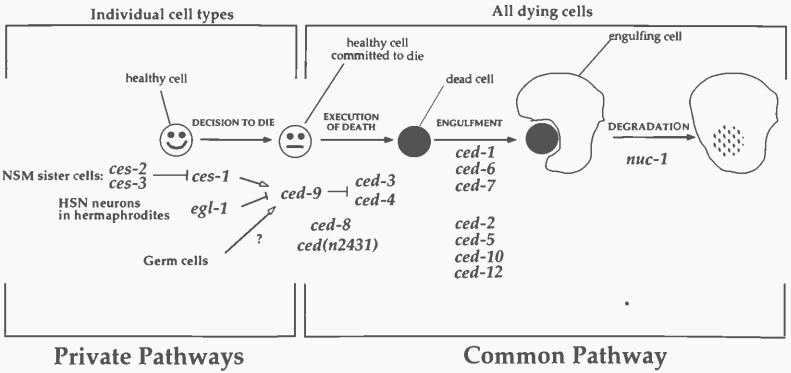

FIGURE 1 A genetic pathway for programmed cell death in *C. elegans*. Mutations in 17 genes affect programmed cell deaths. These mutations divide the process of programmed cell death into four steps; genes that act in the last three steps are common to all programmed cell deaths, whereas genes that act in the first step affect only a few cells. Regulatory interactions deduced from genetic studies are shown. (→) Positive regulation; (—|) negative regulation.

Isolation and Characterization of *ced-9* Suppressors

D. Hoeppner, M. Spector, S. Desnoyers

We have previously shown that *ced-9* is required to protect *C. elegans* cells from death: In the absence of *ced-9*, many cells that normally live undergo PCD, 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 PCD, we have screened for suppressors of the *ced-9(lf)* maternal-effect lethality phenotype. So far, we have isolated more than three dozen suppressors. As expected, many of these are mutations in *ced-3* and *ced-4*. We have also identified one mutation, *ced(n2431)*, that identifies a new cell death gene. The mutation *ced(n2431)* suppresses the weak loss-of-function alleles *ced-9(n1950 n2161)* and *ced-9(n1653)*, but it is unable to suppress *ced-9* null alleles. Furthermore, *ced(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 play a partially redundant part 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. We plan to continue using this screening strategy and are designing other genetic screens (e.g., screens for dominant suppressors) to identify additional cell death genes.

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

T. Gumienny, M. Hengartner [in collaboration with Erika Hartwieg, Massachusetts Institute of Technology and Eric Lambie, Dartmouth University]

We have recently undertaken an in-depth study of PCD in the germ line of *C. elegans*. Although previous studies of programmed cell death in *C. elegans* have focused on developmental deaths, we have found that PCD also has a major role in the germ line. Germ cells are by far the most common cell type to undergo PCD: During development, 131 somatic cells die; in contrast, more than 300 germ cells die during adulthood. Thus, during an animal's lifetime, more PCDs occur in the germ line than in all the somatic tissues combined.

We have found that most, but not all, mutations that affect PCD in the soma also affect germ cell death. *ced-3* and *ced-4* are both required for germ cells to die, 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 PCD only in specific somatic 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 specifically affect PCD in the germ line. Characterization of the genes identified by these mutations promises to further our understanding of how particular cells regulate activation of the death machinery.

Genes Required for the Engulfment of Apoptotic Deaths

Q. Liu, T. Gumienny

One of the consequences of activation of the death machinery is the generation of a signal by the dying cell that allows neighboring cells to rapidly engulf the apoptotic cell and remove it from the organism. Swift elimination of dying cells by phagocytosis is an important aspect of the apoptotic process: In the absence of phagocytosis, secondary necrosis of the apoptotic bodies results in lysis of the dead cells, leading to inflammation and possibly auto-immune disease.

In *C. elegans*, mutations in several genes result in persistent corpses (Fig. 1). In these mutants, many dying cells fail to be engulfed and remain visible within the animals for many hours or even days. We are characterizing several of these genes at the genetic and molecular levels, in the hope of understanding the nature of the engulfment-promoting signals and their effect on neighboring cells.

Reverse Genetic Studies of Programmed Cell Death

Q. Liu [in collaboration with Kristin White, Harvard University, and Hermann Steller, Massachusetts Institute of Technology]

A number of cell death genes have been identified in other organisms, such as mammals and *Drosophila*.

The observation that the pathway for PCD is conserved between nematodes and mammals 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. For example, we have found that the *Drosophila* gene *reaper* can induce cell death in *C. elegans*, and that *reaper*-induced death requires *ced-3* and *ced-4* function. Studies of this type promise not only to confirm that the genes under study interact with, or are 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 cell death pathway by testing the effect of the transgenes in the various cell death mutant backgrounds available in *C. elegans*.

Nervous System Function and Development

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

Genes Required for Axonal Outgrowth

S. Tharin

Genetic screens have identified a large number of genes required for axonal outgrowth, fasciculation of processes into bundles, and neuronal pathfinding in *C. elegans*. Such genes might identify molecules important for the transduction of signals from the extracellular matrix to the growth cone or for the stabilization of growth cones in response to such signals.

We have concentrated our attention on the gene *unc-69*, which causes a severe uncoordinated phenotype. We have found that *unc-69* function is required for proper axonal fasciculation and outgrowth, as *unc-69* mutants are seriously defective in both processes. We have cloned the *unc-69* gene and found that it encodes a 108-amino-acid protein. Although the predicted UNC-69 protein does not contain any obvious catalytic domain, the central region of UNC-69 has a high likelihood of forming a coiled-coil structure. Reporter fusion experiments suggest that UNC-69 is most abundant in the major nerve fascicles in *C. elegans*, known as the nerve ring and the ventral nerve cord. The *unc-69* expression pattern is thus consistent with the hypothesis that UNC-69 functions in neuronal processes to promote axonal outgrowth. Recently, we found that *C. elegans* UNC-69 shares a high degree of sequence similarity (over 70% identity in the coiled-coil domain) to a set of human expressed sequence tags (ESTs). We are now trying to determine whether conservation between *C. elegans* UNC-69 and this putative mammalian homolog extends to the functional level.

Learning and Memory

S. Tharin [in collaboration with John Connolly, Tim Tully, Cold Spring Harbor Laboratory, and Derek van der Kooy, University of Toronto, Canada]

We have recently started a research program on the molecular basis of associative learning and memory in *C. elegans*. We are pursuing this project in collaboration with the Tully and van der Kooy laboratories. Our first step will be to set up an extensive genetic screen based on a classical conditioning paradigm developed by Derek van der Kooy. Mutants identified as defective in acquisition, short-term memory, or long-term memory will be characterized at the behavioral, genetic, and molecular levels.

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GENETICS

Last year, we saw the continued spectacle of dramatic discovery of genes involved in human disease. Not only has the list of cancer genes grown more complete, but also entirely new metabolic pathways are being uncovered with, for example, the finding of a gene involved in human obesity. The academic geneticist can hardly fail to notice that some of the most exciting developments are not occurring in his domain but in either private or public companies. This was not previously the case. Some have derided this development and asked how we can compete with well-funded, huge corporate teams. Such complaint is misguided and the taxpayer/patient probably applauds the infusion of new funding in genetics. During the last year, a singular development took place that has to some extent leveled the playing field for all. In an effort to head off an attempt by one small company to "corner the genome market," a large pharmaceutical house funded a cDNA sequencing effort at Washington University. This work has reached some maturity and provides, to the international world of science, essentially free of charge and commercial restraint, a DNA sequence bank consisting of at least fragments of most human genes. It proves to be extraordinarily useful and cries foul at the complaint that private enterprise is taking over the study of our genes. The work of the geneticists at Cold Spring Harbor Laboratory is described below.

EUKARYOTIC CELL CYCLE CONTROL

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

During the course of the year, Hui Zhang accepted a faculty position at Yale University, Scott Davey took a faculty position at Queens University, Kingston, Canada, and Doug Demetrick returned to Canada, taking a position as Assistant Professor at the University of Calgary. Brad Nefsky went to the laboratory of Nancy Walworth at UMDNJ and David Casso graduated and became a postdoctoral fellow in the laboratory of Tom Kornberg at the University of California, San Francisco. T.K. Kim joined the laboratory of Tom Maniatis at Harvard, Craig Gawel and Diane Lombardi departed to new positions, and Gretchen Hannon left to have a baby. We were joined by Dr. Roberta Maestro, a visiting scientist from Italy, and Greg Hannon was promoted to Staff Investigator.

Role of $p16^{INK4a}$ in Tumor Development and Cell Mortality

M. Serrano, D. Beach

The cell cycle inhibitor $p16^{INK4a}$ is inactivated in many human tumors and in families with hereditary melanoma and pancreatic cancer. To test directly the impact of $p16^{INK4a}$ inactivation on tumorigenesis, we have generated in collaboration with Dr. Ronald DePinho's laboratory (Albert Einstein College of Medicine, New York) a mouse strain carrying a targeted deletion of the INK4a gene. INK4a-deficient mice are viable but develop spontaneous tumors at an early age (7 months) and are highly sensitive to carcinogenic treatments. Histological analysis of the

tumors, done by Dr. Carlos Cordon-Cardo (Memorial Sloan-Kettering Cancer Center, New York), indicates that most of the tumors consist of sarcomas and lymphomas. At the cellular level, INK4a-deficient primary fibroblasts give rise to immortal cell lineages with high efficiency. Interestingly, fibroblasts from INK4a-deficient mice become tumorigenic following the introduction of the activated Ha-*ras* oncogene. This finding is in contrast to that of normal INK4a$^+$ fibroblasts that require the introduction of at least two cooperating oncogenes to become tumorigenic, and it could be the basis for the high incidence of fibrosarcomas in INK4a-deficient mice. INK4a-deficient mice could provide a useful animal model to study the development of these malignancies. Alterations in the INK4a gene are common in human lymphomas and sarcomas. Altogether, our results directly demonstrate that the INK4a locus functions to suppress neoplastic growth.

CDC25 Phosphatases, Signal Transduction, and Cancer

K. Galaktionov, D. Beach

The *ras* and *raf1* proto-oncogenes transduce extracellular signals that promote cell growth. cdc25 phosphatases activate the cell division cycle by dephosphorylation of critical threonine and tyrosine residues within the cyclin-dependent kinases. We have shown that cdc25A phosphatase associates with raf1 in somatic mammalian cells and in meiotic frog oocytes. Furthermore, cdc25A phosphatase can be activated in vitro in a Raf1-dependent manner. This observation uncovers an evolutionarily conserved link between the cell cycle and signal transduction and suggests that activation of the cell cycle by the ras/raf1 pathways might be mediated in part by cdc25.

In rodent cells, human cdc25A or cdc25B but not cdc25C phosphatases cooperate with either Ha-*ras* (Val-12) or loss of Rb1 in oncogenic focus formation. Such transformants are highly aneuploid, capable of growth in soft agar and form high-grade tumors in nude mice. The ability of cdc25 phosphatases to show oncogenic cooperation with either oncogenic *ras* mutants or Rb1 deletion mutants underscores the potential significance of cdc25 overexpression in the development of human malignancies. In support of this suggestion, cdc25B was found to be highly expressed in 32% of the primary breast cancers. Tumor-specific expression of the cdc25B in human breast carcinomas correlates with less favorable prognosis and survival of the patients. Our results suggest that alterations in the function of cdc25A and cdc25B by overexpression might promote oncogenic transformation in vivo and further suggest that cdc25 phosphatases (A and B) are novel potential oncogenes.

14-3-3 Proteins Associate with cdc25 Phosphatases

D. Conklin, D. Beach

The potentially oncogenic cdc25 phosphatases have key roles in cell cycle progression by activating cyclin-dependent kinases. Cyclin-dependent kinases are subject to inhibitory phosphorylations of threonine and tyrosine residues (Thr-14 and Tyr-15) that inhibit kinase activity and consequently cell cycle progression. The cdc25 phosphatases remove these inhibitory phosphate groups, activating the kinase and bringing about cell cycle progression. Two members of the 14-3-3 protein family have been isolated in a yeast two-hybrid screen designed to identify proteins that interact with the human cdc25A and cdc25B phosphatases. Genes encoding the human homolog of the 14-3-3ε protein and the previously described 14-3-3β protein have been isolated in this screen. 14-3-3 proteins comprise a family of well conserved eukaryotic proteins that were first isolated in mammalian brain preparations and that possess diverse biochemical activities related to signal transduction. Recently, 14-3-3 proteins have been found to associate with a number of oncogene products, including Raf1, Bcr-abl, and middle T antigen. We have found that cdc25 and 14-3-3 proteins physically interact both in vitro and in vivo. 14-3-3 protein, however, does not affect the phosphatase activity of cdc25A. Instead, it appears to facilitate the association of cdc25 with Raf1 in vivo, playing an organizational part in linking mitogenic signaling and the cell cycle machinery.

Identification of Proteins That Interact with Cyclin G

K. Okamoto, D. Beach

The tumor suppressor protein p53 is regarded as one of the most important tumor suppressors. Alterations

of p53 are among the most common genetic changes found in human cancers. The importance of these changes is implied by experiments with transgenic mice in which both alleles of p53 have been disrupted. These animals show a greatly increased frequency of cancer. It has been demonstrated that p53 is involved in many biological functions, including cell cycle arrest, apoptosis, genomic instability, neural development, meiosis, and stress response. Since p53 is known to function as a transcription factor, the plethora of biological phenomenon p53 is involved in may be explained by a variety of transcriptional target genes which p53 regulates. Although several transcriptional target genes of p53 have been identified (including *p21*, *mdm2*, *mck*, *gadd45*, and *bax*), it is unlikely that these genes can fully explain the function of p53. Many crucial target genes of p53 may still remain unidentified.

To identify other transcriptional targets of p53, we have used a polymerase chain raction (PCR)-based differential screening method, differential display. We have found that one of the identified cDNAs encodes the mouse homolog of rat cyclin G. The expression of cyclin G was induced under a number of different conditions which are known to activate the p53 pathway. Using DNA-binding and transient transfection assays, we have demonstrated that the cyclin G gene is a transcriptional target of p53.

To understand the biological role of cyclin G, we searched for proteins that can interact with cyclin G. Through the screening of the two-hybrid cDNA library, we have identified two positive cDNAs. Sequence analysis showed that both of them encode regulatory subunits of protein phosphatase 2A (PP2A). We have demonstrated that cyclin G and these proteins can interact both in vivo and in vitro, and these interactions are unique among mammalian cyclins. Projects are under way to understand the biological role of these proteins in the context of a p53-mediated pathway.

p21 Deficiency Compromises Radiation-induced Cell Cycle Arrest

G.J. Hannon, D. Beach

p21 is a dual inhibitor of cyclin-dependent kinases and PCNA (proliferating cell nuclear antigen), both of which are required for cell proliferation. The tumor suppressor, p53, a key mediator of the cellular response to DNA damage, regulates the transcription of the p21 gene. Thus, p21 was implicated as a downstream effector of p53 in radiation-induced cell cycle arrest and apoptosis. p21 has also been touted as a component of cellular senescence and as a mediator of cell cycle withdrawal following differentiation.

In collaboration with Tyler Jacks at the Massachusetts Institute of Technology and Jeff Gordon at Washington University, we investigated the role of p21 in these processes using chimeric mice composed partly of p21+/+ and partly of p21–/– cells. Immunohistochemical staining indicated that p21 deficiency did not affect terminal differentiation of the four major intestinal epithelial cell types. In addition, loss of p21 did not prevent p53-dependent cell death. However, p21-deficient fibroblasts were impaired in their ability to undergo cell cycle arrest following DNA damage. Our results not only suggest that p21 is a major component of the mechanism by which p53 controls cell cycle progression, but also indicate the existence of additional p53-regulated growth control pathways.

A significant effort during the last year has been devoted to the development of a system that may allow the cloning of genes by genetic complementation in mammalian cells. Complementation screening has proven a highly effective way of dissecting biological processes in yeast. Investigators working in mammalian systems have long suffered for the lack of a similar approach.

Along with Doug Conklin and Peiqing Sun, we have devised a system based on replication-deficient, recombinant retroviruses that allows extremely high gene transfer efficiencies into a wide variety of mammalian cell types. By linking the gene whose expression is desired to a marker gene in a bicistronic message, the percentage of drug-resistant cells that also express the gene of interest has been greatly enhanced. Furthermore, recombinase-based strategies allow excision of the provirus both in vivo and in vitro. This allows us to test the dependence of any phenotype on the integrated retrovirus and also provides a convenient way to recover an integrated provirus from the host genome. Work now in progress will apply this new technology to a variety of biological problems.

Regulation of the Cdc10/Sct1 Transcription Complex Requires the Cooperation of the Cdc2 and Ran1 Protein Kinases

M. Caligiuri, D. Beach

In the fission yeast, *Schizosaccharomyces pombe*, the execution of Start and consequent commitment to the mitotic cell cycle require the activities of the Cdc2 protein kinase and the Cdc10/Sct1 transcription complex. Inactivation of any of these genes leads to G_1 arrest and activation of the mating pathway under appropriate nutritional conditions. Cells that lack *sct1*, unlike those that lack *cdc2* or *cdc10*, will conjugate even when grown on rich media and thus bypass the normal requirement of nutritional deprivation for the activation of the mating pathway. This phenotype is reminiscent of that which results from the loss of another cell cycle regulator, the Ran1 protein kinase, which functions to prevent the activation of the mating pathway under conditions that promote vegetative growth. We have been studying the relationship between these proteins to characterize, at the molecular level, the events that lead to passage through the G_1/S-phase transition and commitment to cell division.

The Cdc10/Sct1 complex activates the expression of at least three genes required for DNA replication; dissociation of this complex leads to a loss of DNA-binding activity and consequent cell cycle arrest. We have found that the formation of this heteromeric complex requires both the Cdc2 and Ran1 protein kinases. Inactivation of either of these kinases leads to the dissociation of the Cdc10/ Sct1 complex. Analysis of the primary structure of Cdc10 revealed a single canonical Cdc2 phosphorylation site that is essential for Cdc10 function. We have found that substitution of the serine within this site (Ser-196) with alanine leads to inactivation of Cdc10, whereas substitution with aspartic acid, S196D, restores activity. The phosphorylation of this site is therefore likely to be required for Cdc10 activity. Metabolic labeling experiments have demonstrated that this site is, in fact, phosphorylated in vivo. We have found that the expression of Cdc10 S196D alleviates the requirement of Cdc2 for Cdc10/Sct1 complex formation, suggesting that Cdc2 phosphorylates this site. Additional evidence indicating that Cdc10 may be a substrate of Cdc2 comes from our observation that Cdc10 binds to the CLN3-like cyclin, Puc1. Interestingly, the association between Cdc10 and Puc1 requires Ran1, but not Cdc2. Since Ran1 is required for Puc1 (and presumably Cdc2) to bind Cdc10, we hypothesized that conditions which render Cdc10/ Sct1 complex formation independent of Cdc2 (i.e., expression of Cdc10 S196D) might also obviate the requirement for Ran1 for this process. We have found this prediction to be true and therefore suggest that Ran1 functions to promote vegetative cell growth by activating Cdc10/Sct1-dependent transcription through Puc1/Cdc2 leading to DNA replication.

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CELL CYCLE CONTROL IN *SACCHAROMYCES CEREVISIAE*

B. Futcher J. Donovan B. Schneider T. Volpe
D. Germain G. Sherlock H. Wijnen
K. Hidaka B. Steiner Q.-H. Yang

Our main interest continues to be regulation of Start and mitosis in *Saccharomyces cerevisae*. We are most interested in the tethering of division to cell growth and in the generation of basic cell cycle oscillation. Many key cell cycle events are regulated by protein kinase complexes formed between Cdc28 and a cyclin. There are now more than a dozen known yeast cyclins, and probably more to come. These fall into two broad groups: the G_1 cyclins, including Cln1, Cln2, and Cln3, which regulate Start, and the mitotic, B-type cyclins Clb1, Clb2, Clb3, and Clb4. Two other cyclins, Clb5 and Clb6, are very important for DNA replication, but they also have roles at Start and perhaps also in early mitosis.

A second interest is yeast telomerase. This year, we found telomerase activity in yeast and found that Est1 is associated with the activity.

Substrates of the Ubiquitin-conjugating Enzyme Ubc9

J. Donovan

UBC9 encodes a ubiquitin-conjugating enzyme, and we have previously shown that it is important for degradation of mitotic cyclins (Seufert et al. 1995). A temperature-sensitive *ubc9* mutant arrests and dies in G_2 or M phase. However, cyclins lacking a destruction box, and so not degraded, do not cause lethality. We are investigating whether the lethality of the *ubc9* mutant is due to lack of degradation of some other mitotic protein, for instance, the hypothetical "chromosome glue" protein thought to hold sister chromosomes together until anaphase.

Mitotic Checkpoints and Proteolysis

D. Germain

DNA damage causes a checkpoint arrest in G_2 phase. Phenotypically, the arrest resembles the arrest in a *ubc9* mutant. We wondered whether the mechanism of the checkpoint arrest is failure of some essential proteolysis. Therefore, we looked at Clb5, which is one of the known substrates of Ubc9, to see if its turnover was inhibited by UV radiation. Clb5 was indeed stabilized by UV radiation. This suggests that the checkpoint works, at least in part, by inhibiting the activity of Ubc9, and this prevents progress into mitosis.

Yeast Vectors for Rapid Disruption, Tagging, or Promoter Insertion

B. Schneider, W. Seufert, B. Steiner, Q.H. Yang

We have developed a rapid new method for tagging yeast proteins. We have built a plasmid carrying *URA3* flanked immediately on each side by direct repeats of the triple hemagglutinin (HA) tag. Polymerase chain reaction primers are used to amplify this cassette, and homology with the gene of interest is built into the 5′ ends of the primers. Yeast are transformed with the amplified fragment, which then integrates into the gene of interest, giving a Ura^+ phenotype. Finally, we put the transformants on 5-FOA plates, selecting for Ura^- cells. The two directly repeated tags recombine with each other, popping out the *URA3* gene, and leaving one copy of the triple HA tag in the desired position. A minor modification gives us an equivalent method of disrupting genes and recovering a Ura^- strain. We have built plasmids allowing tagging with a triple HA epitope or a triple *myc* epitope; we have also built a plasmid allowing insertion of the *GAL1,10* promoter.

The Cdk Inhibitor Sic1 Links S Phase to Start

B. Schneider, Q.-H. Yang

Sic1 is an inhibitor of Cdc28–mitotic cyclin complexes, but it does not inhibit Cdc28–G_1 cyclin complexes. In yeast, both S phase and M phase depend on Cdc28-mitotic cyclin activity, and so Sic1 must be inactivated before S and M can proceed. Sic1 is lost shortly after Start, and this loss depends on the ubiquitin-conjugating enzyme Cdc34. We find that Sic1 becomes phosphorylated at Start and that this phosphorylation depends on the Cdc28–G_1 cyclin complexes. In the absence of the G_1 cyclins, Sic1 is not phosphorylated and also not destroyed. This suggests that Sic1 is a substrate of Cdc28 at Start and that the phosphorylation targets Sic1 for proteolysis by the ubiquitin system. Strikingly, inactivation of Sic1 is the only essential function of the G_1 cyclins Cln1, Cln2, and Cln3, because whereas a *cln1 cln2 cln3* triple mutant is dead, a *sic1 cln1 cln2 cln3* quadruple mutant is alive (Fig. 1).

Substrates of Cdc28

G. Sherlock

We have done a computer screen of the *S. cerevisiae* genome to find proteins with multiple clustered consensus sites for Cdc28 phosphorylation. Many of the proteins found in this screen are known to have a role in the cell cycle. We are mutating the sites in some of these proteins to see if there is a phenotype. So far, we have knocked out sites in Cin8, a kinesin-like motor protein, and Cdc6, a protein involved in initiation of DNA synthesis. The *cin8* site mutant has no obvious phenotype. However, the *cdc6* site mutant does have phenotypes. The mutant is sick and appears to have more Cdc6 protein than normal. Although we do not yet know whether it is a lack of phosphorylation that accounts for these phenotypes, it is possible that phosphorylation helps target Cdc6 for proteolysis, as is true for a number of other proteins.

Analysis of *WHI3*: A New Size Control Gene

T. Volpe

The *whi3* mutation was isolated some years ago by Rob Nash, and we are continuing its characterization. Like other *whi* mutants, it gives a small-cell phenotype, which may be because *whi3* mutants overexpress *CLN1* and *CLN2* G_1 cyclins. Overexpression of *WHI3* turns off *CLN1* and *CLN2* and is lethal. How-

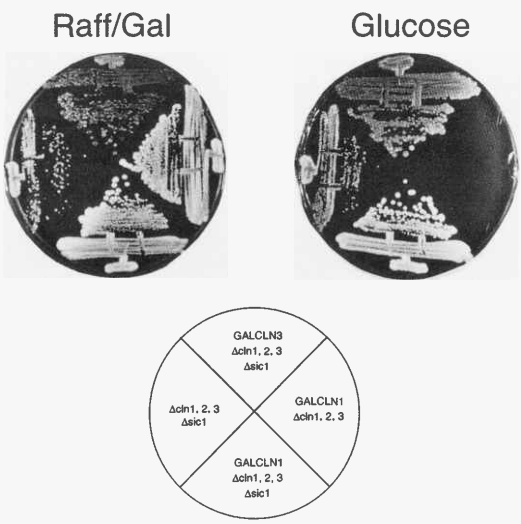

FIGURE 1 A *sic1* deletion rescues the lethality of the *cln1 cln2 cln3* triple mutant.

ever, the reason for the lethality is not the lack of *CLN1* and *CLN2* expression, because when *CLN2* is expressed from a heterologous promoter, this fails to rescue the lethality of *GAL-WHI3*. The Whi3 protein contains RNP-1 and RNP-2 RNA-binding motifs but otherwise has no homology with other proteins. We are trying to understand the mechanism of *WHI3* action.

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. The Swi4 and Mbp1 DNA-binding proteins each forms a complex with another protein called Swi6; these two complexes are called the SBF or MBF transcription factors. Genetic results argue that Cln3 somehow acts through Swi6 to activate the transcription factor. We are trying to understand the mechanism of this.

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 and 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 oligos. When the sequence of the template in the telomerase RNA is changed to include dG residues, the activity coprecipitated with Est1 begins to incorporate dC residues.

Curiously, there seem to be two telomerase-like activities. They are distinguishable in that one is specific for telomeric primers ending in GGG, whereas the other recognizes all yeast telomeric sequences. The "GGG" activity is completely dependent on the presence of the *TLC1* gene, which encodes the telomerase RNA, whereas the other activity is not dependent on *TLC1*. There may be two telomerases with different template RNAs, or the second activity may be some other kind of activity.

Since Est1 appears to be a component of the complex, we have done a two-hybrid screen to find other protein components. A number of different genes have been identified, and these are being characterized. Finally, a homolog of Est1 was recently identified by the yeast genome sequencing project. We are analyzing this homolog and its relationship to Est1 and to telomerase.

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REGULATION OF GROWTH AND CELL CYCLE COMMITMENT IN BUDDING YEAST

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

Our research mostly 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 proteins. Protein phosphatases are proteins that remove phosphate groups from phospho-serine, phospho-threonine, or phospho-tyrosine residues in other proteins. In many cases, removal of the phosphate activates the protein substrate. In other cases, removal of the phosphate inactivates the protein substrate. In my lab, we study three protein phosphatases: type 1 (PP1), type 2A (PP2A), and SIT4. All three of these phosphatases remove phosphate groups primarily from phospho-serine and

phospho-threonine residues. Most of our effort is focused on the SIT4 phosphatase. We have found that SIT4, which is a type-2A-related phosphatase, 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. 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. 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.

A. SAP protein data

	pI	Glu E	Asp D	Asn N	total amino acids	predicted mass (kDa)
SAP190	3.91	116	118	74	1,033	117.0
SAP185	4.01	127	107	79	1,058	121.3
SAP155	4.27	77	96	113	1,000	114.8
SAP4	4.35	62	78	55	818	94.7

B. Percent Identity

	SAP190	SAP185	SAP155	SAP4
SAP190	—	42%	26%	22%
SAP185		—	14%	21%
SAP155			—	36%
SAP4				—

C. Similarity Tree

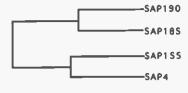

FIGURE 1 (*A*) SAP protein data; (*B*) percent identity; (*C*) similarity tree.

Function and Regulation of the SIT4 Phosphatase during the Cell Cycle

M. Luke, F. Della Seta, C.J. Di Como, K. Arndt

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 (when SIT4 is required for G_1 cyclin expression), SIT4 associates in separate complexes with three high-molecular-mass phosphoproteins termed SAP155, SAP185, and SAP190 (*S*it4-associated *p*rotein, apparent mass in kilodaltons). Near or just 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-polyacrylamide gel separation. R. Kobayashi (see Structure and Computation Section) 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. Moreover, we also identified two additional *SAP* genes in budding yeast by homology: *SAP185* from the budding yeast genome project and another *SAP* gene by polymerase chain reaction (PCR) using degenerate primers. We have shown that *SAP185* encodes a 185-kD protein that associates with SIT4. We currently do not know if SAP4 associates with SIT4, although we suspect that it does. Molecular information for the four SAPs is shown in Figure 1. All four SAPs are acidic.

With the *SAP* genes in hand, we could determine if the SAPs function positively with SIT4 (and turn SIT4 on near Start) or if they function negatively with SIT4 (and turn SIT4 off near Start). Three lines of evidence show that the SAPs function positively with SIT4. First, all four *SAP* genes in high copy number suppress the temperature-sensitive phenotype of *sit4-102* strains. Second, the phenotypic effects due to the loss of all four *SAP* genes are identical to the loss of SIT4. Third, in terms of the genetic interactions with deletion of *CLN3*, *BEM2*, *SIS2*, or *PHO85*, the loss of the *SAP*s is the same as the loss of *SIT4*. These findings indicate that the SAPs are required for SIT4's cellular function. The loss of the SAPs is not at all similar to the effects due to overexpression of SIT4.

By sequence similarity, the SAPs fall into two groups: the SAP4/SAP155 group and the SAP185/SAP190 group. The effects due to deletion of various combinations of the *SAP* genes show that this se-

quence similarity is also a functional grouping of the SAPs. Further analysis shows that the SAPs (and hence SIT4 also) are providing two functions: one function provided by SAP185 and SAP190 and another function provided by SAP155 (perhaps along with SAP4). Deletion of *SAP185* by itself or deletion of *SAP190* by itself causes no decrease in the growth rate of the cells. However, deletion of both *SAP185* and *SAP190* causes a slow-growth-rate phenotype. Importantly, although either *SAP185* or *SAP190* on either a low-copy-number *cen* plasmid or a high-copy-number plasmid restored the growth rate of a Δ*sap185* Δ*sap190* strain to wild type, neither *SAP4* nor *SAP155* could stimulate the growth rate of this strain. Therefore, SAP155, even when overexpressed, cannot provide the function provided by SAP185/SAP190. In addition, the overexpression of SAP185 or SAP190 cannot stimulate the slow growth rate of Δ*sap155* strains. Therefore, the SAPs are providing at least two cellular functions.

We have prepared antibodies that specifically recognize SAP155, SAP185, or 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? Interestingly, SAP155, SAP185, and SAP190 become hyperphosphorylated in the absence of SIT4 function. How is the association of SIT4 with SAP155, SAP185, and SAP190 regulated? Via phosphorylation of the SAPs? How do growth signals and cell cycle signals regulate the association of SAP155 and SAP190 with SIT4?

We have found that the amount of the SAPs that are associated with SIT4 is regulated not only by cell cycle position, but also by cellular growth signals: by carbon source (glycerol/ethanol versus glucose), by the presence or absence of amino acids in the growth medium, and during saturation of the cultures. Possibly, the SAPs are involved in transducing nutrient growth signals via their association with SIT4, thereby linking nutrient growth signals with G_1 cyclin expression, bud formation, and other late G_1 processes. The elucidation of such a signal transduction pathway will require the determination of how the association of the SAPs with SIT4 is regulated and what downstream processes are regulated by the SAPs and SIT4.

TAP42: An Essential 42-kD Protein That Interacts with Both SIT4 and the Type-2A Catalytic Subunits

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 *sit4-102* mutant. From this scheme, we isolated three genes. One gene is *SAP155*, which encodes the 155-kD subunit of SIT4 (see above). 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. In addition, suppression of the *sit4-102* strain by overexpression of HCS26 requires *PHO85*, suggesting that an increase in the HCS26/PHO85 kinase activity is required. We have two models to explain why overexpression of HCS26 does suppress, whereas overexpression of CLN2 does not suppress, the *sit4-102* mutant. In model no. 1, the HCS26/-PHO85 kinase can phosphorylate substrates that CLN2/CDC28 cannot. In model no. 2, the HCS26/-PHO85 kinase does not require SIT4, whereas the CLN2/CDC28 kinase does require SIT4.

The third gene is a previously unidentified gene which we term *TAP42* (for *t*wo A phosphatase-*a*ssociated *p*rotein) because it encodes a 42-kD protein that associates with both SIT4 and the type-2A catalytic subunit (we also isolated *TAP42* as a gene that in high copy number suppressed the temperature-sensitive phenotype of a temperature-sensitive *pp2a* mutant; see below). The TAP42 protein has similarity to the mouse α4 protein, which becomes phosphorylated in response to activation of the B-cell receptor. The budding yeast *TAP42* gene is essential, and germinating Δ*tap42* cells arrest as four to eight unbudded cells. Immunofluorescence microscopy was used to show that TAP42 is enriched in the nucleus, but it is also present in the cytoplasm.

The association of TAP42 with SIT4 does not require the SAP proteins. Moreover, we have not been

able to find any TAP42 in SAP immunoprecipitates. These findings show that TAP42/SIT4 complexes are distinct from SAP/SIT4 complexes. In addition, association of TAP42 with PPH21 or PPH22 (the type-2A PPase catalytic subunits) does not require TPD3 (an A-like subunit) or CDC55 (a B-like subunit). Together, these findings indicate that TAP42 interacts with the catalytic subunit of SIT4 (type-2A-related PPases) and with the type-2A catalytic subunits and that this interaction does not require any of the known phosphatase subunits. Moreover, this TAP42 interaction is specific to these phosphatases because TAP42 does not coimmunoprecipitate with the type-1 phosphatase catalytic subunit.

Genetic interactions and in vivo tests indicate that TAP42 functions positively with SIT4 and with the two PP2A catalytic subunits. We propose that the TAP42/phosphatase interactions result in a new activity that is not present in TAP42 by itself and in SIT4 or the PP2A catalytic subunits by themselves. Possibly, TAP42 might direct the phosphatases to dephosphorylate particular substrates.

Because rapamycin arrests cells in late G_1, and temperature-sensitive *sit4* mutants arrest in late G_1, we have been interested in a possible connection between SIT4 and the signaling pathway (TOR1/TOR2) that is inhibited by rapamycin. We have isolated one temperature-sensitive allele of *TAP42*. Interestingly, similar to the *TOR* mutants that give rapamycin resistance, we have found that the temperature-sensitive *tap42* mutant is almost completely resistant to rapamycin. Moreover, rapamycin rapidly causes the dissociation of TAP42 from SIT4 (but it does not decrease the SAP155/SIT4 association). These findings raise the possibility that the TAP42/SIT4 interaction is a possible downstream process in TOR signaling.

Future experiments will be directed at further elucidation of the TAP42/phosphatase interaction for cell cycle progression.

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 although 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* which 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, importantly, LAS1 becomes hyperphosphorylated in *sit4* mutants. Therefore, LAS1 might be a substrate of SIT4.

Our next goal is to determine if SIT4 can dephosphorylate LAS1 in vitro (the LAS1 for these experiments will be isolated from a *sit4* mutant and will be hyperphosphorylated). In addition, we will try to identify genes involved in bud initiation that might be regulated by LAS1.

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

F. Lin

PP2A (or type 2A) is a major class of protein phosphatase. *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.

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 or the removal of an inhibitor of the complex. 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.

To help understand the cellular functions of PP2A, we isolated two high-copy-number suppressors of the temperature-sensitive phenotype of *pph21-102* cells. One of these is *SBE2* (which is required for bud emergence) and the other is *TAP42*. As mentioned above, TAP42 is an essential protein that physically interacts with the catalytic subunit of PP2A and with SIT4 (a type-2A-related phosphatase). The interaction of TAP42 with PP2A does not require the A (TPD3) or the B (CDC55) subunit of PP2A. We are in the process of determining the cellular functions of TAP42. For SBE2, we are determining if, like TAP42, it also physically interacts with PP2A. In addition, we are determining if SBE2 might be a substrate of PP2A. The isolation of SBE2 as a suppressor may be related to the role of PP2A in bud growth.

Identification of the Proteins That Interact with the Type-1 (PP1) Protein Phosphatase Catalytic Subunit

E. Tegins

Budding yeast has a single known type-1 phosphatase catalytic subunit. This protein is essential. We have found that the epitope-tagged type-1 catalytic subunit coimmunoprecipitates with eight major proteins. We purified these proteins by large-scale immunoprecipitation followed by electrophoresis through SDS-polyacrylamide gels. Peptides from these proteins are currently being sequenced by R. Kobayashi (see Structure and Computation Section). From the complete sequence of the budding yeast genome (due in very early 1996), we will be able to use PCR to rapidly obtain the open reading frame for each type-1 phosphatase-associated protein. These clones will be used both to delete the gene encoding each protein and to overexpress each protein.

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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. Mittal, D.J. Morrison, P.S. Pendergrast, F.C. Pessler, C.L. Sadowski, S. Sepheri, M.-W. Wong

The human small nuclear (sn)RNA genes share very similar promoters, yet some of them are transcribed by RNA polymerase II, whereas others are transcribed by RNA polymerase III. We are characterizing the *trans*-acting factors involved in transcription of the RNA polymerase II and III snRNA genes and comparing them to the transcription factors involved in RNA polymerase II transcription of mRNA-encoding genes and RNA polymerase III transcription of typical RNA polymerase III genes such as the adenovirus 2 (Ad2) VAI gene. As described in more detail below, we find that the RNA polymerase II and III snRNA promoters share transcription factors that are not used by other types of promoters. By identifying the members of the snRNA transcription initiation complexes and comparing them to factors used in transcription from other promoters, we can address how RNA polymerase specificity is determined.

The promoter of human immunodeficiency virus type 1 (HIV-1) can direct the synthesis of two classes of RNA molecules: full-length RNAs that extend through the entire transcription unit, and short RNAs that end approximately 60 nucleotides downstream from the transcription start site, just 3′ of a stable stem-and-loop structure that encompasses the first 59 nucleotides of the RNA. Synthesis of the short transcripts is dependent on a bipartite element, the inducer of short transcripts (IST), which is located downstream from the transcription start site. Mutations in IST reduce the transcriptional output of the HIV-1 promoter by selectively debilitating the synthesis of short transcripts while leaving the synthesis of full-length transcripts unaffected. Thus, IST activates transcription from the HIV-1 promoter, but the RNA molecules resulting from this transcription activation are short. IST, then, is not a termination signal. Rather, it appears to activate the formation of transcription complexes that are incapable of efficient elongation. We are interested in characterizing the biochemical mechanisms that govern IST function.

Characterization of the SNAP45 Subunit of $SNAP_c$

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

The basal RNA polymerase II snRNA promoters consist of only one essential element, the proximal sequence element or PSE. The basal RNA polymerase III snRNA promoters contain in addition a TATA box. We have identified and purified a factor we refer to as the SNAP complex ($SNAP_c$) that recognizes the PSE and is required for both RNA polymerase II and III transcription from snRNA promoters. This factor consists of four tightly associated subunits, SNAP43, SNAP45, SNAP50, and SNAP190, as well as the TATA-box-binding protein, which appears to be more loosely associated. We have previously obtained peptide sequences for some of these subunits and cDNAs corresponding to the SNAP43 subunit. We have now isolated a cDNA corresponding to the SNAP45 subunit. Antibodies directed against the cloned protein retard the mobility of the $SNAP_c$-PSE complex in an electrophoretic mobility shift assay, indicating that it is indeed part of $SNAP_c$. SNAP45 is exceptionally proline-rich; in fact, prolines are, together with alanines, the most abundant amino acids, each representing 14% of the total number of residues. Database searches revealed no striking homologies with any available protein sequence, but the protein contains an intriguing motif constituted by proline residues spaced identically as in the heptade repeat present at the carboxyl terminus of the large subunit of RNA polymerase II.

SNAP45 is specifically coimmunoprecipitated in nondenaturing immunoprecipitations from nuclear extracts performed with anti-SNAP43 antibodies, and reciprocally, SNAP43 is specifically coimmunoprecipitated with anti-SNAP45 antibodies. Thus, these two proteins are part of the same complex, although they do not appear to interact directly with each other

as determined by GST pull-down experiments. However, both proteins interact directly with TBP.

To determine whether the SNAP45 subunit is required for transcription of snRNA genes by both RNA polymerases II and III, we depleted a nuclear extract with anti-SNAP45 antibodies attached to beads and tested the depleted extract for its ability to direct transcription from the RNA polymerase II U1 and RNA polymerase III U6 snRNA promoters. Transcription from both promoters was debilitated, whereas transcription from the RNA polymerase II adenovirus 2 major late promoter and from the RNA polymerase III VA1 promoter was unaffected. Thus, the SNAP complex appears to be required specifically for transcription of genes that contain a PSE. In addition, these data show that if slightly different versions of the SNAP complex are involved in RNA polymerase II and III transcription from snRNA promoters, they both contain the SNAP43 and the SNAP45 subunits.

Characterization of the SNAP50 Subunit of $SNAP_c$

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

We have recently isolated a full-length cDNA clone corresponding to the 50-kD subunit of $SNAP_c$. SNAP50 interacts with both SNAP43 and SNAP45 in GST pull-down experiments. As soon as anti-SNAP50 antibodies become available, we will be able to test the role of SNAP50 in RNA polymerase II and III transcription of human snRNA genes.

Characterization of the SNAP190 Subunit of $SNAP_c$

M.-W. Wong, R.W. Henry, R. Kobayashi, N. Hernandez

In our original $SNAP_c$ preparation, which involved several chromatographic steps, we detected a 190-kD polypeptide whose abundance peaked with DNA-binding activity. However, this protein appeared to be substoichiometric with SNAP43, SNAP45, and SNAP50. To determine whether SNAP190 is indeed part of $SNAP_c$, we purified $SNAP_c$ in just three steps, using immunoprecipitation with either anti-SNAP43 or anti-SNAP45 antibodies as the last purification step. In each case, we could detect a 190-kD polypeptide that specifically coimmunoprecipitated with both SNAP43 and SNAP45. It is therefore clear that this polypetide is indeed part of $SNAP_c$. We have scaled up the purification to obtain enough material for microsequencing.

Characterization of TFIIIB

R. Mital, R. Kobayashi, N. Hernandez

TFIIIB is a poorly characterized fraction required for transcription by RNA polymerase III. We have previously shown that the TFIIIB fraction contains two separable activities. One of these is a TBP-containing complex and is required for RNA polymerase III transcription of genes with internal promoter elements but not for transcription of the human U6 gene, whose promoter is entirely located in the 5′-flanking region.

We have purified this TBP-containing complex to near homogeneity. It contains TBP and a tightly associated protein of approximately 90 kD. We obtained the peptide sequence for this 90-kD polypeptide and used the information to isolate several cDNAs that encode two protein sequences highly related to BRF1, the TBP-associated, 67-kD subunit of yeast TFIIIB. One of these sequences is (nearly) identical to that of TFIIIB90, a TBP-associated protein involved in RNA polymerase III transcription recently cloned by the R. Roeder and colleagues. The other sequence encodes a protein (TFIIIB90b) that is four amino acids longer than TFIIIB90 and diverges from TFIIIB90 in the first nine amino-terminal residues. Intriguingly, TFIIIB90b does not contain a cystein that is part of a putative zinc finger present in the first 30 amino acids of TFIIIB90 and conserved in yeast BRF1 as well as in the related protein TFIIB.

We have generated anti-bodies directed against several peptides common to TFIIIB90 and TFIIIB90b, as well as against the divergent amino termini. Together, the TFIIIB90- and TFIIIB90b-encoding cDNAs and the antibodies will be invaluable reagents to determine whether these proteins have a role in U6 transcription.

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) which 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 have shown that on a probe containing adjacent octamer and PSE sites, the POU domain of Oct-1 binds cooperatively with PTF, a factor that appears to be related or identical to the SNAP complex. In collaboration with M. Cleary and W. Herr (see Transcriptional Regulation in the Tumor Viruses Section), we have pursued this observation.

The POU domain is a bipartite DNA-binding domain consisting of a POU-homeo (POU_H) domain and a POU-specific (POU_S) domain joined by a flexible linker. We showed that the Oct-1 POU domain, but not the related pituitary Pit-1 POU domain, can facilitate the binding of $SNAP_c$ to the PSE by increasing its on-rate. The effect depends largely on one of many amino acid differences between the Oct-1 and Pit-1 POU domains. Thus, exchange of the glutamic acid at position 7 in the Oct-1 POU_S domain for its Pit-1 counterpart, an arginine, inactivated the ability of the Oct-1 POU domain to recruit $SNAP_c$ to the PSE. Reciprocally, substituting the arginine at position 7 in the Pit-1 POU_S domain with the glutamic acid present at the corresponding position in the Oct-1 POU_S domain imparted onto Pit-1 the ability to recruit $SNAP_c$. The effect is most probably due to protein-protein interactions, as an antibody that recognizes the amino terminus of the Oct-1 POU_S domain disrupts the ability of the Oct-1 POU domain to recruit $SNAP_c$ to the PSE. Remarkably, the abilities of wild-type and mutant POU domains to enhance snRNA gene transcription in vitro correlated directly with their abilities to recruit $SNAP_c$ to the PSE. Transcriptional activators usually consist of separable DNA-binding and activation domains. Our results show that the ability to activate transcription, a property generally thought to be the exclusive hallmark of activation domains, can also be displayed by a DNA-binding domain.

Characterization of a Novel PSE-binding Activity

E. Ford, N. Hernandez

In nuclear extracts, we can detect, in addition to $SNAP_c$, a second PSE-binding complex of slower mobility. We have characterized this complex and shown that it consists of $SNAP_c$ and Oct-1. Thus, although Oct-1 on its own cannot bind to the DNA probe used in these experiments, it can associate with the $SNAP_c$-PSE complex. We are characterizing the protein-protein and protein-DNA interactions that stabilize the Oct-1-$SNAP_c$-PSE complex on this probe. In particular, we are determining whether Oct-1 contacts DNA and which of the $SNAP_c$ subunits it contacts directly.

Characterization of Human RNA Polymerase III

S. Sepheri, N. Hernandez

To reconstitute RNA polymerase III transcription in a completely defined system, we need highly purified RNA polymerase III. In addition, to study protein-protein interactions that lead to the recruitment of RNA polymerase III to specific promoters, we need antibodies directed against human RNA polymerase III. Such antibodies are presently not available. To generate anti-human RNA polymerase III antibodies that may be useful both for the purification of RNA polymerase III and for the study of RNA polymerase III recruitment, we have isolated a partial cDNA clone corresponding to the largest subunit of human RNA polymerase III.

We are currently trying to obtain full-length cDNA clones as well as using the sequence information we already have to generate anti-peptide antibodies. If such antibodies recognize the native enzyme, we will use them to study protein-protein interactions between RNA polymerase III and different RNA polymerase III transcription factors. We will also attempt to develop a simple protocol for the purification of RNA polymerase III.

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.

Identification and Purification of IST_{BF}, a Factor That Specifically Recognizes the IST

F.C. Pessler, N. Hernandez

We have identified an IST-binding factor (IST_{BF}) whose binding to wild-type and mutated versions of the IST correlates well with short transcript formation. IST_{BF} has been purified to near homogeneity and consists of a single polypeptide whose size is identical to that of a polypeptide which can be specifically cross-linked to the IST in partially purified fractions. This factor binds to a number of cellular promoters in addition to the HIV-1 promoter.

As a first step to show directly that IST_{BF} is indeed required for formation of short transcripts in the HIV-1 LTR, we have developed an in vitro transcription system in which the synthesis of short transcripts is dependent on IST. Short transcripts are synthesized by RNA polymerase II as determined by α-amanitin sensitivity studies, and they are not generated by constructs containing mutations in the IST. We can now determine whether depletion of IST_{BF} results in specific inhibition of the formation of short transcripts.

Isolation of cDNAs Corresponding to IST_{BF}

D.J. Morrison, R. Kobayashi, N. Hernandez

To elucidate the function of IST_{BF} in formation of short transcripts, we need to obtain corresponding cDNAs such that we can overexpress the protein and use it for functional studies and to raise specific antibodies.

As a first step toward this goal, we have scaled up the purification of IST_{BF} and obtained the peptide sequence. We are using the sequence information to design degenerate primers for use in polymerase chain reactions (PCRs). If we obtain a specific PCR fragment, we will use it as a probe for the screening of cDNA libraries.

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

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

HIV-1 is regulated in large part by a viral activator known as Tat. Tat recognizes an RNA target named TAR and is encoded close to the transcription start site. Whereas IST appears to direct the formation of transcription complexes that are incapable of efficient elongation, Tat appears to have the opposite effect: In the presence of Tat, the number of full-length transcripts is greatly increased and the number of short transcripts is decreased, suggesting that Tat counteracts the activity of IST by promoting the formation of transcription complexes that are capable of efficient elongation.

The HIV-1 promoter contains a TATA box. To determine whether the TATA-box-binding protein (TBP) is required for synthesis of both short and full-length transcripts in the absence and presence of Tat, we used an in vivo assay based on a triple-amino-acid substitution in TBP. This mutation, first described by Dr. Struhl and colleagues, creates an altered specificity TBP (TBP_{AS}) that can bind to both the wild-type TATA box and a mutated TATA box. Reporter promoters containing the altered TATA box can be used to test the effects of mutations in other parts of the protein on transcription. We inserted the altered TATA box in the HIV-1 promoter and showed that synthesis of both short and long transcripts was debilitated. Cotransfection of a TBP_{AS}-expressing vector restored the synthesis of short and long transcripts in the absence of Tat as well as activation by Tat, indicating that TBP bound to the TATA box is required for all of these processes. Full-length human

TBP could be replaced by the carboxy-terminal DNA-binding domain of human TBP and by full-length yeast TBP, indicating that the TBP residues important for all transcription from the HIV-1 LTR reside in the carboxy-terminal domain of TBP and are conserved between yeast and humans. Various mutations in TBP affected the synthesis of short and full-length transcripts as well as Tat activation similarly, and the effects correlated with the previously described effects of these mutations on binding of TBP to the largest subunit of TFIID, $TAF_{II}250$, which is essential for the assembly of the TFIID complex.

Together, these data suggest that if the HIV-1 promoter generates short and full-length transcripts by assembling different transcription complexes, these transcription complexes both use TBP similarly, probably as part of TFIID.

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TELOMERASE BIOCHEMISTRY AND REGULATION

C.W. Greider C. Autexier, A.A. Avilion, M.A. Blasco, K. Buchkovich, K. Collins, J. Hemish, A. Kass-Eisler, S. Le, R. Pruzan, N. Sareen, S.K. Smith

Telomeres were defined in the 1930s as essential components at chromosome termini that stabilize chromosomes against degradation and recombination. Around the same time, chromosomal rearrangements were shown to be prevalent in tumors and were suggested to play a part in cancer development. In the last 5 years, the connection between telomere function, chromosome rearrangements, and cancer pro-gression has begun to be elucidated. Two observations set the stage for the recent rapid progress on telomere function in cancer and aging: Telomeres shorten with progressive rounds of cell division in vivo and in vitro and telomere length is shorter in tumor tissues than in normal adjacent tissue. The mechanism of telomere length regulation in eukaryotes has been established from work in a number of labs using single-cell eukaryotes such as ciliates and yeast as experimental organisms. Telomeric DNA consists of tandemly repeated simple G-rich sequences in a wide variety of eukaryotes. The exact sequence of the repeats varies between species, for example, TTGGGG in *Tetrahymena* and TTAGGG in mammals. Since conventional DNA polymerases require an RNA primer and polymerize only in the 5′ to 3′ direction, chromosomes cannot be fully copied at their ends and thus are predicted to shorten after each duplication. In single-cell eukaryotes, telomere length is maintained by the de novo addition of telomere repeats onto chromosome ends. Thus, telomere length reflects a dynamic balance between telomere shortening and telomere lengthening.

Telomerase is the ribonucleoprotein DNA polymerase that elongates telomeres. Telomerase, first characterized in *Tetrahymena*, consists of both proteins and an essential RNA that provides the template for the synthesis of telomere repeats. The *Tetrahymena* telomerase RNA contains nine nucleotides, 5′-CAACCCCAA-3′, that allow synthesis of telomeric TTGGGG repeats. Alterations in the sequence of this template region alter the sequence of telomere repeats in vivo and in vitro. The telomerase RNA component has been isolated from several

ciliates in addition to *Tetrahymena*, from two yeasts, and last year, we reported the cloning of the human and mouse telomerase RNA components.

In 1990 we showed that telomere length shortens as primary human cells divide in culture. This observation suggested that telomerase may be absent from primary cells and that telomeres may shorten due to the end replication problem. Subsequent work from a number of labs including our own has shown that many primary cells and tissues lack telomerase activity. The lack of detectable telomerase activity in primary tissues may explain the short telomeres in tumor cells compared to normal tissue. During the multiple cell divisions a tumor must undergo, in the absence of telomerase, telomeres are expected to shorten. When primary cells are immortalized after transfection with a variety of viral oncogenes, telomeres initially shorten and no telomerase activity is detected. Just before and during crisis, many end-to-end associations of chromosomes and ring chromosomes are found, suggesting telomere defects. After crisis, both telomere length and the karyotype are stabilized and telomerase activity is detected. These experiments suggested that there is a strong selection at crisis for cells that can re-express telomerase and maintain telomere length. Furthermore, the results suggest that telomerase activity may be required for the growth of immortalized cells. In the absence of telomerase, telomeres may shorten, and cells may re-enter crisis and die.

In the past year, we have continued our detailed biochemical analysis of the *Tetrahymena* telomerase enzyme to understand the mechanism of this unusual DNA polymerase. In addition, we have expanded our studies to elucidate the regulation of mammalian telomerase in tumorigenesis and in normal development using the mouse as a model system.

Functional Dissection of the *Tetrahymena* Telomerase RNA

C. Autexier

Using an in vitro reconstitution assay in which *Tetrahymena* telomerase activity can be recovered from micrococcal nuclease-treated protein extracts and in vitro transcribed telomerase RNA, we have previously identified two functional domains within the nine-nucleotide "template," a template, and an alignment region. In the past year, we continued to use the in vitro reconstitution assay to study the functional requirements of the telomerase RNA. First, mutations in the template and alignment regions were analyzed to characterize further the function of the residues in these regions. Second, mutants in a conserved region upstream of the template were tested. Third, deletions and changes were made in the telomerase RNA to test the function of predicted secondary structures. Results indicate that the template region can be increased and decreased in length, suggesting a flexibility in the sequence of the template domain. Circular permutations of the template region suggest that the telomerase RNA can have different alignment regions, more 3′ to the previously defined alignment region. The relaxed specificity and flexibility of the alignment and template domains of telomerase RNA in the recognition and elongation of substrate DNA may be important in vivo. Telomerase directly adds telomeric DNA de novo onto nontelomeric sequences during developmentally controlled chromosome healing in *Tetrahymena* and in mammalian cells. Perhaps, in vivo, nontelomeric or non-G-rich substrates align with the 3′-flanking region of the alignment region or alternative regions of the RNA.

There is a six-nucleotide sequence 5′-(CU)GUCA-3′, two residues upstream of the template region, which is conserved among the ciliate telomerase RNAs. Changing the spacing (normally two residues) between the template region and this conserved region (42+U) moved the 5′ boundary of the template more 5′ (arrow in Fig. 1), suggesting that the location of this conserved sequence is important in determining the 5′ boundary of the template. For example, deleting a spacer residue (residue 42) resulted in the exclusion of a residue normally part of the template (43). Changing the sequence of the spacer residues did not affect the 5′ boundary of the template (42G). Mutating the sequence of the conserved region (38–40AGU) resulted in the aberrant regulation of the 5′ template boundary, suggesting that some sequence-specific interactions between this conserved region and either RNA or protein are important in preventing polymerization beyond the 5′ end of the template. This is the first identification of a functional region of the telomerase RNA, other than the template domain.

Deletions potentially disturbing predicted higher-order structures at the 5′ (8-base deletion) and 3′ ends (13-base deletion) of the RNA do not affect

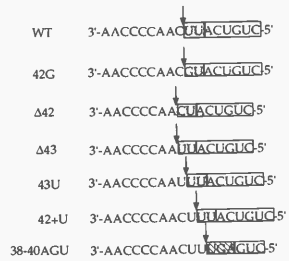

FIGURE 1 The position and sequence of the conserved sequence determine the 5′ boundary of the template. The nine-nucleotide template and alignment domains (residues 43–51 in the full-length wild-type *Tetrahymena* telomerase RNA) are shown with the upstream conserved region 5′CUGUCA-3′ (residues 35–40; *open box*) and the spacer residues (residues 41 and 42; *stippled box*). The sequence is written in a 3′ to 5′ direction. An arrow indicates the 5′ boundary of the template. The wild-type sequence is compared to the sequence of mutant RNAs. In the 38–40AGU mutant, the mutated sequence (*hatched box*) is shown.

template specificity but alter the efficiency of the elongation activity of telomerase, as compared to wild type. Work is currently in progress to address the function of other predicted secondary structures. Terminal and internal deletions of conserved stem-loop structures and sequences are being analyzed. Understanding the role of the RNA component of telomerase is critical in understanding the role of telomerase in DNA replication in both normal and cancer cells.

Utilization of Ribonucleotides and RNA Primers by *Tetrahymena* Telomerase

K. Collins

In vitro, *Tetrahymena* telomerase can elongate single-stranded DNA primers processively: One primer can be extended by multiple rounds of template copying before product dissociation. Telomerase will incorporate dNTPs or ddNTPs and will elongate any G-rich, single-stranded primer DNA. In this past year, we described a new activity of the *Tetrahymena* telomerase; we showed that *Tetrahymena* telomerase can incorporate a ribonuceotide, rGTP, into elongation products. Synthesis of the product d(TT)r$(GGGG)_n$ was processive, suggesting that the chimeric product remained associated with the enzyme both at the active site and at a second, previously characterized, template-independent product-binding site. As predicted by this finding, RNA-containing oligonucleotides served as primers for elongation. More than three nucleotides of RNA at a primer 3′ end decreased the quantity of product synthesis but increased the affinity of the primer for telomerase. Thus, RNA-containing primers were effective as competitive inhibitors of DNA primer elongation by telomerase (Fig. 2). These results support the possible evolutionary origin of telomerase as an RNA-dependent RNA polymerase.

Regulation of Human Telomerase Activity and RNA Levels

A. Avilion [in collaboration with Jyothi Gupta and Dr. Silvia Bacchetti, McMaster University, and Drs. Mieczystaw Piatyszek and Jerry Shay, University of Texas Southwestern Medical Center at Dallas]

We followed the levels of human telomerase RNA (hTR) during cellular immortalization in vitro. In collaboration with Dr. Silvia Bacchetti's group, we used a sensitive polymerase chain reaction (PCR)-based assay for telomerase activity to reexamine telomerase activity in the HEK cells described above that had been transfected with SV40 T antigen. The precrisis cells did not have detectable telomerase activity, whereas the postcrisis cells had significant levels of telomerase activity. In human B cells infected with Epstein-Barr virus (EBV), telomerase activity was also elevated postcrisis. In both cases, however, the hTR levels were initially high and did not increase significantly after crisis. We further looked at 22 different human tumor samples and 6 normal tissues in collaboration with Dr. Jerry Shay's group and found that the level of the telomerase RNA did not parallel the level of telomerase activity in these samples. In all cases, tumors that had telomerase activity had detectable levels of hTR. However, some tumors with very low levels of activity had very high levels of hTR (Fig. 3). These discrepancies indicate that hTR levels cannot be used as a marker for the presence of telomerase activity.

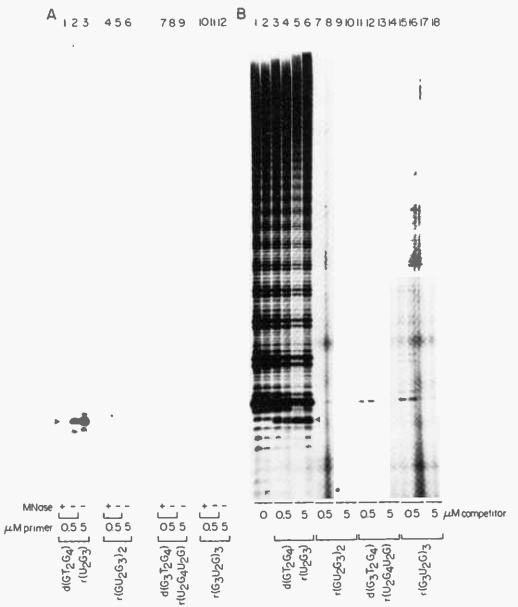

FIGURE 2 Elongation of $d(G_3T_2G)_3$ in the absence or presence of competitor RNA and chimeric RNA/DNA primers. (*A*) Elongation of RNA and chimeric RNA/DNA primers alone. Reactions were mock-treated (–MNase) or treated (+MNase) with micrococcal nuclease before inactivation of the nuclease and addition of primer to reactions. Primers were assayed at either 0.5 or 5 mM concentration. (*B*) Elongation of $d(G_3T_2G)_3$ in the absence (lanes *1,2*) or presence of competitor RNA and chimeric RNA/DNA primers. Reactions were done in duplicate and loaded in adjacent gel lanes. All reactions contained 0.5 mM $d(G_3T_2G)_3$ with the indicated competitor RNA or chimeric RNA/DNA primer at 0.5 or 5 mM concentration. Reactions were precipitated and electrophoresed on an 11% acrylamide, 7 M urea gel. (*A,B*) From the same gel and exposure. Arrowheads indicate the migration of the predominant product from elongation of the 12-nucleotide chimeric primer $d(GT_2G_4)r(U_2G_3)$.

Differential Regulation of Telomerase Activity and Telomerase RNA during Multistage Tumorigenesis

M. Blasco [in collaboration with M. Rizen and D. Hanahan, University of California, San Francisco]

To determine when telomerase is activated during tumor development and progression, we examined telomerase activity and mouse telomerase RNA (mTR) expression in two well-characterized transgenic mouse models of multistage tumorigenesis, developed by Doug Hanahan's lab: K14-HPV-16 transgenic mice and RIP1-TAg2 transgenic mice. These mouse models allow examination of many independent tumors at different stages in tumor progression from genetically identical individuals. K14-HPV-16 transgenic mice express the early region of the hu-

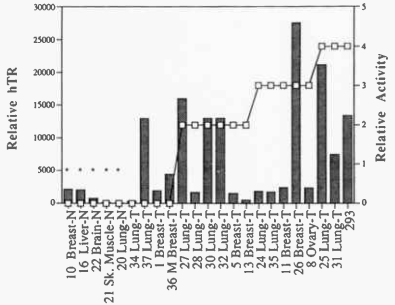

FIGURE 3 Human telomerase RNA levels in tumor vs. normal tissues. Total RNA was made from different tissues, and 25 μg of each was analyzed by Northern blot analysis. The asterisk indicates normal tissues tested, whereas the other tissues are from tumors. The graph shows the relative amounts of telomerase RNA (Relative hTR) in different tumors normalized to the amount of 5S RNA. Telomerase activity levels plotted on the right with a 0, for no activity, or 1–4 to indicate the relative level of telomerase activity detected. Extracts that showed activity at 0.06 μg were designated 4 for telomerase activity. Extracts that had weak activity at higher concentrations (6 μg) were designated 1 for weak activity, 2 for strong activity at 6 μg, or 3 for strong activity at 0.6.

TABLE 1 Telomerase Activity during Multistage Tumorigenesis in Transgenic Mice

Transgenic system	Stage	Telomerase positive samples/total tested
Islet cell carcinoma (RIP1-TAg2)	normal islets[a]	0/1
	angiogenic islets[b]	0/1
	islet tumor	13/17
Squamous cell carcinoma (K14-HPV-16)	hyperplastic back skin	0/5
	dysplastic ear	0/6
	peritumor dysplasia	0/2
	well differentiated-SCC	0/3
	moderately differentiated-SCC	1/2
	poorly differentiated-SCC	2/2
	lymph nodes[c]	2/3

[a]Pool of islets from ten nontransgenic mice.
[b]Pool of angiogenic islets from six transgenic mice.
[c]The lymph node from mouse 64395 was demonstrated by histology to contain a metastatic lesion. The nodes from mouse 62015 were found to be "reactive," but no metastatic cells could be detected by histology.

man papillomavirus type 16 (HPV-16) in basal keratinocytes, resulting in multistage induction of squamous cell carcinomas of the epidermis that show the histological hallmarks of squamous epithelial cancer in humans. Initially, diffuse hyperplastic lesions develop, which are followed by dysplastic lesions, papillomas, and subsequently two classes of carcinoma, well differentiated (WD-SCC) and moderate to poorly differentiated squamous cell carcinoma (M/PD-SCC). In the second model, RIP1-TAg2 mice develop pancreatic β cell islet carcinomas along a multistage pathway elicited by expression of the SV40 T-antigen oncogene. The pathway models the progression of human carcinoma-in-situ lesions; the stages arise sequentially and are statistically separable. Initially, uniform expression of the T-antigen oncogene elicits focal hyperproliferation in a subset of the islets, and then a subset of these hyperplastic islets switch on angiogenesis and a few progress to solid tumors.

In both models of multistage tumorigenesis, telomerase activity was detected only in late-stage tumors (Table 1). In contrast, mTR levels were up-regulated in the early preneoplastic stages and further increased during progression. The mTR levels did not parallel the levels of telomerase activity, and a subset of tumors lacked telomerase activity and yet expressed telomerase RNA. These results suggest that although telomerase RNA is required for enzyme activity, the regulation of telomerase activity is separable from expression of its RNA component. Together with the results from human cells, this suggests that the level of telomerase RNA cannot be used as a marker for the presence of telomerase activity during tumor progression.

Surprisingly, when we examined mTR levels in histologically normal tissue from transgenic and nontransgenic parental mouse lines, we found that mTR levels were significantly higher in the transgenic animal tissues. In the K14-HPV-16 transgenic mice, mTR levels in normal back skin, ear, and lymph node were markedly higher than those in a nontransgenic control parental FVB/n mouse. Brain tissue from both control and the transgenic mice showed very low levels of mTR as expected since brain does not express the HPV-16 oncogene or have detectable telomerase activity (Fig. 4). Similarly, when we compared mTR levels in histologically normal islets from RIP1-TAg2 transgenic mice and control parental nontransgenic C57BL/6 mice, mTR was detected in the RIP1-TAg2 transgenic islets but not in the control

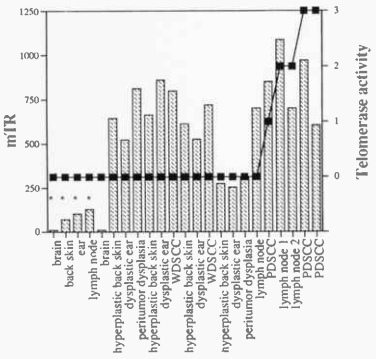

FIGURE 4 mTR and telomerase activity levels during tumor progression in K14-HPV-16 transgenic mice and in normal mice. Northern blots were done using RNA isolated from various stages of tumor progression in four K14-HPV-16 transgenic mice and from normal tissues from a parental nontransgenic strain (asterisk). The amount of mTR in each lane was normalized to the amount of 5S RNA and quantified using a Fuji Phospholmager. The level of mTR is expressed in arbitrary units. Telomerase activity was also assayed in the same tissue samples from transgenic and nontransgenic mice. Activity was classified into four levels according to the amount of activity detected at two different tissues extract dilutions: (0) no detectable activity; (1) low activity visible only at high extract concentrations; (2) moderate activity seen at high and low protein concentrations; (3) high activity when strong activity signal was detected on short exposures at all protein concentrations tested.

mouse islets. These observations suggest that up-regulation of mTR in the preneoplastic stages of tumor formation may be a direct consequence of oncogene expression.

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PLANT GENETICS

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Molecular and Genetic Analysis of the Maize Indeterminate Gene

J. Colasanti, V. Sundaresan

The life cycle of higher plants consists of a vegetative phase and a reproductive phase. Very little is known about the mechanisms that mediate the transition from vegetative to reproductive growth. To better understand how this process is controlled at the molecular level, we are analyzing the *indeterminate1* gene (*id1*) of maize, which is required for the vegetative to reproductive transition. Mutants that are defective for normal *Id1* gene function are unable to undergo normal reproductive development; i.e., *id1* mutants continue to form leaves long after normal plants have flowered and, given enough time, severe *id1* mutants form aberrant floral structures with vegetative characteristics. Therefore, *Id1* is required for the transition to reproductive development in maize and, possibly, in all higher plants. In a previous report, we described the isolation of a transposon-tagged *id1* allele, *id1-m1*, that is the result of a *Ds2* transposable element insertion into the *Id1* gene. The sequence near the *Ds2* insertion encodes a putative zinc finger DNA-binding motif, similar to regulatory proteins found in several other species. In addition, we demonstrated that the severity of the *id1-m1* phenotype is alleviated by the presence of an *Ac* element, which provides transposase function and promotes *Ds* element transposition. The absence of defined sectors of revertant tissue suggests that the *Id1* gene can act non-cell autonomously; i.e, the ID1 protein, or a target of the ID1 protein, can diffuse from one location to another to mediate its function. Here, we report further evidence for this result, and we show preliminary findings about the *Id1* gene structure and expression pattern.

EFFECT OF *AC* DOSAGE ON *Id1-m1* MUTATION

It was observed that *id1-m1* mutants that have *Ac* elements in the background had a less severe *id1* phenotype relative to *id1-m1* siblings with no *Ac*. This characterization has been extended by analyzing the effect of *Ac* dosage on flowering time of *id1-m1* mutants. The *Ac* transposable elements of maize exhibit a negative dosage phenomenon for transposition. For example, the *bz2-m2* allele, the result of a *Ds2* insertion into the aleurone pigmentation gene, *Bz2*, will exhibit large revertant sectors on the kernel in the presence of one *Ac* element. However, the aleurone sectors are smaller and less abundant with increasing copies of *Ac*, demonstrating that higher doses of *Ac* cause *Ds* excisions to occur later and less frequently during development. We wanted to determine if the *id1-m1* phenotype exhibited a similar negative dosage effect. An *id1-m1 bz2-m2* homozygous plant that carried one *Ac* was self-crossed, and a family of kernels was obtained that segregated one *Ac* element (large aleurone spots), two to three *Ac* elements (tiny spots), or no *Ac* elements (no spots). As reported previously and shown in Figure 1, kernels with no spots (0 *Ac*) had the most severe *id1* phenotype; i.e., they made many more leaves than wild-type plants and they formed reproductive structures with vegetative characteristics. However, plants grown from kernels with large spots gave rise to *id1* mutants that flowered relatively early (although later than wild type), but tiny-spotted kernels gave rise to plants with an *id1* phenotype only slightly less severe than the 0 *Ac* mutant siblings (Fig. 1). This observation suggests that different doses of *Ac* result in different levels of functional *Id1* gene expression.

Presumably, the plants from large-spotted kernels have large somatic sectors of normal plant tissue with restored *Id1* function, and small-spotted kernels give rise to plants with smaller sectors of *Id1* tissue. In addition, in support of the suggestion that *Id1* acts non-cell autonomously, revertant sectors of normal flowering tissue on *id1* mutant plants have never been observed. Rather, the whole plant uniformly flowers earlier or later, depending on *Ac* dosage. These observations suggest a possible mechanism where the *Id1* gene product is produced in revertant sectors of the leaves and is transmitted to the shoot apex, or,

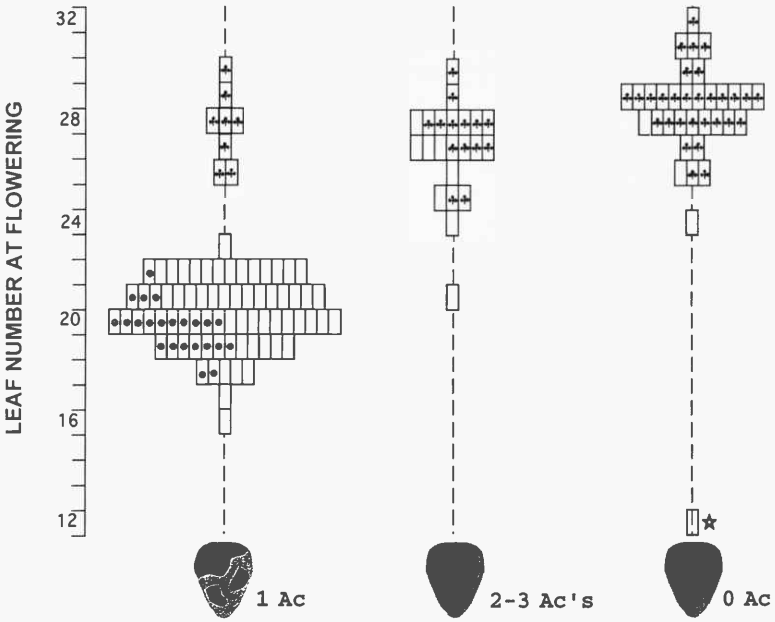

FIGURE 1 Effect of *Ac* dosage on *id1-m1* flowering time. Kernels homozygous for *id1-m1* that carried either one *Ac* element (large spots), two to three *Ac* elements (small spots), or no *Ac* elements (no spots) were planted, and the flowering time of the resulting plants was recorded as a function of leaf number. Each rectangular box represents one plant. Boxes with dots in them represent plants with less severe *id1* phenotypes with relatively normal flowers that could be crossed. Boxes with clover symbols represent plants with severe *id1* mutant phenotypes that exhibited aberrant floral development and extensvie reversion to vegetative growth. One plant from the "0 Ac" plants (box near five-pointed star) flowered at the same time as wild-type plants with 12 leaves and it had completely normal flowers. This plant is the germinal revertant described in the text.

alternatively, by regulating the production of a signal that diffuses to the shoot apex, to mediate the transition from vegetative to reproductive growth. Furthermore, the observation that *id1-m1* mutants with a higher *Ac* dose have a mutant phenotype almost as severe as plants with no *Ac* reveals that critical levels of *Id1* gene product are required to mediate the transition to flowering.

IDENTIFICATION OF A GERMINAL REVERTANT

Further proof that the cloned sequence encodes a part of the *Id1* gene was obtained by the isolation of a germinal revertant. As described above, some *Ac*-bearing *id1-m1* mutant plants flowered with a sufficiently normal phenotype that they could be crossed to each other, thereby generating *id1-m1* homozygous kernels. The progeny of these crosses segregated spotted and bronze kernels in a ratio which suggested that a single *Ac* element was present in the original parents; i.e., 3/4 spotted kernels, 1/4 bronze kernels. When these kernels were planted, all but one plant exhibited the *id1* phenotype, and as observed in the above experiment, spotted kernels give rise to plants with a less severe phenotype than plants from bronze kernels. Among the plants derived from bronze kernels (0 *Ac*), a single exception was found that had a completely wild-type phenotype; i.e., it made as many leaves and flowered at the same time, with completely normal flowers, as its wild-type siblings (Fig. 1). We assume that this is a true revertant allele because the parent of this plant was crossed in the field more than 5 weeks after all wild-type plants had shed out, eliminating the possibility of contaminating pollen. In addition, since there is no *Ac* element present in this plant to cause somatic reversion, it is not the result of a very large somatic sector. Southern blot analysis of this plant showed that it had both the 4.2-kb band (a *Sac*I genomic fragment with the *Ds2* element insertion) and the 2.9-kb band that hybri-

dized to *Ds2* flanking DNA, consistent with excision of *Ds2* from one of the *id1-m1* loci. Whereas Southern analysis showed that all the other plants from bronze kernels had a single 4.2-kb *Sac*I band, typical of *id1-m1* homozygous mutants, one bronze kernel plant that had a severe *id1* phenotype had both 4.2-kb and 2.9-kb bands, as was found in the putative germinal revertant.

The DNA flanking the former *Ds2* insertion site in both of these plants was amplified by PCR, cloned, and sequenced. The *id1* mutant plant had a footprint identical to a previously described stable null allele, *id1-X*, and therefore is the result of an imprecise germinal excision (Annual Report, 1994). The putative revertant plant, however, had no footprint; i.e., it had the same sequence as the wild-type allele. Given that contamination with normal pollen is highly improbable since all normal plants had shed out long before, we assume that this new allele is a germinal revertant that has restored *Id1* function. It is possible that, since the *Ds2* element is inserted into an exon very close to the zinc finger consensus region (see below), an excision that causes an insertion or deletion of even one amino acid could result in a defective gene product, and only precise excisions can restore gene function completely. In addition to revertant alleles, it is possible that *Ds2* excision from *id1-m1*, or its reinsertion into other parts of the *Id1* gene, will result in the generation of new *id1* mutant alleles that will give clues about how the *Id1* gene product functions.

DETERMINATION OF THE *Id1* TRANSCRIBED REGION

Analysis of the structure of the *Id1* gene and its pattern of expression will reveal something about how this gene acts to mediate the conversion of a vegetative meristem to a reproductive meristem. We have not been able to identify *Id1* mRNA by Northern blot analysis, perhaps because it is expressed at very low levels. In lieu of obtaining or constructing cDNA libraries derived from many different plant tissues and screening for cDNA clones by hybridization, the technique of RT-PCR was used to ascertain (1) in which tissues the *Id1* gene is expressed most abundantly, relative to other tissues, and (2) the regions of genomic sequence that are transcribed. The 2.9-kb genomic region flanking the *Ds2* element was sequenced, and primers complementary to both strands were synthesized along its length at 200–400-bp intervals.

Reverse transcriptase was used to synthesize cDNA from poly(A)$^+$ mRNA obtained from shoot apex, young leaf (from plants with 8–10 leaves), and root tissue. Various combinations of primers were used in PCRs to map transcribed regions. The initial selection of primer pairs was based on potential open reading frames (ORFs) that were found in the genomic sequence. This analysis has revealed the following information about the *Id1* transcript: (1) The *Ds2* transposable element is inserted within an exon, 7 bp upstream of an exon/intron junction (Fig. 2). The intron is 1.3 kb in length and is followed by an exon of approximately 500–600 bp. The remainder of the genomic 2.9-kb genomic fragment is intron sequence. (2) RT-PCR confirmed that the *Id1* mRNA is not expressed abundantly in any of the tissues examined. This semiquantitative assessment was made relative to the expression of the maize cell division cycle gene, *cdc2*, which is present at easily detectable levels in all tissues tested. The *Id1* mRNA is found at its highest levels in young leaf tissue. Shoot apices were second in abundance for *Id1* expression, but the tissue used for the isolation of RNA had a small amount of surrounding leaf tissue, and therefore is not composed entirely of apical cells. *Id1* mRNA was not detectable in roots. These results support the idea that *Id1* regulates a flowering signal that originates outside the shoot apex, possibly in the leaves.

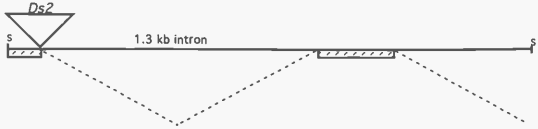

FIGURE 2 Schematic representation of the 4.9-kb *Sac*I (S) genomic fragment. Boxed area represents the known transcribed regions of the genomic clone. The 1.3-kb *Ds2* transposon is inserted 7 bp upstream of the exon/intron junction.

IDENTIFICATION OF *id1-Mum1*, A *Mutator*-TAGGED ALLELE OF *id1*

Recently, a newly identified *id1* allele, *id1-Mum1*, was made available to us by Dr. Guri Johal (University of Missouri, Columbia) who found it segregating in a family of plants that segregated *Mutator* transposable elements. Southern analysis with probes derived from *Ds2*-flanking genomic DNA revealed that *id1* mutant plants from this *Mutator* family contain a large insertion in the 2.9-kb region. Using various primers specific to this genomic region and primers specific to the ends of all known *Mutator* transposons, we have found that *id1-Mum1* has a *Mu* element inserted approximately 700 bp distal to the *Ds2* insertion site of the *id1-m1* allele; i.e., approximately in the middle of the 1.3-kb intron. These results provide further evidence that the cloned fragment contains a portion of the *Id1* gene. The *id1-Mum1* mutation also provides another mutant allele of *id1* which will be used to study *Id1* gene function.

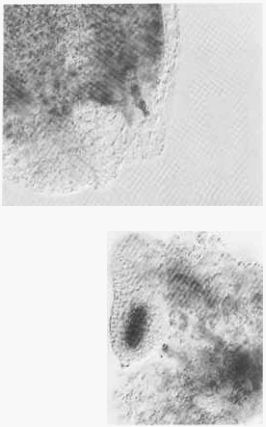

Figure 3 Examples of localized GUS reporter gene expression. (*Top*) Expression confined to suspensor; (*bottom*) expression localized to progenitor cells of root meristem.

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

S. Woody, V. Sundaresan

We are using a gene-trap/enhancer-trap transposon tagging system (Sundaresan et al., *Genes Dev. 9:* 1797 [1995]) to identify and characterize embryo-specific gene expression in *Arabidopsis*. To date, we have produced approximately 20,000 F_1 seeds derived from more than 300 *Ac* x *DsE* (enhancer-trap) or *Ac* x *DsG* (gene-trap) crosses. About 13,000 of these have been grown and harvested, and the F_2 seeds are being screened for new transposant lines at a rate of 400 families per week; in total, we have screened 8500 F_2 families and isolated 1650 transposant lines. These lines, as well as others generated by the Martienssen, Ma, and Grossniklaus labs here at the Laboratory, are being examined for embryo-specific GUS expression and for evidence of *Ds*-induced *emb* (*emb*ryo-defective) mutations.

We have identified eight transposant lines (five gene-trap [GT] and three enhancer-trap [ET] lines) in which the GUS reporter gene of the transposed *DsE* or *DsG* element is expressed primarily in developing embryos. These expression patterns included uniform staining of the embryo, staining restricted to apical or basal domains, and staining confined to groups of cells within the developing embryo (see Fig. 3).

Further characterization of the GUS expression patterns in these lines is ongoing, aimed toward a more precise determination of the developmental stage(s) in which expression is induced and toward identification of the particular cell types in the embryo and other tissues where the tagged genes are expressed. The *Ds* elements in some of these lines have been remobilized by back-crosses to *Ac*-bearing plants; it is possible that short-distance transposition events might create more severe alleles that would cause a mutant phenotype. Finally, TAIL-PCR and RACE-PCR methods are being used to isolate *Arabidopsis* sequences flanking the *Ds* insertion sites; these will be used to probe cDNA and genomic libraries to identify the tagged genes.

The transposant lines are also being screened for *emb* mutants. By inspection of nearly mature siliques, in which transmission of an *emb* allele is expected to result in 25% defective (white or pale green) seeds, we have found *emb* lines at a frequency of approximately 1–2% (>1000 lines screened). Unfortunately, no exceptional phenotypes have been observed so far: In some cases, embryo development is delayed relative to that of phenotypically wild-type siblings but

nevertheless proceeds to maturity. In some lines in which embryogenesis is arrested, the terminal phenotype, while variable, is similar to that observed in many previously described *emb* mutants: arrest at the globular stage, followed by disorganized proliferation of interior cells. Cosegregation of kanamycin resistance (specified by the *Ds* element) and the *emb* phenotype were examined in 11 candidate lines. Consistent cosegregation was observed in 2 lines; results in the other 9 cases were consistent with unlinked segregation of an *emb* locus and kanamycin resistance. Although the presence of multiple *Ds* elements in these lines, only one of which tags an *emb* locus, would yield similar results, another possibility is that some of these *emb* mutants are not tagged with *Ds*.

Of the two lines in which the *emb* loci are potentially tagged, one is an albino mutant and shows no morphological defect indicative of an effect on embryogenesis per se. The other line, ET339, arrests at the globular stage as described above. Although GUS activity is observed in other tissues, we have been unable to detect GUS expression in ET339 embryos. This may indicate that the tagged gene is expressed only transiently or at very low levels during embryogenesis; alternatively, it is possible that the DsE element in ET339 disrupts a locus required for embryogenesis, but the minimal promoter driving GUS expression is responding to an enhancer other than the one that might regulate expression of the *emb* locus. We have crossed ET339 to an *Ac* line to remobilize the element and hope to recover a derivative allele that causes a similar embryogenesis defect but now displays embryo-specific GUS expression prior to or during the globular stage. In the meantime, we continue to screen new and existing transposant lines for additional *emb* mutants.

Transfer of the *Mutator* Elements from Maize to Other Plants

Z. Yuan, V. Sundaresan

The *Mutator* (*Mu*) elements of maize constitute an exceptionally active transposon system with some very unusual characteristics (e.g., see Annual Report, 1992, p.164). A detailed molecular analysis of this transposon system would be greatly facilitated if this transposon system could be transferred from maize into a plant species that is more amenable to transformation. We have now generated transgenic tobacco and *Arabidopsis* plants carrying the element *MuDR*, which is the autonomous regulator of the *Mu* system (Hershberger et al., *Genetics 140:* 1087 [1995]). Modified *MuDR* elements, in which the transcripts are driven by strong promoters, were also introduced into tobacco and *Arabidopsis*. These transgenic plants are being crossed to transgenic plants carrying the nonautonomous element *Mu1*, inserted within a marker gene encoding resistance to streptomycin. Transposition of *Mu1* will be assayed by monitoring excisions, which should result in sectors of the plants that are resistant to streptomycin, and also by screening for new *Mu1* insertions using Southern blots.

Gene-trap and Enhancer-trap Patterns in the *Arabidopsis* Shoot Apex

P. Springer, A. Wells (URP Program), R. Martienssen

The shoot apical meristem (SAM) is a small group of cells at the apex of the plant that gives rise to all postembryonic structures of the shoot. The SAM of *Arabidopsis* is initiated during embryogenesis and is organized into three layers that are maintained during vegetative growth. Little is known about the mechanism by which the SAM is established and maintained, but conventional genetic screens have identified a handful of genes required for meristem differentiation. It is likely that some genes required for SAM function will also be required at earlier stages of development, but these genes would not be identified in seedling screens for meristem mutants because they would die before reaching the seedling stage. Enhancer traps represent a unique way to identify these genes, as the GUS expression pattern is visible in viable heterozygous plants, even if the insertion causes homozygous lethality. We are screening our transposant collection for lines that have GUS expression patterns in the meristem and early leaf primordia. Lines that stain will be examined for lethal phenotypes, as well as for morphological mutations affecting the seedling meristem.

In a pilot screen, 22 of 1300 lines had GUS expression in the shoot apical meristem and 28 lines had GUS expression in leaf primordia. The expression pattern in these lines is being examined in more detail. Examples of some GUS expression patterns

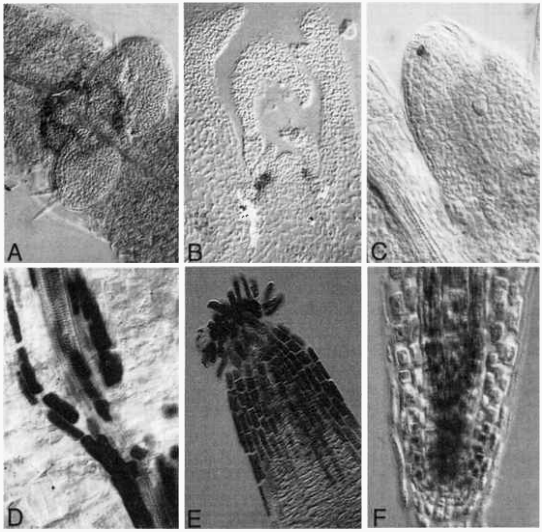

FIGURE 4 Gene-trap and enhancer-trap reporter gene expression patterns in the shoot apex. Seedlings were stained for GUS activity (dark staining) and cleared in ethanol. (*A*) Apical view and (*B*) longitudinal section through the apex of a GT185 seedling. GUS expression is present in a ring of cells surrounding the base of each leaf primordium. (*C*) Leaf primordia; (*D*) shoot-root junction; (*E*) root tip from ET79. GUS activity is localized to individual cells in vascular and root cap tissues that undergo cell death. (*F*) Root tip from *prolifera* showing GUS activity localized to the nucleus in some cells of the root epidermis.

are shown in Figure 4. Line GT185 has GUS expression in a small group of cells surrounding the base of each leaf primordium (Fig. 4A,B), thus marking the boundary between the shoot apical meristem and leaf primordia. ET79 shows GUS expression in a single cell at the tip of each leaf primordium (Fig. 4C). In older leaves, GUS activity expands to several cells in the same position, and expression is found throughout the seedling in individual xylem cells of the vasculature, and in all root cap cells (Fig. 4D,E). These tissues undergo extensive cell death, so that one possible interpretation of this unusual pattern is that this gene may be involved in a cell death pathway.

Because gene traps result in transcriptional and translational fusions, they can be used to determine the subcellular localization of gene products. Last year, we described the cloning and characterization of the *PROLIFERA* (PRL) gene. Since PRL is a member of the MCM2-3-5 family, it is predicted to be nuclear-localized. We have shown that the *prl*::DsG insertion results in a translational fusion that is localized to the nucleus in at least some cells (see Fig. 4F).

Gene-trap Hierarchies in the Shoot Apex

P. Springer, D. Tracey, R. Martienssen [in collaboration with G. Chuck and S. Hake, Plant Gene Expression Centre, Albany]

A powerful application of gene-trap methodology is the placement of individual genes in a genetic hierarchy based on existing regulatory mutations. For ex-

ample, if the expression pattern is altered in the absence of a regulatory gene product, this indicates that the gene in question probably acts downstream from the regulatory function. We have performed crosses between ten ET and GT lines with SAM expression and plants mutant for the previously identified genes *shoot meristemless* (*stm*) and *clavata1* (*clv1*). The *STM* gene is required for the formation of a SAM (Barton and Poethig, *Development 119:* 823 [1993]), whereas the *CLV1* gene is required for meristem organization (Clark et al., *Development 119:* 397 [1993]). Twenty six SAM and leaf primordia expressing lines have also been crossed to plants ectopically expressing the *Arabidopsis KNAT1* gene. *KNAT1* belongs to the *KNOTTED1* family of homeobox genes (Lincoln et al., *Plant Cell 6:* 1859 [1994]) and is expressed in the SAM, but not in the leaf or the root. Ectopic expression of *KNAT1* leads to alterations in leaf cell fate, and direct targets of *KNAT1* are expected to be uniformly expressed (or repressed) in all plant tissues in progeny from these crosses.

Although no direct targets have yet been found by this criteria, we have identified several genes whose expression pattern is altered as a consequence of constitutive expression of *KNAT1*. Ectopic expression of *KNAT1* results in a highly lobed leaf with ectopic stipules at the leaf margin. We have identifed two genes normally expressed at the base of the leaf (GT185 and GT254) that are expressed in the leaf blade in the *KNAT1* overexpresser. These results suggest that ectopic expression of KNAT1 affects pattern elements along the proximodistal axis of the leaf, causing proximal structures to be formed in distal positions. The *PROLIFERA* gene is expressed in proliferating cells and disappears from an expanding leaf primordium in a basipetal pattern (tip to base). In the *KNAT1* overexpresser, *PROLIFERA* is lost from the leaf acropetally (base to tip). These results show that cell division patterns are drastically altered and that the leaf may have become more meristem-like as a result of ectopic KNAT1 expression.

Enhancer-Trap Detection of Stomatal-specific Gene Expression Patterns

Q. Gu, R. Martienssen

Arabidopsis leaves are organized into several distinct tissue types, including upper and lower epidermal layers, photosynthetic mesophyll tissues, and a central reticular vascular system. Several classes of epidermal cells can be readily found in the *Arabidopsis* leaf, including pavement cells, trichome cells, and stomatal guard cells. Pavement cells are jigsaw-shaped and make up the majority of cells within the shoot epidermis, whereas trichomes are regularly spaced epidermal hairs. Two kidney-shaped guard cells and several subsidiary cells form the stomatal complex. Opening of stomates via guard cell turgor facilitates gas exchange during photosynthesis and respiration.

The stomatal complex is formed by virtue of a stereotypical pattern of meristemoidal cell divisions. In the cotyledon, stomata are closely packed, separated by only one or two subsidiary cells. In leaves and stems, however, the spacing pattern of stomatal complexes is not random and they are regularly separated by several pavement cells. The mechanism controlling stomatal patterning in the leaf is not known, but it is likely that cell-cell interactions and lateral inhibition are involved (Bünning, *The growth of leaves.* Butterworths, London [1956]). We are interested in understanding stomatal patterning mechanisms using enhancer-trap mutagenesis. Among the 750 enhancer-trap transposant lines screened in the last year (including 200 lines screened together with the 1995 summer *Arabidopsis* course at CSHL), about 60 lines displayed a tissue-specific or cell-type-specific expression pattern. A handful of these accumulated GUS activity predominantly in the stomatal cells. No stomatal-specific lines were found in the 550 gene-trap lines screened.

GUS expression in ET492 is specifically localized to the guard cells in all organs of the shoot, including cotyledons (Fig. 5B,C), leaves, stems, and floral organs (not shown). Some staining is also present in the root epidermis (Fig. 5B). In contrast, expression in the stomata of ET169 is restricted to hydathodes (Fig. 5A). Preliminary observation suggests that ET169 and ET492 retain normal stomatal spacing. In these cases, the activation of the enhancer-trap reporter gene could result from transposon insertion outside of the coding region. In an attempt to make derivative alleles, we are remobilizing the transposon in these lines by crosses to *Ac* transposase.

In two additional lines, stomatal-specific expression is accompanied by a stomatal defect. GUS expression in ET1967 is detected in cotyledons and leaves, predominantly in the guard mother cells (GMC) as well as in mature guard cells (not shown).

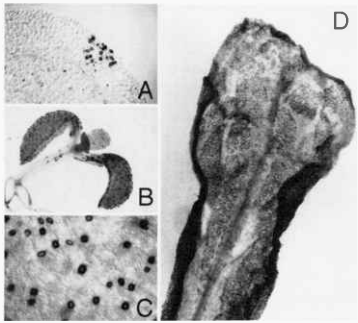

FIGURE 5 Enhancer-trap reporter gene expression in stomatal cells. (*A*) ET169, only those stomata in the hydathode stain in 8-day-old cotyledons; (*B*) ET492, GUS staining in stomatal cells of an 8-day-old seedling; (*C*) ET492. GUS localization in the adaxial epidermis of the cotyledon; (*D*) GUS staining in a leaf of the *patchwork* mutant. Patches of black dots indicate GUS localization in guard cells. In addition, the surface of the leaf is rough and the margins are disorganized.

A putative mutant phenotype resulting in clustering of two to four stomatal complexes is found in this line, but it appears to be conditional and is affected by humidity (not shown). In line ET1370, GUS activity is localized to the stomatal cells in the mature leaf and in vascular tissue of the flower (not shown). This line segregates for a severe mutant phenotype affecting the leaf epidermal surface and flower structure. On the surface of mutant leaves, patches of stomatal cells are intermingled with stomata-free zones. Due to the aberrant distribution of stomatal cells and other cell types in mutant leaves, this mutant line has been named *patchwork* (*pwk*). This phenotype is noticeable in leaves produced during the transition from vegetative phase to reproductive phase. The phenotype persists during the reproductive phase, but juvenile stages show no defects as compared to the wild type. The *pwk* mutant has been backcrossed to wild type, and segregation in the F_2 shows that the *pwk* mutant phenotype is genetically linked to the *Ds* insertion. We are currently screening for genetic revertants and wild-type sectors in the presence of transposase. Molecular cloning of the *PWK* gene will help us to understand its role in cell-cell communication and spacing differentiation. The origin of the patches of stomata-free cells is of particular interest as reporter gene expression is not observed in these mature cells. This may indicate that the gene product acts nonautonomously to affect their differentiation.

Exon Trap cDNA Sequencing

P. Springer, A. Wells (URP Program), C. Yordan, R. Martienssen [in collaboration with M. Lodhi and R. McCombie, Cold Spring Harbor Laboratory]

We are constructing a database of exon trap tags using gene-trap transposon mutagenesis. Each entry will include a reporter gene expression pattern, mutant phenotype, and chromosomal exon sequence fused to the reporter gene in a given line. We are attempting direct sequencing of RACE PCR products from each line to rapidly obtain the exon trap sequence data. Fifteen lines that had strong reporter gene expression were selected for direct sequencing of RACE PCR products. Total RNA was prepared, and nested RACE PCR products were directly sequenced using dye terminators and a variety of primers. Six lines were selected that were known to have insertions into *prolifera* (Springer et al. 1995), or into T-DNA marker genes that resulted in strong reporter gene expression driven by T-DNA promoters (Sundaresan et al. 1995). In four out of five T-DNA insertions, and for *prolifera*, sequence reads of 50–500 nucleotides beyond the *Ds* element were obtained, in one case extending to the start point of transcription. *prolifera* products were predominantly spliced (Springer et al. 1995), whereas T-DNA insertion transcripts were unspliced (T-DNA has no introns). In the remaining nine lines, reporter transcripts were unspliced or multiply spliced (which led to mixed sequence reads). In five of nine lines, sequencing primers from the intron gave a readable sequence which extended beyond the *Ds* element by 30–200 bp. One sequence matched a phosphofructokinase gene (a result confirmed by sequencing cloned PCR products), whereas a second sequence matched an EST from *Caenorhabditis elegans*. One advantage of using cDNA rather than genomic DNA as a substrate for gene-trap sequencing is that an exon sequence, rather than an intron sequence, is always obtained, avoiding ambiguities in identifying the trapped gene. However, given that many products appear to be unspliced or alternately spliced, DNA-based methods may be a more efficient procedure for most lines.

Remobilization of Gene-trap Transposons

J. Montagu, C. Yordan, H. Cross (URP Program),
P. Springer, R. Martienssen [in collaboration with the McCombie laboratory, Cold Spring Harbor Laboratory]

One of the most important goals of *Arabidopsis* genome sequencing is to establish a function for the genes identified. This can be achieved by local saturated mutagenesis using transposable elements. Such methods have been widely used in *Drosophila* and *C. elegans* as well as in maize (Das and Martienssen 1995). In addition, many enhancer-trap transposon insertions have no mutagenic effects because they are inserted near but not within the gene of interest. Remobilization would allow disruption of nearby genes to help determine their function. In a pilot study, we have remobilized the DsG genc-trap transposon at the *prolifera* locus on chromosome IV by crosses to transposase, which is removed by counter-selection in F_2 seedlings. In this case, the DsG transposon is not flanked by the counter-selectable marker, so that short-range transpositions are preferred. Using a strong transposase source, at least 20 new transpositions have been recovered in only 53 doubly resistant plants. This is a high enough frequency to enable PCR-based selection of insertions into genes of interest in pools of a few hundred plants. The complete sequence of a 25-kb contig surrounding the *prolifera* locus (see report from McCombie laboratory) will provide a molecular "target" for larger-scale mutagenesis now in progress.

Role of the *Hcf106* Gene in Chloroplast Membrane Protein Translocation in Maize

A.M. Settles, R. Martienssen [in collaboration with R. Voelker and A. Barkan, University of Oregon; and R. Henry and K. Cline, University of Florida]

Chloroplast biogenesis is regulated by nuclear genes during leaf development and requires the assembly of a large number of nuclear- and chloroplast-encoded gene products in the thylakoid membrane. High chlorophyll fluorescence (*hcf*) mutants affect the biogenesis of the photosynthetic apparatus, and *hcf106* mutants show reduced levels of photosystem I, photosystem II, and cytochrome b_6f membrane protein complexes. *hcf106* membranes have lost their structural heterogeneity and are thought to have a defect in protein translocation. The *hcf106* gene has been cloned by transposon tagging using the *hcf106-mum1* allele, and antisera to Hcf106 have been raised. By sequence analysis, subfractionation, and protease protection, the Hcf106 protein has been shown to be a chloroplast integral membrane protein in both the thylakoid and the inner envelope.

At least three pathways for targeting proteins to the thylakoid membrane have been distinguished in biochemical studies (Cline et al., *EMBO J. 12:* 4105 [1993]). These pathways have different requirements for NTPs, stromal factors, and the pH gradient across the thylakoid membrane (ΔpH). The biochemical requirements of two of these pathways resemble the azide-sensitive general secretory (sec) pathway of bacteria in one case and SRP-mediated translocation across the endoplasmic reticulum in the other. The third pathway is thought to be unique to chloroplasts. In pea, thylakoid targeting of plastocyanin (PC) and the 33-kD subunit of the oxygen evolving complex (OE33) is mediated by the sec pathway. In contrast, the 23-kD and 17-kD subunits of the oxygen evolving complex (OE23 and OE17) require only the ΔpH and are targeted via the third pathway. In-vivo-labeled *hcf106-mum1* homozygous seedlings accumulate precursors or intermediates of OE23 and OE17 but accumulate only mature PC and OE33 (Voelker and Barkan, *EMBO J. 14:* 395 [1995]). This suggests that mutant chloroplasts are defective in the third targeting pathway.

The *hcf106-mum1* phenotype is leaky, perhaps because it is suppressed in cells that lose *mutator* transposon activity. Three derivative alleles were isolated by site-directed transposon mutagenesis (Das and Martienssen 1995). Two of these new alleles are independent of *Mu* activity and behave as nulls. These new alleles have similar protein phenotypes to *hcf106-mum1*, suggesting that the leaky phenotype of *hcf106-mum1* is due to the nature of the ΔpH-dependent pathway, rather than the nature of the allele. We are using the deletion allele, *hcf106-mum3*, for biochemical and genetic analysis of Hcf106 function in wild-type plants. We are testing the interaction of *hcf106* mutants with other *hcf* mutations. In particular, we have crossed *hcf106-mum3* to *tha1*, a mutation in chloroplast SecA (R. Voelker and A. Barkan, pers. comm.). *tha1* has been shown to affect the accumulation of Sec precursors (PC and OE33), but not ΔpH-dependent precursors (OE17 and OE23).

We have begun to examine the biochemical pro-

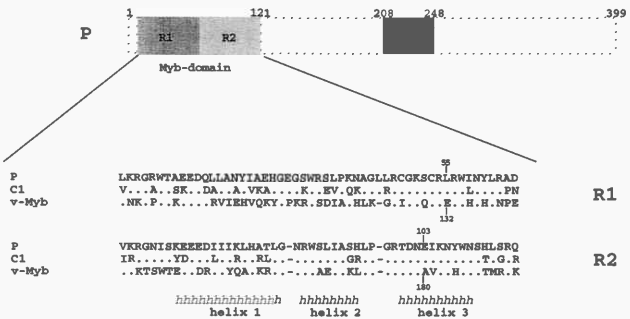

FIGURE 6 Structure of the P protein and sequence comparisons between different Myb domains. The Myb domain of P (*light gray*) is formed by two repeats about 52 residues long each. (*Dark gray*) An acidic sequence able to activate transcription in yeast. The amino acid sequences of the two Myb repeats of the maize P and C1 proteins and the AMV v-Myb protein are shown. Residue identity with P is indicated by a dot. The positions of the α-helices are indicated with *hhhh*, and the positions of the critical residues so far identified as important for the DNA-binding specificity of P and v-Myb are indicated by a number.

perties of the mutant seedlings using in vitro chloroplast import protocols that we have adapted from studies in pea. The preliminary in vitro import data suggest that in mutant *hcf106-mum3* chloroplasts, the ΔpH-specific precursors (OE17 and OE23) are shunted to the Sec pathway normally used by PC and OE33. This is because mutant chloroplasts process OE17 and OE23 precursors with reduced efficiency, and this processing is sensitive to azide, which normally only affects the processing of Sec precursors. Through database searches, we have found that Hcf106 protein shows homology with three bacterial ORFs and an *Arabidopsis* EST. This suggests that the ΔpH-dependent protein translocation pathway may be conserved in evolution, instead of being unique to chloroplasts.

Transcriptional Regulation by P Does Not Require Other Maize-specific Factors

E. Grotewold

The *P* gene encodes a Myb domain protein required for the transcriptional regulation of a subset of the flavonoid biosynthetic genes regulated by C1 (Grotewold et al., *Cell 76:* 543 [1994]), including *C2, CHI1*, and *A1*. C1, however, does not regulate transcription by itself and requires a member of the *R* or *B* gene families (encoding proteins with bHLH domains) for its activity. In agreement with the genetic evidence, P does not require R protein for the regulation of *A1*. Either P protein alone or C1 protein (in the presence of R/B) is able to activate efficiently a minimal 35S promoter containing the high-affinity P-binding sites identified in the *A1* promoter, when transiently expressed in embryonic callus or BMS cells (Grotewold et al., *Cell 76:* 543 [1994]). In addition to these P-binding sites, other *A1* promoter elements are required for normal regulation of *A1* by P and by C1 proteins (Grotewold et al., *Cell 76:* 543 [1994]; Tuerck and Fromm, *Plant Cell 6:* 1655 [1994]), yet those promoter elements are not recognized by P in vitro.

P and C1 share more than 83% identity in their Myb domains (Fig. 6), the region responsible for the interaction between C1 and R (Goff et al., *Genes Dev. 6:* 864 [1992]). Does P require a partner, as C1 does? I decided to approach this problem by investigating the possibility that P would function in a heterologous system, and for that purpose, I have chosen yeast. I introduced a reporter *lacZ* gene downstream from a dimeric high-affinity P-binding site (with a

cyc TATA box) into the yeast chromosome. When P was expressed in yeast cells carrying this reporter construct, a very efficient activation of the reporter was obtained (>130-fold). There was no activation when the plasmid carrying the P cDNA was absent (1-fold), nor when the P-binding sites were replaced by a dimer of a sequence to which P does not bind in vitro.

These observations are a strong indication that P is sufficient to activate transcription from promoters carrying high-affinity P-binding sites. These results are not in contradition with the observation that other promoter elements are important for the regulation by P in plant cells. Yet, they suggest that for promoters carrying high-affinity P-binding sites, there is no need to invoke a P partner.

Not all P-regulated genes have P-binding sites. In fact, I have not been able to identify sequences to which P binds in vitro in the promoter of the *CHI1* gene, which is dependent on the presence of P for its expression in the pericarp (Grotewold and Peterson, *Mol. Gen. Genet. 242:* 1 [1994]). This could possibly mean that P regulates this promoter through a different mechanism, which could be similar to the mechanism by which *A1* is regulated in the absence of high-affinity P-binding sites. These alternative ways of regulation by P could involve the existence of accessory factors that recruit P to promoter elements or that change the DNA-binding specificity of P.

Altering the DNA-binding Properties of Myb Domain Proteins

C.E. Williams (Dowling College), E. Grotewold

The maize *P* gene encodes a Myb domain protein required for flavonoid pigmentation in some floral organs. In plants, a large number of Myb domain proteins have been isolated, and they are involved in a variety of cellular functions through the activation of specific sets of target genes. Myb domains are usually formed by two or three 52-residue-long repeats, containing three α-helices each (Fig. 6). The second and third helices of each repeat form a helix-turn-helix motif, similar to the one found in homeodomain proteins or in the λ repressor. The third helix of each repeat makes contact with DNA. Nuclear magnetic resonance studies carried out on the Myb domain of c-Myb indicate that each repeat recognizes a half binding site, in such a way that the second Myb repeat recognizes the AAC core in a very sequence-specific fashion, whereas the first Myb repeat recognizes the last G of the ATAACGG sequence (Ogata et al., *Cell 79:* 639 [1994]). These studies, however, failed to explain why Myb domains with highly conserved DNA-recognition helices (Fig. 6) bind DNA with different sequence specificities (Grotewold et al., *Cell 76:* 543 [1994]). Despite the fact that P and v-Myb share more than 45% identity in their DNA-binding domains, they bind DNA with different specificities: v-Myb preferentially binds the consensus sequence $^{G}/_{C}{}^{T}/_{C}$AACGG, and the sequence present in the *Mim1* gene promoter (ATAACGG) bound by v-Myb, but not by P, provides a convenient probe to assay v-Myb DNA-binding activity; P preferentially binds the consensus sequence $AC^{C}/_{T}{}^{T}/_{A}$ACC and the sequence present in the *A1* gene promoter (ACCTACCAACC) bound by P, but not by v-Myb, provides a good probe to assay P DNA-binding activity (Grotewold et al., *Cell 76:* 543 [1994]). We approached the problem of how Myb domain proteins with very similar DNA-binding domains direct different patterns of gene expression through the interaction with particular elements in the regulated genes, by using the P and v-Myb proteins as model systems. The corresponding Myb domains are sufficient for these different DNA-binding preferences, as shown by expressing the corresponding regions of P and v-Myb as poly-histidine fusions in *Escherichia coli* and defining the corresponding DNA-binding consensus. These studies provide a convenient framework to identify the particular residues in the Myb domain that are involved in sequence-specific DNA binding.

We first tried to address this question by generating chimera Myb domains between the Myb domains of v-Myb and of P (which have different DNA-binding preferences). When the first Myb repeat of P (Fig. 6, R1) was fused to the second Myb repeat (Fig. 6, R2) of v-Myb (P1Myb2), a chimera Myb domain protein was obtained with novel DNA-binding preferences: It bound both the P-binding sites present in the *A1* promoter and the c-Myb-binding site present in the *Mim1* gene. In addition, a DNA sequence was identified (CTTAACTC) which is only bound by P1Myb2, but not by P or v-Myb. These findings indicate that by combining Myb repeats of proteins with different DNA-binding preferences, novel DNA-binding specificities can be achieved.

We analyzed the effect of residue changes in the DNA-recognition helix of the first Myb repeat. In particular, we focused our attention on the E residue present at position 132 in v-Myb (Fig. 6), since it is the only residue from this recognition helix that was proposed to be in direct contact with DNA, in addition to stabilizing a cooperative interaction of both Myb repeats with DNA (Ogata et al., *Cell 79:* 639 [1994]). In P, as well as in most plant Myb domain proteins, this position is occupied by a L residue. The change of this L residue to E completely abolished binding to the P-binding sites but allowed the mutant P protein to bind very weakly to the Myb-binding sites. When the corresponding change was done on v-Myb (E132 to L), binding to the Myb-binding sites was not affected, but binding to the P-binding sites became significant. These observations indicate (1) that E132 does not have a fundamental role in the cooperative interaction of the Myb repeats, as suggested (Ogata et al., *Cell 79:* 639 [1994]), since v-Myb(E132L) binds the Myb-binding sites with an affinity similar to that of v-Myb, and (2) that L55 in P plays a part in sequence-specific DNA recognition.

Next, we changed particular residues in the DNA-recognition helix of the second Myb repeat of v-Myb to the corresponding residues present in P (Fig. 6). Interestingly, when the three residues that are different in this region in these two proteins were changed in v-Myb to the corresponding residues of P (A, V, and H to E, I, and Y; Fig. 6), binding to DNA was completely lost. Accordingly, the Myb chimera domain Myb1P2 (first Myb repeat of v-Myb fused to the second Myb repeat of P) did not bind DNA either. These results indicate that the corresponding Myb repeats of P and v-Myb are not equivalent, and preliminary data suggest that the presence of the E55 residue in the first Myb repeat, together with the E132 residue in the second Myb repeat, is somehow interfering with proper folding of the Myb domain or binding to DNA, although both positions (55 and 132) seem to be important for sequence-specific DNA binding. These possibilities are currently being tested.

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ARABIDOPSIS FLOWER DEVELOPMENT

H. Ma H. Choi, S. Damanan, H. Fan, R. Farkas, C. Flanagan, A. Hameed, Y. Hu, H. Huang, M. Kim, Y. Mizukami, D. Rosa, P. Rubinelli, R. Salins, R. Thomas, M. Tudor, C. Weiss, E. White

We are interested in understanding the molecular control of cell fates during development, using the small weed *Arabidopsis thaliana* as the experimental system. In particular, we have been characterizing the function of members of a conserved transcription factor gene family (the MADS-box genes) during flower development. Considerable progress has been made in the understanding of the molecular mechanisms controlling early flower development (Coen and Meyerowitz, *Nature 353*: 31 [1991]; Ma, *Genes Dev. 8*: 745 [1994]). Mutations in one of the *Arabidopsis* floral homeotic genes, *AGAMOUS* (*AG*), cause double-flower phenotypes, where the stamens are converted to extra petals and the ovary is replaced by a new flower. The deduced AG protein (Yanofsky et al., *Nature 346*: 35 [1990]) has strong sequence similarity to the DNA-binding domains of transcription factors from humans, suggesting that the AG

protein is also a transcription factor. The conserved region between AG and the transcription factors is called the MADS-box. Other MADS-box genes were isolated using *AG* as a probe and have been designated *AGL1* through *AGL6* for *AG-L*ike. We have continued our analyses of *AG*, *AGL1*, *AGL2*, and *AGL3*.

Because flower development is a complex process, it is likely that many other regulatory genes are also needed. We have recently isolated a new *Arabidopsis* mutant, *fon1* (*f*loral *o*rgan *n*umber), which has an increased number of reproductive floral organs. Furthermore, to identify new genes required for flower development, we are collaborating with Drs. V. Sundaresan, R. Martienssen, and U. Grossniklaus (CSHL) to generate a large number of independent enhancer trap/gene trap transposon insertional lines. In an alternative approach to isolate new genes that function during late floral organ development, we have begun a new project aimed at identifying genes specifically expressed in carpels or stamens. These genes are likely to be involved in cell differentiation during late flower development, and they provide molecular markers for specific cells or tissues of the reproductive organs.

In 1995, in addition to the people previously introduced, several undergraduate students participated in our research. Matt Tudor from Cornell, who has been here twice before, was again extremely productive, carrying out several projects concurrently. Rebecca Farkas from Yale was our URP in the summer, and she did a beautiful piece of work on in situ RNA hybridization of floral genes in *fon1* mutant flowers. Renate Thomas, Rohan Salins, and Sonia Damanan from SUNY Stony Brook, Henry Choi from Columbia, and Michael Kim from Wesleyan were very helpful to our enhancer/gene trap project and other experiments. In addition, Aliyah Hameed from Bryn Mawr participated in our gene trap and subtraction projects, and Elyssa White from Lehigh worked with Catherine Weiss on the localization of the GPA1 protein.

We bid farewell to our postdoc Catherine Weiss, who has taken a job with American Cyanamide. Last Fall, Dominik Rosa, a senior at Commack High, joined us as one of the Partners for the Future, working with Peter Rubinelli on the analysis of stamen-specific cDNA clones. Finally, we welcome our new postdoctoral fellow, Hua-ying Fan. She is a native of Taiwan and just finished her Ph.D. training from New York University.

Generation and Characterization of Enhancer/Gene Trap Transposants

C. Flanagan, Y. Hu, Y. Mizukami, H. Fan, H. Choi, M. Kim, A. Hameed, R. Thomas, R. Salins, S. Damanan, H. Ma

To be able to identify new genes important for *Arabidopsis* flower development, we have participated in the collaboration with three other plant labs here at the laboratory: those of Drs. Sundaresan, Martienssen, and Grossniklaus. We have obtained about 50,000 F_1 seeds from crosses between three different *Ac* lines and four different *Ds* lines. We have planted about 14,000 of the F_1 seeds and have harvested the F_2 seeds from them. Using the NAM/Kan double selection, we have generated about 1500 independent insertional lines, or transposants. Our goal is to generate a total of 4000 transposants, 3000 of which will be obtained before the end of 1996.

We have examined the expression patterns of the *Ds*-borne *GUS* reporter gene in the first 900 transposants from the four labs. The staining patterns have also been examined 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. 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, 7000 of which are specific; Scott et al., *Plant Science* 80: 167 [1991]). Nevertheless, we would like to verify that such a high frequency of anther/pollen-only staining truly reflects the endogenous situation. Even if the anther/pollen patterns are excluded, about 20% of the gene trap and 30% of the enhancer trap lines stain elsewhere in the flower. GUS staining was observed in almost every combination of first, second, third, and fourth whorl floral organs and of early, intermediate, and late stages of flower development, with that in the reproductive organs and at intermediate-to-late stages being the most common. It is interesting that truly constitutive expression was rare. Furthermore, there are many different patterns within single organs, reflecting cell- or tissue-specific GUS expression. For instance, within the gynoecium, various lines stain the stigmatic papillae, style, carpel valves, valve

margins, transmitting tissue, or septum. This wealth in the number and variety of expression patterns within the flower will be a great resource for our investigations of flower development.

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

C. Flanagan, Y. Hu, R. Thomas, H. Choi, 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 characterized extensively its spatial and temporal expression pattern in both wild-type and mutant flowers using RNA in situ hybridization. Our results indicate that *AGL1* expression is restricted to only one type of floral organ, the gynoecium composed of two fused carpels. *AGL1* is expressed in only one of the four floral organs; most of the others are expressed in two (e.g., *AG*) or four (e.g., *AGL2*) types of organs. *AGL1* expression arises late in flower development, detectable at the tip of stage-7 (of 12 floral stages) gynoecia. At stage 10, *AGL1* is expressed in four narrow areas along the length of the inner carpel wall. Later, *AGL1* expression can also be seen in the developing ovule primordia, in the funiculus, and in the outer integument primordia, but not in the inner integument primordia and the nucellus. In mature flowers, *AGL1* expression is primarily in the ovules, along the surfaces of the septum, and as vertical 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 expressed at the chalazal end, near the micropyle, and in the endothelium. *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.

It is known that *AG* is expressed interior to the second whorl in both wild-type and *ag* mutant flowers even though the floral organ identities are different in these two backgrounds. *AGL1* expression occurs in the gynoecium of fused carpels that normally occupy the fourth whorl. Is *AGL1* expression controlled by the identity or the position of the organs? To address this question, we examined *AGL1* expression in *ap2*, *ap3*, and *ag* mutant flowers. In the homeotic mutants *ap2* and *ap3*, *AGL1* is expressed in both normal and ectopic carpels, and in *ag* flowers, *AGL1* is not expressed in the sepals that occupy the fourth whorl, indicating that *AGL1* expression is regulated by the identity, rather than the position, of the organs. It is worth noting that *AGL1* is still expressed in the nectaries of *ag* flowers, indicating that *AG* function is not required for *AGL1* expression in some cells.

The specific *AGL1* expression pattern in the wild type suggests that *AGL1* may have regulatory functions in formation of the anterior-posterior axis of the ovule; nutritional supply to the ovule and embryo sac; pollen tube guidance; structural definition of the carpel margins, which could include such processes as congenital fusion of carpels, septum, and placenta formation, and abscission within the siliques. Analysis in mutant flowers indicates that *AGL1* expression depends on the carpel identity, not on the position of the organs.

Function of the *FON1* Gene in Floral Organ Development

H. Huang, 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 and 1994 Annual Reports). To understand *FON1* function in flower development, we have analyzed in detail the *fon1* mutant phenotype using scanning electron microscopy. The *fon1* mutant plants have normal inflorescence meristems; in addition, the early flower development from stages 1 to 5 in a *fon1* mutant is normal. At stage 6, after the third whorl stamen primordia are formed, while the wild-type floral meristem terminates by forming the gynoecium primordium at the center, the *fon1* floral meristem continues to be active, giving rise to extra stamen primordia. The center of the *fon1* floral primordia does eventually produce carpel primordia, but they are abnormal and often have ectopic stamen primordia or are chimeric. These phenotypes indicate that the *FON1* gene regulates the duration of the floral meristem and the organization of floral organ primordia. We are currently in the process of analyzing phenotypes of double mutants between *fon1* and a number of other floral mutations.

Characterization of *AG* Function in Determination of Floral Meristem Identity

Y. Mizukami, H. Ma

It is known that the *Arabidopsis* reproductive organ identity gene *AG* is also required for the control of floral meristem determinacy, because *ag* mutations and antisense *AG* RNA cause the production of indeterminate double flowers. Meristem determinacy and the production of floral organs instead of lateral meristems are important characteristics of the floral meristem; thus, it is possible that the *ag* mutation affects floral meristem identity. To understand *AG* function in controlling floral meristem identity, we have analyzed the effects of a recessive loss-of-function *ag* mutation and dominant gain-of-function *AG* transgenes. Previously, *ag* mutant flowers of plants grown under short-day photoperiods were shown to produce inflorescence shoots with lateral flowers in the indeterminate center. Our detailed observations revealed that the formation of inflorescence at the center of *ag* mutant flowers is due to the reversion from floral meristems to inflorescence meristems. We also found that the *ag* floral meristem is reversible throughout plant development. These observations demonstrate that *AG* is indeed required for determining floral meristem identity. Furthermore, we found that ectopic *AG* expression in wild-type *Arabidopsis* plants converts apical inflorescence meristems into floral meristems, indicating that *AG* can promote a determinate floral meristem identity.

Identity of the *Arabidopsis* floral meristem is first specified by the floral meristem identity genes, such as *LFY* and *AP1*. *LFY* and *AP1* together control proper *AG* expression, suggesting that *AG* acts downstream from *LFY* and *AP1*; therefore, it is possible that *AG* can replace *LFY* and *AP1* to specify determinate floral meristem identity. To test this, we analyzed the effects of a 35S-*AG* transgene on floral meristem formation in *lfy ap1* double-mutant plants. Whereas in *lfy ap1* plants without the 35S-*AG* transgene all flowers were replaced by indeterminate shoots producing leaf-like organs and lateral meristems, plants ectopically expressing *AG* produced determinate flowers, although early arising flowers had a few lateral floral meristems. This observation indicates that *AG* function requires neither *LFY* nor *AP1* function for promoting floral meristem determinacy. We conclude from these results that, considering the fact that *AG* is normally only expressed at the center of the floral meristem, *Arabidopsis* floral meristem identity is initially specified by genes such as *LFY* and *AP1*, which inhibit lateral meristem formation and activate floral organ identity genes; subsequently, the floral meristem is further defined by *AG*, which specifies reproductive organ identity and controls meristem determinacy.

In Vitro and In Vivo Analysis of AG Functional Domains

Y. Mizukami, H. Huang, M. Tudor, Y. Hu, H. Choi, H. Ma

The AG protein can be divided into five regions on the basis of sequence similarity with other MADS domain proteins (Fig. 1). To assay the contribution of each region to AG function in vitro and in vivo, we generated *AG* constructs that encode amino-terminal and carboxy-terminal truncated AG proteins. Using our previously described in vitro DNA-binding assay system and AG proteins expressed in *Escherichia coli*, we found that the AG MADS domain and the I region are required for DNA binding but that the N, K, and C regions are not. We have also demonstrated that AG binds to DNA either as a homodimer or as a heterodimer with any AGL1, AGL2, and AGL3 proteins. Although the K and C regions, which are found in nearly all plant MADS domain proteins, are not required for DNA binding in vitro, they may affect binding in vivo or have other activities.

To understand the in vivo function of the regions not required for DNA binding, we introduced several AG truncation constructs into wild-type plants and characterized the effects of transgenes. We show that transgenic plants with 35S-*AG* constructs encoding an AG protein lacking the amino-terminal domain produce *ap2*-like flowers (Fig. 2B) similar to flowers ectopically expressing an AG protein retaining the N domain. This suggests that the N region is not required for producing *ap2*-like flowers. Transgenic plants with 35S-*AG* constructs encoding an AG protein lacking the C region produced *ag*-like flowers (Fig. 2C), indicating that the carboxy-terminal truncation behaves like a dominant negative mutation. The plants carrying a transgene encoding AG protein lacking both K domain and C regions had flowers with more stamens and carpels (Fig. 2D), as seen in *ag-1*/+ heterozygous plants, suggesting that this trun-

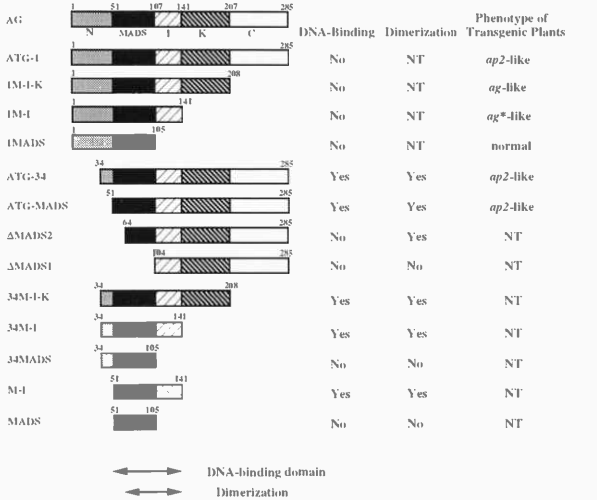

FIGURE 1 Analysis of AG functional domains. The AG protein and its truncated forms are shown schematically, with each of the five regions represented by a box: (N) amino-terminal region; (MADS) MADS domain; (I) I region; (K) K domain; (C) carboxy-terminal region. The numbers indicate the boundaries of the regions and the positions of the AG truncations. The double-headed arrows indicate the AG regions for DNA binding and dimerization. NT indicates not tested. The reason for the failure to bind DNA by the proteins starting at position 1, which is not the native amino terminus of AG, is not known, although it is possible that the artificial amino terminus may have an inhibitory effect. Position 34 corresponds to the amino termini of several MADS domain proteins. Phenotypes: (*ap2*-like) conversion of sepals and petals to carpels (or carpelloid sepals) and stamens (or staminoid petals); (*ag*-like) conversion of stamens and carpels to petals and sepals, and/or indeterminacy; (*ag**-like) increased number of reproductive organs.

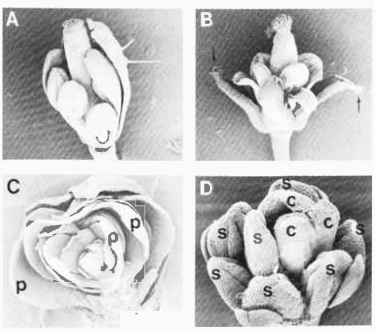

FIGURE 2 Phenotypes of transgenic flowers with AG truncations. Shown are scanning electron micrographs. (*A*) Wild type; (*B*) ATG-MADS, lacking the N region. Note that the first whorl organs have stigmatic papillae (arrows), characteristic of carpels. (*C*) 1M-I-K, lacking the C region. The flower is indeterminate and exhibits conversion of stamens and carpels to petals (p) and sepals. (*D*) 1M-I, lacking both the K domain and C region. The flower has seven rather than six stamens (s) and three unfused carpels (c) instead of two.

cated AG protein can only partially inhibit normal AG function. These results demonstrate that the K domain and the C region both have important yet distinct in vivo functions.

Isolation of Organ-specific cDNAs by Subtractive Hybridizations

P. Rubinelli, Y. Hu, M. Kim, A. Hameed, D. Rosa, H. Ma

We have carried out further characterization of the anther-specific clones we isolated using a subtractive hybridization procedure, as described in previous annual reports. We have performed two sets of experiments. The expression pattern of several genes was analyzed using RNA in situ hybridizations. We found that among these anther-specific genes, four (A7, A20, A26, A27) have highly localized expression in the tapetum cell layer, which surrounds the developing pollens. Two other genes (A18, A21) are expressed only in the pollen grains within the flower. We have isolated full-length cDNAs for these six genes. On the basis of the similarity to known sequences, A7 is likely to encode a new lipotransferase, and the predicted A27 protein is probably a glucosidase. A20 and A26 encode glycine-rich proteins; glycine-rich sequences have been found in structural proteins, such as elastin and plant cell wall proteins. Both A18 and A21 encode proteins with novel sequences.

These clones represent genes that are specifically or predominantly expressed in anthers. Because the anther has several specialized cell types, some of the genes are likely to be important for the differentiation and/or function of some of these cells. The tapetum layer is important for pollen development, and thus some of the tapetum-specific genes may be needed for pollen development. For example, during pollen development, complex lipoproteins are deposited on the surface of the pollen; it is possible that the A7 lipotransferase plays a part in this process. Lipotransferases have previously been found to be anther-specific, but the A7 sequence suggests that it may be a new enzyme, perhaps having a unique activity. As the pollen matures, the tapetum cells die and degenerate, and it is believed that hydrolytic enzymes are required for this process. The sequence of A27 suggests that, being a potential glucosidase, it could participate in the developmentally regulated cell death of tapetum cells. Further studies, such as those using transgenic plants, are needed to help provide clues to the function of these genes.

Biochemical Characterization of the G Protein GPα1

C. Weiss, E. White, H. Ma

We have continued our characterization of the subcellular localization of GPα1 using fractionation methods and immunofluorescence. Multiple fractionation experiments and enzymatic assays have determined that GPα1 is associated with the plasma membrane, as well as with the endoplasmic reticulum (ER). This is consistent with the observation from immunofluorescence experiments. The plasma membrane association is similar to the well-characterized G proteins in mammalians cells, and it suggests possible signaling functions for GPα1. In mammalian cells, small and heterotrimeric G proteins have been shown to be associated with the Golgi network, suggesting a role in vesicular trafficking, but only small G proteins (Rab1, Rab2) are known to associate with the ER compartment. The fact that GPα1 is found with ER suggests that in plants, heterotrimeric G proteins may perform additional functions.

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HIGHER-ORDER CHROMOSOME DYNAMICS

T. Hirano M. Hirano

Genomic DNA is organized and packaged into a higher-order structure inside the cell. Virtually all chromosomal events, including DNA replication, gene expression, and chromosome segregation, require faithful regulation of higher-order chromosome dynamics. Our laboratory is particularly interested in the molecular mechanisms of mitotic chromosome condensation, an essential process for maintaining the integrity of genetic information during mitosis. To address this problem, we established a cell-free system derived from *Xenopus laevis* (African toad) eggs in which higher-order chromosome structures can be reconstituted in vitro in a cell-cycle-dependent manner. This unique system provided us with both a simple procedure for chromosome isolation and a powerful functional assay. Using this system, we recently identified two chromosomal polypeptides, XCAP-C and XCAP-E, that have a fundamental role in mitotic chromosome condensation (XCAP stands for X*enopus* chromosome-*a*ssociated *p*olypeptides). Sequence analysis revealed that they share common structural motifs and belong to a recently identified protein family, the SMC family. Genetic studies in other laboratories showed that yeast homologs of XCAP-C and XCAP-E are required for chromosome condensation and segregation in vivo. Other members of this protein family were found to be involved in many aspects of higher-order chromosome structure and function, including dosage compensation and recombinational DNA repair.

We are now focusing our efforts on biochemistry and cell cycle regulation of the chromosome condensation protein complex containing XCAP-C and XCAP-E and are trying to understand how the dynamic behavior of chromosomes is regulated during the cell cycle.

Purification and Characterization of the Chromosome Condensation Protein Complex Containing XCAP-C and XCAP-E

T. Hirano

XCAP-C and XCAP-E were originally identified as major components of mitotic chromosomes assembled in *Xenopus* egg extracts and were subsequently shown to be present in the same protein complex. They share structural motifs common to all members of the SMC family, which include an amino-terminal nucleotide-binding motif (the P-loop) and two long coiled-coil regions. No obvious DNA-binding motif is found in either polypeptide. Moreover, no SMC proteins have been purified to homogeneity from any organism. Thus, although our functional blocking experiments showed that XCAP-C and XCAP-E are required for both assembly and structural maintenance of mitotic chromosomes in vitro, detailed biochemical activities of this class of proteins are currently unknown.

As an initial step toward an understanding of the biochemical processes of mitotic chromosome condensation, we established a purification scheme for the protein complex containing XCAP-C and XCAP-E. In this scheme, we took advantage of immunoaffinity column chromatography using anti-peptide antibodies specific for the carboxy-terminal sequences of either XCAP-C or XCAP-E. Interestingly, a highly purified fraction contained not only XCAP-C and XCAP-E, but also three additional polypeptides. We tentatively named them p150, p130, and p100 based on their apparent molecular weights. Sucrose gradient centrifugation revealed that the

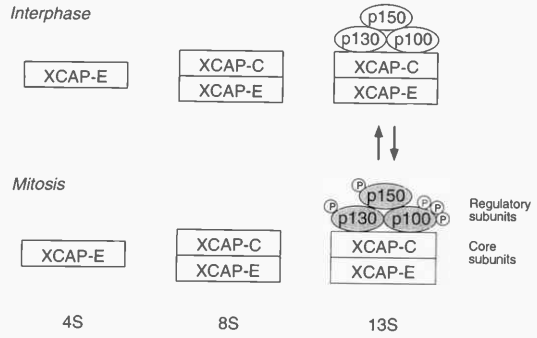

FIGURE 1 Subunit composition and cell cycle regulation of the chromosome condensation protein complex. XCAP-C and XCAP-E exist in multiple forms in *Xenopus* egg extracts: (1) the 4S form contains XCAP-E only; (2) the 8S form represents a heterodimer of XCAP-C and XCAP-E; and (3) the 13S form contains an additional three subunits, p150, p130, and p100. These three subunits, which become phosphorylated in a mitosis-specific manner, may be responsible for the targeting of this complex to chromosomes.

fraction could be further fractionated into three distinct populations: (1) the first 4S peak contained XCAP-E only; (2) the second 8S peak contained XCAP-C and XCAP-E; and (3) the third 13S peak contained XCAP-C, XCAP-E, p150, p130, and p100 (see Fig. 1). No free XCAP-C was detected. Thus, XCAP-C and XCAP-E existed in multiple forms in the egg extracts, and a significant population of them (>50%) was present in a large protein complex containing additional three subunits. Preliminary experiments suggested that, like XCAP-C and XCAP-E, the other three subunits were also recovered as major components of mitotic chromosomes assembled in vitro. They seem to have an important role in cell cycle regulation of the protein complex function (see below). We speculate that the 8S and 13S forms represent a core complex (XCAP-C/E) and a holocomplex (XCAP-C/E plus "regulatory" subunits), respectively, and that both are required for mitotic chromosome assembly in vitro.

Having established the purification scheme described above, we are now in a good position to start characterizing the biochemical activities of this chromosome condensation protein complex. The following specific questions are to be addressed: (1) Does the complex interact directly with DNA? If so, is there any sequence specificity or sequence preference? (2) The XCAP-C and XCAP-E subunits contain a putative nucleotide-binding motif. What is the role of nucleotide binding and hydrolysis in protein function? (3) How do the two activities (DNA binding and nucleotide binding) contribute to changes of higher-order chromosome structure? (4) What is the functional relationship between the 8S and 13S forms?

Cell Cycle Regulation of the Chromosome Condensation Protein Complex

T. Hirano

Chromosome condensation is a cell-cycle-dependent event in the cell. If the protein complex containing XCAP-C and XCAP-E is directly involved in this process, its function should be tightly regulated in the cell cycle. We found that this was the case. We used cell-cycle-specific extracts, namely, mitotic and interphase extracts, and assembled mitotic chromosomes and interphase chromatin structures in vitro. When the two structures were isolated and fractionated on an SDS-polyacrylamide gel, XCAP-C and XCAP-E were detected only in mitotic chromosome fractions, showing that chromosomal targeting of the protein complex is mitosis-specific. In addition, pharmaco-

logical experiments with inhibitors for protein kinases and phosphatases suggested that the targeting is regulated by mitosis-specific phosphorylation. Whereas phosphorylation of XCAP-C and XCAP-E was barely detectable by immunoprecipitation of ^{32}P-labeled extracts, we found that p150, p130, and p100 were heavily phosphorylated in a mitosis-specific manner. No subunit rearrangement was observed between the mitotic and interphase forms. Taken together, we propose that mitosis-specific phosphorylation of the "regulatory" subunits has a key role in recruiting this protein complex to chromosomes during mitosis. The phosphorylated 13S complex may in fact have a higher affinity for DNA. It is also possible, however, that additional or redundant regulatory mechanisms exist by which the protein complex is targeted to chromosomes strictly during mitosis. For example, mitotic chromatin may have a "receptor"-like protein for the phosphorylated complex. Candidates for such a receptor include histones H1 and H3, both of which are known to be hyperphosphorylated during mitosis.

Cloning of cDNAs Encoding the Subunits of the Chromosome Condensation Protein Complex

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

To better understand the structure and function of the chromosome condensation protein complex, we are now cloning cDNAs encoding the newly identified three subunits. We isolated the protein complex by immunoprecipitation and raised mouse polyclonal antibodies against each polypeptide purified from an SDS-polyacrylamide gel. We obtained an antibody specific for p130, which was then used to screen a *Xenopus* ovary cDNA expression library. The full-length cDNA encoded an acidic polypeptide with a calculated molecular mass of 116 kD. No significant homology was found with other proteins available in the databases. One exception was a yeast polypeptide with unknown function that was identified in the genome sequencing project. The homology between the *Xenopus* and yeast polypeptides was relatively weak but was observed throughout the whole molecules. More recently, in collaboration with R. Kobayashi of the CSHL Protein Chemistry Facility, we obtained several peptide sequences for p150 and p100. The information is now being used to design degenerate primers for cloning the corresponding cDNAs by polymerase chain reaction (PCR). Full-length sequence information and antibodies raised against expressed proteins will be valuable tools for further characterization of this protein complex.

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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 using degenerate PCR techniques
- 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, E. Hackborn, J. Heimlich, T. Iwasaki, R. Koskela, M. Mallison, C. Reed, J. Salit, A. Tracy

The focus of our group is in the continued development of a major computer program which 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 epidemiological and genetic analysis to functional analysis of candidate genes.

GT enables users to carry out 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, online 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.

Our work with GT has been funded as a research project for many years, but this year, we converted our research grant into a resource grant. This was done by standard NIH peer review. This new and substantial grant enabled us to establish the CSHL Human Genome Informatics Research Resource, which is the only one of its kind in the United States, perhaps the world. We are working on documentation and training and the other things that need to be done before software can be made generally available.

Our software is in beta test at several major laboratories, including a major cancer research lab involved in gathering mutation and phenotype information from laboratories around the world that are engaged in breast cancer susceptibility gene research. We are scheduled for our first widely available software release in September 1996.

Dana Foundation Consortium on the Genetic Basis of Manic Depressive Disorder

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(2) Johns Hopkins University, School of Medicine, Dept. of Psychiatry
(3) Stanford University, School of Medicine, Dept. of Human Genetics

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 manic depressive disorder. This is the first major program at CSHL involving clinicians, computers scientists, human geneticists, and quantitative biologists.

MDD is a mood disorder consisting of alternating periods of elevated and depressed moods. During these episodes, affected individuals can become seriously impaired both socially and in the workplace. There are three distinct subtypes of related disorders in MDD. The first is Major Depressive Disorder. The lifetime risk of suffering at least one major depressive episode is 10–25% in women and 5–12% in men. Risk is unrelated to ethnicity, income, education, or marital status and is 1.5 to 3 times higher in first-degree biological relatives of people who have major depressive disorder. Concordance is higher in monozygotic twins than in dizygotic twins, indicating at least a moderate genetic component.

The second subtype is Bipolar I (BPI) disorder, which in simplistic terms is indicated when a person suffers at least one manic episode and at least one major depressive episode. Bipolar II is a distinct, recognizable (by a rigorously trained psychiatrist) subtype which again involves at least one major depressive episode but is accompanied by a milder form of elevated mood called hypomania.

Prevalence of BPI varies from 0.4 to 1.6 and is about 0.5% for BPII. Twin and adoption studies show strong evidence of a heritable component. First-degree biological relatives of a BPI have a 4–24% increase for BPI, 1–5% increase for BPII, and 4–24% increase risk for major depressive disorder. Little is known about the relative risk of BPII persons.

MDD appears to exhibit complex genetic features in many ways. Many genes appear to be involved. The disease can manifest itself in different ways depending on the parent origin of disease alleles. Some studies indicate that genetic anticipation is involved in MDD. Our study was designed to reduce the potential genetic complexity of the disease by studying only those families in which the disease is transmitted through only one parental line. Furthermore, only BPI probands were used to screen for study families. We set out to collect 50 such families for genetic analysis.

We have done genotyping on more than half of the 50 families and have found interesting linkage results on two regions on human chromosome 18, one on the p-arm and one on the q-arm. The q-arm linkage finding was most strong in those families where the disease was transmitted by fathers. We will be close to completion of a complete genome scan of all of the families by the end of 1996.

SEQUENCE-BASED ANALYSIS OF COMPLEX GENOMES

W.R. McCombie N. Kaplan M. Lodhi
S. Till A. Johnston
J. Hoffman

The long-term goal of our lab is to develop new tools and strategies for high-throughput DNA sequencing and to use them to analyze the structure of complex genomes. Our program is therefore divided into technology development and sequence analysis components. The technology development effort is developing new technology to increase the speed at which we can analyze complex genomes. The sequencing effort uses what is the best available technology at the time to analyze organisms of biological interest. We have been sequencing the genome of the fission yeast *Schizosaccharomyces pombe*. In this past year, we began a project to sequence human genomic DNA as part of a test project coupled with our technology development efforts. We also expanded our efforts in *Arabidopsis* genome analysis from the characterization of the sequence surrounding transposon insertion sites to *Arabidopsis* genome sequencing.

Technology Development

A. Johnson, M. Lodhi, W.R. McCombie

The ability to determine the sequence of any region of DNA by the use of a custom oligonucleotide to prime a sequence reaction is a powerful tool in many sequencing applications ranging from genome sequencing to the sequencing of particular cDNAs of interest. Most large-scale projects, however, rely on cloning techniques devised to allow sequencing using universal primers (the same primer for each clone sequenced). This is due to the expense, time, and management burden associated with the use of large numbers of custom synthesized oligonucleotides. We have been developing the use of a small number of short modular primers used in combination, rather than standard 18–20-base primers. There are only 4096 possible hexamers. The use of such modular primers was originally developed for sequencing with radioisotopes by Kieleczawa et al. (*Science 258:* 1787 [1992]). This technology if successfully developed will allow us to combine any three hexamers from our library of all possible hexamers to form the appropriate 18 mer in situ to prime a sequencing reaction. This has the potential not only to improve genome sequencing technology, but also to allow rapid and inexpensive sequencing of cDNAs, using hexamer-based primer walking. We have been developing this technology on both single- and double-stranded templates.

Automated DNA Sequencing of Single-stranded Templates Primed with Strings of Hexamers

M.A. Lodhi, W.R. McCombie

Last year, we reported the use of strings of three hexamer primers, instead of one long primer, for sequencing single-stranded M13mp18. Since then, we have made several changes to the protocol. Our current protocol has been tested with a wide variety of hexamer string primers and it has been found to be very robust and produces substantially longer read lengths than we previously obtained. We have used 32 primers composed of 3 hexamers each that were picked from different sites on M13 to test the reliability of the current protocol for sequencing from any site on a single-stranded template. These hexamer strings were chosen from strings initially used successfully in radioisotopic sequencing but which produced varying signal strengths (J. Kieleczawa and B. Studier; pers. comm.). To compare the read lengths obtained with hexamer primer strings, 12 long primers (18 mers) corresponding to a subset of the hexamer strings were also tested in standard Sequenase dye terminator sequencing reactions. The two-cycle Sequenase dye ter-minator reaction starts with incubation on ice for 5 minutes to stabilize the hexamer string on the tem-plate. The reaction is then incubated at room temper-ature for 10 minutes and heated at 65°C for 2 minutes. One unit of Sequenase is added and again incubated at room temperature for 10 minutes. At the end, Pronase is added to destroy the single-strand binding protein used to stabilize the template. The reaction is then precipitated and run on an ABI 373 or 377 DNA sequencer using standard procedures.

The overall success rate of priming with 32 hexamer string primers was 97%, with the failure of only one string (S12). The reason for the failure of S12 is not clear and our attempt to sequence with a corresponding long primer (S12L) also failed. The average read length from reactions successfully primed with 31 hexamer string primers was 461 bases (max = 610 bases) with greater than 99% base-calling accuracy, whereas the average read length obtained with long primers was 492 bases (max = 590 bases) with the failure of only one primer (S12L). To simplify these reactions, we used 15 two-hexamer string primers in our standard hexamer sequencing procedure. The average read length from reactions primed with 14 strings was 465, with the failure of only one string. We believe that the current protocol is robust enough to be used at the end stages of sequencing projects for gap filling and resolving base ambiguities which follows the shotgun sequencing portion of a large-scale sequencing project. It could also be used in any other application involving primer walking on single-stranded templates such as the sequencing of cDNA clones. We are currently testing this technology in the finishing stage of three *S. pombe* cosmids sequenced at different levels of shotgun sequencing with single- and double-stranded templates.

Sequencing with Hexamer Primers on Double-stranded Templates

A. Johnston, W.R. McCombie

In addition to single-stranded templates, we have also applied the principle of hexamer string priming to fluorescence-based sequencing on double-stranded templates. Double-stranded sequencing is advantageous because it enables one to easily obtain sequence information from both strands of a particular DNA fragment. Previous work in this area had only limited applicability to a fluorescence-based approach. During the past year, we have successfully developed a robust hexamer string primer sequencing protocol for double-stranded templates that uses fluorescent T7 (Sequenase) dideoxy terminators. The key to success with these reactions was the linearization of the plasmid clones with a rare cutting restriction enzyme prior to primer extension. This protocol has been tested extensively on the sequencing vector pUC19 with a wide variety of hexamer strings. As part of this work, we have successfully walked around the entire length of pUC19 (2686 bases) on both strands. The success rate of these reactions was 75%, which is comparable to that obtained with custom synthesized long primers and Sequenase dye terminator chemistry. The sequencing of pUC19 on both strands required 28 successful primer walks. Current read lengths vary between 350 and 450 bases with an average accuracy of 98%. We are now incorporating this protocol into the finishing phase of our production sequencing efforts on a human cosmid.

Genome Sequence Analysis

J. Hoffman, A. Johnson, N. Kaplan, M. Lodhi, S. Till, W.R. McCombie

In the past year, we have finished the sequencing of several cosmids from *S. pombe*. One 31-kb segment of cosmid DNA that was finished last year was checked and submitted to GenBank this year. This cosmid, 359, had a region of about 8 kb that had not yet been connected to the larger 31-kb finished sequence. We determined that this was due to a repeat sequence that made automated assembly of this region difficult. We filled this gap in the sequence by using PCR to amplify across the gap using cosmid DNA as the template. We then primer walked across the PCR product to fill the gap in an unambiguous manner. This additional 8 kb of sequence will be submitted to GenBank shortly. Two cosmids in which the shotgun phase of sequencing was completed at the beginning of this year (1198 and 1683) were completed and submitted to GenBank as well. These cosmids, along with the 31 kb of PGAA, represent an 85,837-base-pair contig that was completed and submitted to GenBank in 1995. The next cosmid in this contig is 660. This cosmid is being sequenced and has a single gap of about 1.2 kb (based on PCR analysis) which will be completed shortly. The next cosmid, 800, has been completed and submitted to GenBank. The sequence from cosmid 800 is 40,438 base pairs. This makes a total of 126,275 base pairs of *S. pombe* genomic DNA completed and submitted to GenBank in the past year. In addition, we have begun sequencing the next four cosmids from *S. pombe* chromosome II. A diagram of the current state of this contig is shown in Figure 1.

We have begun analysis of this sequence in collaboration with Michael Zhang in Tom Marr's lab here

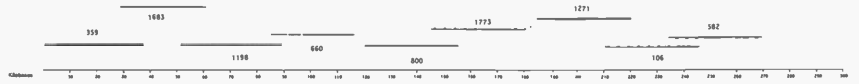

FIGURE 1 Diagram showing the status of sequencing a region of *S. pombe* chromosome II. Solid black bars represent cosmids or partial regions of cosmids which were completed and submitted to GenBank in the past year (such as cosmid 1683). Solid gray bars (such as cosmid 660) represent cosmids or regions of cosmids that are in the final finishing phase of sequencing. Hatched bars such as cosmid 1773 represent cosmids in the shotgun data collection phase of sequencing. The axis shows approximate size of the region in kilobases pairs.

at the Laboratory. The initial computer analysis of the contig represented by cosmids 359, 1198, and 1683 is shown in Figure 2. We will continue working with Dr. Zhang to analyze the additional sequence we are generating and begin carrying out experimental analysis of the sequence based on these predictions. The initial conclusion from this analysis supports the prediction that the *S. pombe* genome is gene-rich, with about one gene every 2 kb. This demonstrates that continued sequencing of the *S. pombe* genome is a cost-effective method for the identification of all of the genes contained in this important model organism.

Our initial efforts in collaboration with the plant biology group (Martienssen and Sundaresan labs, CSHL) was to sequence the insertion sites of transposons inserted in the *Arabidopsis* genome. These results are described in the progress report of the Martienssen lab. This project has expanded into genome sequencing in *Arabidopsis*. We obtained an *Arabidopsis* genomic library from the *Arabidopsis* stock center. This library was composed of 81 "chapters" which were pools of 500 cosmids each. We carried out PCR on these chapters using primers derived from the *PROLIFERA* gene which we had previously sequenced. Positive chapters were plated and 4 x 96 clones from each chapter gridded in a 96-well pattern. Clones from each plate were again screened using the PCR primers from *PROLIFERA*. Positive plates were broken down into column pools, and clones from positive columns were eventually tested to identify *PROLIFERA* containing cosmids. One of these was selected for genome sequencing. The shotgun portion of this sequencing is completed and we are currently filling gaps and finishing the sequence. We have begun an initial analysis on the partially finished sequence. In addition to *PROLIFERA*, we have also identified a gene in this sequence that encodes a protein similar to the *Escherchia coli MutS* gene (mutator). A homolog of this gene is implicated in human colon cancer. The role of this gene in plant growth and development is not yet understood. However, our identification of its position near *PROLIFERA* should enable us to work with the Martienssen lab to identify transposon insertions in this gene, which will be an

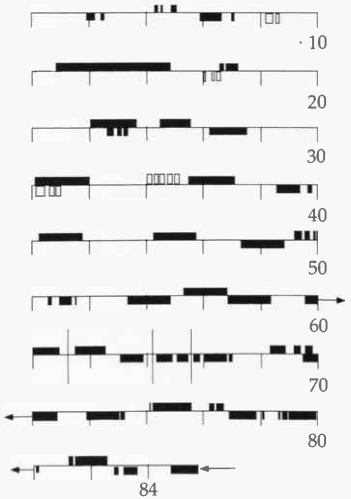

FIGURE 2 Diagram showing predicted genes in an 85,837-base-pair contig from *S. pombe*. Solid boxes indicate predicted genes that have significant matches to genes in the publicly available database. Cross-hatched boxes indicate the predicted structure of genes that are not similar to any previously known gene. Boxes above the axis indicate genes predicted to be transcribed in the forward orientation relative to the contig, and boxes below the axis are predicted to be transcribed in the reverse direction relative to the sequence. Numbers indicate the distance from the most telomeric end of the contig in kilobase pairs. Large perpendicular lines are placed to divide some predicted genes that cluster close to one another (between 60 and 70 kb). Note the presence of a number of predicted genes containing introns.

important step in identifying its function in *Arabidopsis*. We have also identified putative homologs of the gene *Sar1* and a hexose sugar transport protein by analyzing the sequence of this cosmid. In addition to finishing the sequence of this cosmid, we will be expanding our sequencing on the short arm of *Arabidopsis* chromosome IV. We have made arrangements to receive bacterial artificial chromosome clones (BAC) containing large *Arabidopsis* inserts derived from this part of the genome from the European Scientists Sequencing *Arabidopsis* consortium, particularly Mike Bevan (John Innes Center, Norwich England) who heads this group. We will begin sequencing these clones in the coming year.

PROTEIN CHEMISTRY

R. Kobayashi G. Binns P. Kearney
H. Cai C. Kelley
N. Carpino N. Poppito
J. Kahler

This year brought many changes in our group. Camille Kelley joined the protein chemistry core facility and is working in the protein sequencing section. G. Binns remains as Lab Facility Manager for peptide synthesis. Since Dan Marshak moved to Osiris Therapeutics in Baltimore, sections of his two program projects with Memorial Sloan-Kettering Cancer Center and New York University moved to our group this year.

Protein Chemistry Core Facility

C. Kelley, N. Poppito, G. Binns, R. Kobayashi

Collaboration with other scientists at Cold Spring Harbor Laboratory is a major activity in our laboratory. Because internal sequence analysis of proteins has an essential role in the strategy of cloning a specific gene, this technique has been used extensively at Cold Spring Harbor since 1992 and has been very successful. Not only has there been a significant increase in the use of the facility, but also the protein required for internal sequence analysis has been improved to the submicrogram level of protein at the stage of polyacrylamide gel electrophoresis. Since we began using the 1-mm microbore high-performance liquid chromatography (HPLC) column for peptide mapping, it has become routine to obtain sequence information of 10–20 pmoles proteins. Changing the particle size from 5 μm to 10 μm of silica reduces back pressure and enables the HPLC to run without modification. The microbore column has better recovery for a small amount of peptides than the 2.1-mm narrow bore column and thus using the microbore column for peptide mapping became critically important for low amounts of protein. We are enthusiastically expecting to have matrix-assisted laser desorption mass spectrometry in 1996 (shared instrumentation grant for NIH grant is pending). This instrument will strengthen the core facility capability significantly and will be a powerful instrument for analyzing posttranslational modification, which has an important role in macromolecule interactions by modulating the charge, hydrogen-bonding capabilities, and conformational changes of molecules.

Method Development for Protein Sequence Analysis

J. Kehoe, H. Cai, R. Kobayashi

This project is to develop a more sensitive method than Edman degradation by utilizing highly sensitive chemiluminescence detection. To use the chemiluminescence detection system, a chemiluminergic (fluorescence) compound must be introduced into sequencing products. We synthesized two promising fluorescent isothiocyanates to replace the phenylisothiocyanate that had been used in Edman degradation. We are currently examining this new reagent to determine whether it is usable for protein sequencing.

GAP-associated p62 in Chronic Myelogenous Leukemia

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

Chronic myelogenous leukemia is a blood disease caused by an oncogenic tyrosine kinase. A reciprocal translation between chromosome 9 and 22 in a single primitive myeloid progenitor cell activates the abl kinase. In due course, the progeny of this progenitor cell, all of which bear the Philadelphia chromosome, populate the entire hematopoietic system. During an early phase of the disease, the affected cells retain their ability to function normally and their proliferation can be controlled by drug treatment. However, almost without fail, the disease progresses to a fatal stage characterized by undifferentiated, dysfunctional Ph^+ cells.

We hypothesize that the activated abl kinase disrupts signal transduction pathways which control normal myeloid cell development. It accomplishes this by phosphorylating intracellular substrates, some of which might be involved in the regulation of hematopoiesis. Aberrant phosphorylation can lead to the abnormal arrangement and disregulated activity of molecules witnin the cells that contain the Philadelphia chromosome. Our goal is to identify the immediate substrates of the activated abl kinase. In doing so, we hope to unravel the mechanism by which the activated abl causes chronic myelogenous leukemia.

In collaboration with Dr. Bayard Clarkson at the Memorial Sloan-Kettering Cancer Center, we have identified five candidate proteins that appear to be targets of the abl kinase. We have chosen to focus our efforts on one of these proteins, termed p62, because it is the most prominent and consistently tyrosine-phosphorylated molecule that appears in the blood cells of patients during the early stages of the disease. We have demonstrated that in cells which express the activated abl, p62 interacts abnormally with another signaling molecule known as p120 ras GAP. This interaction appears to be due to the presence of phosphotyrosine within the molecule. We intend to address the functional significance of this abnormal interaction. To do so, it is necessary to purify and clone p62. We have purified p62 from tissue culture cells that express the activated abl kinase, obtained partial amino acid sequences of the molecule, and are presently in the midst of cloning the cDNA encoding p62. Our future experiments will address the question of how the abnormal modification of this protein contributes to the pathogenesis of chronic myelogenous leukemia.

Study of 5-HT1A Receptor Signal Transduction Mechanism

P. Kearney, R. Kobayashi [in collaboration with E. Azmitia, New York University]

We set out to study the expression, regulation, and modification of the serotonin (5-HT1A) receptor by developing a model for expression. Our study of the 5-HT1A receptor began with experiments using stably transfected mouse fibroblast cells (LZD-7) expressing the rat 5-HT1A receptor protein. To find a better model and to be able to use the expression system in other mammalian cells, we decided to develop an expression system utilizing the cytomegalovirus (CMV) promoter. The genomic gene for the rat 5-HT1A receptor (P. Albert et al., *J. Biol. Chem. 265:* 5825 [1990]) was used in the construction of the expression system. The gene is intronless, but it contained a larger stretch of noncoding region prior to the start codon which we removed. The clone was then inserted into a plasmid containing the CMV promoter.

Transformed primary human embryonal kidney 293 cells were chosen for the host of our expression system. The CMV promoter is very progressive in these cells, and the natural low basal expression of the 5-HT1A receptor made the cells a good candidate because the correct machinery for receptor interaction would be more likely present. The rat 5-HT1A receptor gene was transiently transfected into human kidney 293 cells via coprecipitation of calcium phosphate and plasmid DNA. Western blot analysis using specific antibodies to the receptor showed high levels of expression and thus the model will be used to study the structure and function of the receptor.

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MACROMOLECULAR CRYSTALLOGRAPHY

X. Cheng J. Horton K. McCloy
L. Jokhan M. O'Gara
Y. Liu R. Ventura
T. Malone R. Xu

Our goal is to determine the structures of a number of biologically important proteins to atomic resolution for a better understanding of their functions in cellular processes. The following two new structures were solved during the 1995 year: the DNA-binding domain of Mbp1, a transcription factor in the yeast *Saccharomyces cerevisiae* that activates the transcription of genes encoding S-phase proteins, and the catalytic domain of casein kinase I from *Schizosaccharomyces pombe* in complex with a protein kinase inhibitor.

Eukaryotic Multiprotein Transcription Factors

We are studying the mechanisms of transcriptional regulation in eukaryotes and are focusing on two systems: the Mbp1-Swi6 complex from the budding yeast *S. cerevisiae* and the VP16-induced complex from herpes simplex virus (HSV) type 1. The Mbp1-Swi6 complex is a transcription factor important for progression from G_1 to S phase in the budding yeast. The HSV-encoded virion protein VP16 associates with two cellular proteins, Oct-1 and HCF, to form a

multiprotein complex that activates HSV immediate-early gene expression. We will determine how these two multiprotein-DNA regulatory complexes are assembled.

Structure of the DNA-binding Domain of Mbp1

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

We have determined the three-dimensional structure of the DNA-binding domain of Mbp1, a transcription factor involved in the regulation of G_1 to S cell cycle progression in *S. cerevisiae*. Mbp1 associates with Swi6 to form a transcriptional activation complex termed MBF (*M*CB *b*inding *f*actor). MBF activates the transcription of many genes encoding S-phase proteins by binding to copies of the MCB element (ACGCGTNA) in the promoters of these genes. Mbp1 consists of three functional regions: (1) a 124-residue amino-terminal region (termed Mbp1$\Delta^{1\text{-}124}$) that binds to the MCB element, (2) a central region that contains copies of the 33-residue ankyrin repeat found in many different regulatory proteins and implicated in protein-protein interactions, and (3) a carboxy-terminal region that is important for interaction between Mbp1 and Swi6.

The structure reveals that Mbp1$\Delta^{1\text{-}124}$ contains a helix-turn-helix DNA-binding motif (Fig. 1). It has a short β-strand amino-terminal to the motif and a pair of antiparallel β-strands carboxy-terminal to the motif. Such an arrangement of secondary structure elements is also found in other helix-turn-helix DNA-binding proteins, including the carboxy-terminal domain of the catabolite gene activator protein (CAP), the globular domain of the linker histone H5 (GH5), and the DNA-binding domain of the hepatocyte nuclear factor-3 (HNF-3)/fork head family of eukaryotic transcription factors. These proteins probably form a subfamily of the helix-turn-helix class of DNA-binding proteins. Aligning the helix-turn-helix

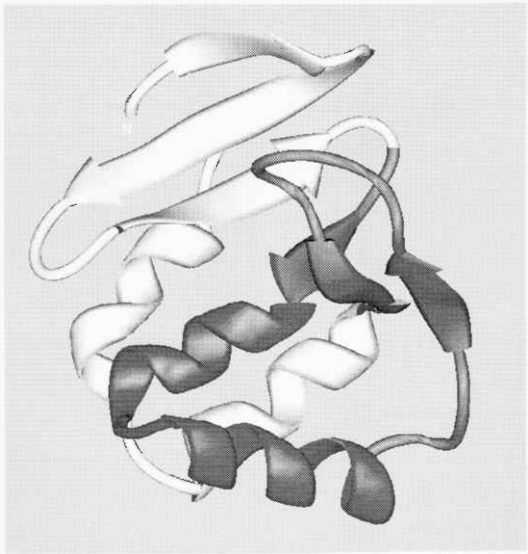

FIGURE 1 Schematic ribbon diagram of the structure of the DNA-binding domain of Mbp1. The similar regions among Mbp1, CAP, GH5, and HNF-3 are shown shaded.

motif of Mbp1 with that of CAP and HNF-3 reveals major groove contact of DNA by the recognition helix within the helix-turn-helix motif, as well as minor groove contact by the loop between the pair of β-strands carboxy-terminal to the motif. The current model includes residues 5–100, and the model is being refined against a dataset to 1.7 Å resolution, which should allow more residues to be included in the model.

Viral Protein VP16

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

The HSV protein 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 immediate early gene transcription by binding to TAATGARAT elements in the immediate early promoters. This binding enables the activation domain of VP16 to interact with the basal transcriptional machinery and activate transcription. Since transcription regulation is intimately related to cell growth, cell cycle control, and oncogenesis, VP16 has been the subject of intensive studies.

We have crystallized the central region (amino acids 48–412) of VP16 which is sufficient for VP16-induced complex formation but lacks the activation domain. This VP16 subunit crystallizes in the orthorhombic space group $P2_12_12_1$, with unit cell dimensions of a = 60 Å, b = 77 Å, c = 83 Å. We have collected X-ray diffraction data in our laboratory with an R-AXIS imaging plate area detector and rotating anode generator. Datasets from $HgCl_2$ and *p*-chloromercuriphenyl-sulfonate-soaked crystals showed mercury site(s) in isomorphous difference Patterson maps. Mercury derivatives constitute the basis of our approach to solving the phase problem. A trial set of phases were then used to calculate a 3 Å resolution electron density map. Regions of the map could be interpreted as α helices. Experiments are under way to improve the interpretability of the map.

To overcome the limitations of our laboratory X-ray source, we used the facilities at beamline X12C at the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory (BNL). More than 45,439 measurements from a single crystal, frozen at a liquid nitrogen temperature of 95K, resulted in 18,757 unique reflections between 20 Å and 2.1 Å with an overall R-merge of 4.8%. The data are 85.8% complete between 20 Å and 2.1 Å with $I/\sigma(I) > 1$.

Casein Kinases

Casein kinases I and II are the major multipotential protein kinases recognizing serine/threonine amino acids situated in an acidic environment in the substrate. The nomenclature is derived from the substrate, dephosphorylated casein, which was used in initial studies to purify the enzymes. The two enzymes appear to be ubiquitous in eukaryotes and have been found in all cell types and species examined. We are studying their structures, functions, and regulations.

Structural Basis for Selectivity of the Isoquinoline Sulfonamide Family of Protein Kinase Inhibitors

R. Xu [in collaboration with G. Carmel and J. Kuret, Northwestern University Medical School]

A large family of isoquinoline sulfonamide compounds inhibits protein kinases by competing with ATP, yet interferes little with the activity of other ATP-using enzymes such as ATPases and adenylate cyclases. One such compound, *N*-(2-aminoethyl)-5-chloroisoquinoline-8-sulfonamide (CKI7), is selective for casein kinase I (CKI) isolated from a variety of sources. We have solved the crystal structure of the catalytic domain of *S. pombe* CKI complexed with CKI7, refined to a crystallographic R-factor of 17.8% at 2.5 Å resolution.

In the CKI-CKI7 structure, the overall folding of CKI and the spatial positions of its ordered side chains closely resemble those found in the CKI-MgATP complex described previously (Xu et al. 1995). The root-mean-square deviation is 0.39 Å when the Cα atoms of the two structures are least squares aligned. In this superposition, CKI7 is found to occupy the same cleft that binds ATP. The isoquinoline ring replaces the adenine ring, and the ethylamine sulfonamide chain of CKI7 points away from the triphosphate moiety of ATP (Fig. 2). Thus, it is structurally confirmed that CKI7 and presumably the entire family of isoquinoline sulfonamide inhibitors bind their respective protein kinases stoichiometrically by competing with ATP for its binding cleft.

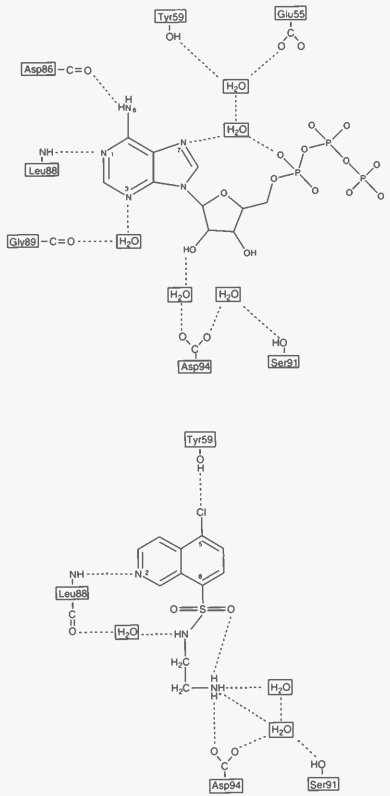

FIGURE 2 Schematic representation showing the specific interactions observed between (*top*) CKI and ATP (pdb code 1csn) and (*bottom*) CKI and CKI7 (pdb code 2csn). For clarity, hydrophobic and van der Waals' contacts are not shown. The ring N2 atom of CKI7 occupies the same position where N1 of ATP is anchored and makes the same hydrogen bond with the main-chain amide of Leu-88, suggesting that this interaction makes an important contribution to the binding affinity of CKI7.

The structure provides new insights into the mechanism of ATP-competing inhibition and the origin of such inhibitors' selectivity toward different protein kinases. Selectivity for protein kinases versus other enzymes is achieved by hydrophobic contacts and hydrogen bonding between the protein kinase and the isoquinoline ring. We propose that the hydrogen bond involving the ring nitrogen-2 atom of the isoquinoline must be preserved but that the ring can flip depending on the chemical substituents at ring positions 5 and 8. Selectivity for individual members of the protein kinase family is achieved primarily by interactions with these substituents.

Casein Kinase II

J. Horton [in collaboration with D. Marshak, Osiris Therapeutics, Inc.]

During this past year, increasing evidence has shown that casein kinase II (CKII) has a critical role in cell regulation and may be involved in tumorigenesis. CKII is known to phosphorylate a variety of proteins. Most of its substrates are nuclear proteins, particularly those that are involved in DNA replication and transcription, whereas some are cytosolic and include those involved in protein synthesis and signal transduction.

CKII is composed of a catalytic α and regulatory β subunit that combine to form a native $\alpha_2\beta_2$ holoenzyme. The amino acid sequences of both subunits are highly conserved in organisms evolutionarily distant as yeast, *Drosophila*, and humans. In fact, its primary sequence is nearly 100% conserved in vertebrates. Among protein kinase families, CKII is more akin to the cyclin-dependent kinase family than any other: This similarity includes sequence homology in both the catalytic and regulatory subunits, the use of GTP as a phosphate donor, and more substantially, a quaternary composition, including catalytic and activating subunits. Recent experiments by D.C. Seldin and P. Leder (*Science 267:* 894 [1995]) have provided strong evidence that disruption of the regulation of the activity of CKIIα contributes to cellular transformation in mice.

We recently improved the quality of CKIIβ crystals and were able to grow larger, more stable crystals of this protein at 16°C. These crystals are primitive, hexagonal with unit cell dimensions of a = 79 Å, b = 79 Å, c = 269 Å, α = β = 90°, and γ = 120°, and they can be cryogenically preserved during data collection. Small crystals (<0.2 mm along edge, <0.06 mm

thick) diffracted to approximately 3.5 Å at the X12C beamline (at the NSLS, BNL).

A larger crystal (>0.5 mm long) has recently been grown. Crystallization conditions are being further refined. To solve the phase problem and determine the structure of CKIIβ, selenomethionyl CKIIβ has been produced.

DNA Methylation

DNA methyltransferases (Mtases) transfer methyl groups from *S*-adenosyl-L-methionine (AdoMet) to specific positions on bases in double-stranded DNA. The DNA Mtases fall into two major classes, defined by the position methylated. The members of one class methylate a pyrimidine ring carbon yielding C5-methylcytosine (e.g., *Hha*I Mtase). Members of the second class methylate exocyclic amino nitrogens, forming either N6-methyladenine (e.g., *Taq*I Mtase or T4 Dam) or N4-methylcytosine (e.g., *Pvu*II Mtase). The Mtases of the two classes were expected to be substantially different from each other, based on the fact that their targets of methyl transfer are very different. Structural analysis, however, has found striking similarities between the DNA Mtases of the two classes.

Universal Catalytic Domain Structure of AdoMet-dependent Mtases

M. O'Gara, K. McCloy, T. Malone [in collaboration with W. Saenger, Freie Universität, Germany]

Our structural comparison has revealed that the catalytic domains of the bilobal proteins, *Hha*I and *Taq*I Mtases, have similar folding with an α/β structure containing a mixed central β-sheet. The functional residues are located in equivalent regions at the carboxyl ends of the parallel β-strands. The AdoMet-binding sites are almost identical and the essential catalytic amino acids coincide. The comparable protein folding and the existence of equivalent amino acids in similar secondary and tertiary positions indicate that many (if not all) AdoMet-dependent Mtases have a common catalytic domain structure. This permits tertiary structure prediction of other DNA, RNA, protein, and small-molecule AdoMet-dependent Mtases from their amino acid sequences.

Structure-guided Sequence Analysis among DNA Amino-Mtases

T. Malone [in collaboration with R.M. Blumenthal, Medical College of Ohio]

Guided by the above common structure, we performed a multiple sequence alignment of 42 amino-Mtases (N6-adenine and N4-cytosine). This comparison revealed nine conserved motifs, corresponding to motifs I–VIII and X previously defined in C5-cytosine Mtases. The amino and C5-cytosine Mtases thus appear to be more closely related than has been previously appreciated. The amino-Mtases could be divided into three groups based on the sequential order of motifs. This variation of the motif order may explain why only two motifs were previously recognized in the amino-Mtases. The Mtases grouped in this way show several other group-specific properties, including differences in amino acid sequence, molecular mass, and DNA sequence specificity. These results have implications for the catalytic mechanisms, evolution, and diversification of this family of enzymes. Furthermore, a comparative analysis of the AdoMet and adenine/cytosine-binding pockets suggests that, structurally and functionally, they are remarkably similar to one another. Whether this actually occurs is currently being explored, as several more DNA Mtases are undergoing crystallographic analysis.

*Pvu*II N4-Cytosine Mtase

M. O'Gara [in collaboration with R.M. Blumenthal, Medical College of Ohio]

The *Pvu*II Mtase recognizes the DNA sequence 5′-CAGCTG-3′. The enzyme acts on the internal C of the sequence and converts the cytosine to N4-methylcytosine, which has been found in a broad range of bacterial species. N4-methylcytosine may present particular advantages to thermophilic bacteria since it is more resistant to heat-induced deamination than cytosine or C5-methylcytosine.

The 38.3-kD *Pvu*II Mtase has been overexpressed in *Escherichia coli* as a mixture of a longer (5%) and shorter (95%) form because in the full-length open reading frame (ORF), there are two initiation sites for translation, 14 amino acids apart. The shorter form alone has been overexpressed and purified. This was

made possible by using the overexpressing construct that lacks the alternative translation initiator, which Gail Adams made in Blumenthal's laboratory. Crystallization trials were performed with the purified shorter form of *Pvu*II Mtase, complexed with its cofactor AdoMet, at a concentration of 20–30 mg/ml. A sparse matrix screening method was used to search for initial conditions for crystallization by the hanging-drop vapor diffusion technique. Crystals were initially observed under two conditions at 16°C. These conditions were refined to produce single crystals that grew to 0.7 x 0.2 x 0.05 mm^3. The crystals diffract to approximately 3.1 Å, but they decay in the X-ray beam and so were frozen for data collection. The protein crystallizes in the space group $P2_1$, with cell dimensions a = 48.75 Å, b = 112.36 Å, c = 59.25 Å, and β = 109.21°.

Because attempts to isolate heavy atom derivatives proved unsuccessful, the selenomethionine derivative protein was overexpressed, purified, and crystallized in an effort to apply the phasing method of multiwavelength anomalous diffraction (MAD). MAD data were collected on one crystal, at three different wavelengths near the Se K absorption edge, at beamline X12C (at the NSLS, BNL). The isomorphous and anomalous difference Patterson maps show a number of peaks, corresponding to possible selenium sites. The maps are currently being interpreted.

Mechanistic Implications of New Crystal Structures for *Hha*I Mtase-DNA-AdoHcy Complexes

M. O'Gara, K. McCloy [in collaboration with R.J. Roberts, New England Biolabs, Inc.]

The refined crystal structures of *Hha*I Mtase complexed with cognate and methylated DNA in the presence of *S*-adenosyl-L-homocysteine (AdoHcy), along with the previously solved binary and covalent ternary structures (Cheng et al., *Cell 74:* 299 [1993]; Klimasauskas et al., *Cell 76:* 357 [1994]), offer a detailed picture of the active site throughout the reaction cycle. This picture supports and extends a proposed mechanism for C5-cytosine methylation that may be general for the whole family of C5-cytosine Mtases. The structures of these two complexes have been refined to crystallographic R-factors of 0.189 and 0.178, respectively, at 2.7 Å resolution. We observe that both unmethylated 2′-deoxycytidine and 5-methyl-2′-deoxycytidine completely flip out of the DNA helix and fit into the active site of the enzyme. The C5 methyl group of the flipped 5-methyl-2′-deoxycytidine is bent out of the plane of the cytosine ring and toward the sulfur of AdoHcy. This unusual position is probably due to the partial sp^3 character at C5 and C6 and to the steric effects of the conserved amino acids Pro-80 and Cys-81. Two water molecules are held near the hydrophobic edge (C5 and C6) of the flipped cytosine by two conserved amino acids (Gln-82 and Asn-304) and the phosphoryl oxygen of the phosphate group 3′ to the flipped nucleotide, and one of them may serve as the general base for eliminating the proton from C5. Protonation of the cytosine N3 during the methylation reaction may involve Glu-119, which itself is protonated via a water-mediated interaction between the terminal carboxyl group of Glu-119 and the amino group of the methionine moiety of AdoMet. This cofactor thus has two key roles in the reaction.

Cell Cycle Proteins

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

We continue our endeavors toward the structural determination of several proteins involved in the cell cycle. Mainly, our goal has been the crystallization of the CDIs (cyclin-dependent kinase inhibitors) p16 and p21, alone and with one of their protein partners, such as the cyclins and PCNA (proliferating cell nuclear antigen). We have encountered many obstacles toward obtaining workable crystals, including protein insolubility, difficulty in purification, and inability of crystallization possibly due to molecular flexibility or a polydisperse character of the protein in solution. We overcame the problem of very low expression of p16 in *E. coli* by fusing it with glutathione-*S*-transferase, and a large amount of the protein has been purified. Unfortunately, no crystal was obtained even after screening several hundred crystallization conditions. Many attempts to solubilize cyclin D1 have also been fruitless.

Pre-mRNA Splicing Factors

R. Xu, L. Jokhan [in collaboration with A. Mayeda and A. Krainer, Cold Spring Harbor Laboratory]

Pre-mRNA splicing machinary involves a complex assembly of small nuclear ribonuclear particles and a number of proteins known as splicing factors. Two proteins, U2AF65 and SF2/ASF, are among the most studied splicing factors to date. Both proteins are required in early splicing complex assembly, essential for 3′ and 5′ splicing site selection, respectively, and are involved in regulating alternative splicing. SF2/ASF belongs to a family of splicing factors that have a characteristic serine-arginine repeat (termed SR proteins) at their carboxyl termini and a conserved RNA-recognition motif (RRM) near their amino termini. However, U2AF65 consists of one serine-arginine repeat followed by three RRMs. In an effort to understand how these pre-mRNA splicing factors achieve their specific functions from a structural point of view, we have purified U2AF65, and some members of the SR proteins. A search for crystallization conditions is currently under way.

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STRUCTURAL BIOLOGY

L. Joshua-Tor

Our focus is to study the molecular basis for regulatory processes in terms of molecular recognition. We are using tools of structural biology and biochemistry in a combined approach to look at proteins and protein complexes associated with these processes. X-ray crystallography, our primary technique, enables us to obtain a very accurate three-dimensional structure of our proteins and the interactions in which they are involved. We use biochemistry to study properties predicted by the structure and to direct our structural studies in an iterative manner. We are also combining information from molecular biology and genetics in collaborative efforts to study their functions.

The Yeast Bleomycin Hydrolase, Gal6

L. Joshua-Tor [in collaboration with W. Zheng and S.A. Johnston, Southwestern Medical Center]

Resistance to anti-neoplastic drugs is a central problem in the treatment of human cancers. Bleomycin hydrolase was first recognized due to its ability to detoxify the anti-cancer drug bleomycin and to limit its use. Tissues with low levels of this enzyme, particularly lung, are very sensitive to bleomycin, and tumors with high levels of the enzyme become resistant to the drug. Bleomycin hydrolase is found in

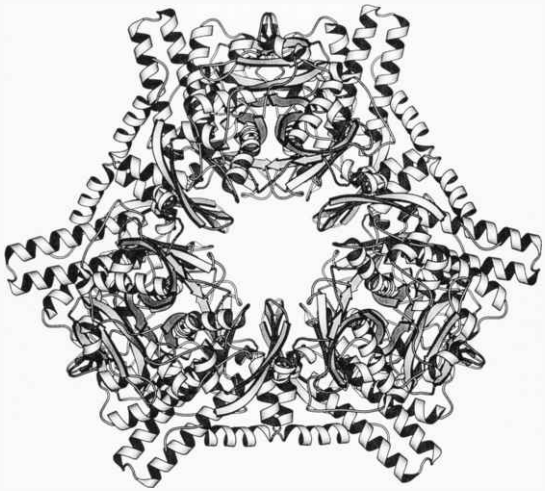

FIGURE 1 Three-dimensional structure of the Gal6 hexamer. The six subunits are arranged in a ring with 32-point group symmetry that creates a prominent central channel along the threefold axis of the molecule.

all mammals, birds, and reptiles and in all tissues tested. The recent discovery of homologs of this protein in yeast and bacteria implies a conserved cellular function for this protein; yet, the only known natural substrate, bleomycin, is almost never seen by any of these cells. The yeast homolog Gal6 is an intracellular, primarily nuclear, protease that binds DNA and acts as a repressor in the expression of *gal* genes.

The cellular function of this ubiquitously expressed protein is still unknown. In recent years, there has been considerable interest in intracellular proteases, since they seem to be involved in important regulatory processes. Bleomycin hydrolase, with its many functions, including its unusual DNA-binding activity, adds another dimension of complexity to these proteases.

We have recently solved the three-dimensional structure of the yeast bleomycin hydrolase, Gal6, a 300-kD hexamer, by X-ray crystallography. The structure was surprising and remarkable in that it revealed a hexameric structure in which the protease and DNA-binding activities are structurally intertwined (see Fig. 1). This was highly unexpected since, in the few cases known so far in which more than one activity resides in a single protein, the different activities tend to reside in separable domains of the protein, whereas in this case they are inseparable and influence each other. We are now trying to understand the functional coupling of these two activities in Gal6, its DNA recognition, and the determinants of recognition and hydrolysis of the anti-cancer drug bleomycin.

The most accurate way of understanding how Gal6 binds single-stranded DNA and the effect on the protein conformation is to obtain a co-crystal structure of the complex of Gal6 with a single-stranded oligonucleotide that we know it binds. Crystals of this complex which diffract to a moderate resolution have been obtained, and we are now improving crystallization conditions and pursuing the structure determination. On the basis of the crystal structure of the native protein, we have also prepared a mutant of Gal6 deficient in its protease activity that will enable

us to study a stable complex between the protein and bleomycin. These studies will foster an understanding of how bleomycin hydrolase functions in tumor drug resistance and provide insight into its basic cellular functions.

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ENCODED COMBINATORIAL LIBRARIES

H.P. Nestler D.L. Dong
R. Liu
R. Sherlock

Encoded combinatorial "One-bead-One-Structure" libraries have opened a new dimension for the study of intermolecular interactions of synthetic receptors of low molecular weight with chemical and biological ligands. Although biological research has known the use of pools of proteins or genes for some years, the use of complex mixtures of small organic molecules has been hindered by the problems of purifying active compounds and of elucidating their structures. "Split synthesis" solves part of this problem by creating "One-Bead-One-Structure Libraries" that have the individual library members physically separated on beads of a solid support resin. Split synthesis is characterized by iterative cycles of splitting the resin into portions and reacting each portion with a different set of reagents. After completion of the reactions, the portions of the resin are combined and mixed thoroughly. The resin is then redistributed for the next synthesis cycle, thus yielding a combinatorial increase of the diversity of structures. As each bead reacts with only one set of reagents per synthesis cycle, each bead carries an individual structure which is determined by the series of reactions that occurred during the synthesis. Active library members can be identified by selecting beads detected in assays performed on the beads. Nevertheless, the problem of structure determination for the compounds thus isolated restricts split synthesis libraries to the incorporation of single-chain sequencible oligomers, such as peptides or oligonucleotides.

The molecular tagging of the individual beads in the libraries was a breakthrough that lifted the restriction for structures that can be included in the combinatorial libraries: Easily detectable molecular tags are attached to the resin beads as they proceed through the split synthesis during the library construction, thereby recording the reaction history of each individual bead. After the screening of the library, the molecular tags can be cleaved from each of the selected beads and analyzed to report the structures of the library members on each of these beads.

In our initial studies, we have elaborated encoded combinatorial libraries of low-molecular-weight receptors with branched peptidic structure and have shown examples of highly specific interactions between these receptors and small peptide ligands. We intend to explore these systems to derive small molecules that bind proteins of physiological importance at specific functional sites.

Synthesis of Encoded Combinatorial Receptor Libraries

H.P. Nestler, R. Liu, R. Sherlock

We have set up our laboratory to generate, assay, and analyze encoded combinatorial libraries. The starting materials necessary for the synthesis and encoding of libraries have been prepared. As our initial studies have shown that simple molecules displaying two short peptide or peptide-like chains can provide reasonable affinities and selectivities toward peptide substrates, we will base our first investigations on libraries that contain receptors with forceps and multi-arm designs. To avoid obstacles during library generation, the synthesis schemes will mostly utilize peptide chemistry, which is well-developed. Furthermore, the variety of available peptide and peptide

analog building blocks will allow for sufficient diversity in our libraries. The generated libraries will be screened for molecules that interact with proteins of physiological importance. Two envisioned physiological targets are the binding and modulation of Ras proteins, and the interaction with proteins that are involved in the decision point of programmed cell death.

Small Synthetic Receptors That Recognize Peptides

H.P. Nestler [in collaboration with W.C. Still, Columbia University, New York, and B. Salom and C. Gennari, University of Milano, Italy]

Conformational analyses of vinylogous sulfonyl peptides (VSPs), a new class of peptide analogs, show that even the vinylogous sulfonyl tripeptides adapt a well-ordered three-dimensional structure. Therefore, we hoped that short single-chain VSPs might show distinct intermolecular interactions.

Encoded combinatorial libraries provide us with a simple test system for the preliminary examination of the binding capacity of receptors with novel design: Individual examples of potential receptor families are synthesized and screened against a combinatorial library of about 50,000 derivatized tripeptides. We use the affinity and the selectivity that we find in this assay as an index for the capacities of the new receptor class.

Although none of the five single-chain VSPs that we prepared showed affinity to members of our tripeptide library, we were able to obtain molecules that bind selectively to peptides by joining two VSP chains with a spacer, thus generating molecules with a forceps-like architecture. A prime question in our investigation of VSPs was to understand how much the substitution of peptides by peptide analogs effects the binding profile of otherwise similar receptors. We therefore synthesized the peptidic analog of one of our forceps molecules. This branched peptide also binds to members of our tripeptide library selectively: Whereas the strength of the interactions lies within the same range as the affinity of the VSP molecule, the selectivity profile has changed completely.

Encouraged by these results, we developed a synthetic method that will enable us to incorporate the VSP-building blocks into encoded combinatorial libraries, and thus make them available to further evaluation of their biological potential.

Screening of Combinatorial Libraries with GFP-fusion Proteins

H.P. Nestler, D.L. Dong [in collaboration with M.H. Wigler, Cold Spring Harbor Laboratory]

In the past, secondary antibodies coupled to alkaline phosphatase have been used for detection of interactions between proteins and the beads. However, there are several drawbacks to this protocol. First, some library members may bind to the secondary antibody or its conjugate. Second, the procedure is cumbersome and requires multiple washings during which the protein binding to beads may become diminished. Third, after alkaline phosphatase staining, the beads cannot be reused since it is not possible to wash the precipitated colored substrate away. Therefore, we have tested whether we could detect the binding of proteins to beads using fluorescent fusion proteins.

For this purpose, we utilized a stable and intensively fluorescent mutant of the green fluorescent protein (GFP) as a marker. GFP was originally isolated from the jellyfish *Aequorea victoria* and fluoresces green upon stimulation by blue light. We expressed the mutant GFP in *Escherichia coli* as a T7 epitope fusion protein carrying a His tag that allowed for the easy purification. The purified protein was first cross-linked to several types of supports to establish the feasibility for the fluorescence assay. In the second stage, antibodies to the T7 epitope were cross-linked to Tentagel beads: We were able to identify the beads carrying antibody easily under a fluorescence microscope after they had been incubated with the GFP-T7 fusion protein.

A series of GFP fusion proteins (including Ha-Ras, Rsb2, and functional domains of Ras) were constructed and expressed in *E. coli* and are available for the screening of combinatorial libraries of small molecules.

Microradiographic Screening of Combinatorial Libraries

H.P. Nestler, R. Sherlock, D.L. Dong [in collaboration with H. Wennemers and W.C. Still, Columbia University, New York, and M.H. Wigler, Cold Spring Harbor Laboratory]

We have had good success screening encoded combinatorial libraries for receptors to peptides using peptides coupled to dyes as ligands, when these as-

says have been conducted in organic solvents. However, we have had difficulties applying this straightforward protocol in aqueous phase. Although the labeling of proteins with hydrophilic dyes, such as fluorescein and rhodamine, is a commonly used protocol for biochemical studies, members of the libraries binding the dye instead of the peptide ligands have predominated in our assays, thus interfering with efficient screening.

Radioactive labeling is an elegant approach to tag compounds without altering their molecular structure, and the use of ^{14}C as radioisotope allows for the general incorporation of radioactivity into organic compounds. We could show that individual radioactive beads can be easily identified by microautoradiography. To prove the efficacy of our approach, we radioactively labeled a previously described, dye-conjugated receptor using a ^{14}C-linker between the receptor and the dye. A bead-supported tripeptide library was equilibrated with a solution of the receptor and assayed by microradiography. Only beads that had picked up color showed a characteristic black halo after exposure and photographic processing. As the signal intensity in the microradiographic process can be increased easily by extending the exposure times, our method promises to trace unambiguously even very weak interactions that might escape detection using X-ray film or the phosphoimager techniques.

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The marriage between vertebrate and invertebrate neuroscience is flourishing, nourished by a healthy diet of molecular biology. Electrophysiological work by the Zhong lab has defined for the first time a clear role for the Ras/Raf/MAP Kinase pathway in neuronal function. Consistent with this observation, behavioral experiments in the Silva lab have shown defective learning in mice mutant for the NF1 gene, which appears to participate in the Ras/Raf pathway and which produces heritable learning disabilities in children. Thus, work in flies and mice once again has converged to yield important insight to human cognitive function.

The Silva, Malinow, and Cline labs continue to elucidate the role of CaMKII in neural plasticity. In part by using a recombinant gene delivered to frog neurons by vaccinia virus vectors, Cline et al. have shown that CaMKII functions both pre- and postsynaptically to regulate arbor growth during development of the visual system. In vertebrates, Malinow et al. have discovered a novel process, calcium-evoked dendritic exocytosis, which also is mediated by CaMKII and which may have a role in synaptic plasticity. Finally, the Silva lab has extended its seminal work on CaMKII and synaptic plasticity in the mouse hippocampus by showing that this enzyme mediates synaptic plasticity in the cortex.

The Enikolopov lab has recently shown that, in addition to its role in regulating the NGF-mediated switch from proliferation to cytostasis, nitric oxide (NO) protects differentiated neurons from apoptosis. Using pharmacological agonists and antagonists of NO and transgenic flies carrying three different versions of *Drosophila* nitric oxide synthase (NOS)—two of which were generated by Dr. Michael Regulski in the Tully lab—Enikolopov et al. also have shown that NO regulates the number of mitotic cell divisions during adult leg development. These elegant in vivo experiments finally have demonstrated a clear role for NO during development.

Molecular cloning of new genes involved with associative learning in *Drosophila* reached an important milestone in the Tully lab. The learning defect in mutant *linotte* flies has been eliminated by inducing the expression of a *lio*$^{+}$ transgene in adults. This constitutes the first "rescue" of a learning defect via gene therapy. The Tully and Yin labs also continue their work on the role of CREB during memory formation. The Yin lab has concentrated its efforts on the regulation of endogenous CREB function during long-term memory formation, and both labs are applying molecular techniques to identify the downstream genes involved with long-term memory formation.

We have said farewell to two neuroscientists this year: Dr. Hiro Nawa has moved to Niigata University, Japan, as Professor of Neuropharmacology, and Dr. Dan Marshak has moved to Osiris Therapeutics, Baltimore, as Senior Vice President of Research and Development. To balance these losses, however, Dr. Jerry Yin accepted a Senior Staff Investigator position at CSHL to continue his work on CREB and memory.

MOLECULAR NEUROBIOLOGY OF LEARNING AND MEMORY IN *DROSOPHILA*

T. Tully C. Jones, G. Bolwig, S. Pinto, K. Velinzon, M. Del Vecchio, J. Connolly (Cambridge), M. Regulski, J. Dubnau, J. Christensen, Z. Asztalos, N. Arora (URP), J. DeZazzo, H. Tobin, B. Svedberg, R. Mihalek, C. Hogel (Wellington College)

Our work on the molecular mechanism(s) of memory formation during the last 2 years has established two important points. First, memory retention is composed of several genetically distinct temporal phases.

In particular, a permanent, long-term memory (LTM) coexists for a few days in parallel with a slow-decaying anesthesia-resistant memory (ARM). Second, the consolidation of early memory into LTM is regulated by the relative amounts of activator and repressor isoforms of the CREB transcription factor(s). Too much CREB repressor blocks and too much CREB activator enhance the formation of LTM.

We continue this genetic analysis of memory formation in *Drosophila* with further experiments on CREB transgenic flies and with behavioral evaluations of disruptions of other genes involved with this form of olfactory learning. Of note is our "rescue" of the learning/memory defect of *linotte* (*lio*) mutants by inducing the expression of a *lio*$^+$ transgene 3 hours before training. More generally, this represents the first "cure" of a learning disability via gene therapy in any species.

dCREB2-mediated Enhancement of LTM Depends on Associative Learning

T. Tully, M. Del Vecchio, H. Tobin, J. Dubnau
[in collaboration with J. Yin, Cold Spring Harbor Laboratory]

Genetic dissection of memory formation after olfactory learning has revealed that a newly acquired experience is processed sequentially first through short-term memory (STM) and then through middle-term memory (MTM). At this point, processing branches into two parallel paths: one that yields anesthesia-resistant memory (ARM) and one that yields long-term memory (LTM). With this general view, two aspects of LTM become clear. First, LTM is formed in normal flies only after ten repetitions of the usual training session. In addition, these ten training sessions must be spaced by at least a 10-minute rest interval to form maximal LTM. If no rest interval occurs during repetitive training (massed training), then no amount of training sessions will produce LTM. Thus, spaced training is necessary to form LTM. Second, all earlier memory phases (STM, MTM, and ARM) decay away within 4 days after ten massed or spaced training sessions. LTM after ten spaced training sessions, however, shows no decay for at least 1 week. Thus, 7-day retention is a direct measure of LTM unobscured by earlier memory phases.

Transgenic flies expressing copious amounts of CREB repressor do not form LTM even after ten spaced training sessions. In contrast, transgenic flies overexpressing CREB activator form maximal LTM after only one training session. The latter gain-of-function effect constitutes a strong argument that CREB acts as a molecular switch during LTM induction. Implicit to this argument, however, is the notion that the CREB switch is activated in response to an associative learning event. Alternatively, induced expression of CREB activator 3 hours before training simply may produce an up-regulation of downstream CREB target genes, which then "prime" the associative learning process, rather than the LTM process per se.

We tested this notion by exposing normal flies or transgenic flies expressing CREB activator (CREB-a) to *unpaired* presentations of the odors and electroshock stimuli. Tully and Quinn (*J. Comp. Physiol. 157:* 263 [1985]) have shown that a 45-second interval between the presentation of odors and electroshock is sufficient to eliminate associative learning. With this procedure, then, flies experience odors and shock, but the stimuli are not associated in time. After ten spaced but unpaired training sessions, both normal and CREB-a transgenic flies showed no LTM, as measured by 7-day memory retention. Thus, induced expression of CREB-a in transgenic flies did not produce enhanced LTM by nonspecifically "priming" the associative learning process. Ten spaced but unpaired training sessions followed by one paired training session in normal flies also produced no LTM. Thus, experience with odors and shock in the absence of a temporal association between them did not "prime" normal flies to induce LTM after one subsequent paired training session. CREB-a transgenic flies, in contrast, showed enhanced LTM after similar training.

Currently, we are advancing our understanding of the molecular mechanism of the CREB LTM switch by assessing which serine/threonine phosphorylation sites on CREB-a are involved with the enhanced LTM effect. We also are expressing CREB-repressor in restricted regions of the adult brain to determine where LTM is stored. Finally, we are searching for CREB downstream genes, the expression of which is regulated during LTM formation.

Identification of New Genes Involved with Associative Learning

T. Tully, R. Mihalek, C. Jones, S. Pinto, J. Christensen, G. Bolwig, J. DeZazzo, B. Svedberg, K. Velinzon

In September 1991 when our group arrived here at the Laboratory, we brought with us four learning/memory mutants identified from a mutagenesis that we initiated at Brandeis University. Genetic and cytological analyses revealed that these four mutants defined four autosomal genes, *linotte*, *latheo*, *nalyot*, and *golovan*. To date, we have cloned and definitively identified the transcription units of *linotte* and *latheo* via rescue of their mutant learning/memory defects after induced expression of lio^+ or lat^+ transgenes (see Fig. 1 for results from *linotte* experiments).

DNA sequencing of the *lio* and *lat* genes indicates that each encodes a novel protein. Thus, we have achieved one major long-term goal: discovery of new genes involved with learning and memory. The next step in this analysis is an arduous one. We must pursue molecular, genetic, and biochemical clues to understand the biological function of these new genes. To this end, preliminary data indicate that associative learning is disrupted much more severely in *rut;lio* double mutants than in either single-gene mutant, suggesting that *rut* and *lio* define two different "input" paths to the associative process. We also have determined that the *lat* gene product interacts physically with tyrosine hydroxylase, suggesting a modulatory role for *lat* in neurotransmitter synthesis.

The transcription units of *nalyot* and *golovan* have been tentatively identified. The *nalyot* and *golovan* mutations (as well as the *latheo* and *linotte* mutations) were generated by transposon insertions into the genome. Cloning of the *nalyot* transposon revealed that it was inserted in an intron of the *Adf-1* gene, which encodes an HTH transcription factor that binds to the promoter regions of *Alcohol dehydrogenase* (*Adh*) and *Dopa decarboxylase* (*Ddc*). Cloning of the *golovan* transposon revealed that it was inserted 70 bp 5′ of *extra machrochaetae* (*emc*), a negative regulator of the *daughterless*, *achaete*, and *scute* transcription factors, which are involved in sex and neuronal determination.

Planned rescue experiments will determine whether disruptions of these transcription units are responsible for the learning/memory defects of *nalyot* or *golovan* mutants. Induced expression of the rescuing transgenes at various times throughout development will determine whether the learning/memory defects derive primarily from their function during associative learning or secondarily from maldevelopment.

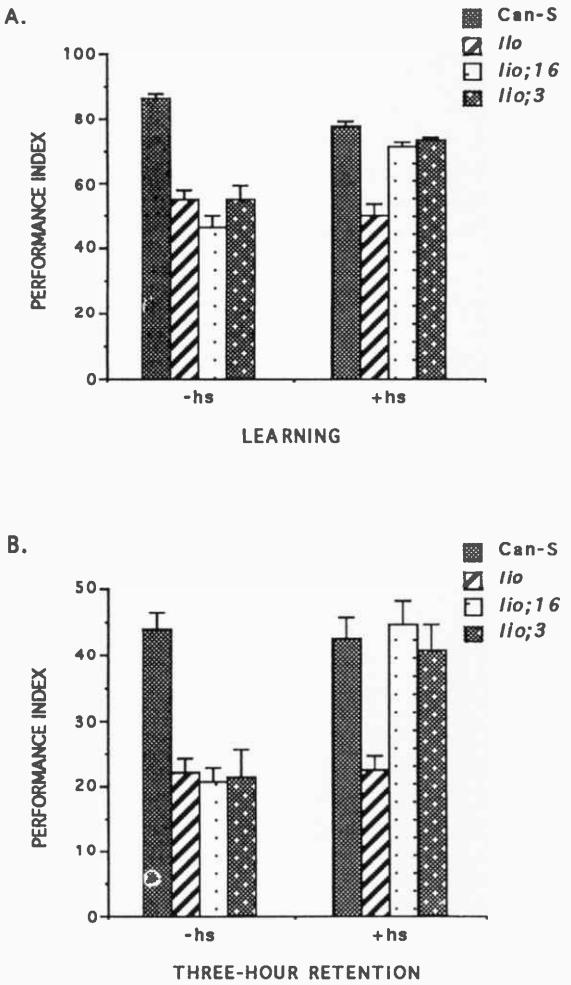

FIGURE 1 Rescue of the *linotte* (*lio*) learning (*A*) and 3-hour memory retention (*B*) defects after induced expression of a lio^+ transgene. Before heat shock induction (*-hs*), two independently derived *hs-lio*$^+$ transgenic lines (*lio;16* and *lio;3*) yielded olfactory learning and memory scores similar to those of the original *linotte* (*lio*) mutant, all three of which were significantly lower than those of normal (*Can-S*) flies. When trained 3 hours after heat shock, however, the learning and memory scores of both transgenic lines were no different from those of normal flies. These data indicate that the *linotte* gene functions acutely during adult olfactory learning.

Characterization of Extant Mutants in Signal Transduction Pathways

T. Tully, M. Del Vecchio, C. Hogel [in collaboration with D. Kalderon, Columbia University, and K. Kaiser, University of Glasgow]

Previous experiments on several extant mutants have outlined the involvement of the cAMP cell signaling pathway in olfactory associative learning. Mutations in *dunce*, *rutabaga*, and *DC0* all disrupt olfactory learning, and the corresponding genes encode a cAMP phosphodiesterase, an adenylyl cyclase, and a catalytic subunit of cAMP-dependent protein kinase (PKA), respectively. In collaboration with Dr. K. Kaiser, we have extended this behavior-genetic analysis of the cAMP pathway by showing that mutations in the RI regulatory subunit of PKA also disrupt olfactory learning.

We have studied a temperature-sensitive allele of *DC0* in collaboration with Dr. D. Kalderon. This mutation affects olfactory learning and memory at both permissive and restrictive temperatures. The temperature-shift-specific effect on memory formation, however, appears to correspond specifically with the MTM phase. Consistent with this observation, the temperature-shift-specific memory decay curve of $DC0^{ts}$ mutants is similar to that of *amnesiac* mutants, which we previously have shown also to disrupt MTM.

We also have studied *turnip* mutants, which were isolated as defective in olfactory learning and have been reported to be defective in phospholipid-dependent protein kinase (PKC). We have shown that task-relevant assays for sensorimotor responses are abnormal in these mutants. In short, *turnip* flies are sluggish. Such an effect precludes the notion that *turnip* (and PKC activity) underlies olfactory associative learning in fruit flies.

Behavioral Properties of Learning and Memory in Normal and Mutant Flies

T. Tully, N. Arora, Z. Asztalos, C. Jones, J. Connolly [in collaboration with D. Yamamoto, Mitsubishi Kasei, and K. O'Kane, Cambridge]

Genetic dissection of the behavioral properties of learning and memory progresses. To complement our olfactory habituation assay, we have developed an olfactory sensitization assay. Thus, we now can study various mutants in both of these forms of nonassociative learning and compare such results with those from olfactory associative learning. In collaboration with Dr. D. Yamamoto, for instance, we have shown normal dishabituation but no sensitization in mutant *fickle* flies, thereby demonstrating a genetic dissection between these two behavioral phenomena. Moreover, habituation in *fickle* mutants is faster than normal, which is consistent with the long-standing hypothesis that habituation results from a "two-opponent process." Further behavioral analyses of *fickle* mutants will reveal whether associative learning also is defective.

We also have shown a disruption of olfactory associative learning when a constitutively active $G_{s\bullet}$ protein is expressed in the mushroom bodies. Olfactory learning is normal when $G_{s\bullet}$ is expressed in other anatomical regions of the central brain. This result confirms other studies implicating the mushroom bodies in olfactory associative learning and demonstrates for the first time an involvement of G protein in the underlying second-messenger cascade. In contrast, olfactory habituation is normal even when mushroom bodies are chemically ablated, indicating that the anatomical focus for olfactory habituation lies elsewhere.

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LONG-TERM MEMORY FORMATION IN *DROSOPHILA*

J. Yin J. Wallach
M. Stebbins
H. Zhou

The highlight of our inaugural year was a paper describing the enhancement of long-term memory (LTM) formation after induction of the *Drosophila* CREB activator. This paper followed our previous finding that induced expression of the CREB blocker prevented LTM formation, and together provide strong evidence for the crucial involvement of this transcription factor in LTM formation. Our work now focuses on three aspects of CREB: (1) What signal transduction pathways "upstream" of CREB are activated during learning, and how do they affect the activity/amounts of CREB? (2) Where does all of this occur in the brain? (3) What molecular cascade occurs "downstream" from CREB after it is activated and begins the process of LTM formation?

Upstream Activation of CREB

H. Zhou, J. Yin

Two experiments demonstrate that changing the amounts of the fly CREB isoforms has dramatic effects on long-term memory formation. If a naturally occurring, dominant-negative "blocking" form of CREB is induced just prior to behavioral training, it specifically and totally prevents the formation of LTM, without affecting other phases of memory. Conversely, if an activator is induced prior to training, formation of LTM is accelerated, resulting in full levels of LTM after just a single training trial, instead of requiring the usual ten trials. These transgenic manipulations demonstrate the pivotal role of CREB in the process of LTM formation and suggest that some aspect of the amount of activator protein is rate-limiting. They also suggest that the activator/blocker (A/B) ratio is important for full levels of LTM formation.

To complement our work on changing the amounts of activator and blocker proteins, we have initiated studies testing the effects of phosphorylation on the activities of each isoform. Sequence comparisons between the fly CREB gene and its mammalian counterparts indicate that only two regions of the molecule have a significant amount of one-to-one amino acid conservation: the carboxy-terminal basic region–leucine zipper (dimerization and DNA-binding region) and the internal phosphorylation region. Examination of the phosphorylation domain shows that a number of residues which are known to be substrates of various kinase pathways are conserved. These conserved residues include putative phosphorylation sites for calcium/calmodulin kinase II, glycogen synthase kinase-3, and casein kinase, in addition to the well-characterized cAMP-dependent protein kinase site (Ser-133). These kinases may represent the nuclear end of signal transduction pathways which are activated (and cross-talk) during learning and are involved in the regulation of CREB activity. We have mutated these conserved serine/threonine residues to the nonphosphorylatable residue alanine in both of the inducible activator and blocker genes, made transgenic flies, and are ready to test them for behavioral effects.

Two mutations are of particular interest. Mutation of Ser-133 on the mammalian blocker molecule increases its activity, implying that phosphorylation of the blocker may normally decrease its activity. Therefore, the same kinase may activate the activator isoform and decrease the activity of the blocker protein by phosphorylating Ser-133 on each respective molecule, providing a very efficient way to change the A/B ratio.

Work from Tim Tully's lab here at the Laboratory shows clearly that two behavioral parameters are essential to induce full levels of long-term memory: repetitive training and rest intervals between each of the training trials (spaced vs. massed training). This requirement for a rest interval may be quite universal, since it is part of the standard protocol for induction of protein-synthesis-dependent long-term facilitation in *Aplysia* and long-lasting long-term potentiation (L-LTP) in hippocampal slices. Recently, it has been shown that in certain cell lines, CREB activity can be inhibited by CaM kinase II-mediated phosphorylation of a conserved Ser-142 residue. This inhibition is "dominant" to the activating phosphorylation that oc-

curs on Ser-133. This finding provokes the intriguing hypothesis that during each training trial, neurons in the LTM circuit are activated, Ca^{++} influx occurs, a CaM kinase family member is activated, and CREB is inactivated through Ser-142 phosphorylation. During the rest interval which defines spaced training, the appropriate phosphatase dephosphorylates Ser-142, allowing activation of the molecule if Ser-133 is still phosphorylated. This molecular model provides a simple explanation for the seemingly universal requirement for a rest interval in protein-synthesis-dependent, CREB-dependent processes in neurons. The direct prediction of this model is that the Ser-142 to alanine mutation on the activator transgene will allow LTM formation after massed training. We are currently testing this hypothesis, along with the involvement of other nuclear kinases predicted from the sequence conservation.

The Long-term Memory Cells

J. Wallach, H. Zhou, J. Yin

We believe that the cells which participate in long-term memory formation are likely to be a very small subset of the neurons in the brain. Therefore, the identification of "downstream" target genes of CREB may be difficult due to a signal-to-noise problem, unless these genes are totally unexpressed in untrained flies. The identification of the "memory cells" not only is an intrinsically interesting problem, since they represent the "engram," but may also contribute significantly to efforts to identify "downstream" genes. Our current efforts are directed at the identification of these cells, with the eventual goal of their enrichment or purification, facilitating molecular analysis.

We are using CREB-responsive transcriptional reporters to identify cells in which CREB is activated during long-term memory formation. To facilitate comparison of different transgenes, we have tested the efficacy of "insulator" elements first described by Paul Schedl's lab. When these discrete pieces of DNA flank an eye color gene, independent transgene insertions show the same levels of expression, indicating that the insulators are overcoming the effects of (random) "position effects" on transgene expression. We have made five independent transgenic lines that contain three wild-type CRE (3xCRE) sites in front of a truncated promoter and the β-galactosidase gene. When adult heads from these lines are sectioned and developed for β-galactosidase staining, the same complex pattern of staining is seen for all lines, implying that the insulators are functioning. When transgenic lines are isolated that contain reporters containing 6, 9, or 15xCRE sites, the staining pattern is a superset of that seen with the 3xCRE reporter. In addition, the staining in the cells that are common to all of the reporters is darker. These results imply that by increasing the number of binding sites, a more sensitive reporter is created and more and more cells are recruited to the staining pattern. This result suggests that this set of transgenic reporters may represent reporters which have increasing sensitivity to CREB activation and will be useful in the effort to detect CREB activation during the formation of long-term memory. In principle, this strategy can be applied to the mouse system, and we have begun to test its feasibility.

Spatial/Temporal Regulation of Expression

M. Stebbins, H. Zhou, J. Yin

One of the most important problems in transgenic analysis is the refinement of current technology to achieve greater specificity in the induction of transgenes. Spatial (cellular), temporal, and subcellular regulation of the delivery of transgenic products is required for better analysis of complex phenotypes. We have begun the process of combining spatial and temporal regulation of genes. The currently available heat shock promoter system for temporal control of gene expression, while rapid and reproducible, suffers from the flaw that many processes, including learning and memory formation, are affected by the heat shock itself. To avoid this problem, we are trying to combine the recently developed tetracycline-inducible system with the existing enhancer-trap system to achieve spatial-temporal regulation of transgene expression.

The tetracycline-inducible system has been shown to work in transgenic plants and mice. However, one of the problems with the existing systems, whether "forward" (add tetracycline to induce genes) or "reverse" (remove tetracycline to induce genes), is that the expression levels of the *trans*-activator are very low. Analysis of the *trans*-activator RNA reveals that most of it is cryptically spliced into non-productive forms. Mutations that destroy the cryptic

splicing sites allow high levels of RNA and protein expression. We have combined these changes with the recently described "forward" system and are currently generating the appropriate constructs and transgenic flies to test the system. In addition, by placing the *trans*-activator under the control of gal4 binding sequences (which are activated by gal4 genes under control of spatially defined enhancers), it is possible to limit induction spatially. The feasibility of this further refinement is under investigation.

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NEURAL BASIS OF LEARNING AND MEMORY

Y. Zhong L. Luo Y. Wang
F. Guo N. Wright

Our research interest is to understand the neural basis of learning and memory. Our approach is to analyze *Drosophila* learning and memory mutants. During the last two decades, a number of *Drosophila* mutants have been isolated on the basis of their poor performance in a classic conditioning task. Some of these mutations affect learning, and other mutations affect memory. How functions of the nervous system are altered in these mutants must be elucidated in order to gain insight into the neural basis of learning and memory. However, electrophysiological recordings from the *Drosophila* brain have been impossible because of the small size of the fly's brain. In facing such a challenge, we are taking three different approaches.

First, we want to determine the cellular functions of these mutant genes in the nervous system, specifically for their roles in neurotransmission. To address this question, we will study not only the synapses involved in learning and memory, but also preparations that are more accessible to electrophysiological recordings, such as neuromuscular junctions and cultured neurons. Second, we are trying to overcome the technical difficulty of electrophysiological recordings due to the small-sized brain and neurons and thus be able to study how synaptic transmission and synaptic plasticity are affected in these mutants at synapses that have been shown to be critical for fly learning and memory. Third, we are exploring the possibility of turning the disadvantage of the small-sized brain into an advantage by developing an optic recording method, which may allow us to study neural network properties that are important for learning and memory.

Our work in the last year has focused mainly on the first approach, but we have made significant effort toward the development of preparations suitable for the other two approaches, as further detailed below.

Neuropeptidergic Transmission: Involvement of Ras and Rut-Adenylyl Cyclase

F. Guo, Y. Zhong

The *rut* gene, isolated as a learning and memory mutant, encodes a Ca^{++}/CaM-sensitive adenylyl cyclase (Rut-AC). Biochemical study by M.S. Livingstone has shown that Rut-AC is not involved in the cAMP synthesis activated by monoamine transmitters, such as dopamine, 5-HT, and octopamine. We therefore examined the possibility that Rut-AC may be activated by a neuropeptide. Our study has demonstrated that at the larval neuromuscular junction, a pituitary adenylyl cyclase-activating polypeptide (PACAP38)-like neuropeptide activates both Rut-AC and Ras/Raf pathways to modulate K^+ currents synergistically. PACAP38-induced 100-fold enhancement of K^+ currents was eliminated in *rut*, *ras*, and *raf* mutants (Fig. 1). Enhancement was also induced when both pathways were coactivated by ex-

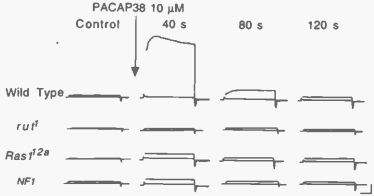

FIGURE 1 PACAP38-induced enhancement of K^+ currents is abolished in *rut*, *Ras1*, and *NF1* mutants. The arrow indicates focal application of PACAP38 (10 μM). "Control" current traces were recorded before application of PACAP38. The time in seconds after pressure-ejection of PACAP38 is indicated at the top of current traces. Data were recorded with the bath saline containing 1 mM Ca^{++}.

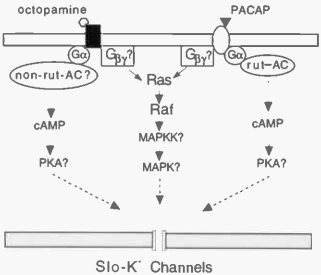

FIGURE 2 A model of dual signal transduction pathways activated by PACAP38-like neuropeptide and octopamine for modulation of K^+ currents. Question marks indicate molecules whose functions have not been tested in the PACAP38-induced modulation. Dashed arrows indicate that where the cAMP and Ras/Raf pathways converge, to channels directly or to an intermediate target, remains to be determined.

pressing the active *raf* transgene and adding cAMP. The result not only indicates that Rut-AC is activated by the neuropeptide, but also suggests that the Ras/Raf pathway is essential for neurotransmission. This suggestion is further supported by our study of octopamine transmission.

Octopamine is an insect neurotransmitter and is present at the *Drosophila* neuromuscular junction. Our recordings revealed that octopamine also induced 100-fold enhancement of K^+ currents, but it did so by activating the non-Rut-AC and Ras/Raf pathways. Octopamine-induced response is normal in the *rut* mutant. On the basis of these studies, we have proposed the model depicted in Figure 2. Both the PACAP38-like peptide and octopamine activate the Ras/Raf pathway via the $G_{\beta\delta}$ complex, and each transmitter activates different types of adenylyl cyclase via the G_{α} subunit. The two coactivated pathways then modulate K^+ currents synergistically. We are now trying to understand how the Ras/Raf pathway is activated by G-protein and how K^+ currents are modulated by the two pathways. One obvious implication from this study is the possibility of the involvement of the Ras/Raf pathway in learning and memory. This possibility is further delineated by the finding that NF1 is involved in PACAP38-like peptide transmission. NF1 is the neurofibromatosis 1 gene, a frequently encountered neurogenetic disorder. About 30–40% patients show a specific learning disability. Although it has been demonstrated biochemically and genetically that NF1 has Ras GAP activity, its physiological function remains to be elucidated. Our experiment revealed that PACAP38-like neuropeptide transmission is eliminated by mutations of the *Drosophila* NF1 homologous gene (see Fig. 1). We will continue to investigate the mechanism of the NF1 involvement.

Cloning the Gene Encoding PACAP38-like Neuropeptide

L. Luo [in collaboration with L. Pena, Sloan-Kettering Cancer Center]

The result that PACAP38-like neuropeptide transmission is defective in *rut* and *NF1* mutants raises the possibility 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.

After failing to isolate the *Drosophila* homolog of the vertebrate PACAP38 peptide by using polymerase chain reaction (PCR), we are now purifying the *Drosophila* PACAP38-like peptide on the basis of cross-immunoreactivity with vertebrate antibody. Western analysis has identified a PACAP38 immuno-

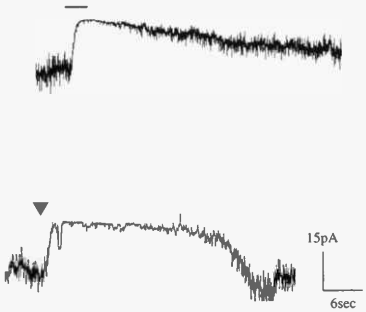

FIGURE 3 5-HT and Amn peptide-induced membrane currents in cultured CNS neurons. The top current trace is an example of 5-HT response. The bottom current trace is induced by Amn peptide. For these recordings, the membrane voltage was clamped at -80 mV. The bar over the current traces indicates the period during which transmitters were perfused. Bar, vertical 20 pA/horizontal 4 s.

reactive band with a size similar to that of PACAP38, which was highly enriched by high-performance liquid chromatography (HPLC) purification. We are going to test the physiological function of this peptide and then obtain the sequence of the peptide for cloning the gene.

Neurotransmission in Cultured CNS Neurons

N. Wright

To extend a similar research of the neuromuscular junction into CNS neurons, we studied responses of identified cultured CNS neurons to various neurotransmitters. Currently, analysis is focusing on 5-HT and the neuropeptide encoded by the memory mutant gene *amnesiac* (*amn*). Both 5-HT and the Amn peptide induced similar responses: a hyperpolarization followed by modulation of K^+ currents. This hyperpolarization is caused by closing an Na^+-dependent leakage current (Fig. 3) gated via cation channels whose reversal potential is approximately 0 mV and whose conductance is 24 pS. This study provides a foundation for further investigation of whether and how other learning and memory mutant genes are involved in these central neurotransmissions.

Synaptic Transmission in the Mushroom Body

Y. Wang, N. Wright

Several lines of evidence have indicated that the mushroom body is the CNS structure carrying out olfactory-related associative learning in insects. By

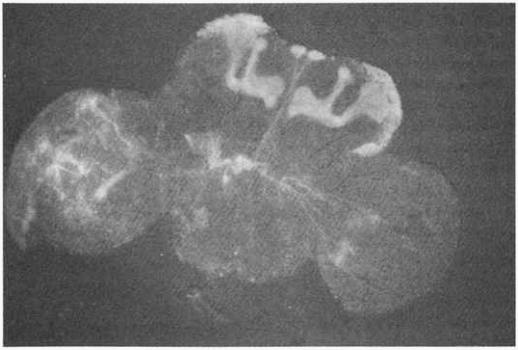

FIGURE 4 Mushroom body neurons revealed by the expression of green fluorescence protein. In this whole-brain preparation, mushroom body perikarya, calyx, and α, β, δ lobes are clearly identifiable by green fluorescence.

expressing green fluorescence protein in the GAL4 enhancer-trap lines, we have been able to identify mushroom body neurons in a whole-brain preparation (Fig. 4). This has allowed us to apply extracellular and intracellular recordings to these small neurons (3–8 μm). Although action potentials were recorded from these neurons, the recording was very difficult. We are now experimenting to determine whether using the brain slice and the patch-clamp method will make the recordings more accessible.

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CELLULAR MECHANISMS OF ACTIVITY-DEPENDENT NEURONAL DEVELOPMENT

H. Cline B. Burbach, J. Edwards, I. Miloslavskaya, E. Pinches (URP), S. Witte, Y. Wu, D.-J. Zou

The role of neuronal activity in shaping circuits within the brain has been apparent since the pioneering work of Hubel and Weisel, who demonstrated that visual experience governs the formation of synaptic connections within visual cortex. Despite the widespread recognition that the developing brain can be influenced by activity, we have little understanding of the how neurons translate "experience" into modified neuronal growth and the strengthening of synaptic connections.

The focus of my research is to determine the cellular mechanisms by which neuronal activity modifies the development of structure and function in the central nervous system (CNS). We perform our studies using the developing retinotectal projection of the frog *Xenopus*. I have recently applied several analytical approaches to this system which allow us to assess and manipulate the coordinated development of neuronal morphology and synaptic transmission in the CNS. One approach is to use imaging of single retinal axons or tectal neurons in the intact anesthetized animal to observe their morphological development for up to 5 days in vivo with time-lapse confocal microscopy. A second approach is to use a whole brain preparation to take whole-cell patch clamp recordings from neurons at different stages in the process of maturation. We can record from tectal neurons as they receive their first synaptic contact from retinal afferents and track the development of the synaptic connections as they mature. This is one of the only experimental systems that permits direct recordings of synapse formation and maturation in the intact CNS. Furthermore, I have developed the ability to use viral vectors to deliver genes of interest into frog neurons. We are now using this range of techniques to test the hypothesized roles of synaptic activity and activity-regulated proteins in the coordinated development of the structure and function of the CNS.

My research has been directed toward testing the following working hypothesis: Retinal axons form initial synapses with tectal neurons, and these initial synapses are only maintained if their activity is correlated with other converging synapses. The selection process for recognizing and maintaining correlated synapses is based on NMDA receptor activity, which requires significant depolarization in order to relieve the voltage-dependent magnesium block of the associated channel. The resultant calcium influx through NMDA channels into tectal cell dendrites is hypothesized to trigger biochemical events that result in the stabilization of the coactive synapses. The hypothesis

predicts that the structural and functional dynamics of the system should be modified in an NMDA receptor- and calcium-dependent fashion.

Dynamic Growth of Normal Axons

H. Stier, I. Miloslavskaya, S. Witte, H. Cline

To test for rapid activity-dependent changes in neuronal morphology, we needed to observe directly the dynamic rearrangements of retinal axon arbors and tectal cell dendrites growing with the intact animal. Albino *Xenopus* are ideally suited for in vivo imaging studies. Because the animal is transparent, the CNS can be observed in the intact anesthetized animal by placing it on the stage of a laser scanning confocal microscope. We take confocal images of single axons or tectal neurons labeled with the fluorescent dye, DiI. Use of the confocal microscope permits us to acquire high-resolution images of the three-dimensional structure of the growing neurons. By taking images at intervals ranging from several minutes to several days, we have collected data on the continuum of events underlying the elaboration of the arbor.

Changes in neuronal structure can contribute to the plasticity of neuronal connections in the developing and mature nervous system; however, the expectation that changes in neuronal structure would occur slowly precluded many from considering structural changes as a mechanism underlying the synaptic plasticity that occurs over a period of minutes to hours. We have taken time-lapse confocal images of retinotectal axon arbors to determine the time course, magnitude, and distribution of changes in axon arbor structure within living *Xenopus* tadpoles. Images of axons were collected at intervals ranging from 3 minutes, 30 minutes, and 2 hours over total observation periods up to 8 hours. Branch additions and retractions in arbors imaged at 3- or 30-minute intervals were confined to shorter branches. Sites of additions and retractions were evenly distributed throughout the arbor. The average lifetime of branches was about 10 minutes. Branches of up to 10 μm could be added to the arbor within a single 3-minute observation interval. In arbors imaged at 2-hour intervals over a total of 8 hours, morphological changes included longer branches tens of microns in length. An average of 50% of the total branch length in the arbor was remodeled within 8 hours. The data suggest that the elaboration of the arbor occurs by the random addition of branches throughout the arbor, followed by the selective stabilization and elongation of a small fraction of the new branches and the retraction of the majority of branches.

Growth cones have been implicated in branch formation and process extension; however, these structures have not been observed in retinal axon arbors as often as might be expected given the high degree of branch addition and elongation in these arbors. One possibility is that growth cone structure may be transient and previous observations at intervals of 1 hour or more may have been too infrequent to detect specializations at the growing branchtips. Observations of arbors at 3-minute intervals show rapid changes in the structure of branchtips, including transitions from lamellar growth cones to more streamlined tips, growth cone collapse, and re-extension. Simple branchtips that may not be readily identifiable as growth cones in single images appeared motile and capable of exploratory behavior when viewed in time-lapse movies. These data indicate that branchtip morphology is dynamic in vivo and suggest that identification of tips as "growth cones" requires a more functional analysis of motility, rather than an analysis of structure at a infrequent time points.

Calcium Imaging in Retinal Arbors

J. Edwards, H. Cline

Now that we have documented the rates of morphological changes in retinal axon arbors and in growth cones, we have begun to test whether changes in $[Ca^{++}]_i$ correlate with neuronal growth and growth cone navigation in vivo, and whether experimentally decreasing $[Ca^{++}]_i$ changes these aspects of neuronal development. We have been able to image changes in $[Ca^{++}]_i$ in the growing axon arbors and growth cones in vivo. Our initial experiments in this project indicate that branch additions are spatially and temporally correlated with transient increases in local calcium.

Development of Glutamatergic Retinotectal Synapses

G.-Y. Wu, H. Cline [in collaboration with R. Malinow, Cold Spring Harbor Laboratory]

The development of glutamatergic synapses in the CNS has been difficult to study because most experi-

mental systems are relatively intractable during the period of synaptogenesis. To test the hypothesis that the maturation and stabilization of retinotectal synapses require NMDA receptor activity, and subsequent calcium-dependent events, we developed a preparation of the isolated intact tadpole brain in which we can examine developmental changes in synaptic connections. The tadpole optic tectum is particularly advantageous for the study of neural development because new neurons are constantly being generated in the caudal region of the tectum. Consequently, at any time in tadpole development, there exist neurons in the tectum at a wide range of developmental stages, arranged conveniently along the rostrocaudal axis of the tectum. Neurons in the caudal part of the tectum have recently differentiated: They are morphologically simple and have just received their first retinotectal synaptic contacts. Neurons in rostral tectum are relatively mature: They are morphologically complex, they have many retinotectal inputs, and they process visual information. We have taken whole-cell patch clamp recordings from tectal neurons at all stages of maturation present in the tectum of stage 44–50 tadpole brains.

G.-Y. Wu analyzed evoked retinotectal synaptic currents recorded from tectal neurons at different positions along the rostrocaudal axis of the tectum. Retinotectal evoked synaptic currents recorded from neurons in rostral tectum show classical glutamatergic voltage sensitivity and pharmacology, indicating that both the NMDA and non-NMDA (or AMPA)-type glutamate receptors are present on the mature neurons. However, our recordings from younger neurons in caudal tectum suggest that the first glutamatergic retinotectal synapses are mediated purely by NMDA receptors and that synapses develop progressively from pure NMDA responses to gradually acquire mixed AMPA/NMDA responses. One interpretation of these data is that the maturation of glutamatergic synapses involves the addition of AMPA receptors to preexisting synapses where synaptic transmission was initially mediated solely by NMDA receptors.

Alternative hypotheses are that the maturation process that we observe is due to the development of the presynaptic mechanism of neurotransmitter release or changes in the rate of removal of glutamate from the synaptic cleft. We have begun additional experiments to determine whether AMPA receptors are present in the membrane of immature neurons in caudal tectum.

A Signal Transduction Mechanism Mediating Neuronal Maturation

D.J. Zou, G.-Y. Wu, I. Miloslauskaya, B. Burbach, H. Cline

Calcium entry through the NMDA receptor might trigger the stabilization of synaptic connections and neuronal morphology, by activating calcium-sensitive protein kinases such as calcium calmodulin-dependent kinase type II (CaMKII). CaMKII is a developmentally regulated protein kinase implicated in control of neuronal growth, synaptogenesis, and synaptic plasticity. CaMKII is activated by calcium entry through NMDA receptors and has several substrates relevant to neuronal development, including cytoskeletal proteins and the AMPA receptor. We are testing the hypothesis that CaMKII activity in tectal neurons may be part of the signal transduction pathway that mediates activity-dependent changes in synaptic physiology and neuronal structure.

Control of Synaptic Maturation by CaMKII

G.-Y. Wu, H. Cline [in collaboration with R. Malinow, Cold Spring Harbor Laboratory]

To test the role of CaMKII in the maturation of retinotectal glutamatergic synapses, we have increased CaMKII activity selectively in tectal neurons by infecting them with a recombinant vaccinia virus carrying the gene for the constitutively active truncated form of CaMKII (tCaMKII). The truncated CaMKII is no longer regulated by calcium/calmodulin. Expression of tCaMKII in tectal neurons increases the calcium/calmodulin-independent CaMKII activity by about 30%. G.-Y. Wu has taken patch clamp recordings from infected tectal neurons located in either mature (rostral) or immature (caudal) tectum. Our results indicate that expression of tCaMKII transforms synaptic responses in neurons from caudal tectal that normally have a low AMPA/NMDA ratio into responses that are comparable to those of mature neurons from rostral tectum, assessed by a smaller fraction of pure NMDA synapses and a larger AMPA/NMDA ratio. We found no neurons with pure NMDA responses in animals expressing tCaMKII. In control experiments, recordings from neurons that

were infected with a recombinant virus carrying only the gene for the marker protein, β-galactosidase, show that synaptic transmission was indistinguishable from that of uninfected neurons. These exciting data suggest that calcium entry through the NMDA receptor in young neurons triggers the addition of or uncovers functional AMPA receptors at postsynaptic sites.

Control of Neuronal Growth by CaMKII

G.-Y. Wu, D.-J. Zou, I. Miloslavskaya, B. Burbach, H. Cline

Developing neurons must coordinate their morphological and functional development. If CaMKII activity were involved in the activity-dependent control of neuronal growth as well as synapse maturation, this may be a pathway by which neurons could coordinate various aspects of their development. Here again, we used a recombinant vaccinia virus containing the gene for tCaMKII to increase kinase activity selectively in tectal neurons without directly changing the kinase activity in the presynaptic retinal axons. D.-J. Zou has taken in vivo time-lapse confocal images of retinal axons to observe directly the effect of increased postsynaptic CaMKII activity on the elaboration of axonal morphology. These experiments have demonstrated that elevated tectal cell CaMKII activity results in retinal axons that have simpler morphologies due to a selective retraction of short branchtips.

G.-Y. Wu has also examined the effect of increasing CaMKII activity on the development of tectal neuron morphology. Our results indicate that neurons expressing tCaMKII have simpler morphologies than normal neurons. In contrast to the effect of elevated tectal cell CaMKII activity on presynaptic axon arbor growth, it appears that the elevated CaMKII activity in tectal cells specifically prevents the addition of new branches and the elongation of preexisting branches, but does not cause retraction of branches.

These observations on neuronal morphology, taken together with the electrophysiology data, in which it appears that elevated CaMKII promotes the maturation of synapses, suggest the following type of coordinated regulation of neuronal structure and function: When NMDA receptors are active and there is calcium influx, the resultant increase in CaMKII activity has two distinct effects on the tectal cell. One is to promote the maturation of the synaptic physiology and the other is to deter additional growth of the dendrite at the local site where CaMKII activity was elevated. As a result of the decreased branch addition and branch extension in the tectal neurons, the retinal axons retract the newly added short branchtips that have failed to establish synaptic contacts with target neurons. In our experiments, we increase CaMKII activity throughout the tectal cell, but in the normal neuron, NMDA receptor activity, calcium influx, and CaMKII activity would occur at discreet sites within the arbor corresponding to sites of converging inputs. This would result in highly localized control of synapse maturation and neuronal growth.

Activity-regulated Genes

H. Cline, B. Burbach [in collaboration with P. Worley, Johns Hopkins University, and E. Nedivi, The Weismann Institute]

In addition to allowing us to examine the role of CaMKII in neuronal development, the gene-transfer method with vaccinia opens up the possibilities of testing the postulated roles of several activity-regulated proteins in neuronal plasticity. I have established collaborations with Paul Worley at Johns Hopkins University and Elly Nedivi at the Weismann Institute, each of whom has isolated several genes and their protein products that are regulated by neuronal activity and during development. We are beginning to make recombinant vaccinia virus constructs with several of these genes. These collaborations will test the roles of these proteins in activity-dependent neural development and plasticity in vivo.

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THE BIOLOGY OF LEARNING AND MEMORY

A. Silva P. Chen, J. Coblentz, N. Fedorov, J. Kogan, Y. Cho, E. Friedman, K.P. Giese, R. Lundston

Our laboratory is studying the mechanisms of learning and memory. Our working hypothesis is that changes in cell-to-cell communication (synaptic plasticity) in the brain are at the heart of learning and memory and that pathological conditions as diverse as epilepsy, learning disabilities, and memory deficits could result from deregulation of these mechanisms. Most of our studies have been focused on the function of the hippocampus, a structure that is critical for the formation of new memories in humans and mice. I summarize below our efforts during the past year to uncover the molecular, physiological, and behavioral mechanisms involving the acquisition and storage of information in the hippocampus.

THE HIPPOCAMPUS IS INVOLVED IN SPATIAL LEARNING AND IN COGNITIVE PLANING IN MICE

Studies in rats have demonstrated that lesions of the hippocampus disrupt learning of spatial information. For example, rats with hippocampal lesions are unable to learn and remember the location of an escape platform in a maze. Similarly, in this last year, we have demonstrated that mice with hippocampal lesions are profoundly impaired in spatial learning tasks. Even small lesions of the hippocampus (>20%) completely abolish spatial learning while not affecting performance in other control learning tasks. These results were important because they confirmed that it is justified to look for electrophysiological deficits in mutant mice with specific spatial learning impairments.

Surprisingly, we found that mice with hippocampal lesions that were first trained to swim to a marked platform (we marked it with an object on it) were subsequently able to find an unmarked platform by using distal objects in the walls of the room. Thus, mice with prior training on the maze seemed to be able to learn spatial information despite the lesion of the hippocampus. This unexpected result indicates that the hippocampus may be required to design cognitive strategies for solving complex problems and that it may not be essential to learn spatial information itself. This finding has important implications for the design of therapies for human patients with temporal lobe lesions. These patients normally show a profoundly incapacitating amnesia with very few available therapies.

GENERATION OF A POINT MUTATION IN THE CALMODULIN-BINDING DOMAIN OF αCAMKII

Gene-targeting studies of learning and memory have been based on mice with complete or partial deletions of chosen genes. However, to test specific functional hypothesis, it is often very useful to alter instead specific functional domains of an otherwise intact gene. For example, studies based on mutants with a complete deletion of the αCaMKII indicated that this enzyme has a key role in the modulation of both short- and long-term synaptic plasticity, as well as in learning and memory. To further explore the function of this enzyme in neuronal cells, we have generated a mouse with a single-amino-acid mutation in a critical domain of this kinase. At basal calcium levels, unphosphorylated CaMKII is inactivated in less than a second, whereas autophosphorylated CaMKII might remain active for minutes or even hours. After autophosphorylation at Thr-286, the affinity of the enzyme for Ca/CaM increases 1000-fold. Previous biochemical studies showed that the substitution of Thr-286 by alanine ($\alpha CaMKII_{T286A}$) does not disturb enzyme function in high Ca/CaM, but blocks its ability to trap Ca/CaM and to remain active without increased Ca/CaM levels. Using the Cre/Lox system, we have generated this mutation in embryonic stem cells and have derived the mutant mice. With this mutant, we are currently studying how the biochemistry of this enzyme affects the electrophysiological properties of neuronal cells. For example, we postulated that the increase in neurotransmitter release observed in the $\alpha CaMKII^{+/-}$ mutants (mice heterozygous for a null mutation of the αCaMKII) is due to less calmodulin trapping caused by the partial loss of αCaMKII. Consequently, the $\alpha CaMKII_{T286A}$ mutant should have enhanced release because the calmodulin-binding domain is impaired. This and other related mutants will provide an array of related physiological phenotypes that will be useful to test hypotheses about the function of this enzyme in the modulation of synaptic plasticity and in learning and memory.

REQUIREMENT FOR αCaMKII IN EXPERIENCE-DEPENDENT PLASTICITY OF MOUSE CORTEX

In collaboration with Kevin Fox's group at the University of Minnesota, we have recently analyzed the impact of the αCaMKII mutation on experience-dependent plasticity in the somatosensory cortex, where neurons modify their response properties according to changes in sensory experience. We previously showed that LTP is affected in αCaMKII homozygous animals, both in the hippocampus and in the neocortex. To determine whether CaMKII and/or LTP were involved in experience-dependent neocortical plasticity, we studied changes in neocortical responses after changing patterns of tactile impute to the barrel cortex. Our findings showed that despite the lack of this kinase with a prominent role in synaptic plasticity, the neocortical responses of young mutants to changes in tactile inputs are normal. In contrast, adult mice are profoundly abnormal in these responses, suggesting that the CaMKII is necessary for the induction or the expression of plasticity in adults.

Interestingly, studies done in collaboration with the laboratory of Michael Stryker at the University of California, San Francisco, revealed evidence that αCaMKII may have a role in neocortical plasticity of visual cortex even in young animals. In this case, the sensory deprivations that trigger the plasticity changes involve shorter periods than in the experiments with the somatosensory cortex, and therefore, they may be a more stringent test for neocortical plasticity in these mutants. It is important to note that in either the somatosensory or the visual cortex, we did not find evidence for gross developmental abnormalities in these mutants: Both the topography and columnar organization of the somatosensory and visual cortices appear to be normal in the mice. Our results show that in adult mice, just as in adult monkeys and humans, changes in sensory perception can drive neuroanatomic changes in neocortical structure and that the plasticity of these events is dependent on αCaMKII

SPACED TRAINING RESCUES THE LONG-TERM POTENTIATION AND THE MEMORY DEFICITS OF MICE MUTANT FOR THE cAMP-RESPONSIVE ELEMENT BINDING PROTEIN (CREB)

Studies in *Aplysia*, *Drosophila*, and mice indicate that the transcription factor CREB has a key role in both long-term synaptic plasticity and long-term memory. Recent findings in *Aplysia* and *Drosophila* suggest that CREB activity levels are rate-limiting during learning, so that increases in its activity obviate the requirement for multiple spaced training sections and thus facilitate the induction of long-term memory. This year, we confirmed that the CREB mutation has a dramatic impact on long-term memory formation in mice.

Mice with an intact CREB gene can remember the context in which they receive a foot shock with only a single trial. The memory of this experience is not only robust, but also long-lasting. Despite intact nociceptive reactions, the same single trial can trigger a memory that lasts no more than 60 minutes in mutants lacking the α and the δ CREB proteins. Not even five trials given 1 minute apart, which in wild-type mice induces maximum performance levels in contextual memory tests, can compensate for the profound amnesia of these mutants. Surprisingly, as little as two spaced trials can trigger almost normal 24-hour contextual memory in the mutants (see Fig. 1) This result is consistent with a model, also suggested by findings in *Aplysia* and *Drosophila*, claiming that CREB activity levels are rate-limiting during learning and that increases in its activity can under certain conditions obviate the requirement for spaced training in the induction of long-term memory.

The electrophysiological analysis of the CREB mice reverberate the behavioral findings summarized above: In hippocampal slices from wild-type mice, spaced tetanization is not required to induce stable L-LTP: Either spaced (10-min ITIs) or massed (1-min ITIs) can induce similar levels of stable 7-hour LTP. In contrast, only spaced tetanization can induce normal L-LTP in CREB mutants. Massed tetanization results in smaller L-LTP that by 5–7 hours is indistinguishable from L-LTP induced in the presence of a protein-synthesis inhibitor (see Fig. 2). The provocative parallels between the behavioral and the electrophysiological results in mice, as well as related electrophysiological results in *Aplysia* and behavioral results in *Drosophila*, indicate that CREB transcription is required for long-term synaptic changes involved in memory.

THE INVOLVEMENT OF THE NF1/*ras* PATHWAY IN LEARNING

Children with neurofibromatosis type 1 (NF1) show clear learning disabilities of unknown etiology. These cognitive deficits have a devastating economic and personal cost. Because of the difficulty with studying this problem in humans, we decided to study mice

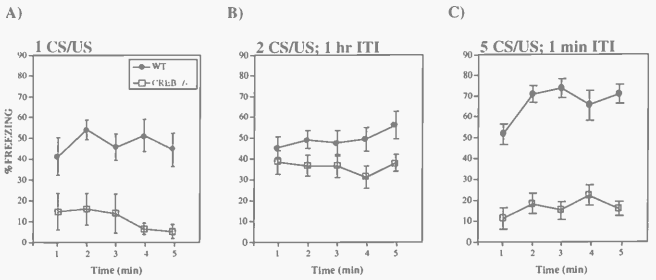

FIGURE 1 24-hour contextual memory triggered with three different conditions. (*A*) CREB$^{-/-}$ mice and littermate controls were trained with a single trial, and contextual conditioning was tested 24 hr later. Shown here are the results for each minute of a 5-minute observation period. (*B*) CREB$^{-/-}$ mice and controls were trained with two trials with 1-hr interval between trainings. (*C*) CREB$^{-/-}$ mice and controls were trained with five trials with 1-minute intervals. The results show that CREB$^{-/-}$ mutants have a profound deficit in long-term memory that can be compensated for with additional spaced training, but not with massed training.

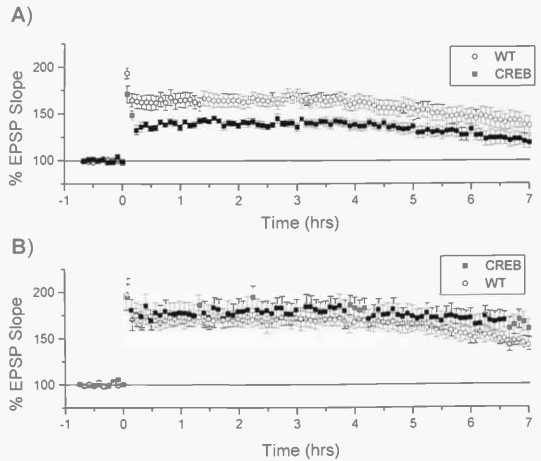

FIGURE 2 Effect of interval in 7-hr LTP of CREB$^{-/-}$ mutants and controls. (*A*) Hippocampal field EPSPs were collected every 5 min in the CA1 region (stratum radiatum) of slices from wild-type and CREB$^{-/-}$ mutants. LTP was induced with three tetanic trains of ten theta bursts with a *1-min* interval between each tetanus. (*B*) Hippocampal field EPSPs were collected every 5 min in the CA1 region (stratum radiatum) of slices from wild-type and CREB$^{-/-}$ mutants. LTP was induced with three tetanic trains of ten theta bursts, but in this case, we used *10-min* intervals between each tetanus. These results indicate that similar to memory, spaced but not massed tetanization can compensate for the LTP defect of the CREB$^{-/-}$ mutants.

with a genetic lesion similar to that present in NF1 patients. We started by determining whether there were parallels between the behavioral phenotypes of mice and humans. Since NF1 is highly expressed in the hippocampus, we concentrated our efforts on learning and memory tasks sensitive to lesions of this structure. Our studies showed that NF1 heterozygotes have highly specific spatial cognitive learning deficits, even though we and others could not identify any significant brain neuroanatomic pathology. It is important to note that NF1 mice, just as NF1 patients, do not have a complete loss of spatial cognitive abilities. Instead, we found that they need much more training than littermate controls to reach comparable levels of performance (see Fig. 3). Control tests showed that vision, motor coordination, and motivation are normal in these mice. Interestingly, just as with NF1 patients, memory seems to be unaffected by the NF1 mutation in mice, since tests showed that once trained, the NF1 mutants retain spatial information as well as controls. Since NF1 is known to regulate *ras*, we also tested directly whether the mutation

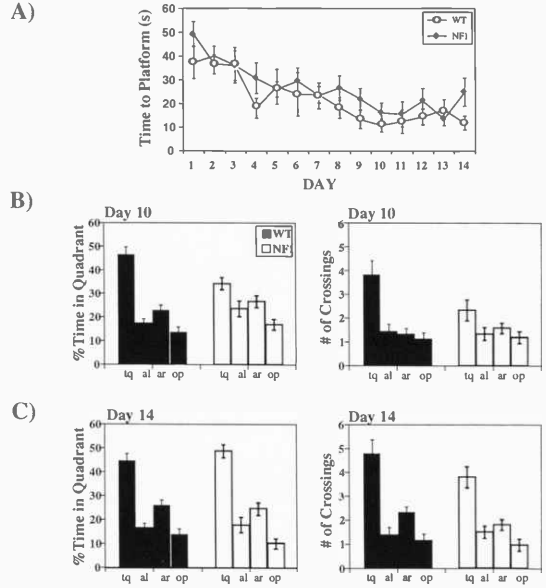

FIGURE 3 Performance of NF1$^{+/-}$ mutants and controls in the water maze. (*A*) Two trials each day training in the hidden platform version of the water maze. The mice were given two trials each day for 14 days. Both NF1$^{+/-}$ mutants and controls improved throughout training, but controls were significantly better than mutants (ANOVA; $p<0.05$). (*B*) Probe trial given after 10 days of training. The controls show greater selectivity for the training quadrant (*left panel;* ANOVA $p<0.001$) and they cross the exact site of the platform (*right panel;* ANOVA $p<0.001$) more often than controls. (*C*) Probe trial given after 14 days of training. Both controls and NF1$^{+/-}$ mutants show similar selectivity for the training quadrant (*left panel;* ANOVA $p>0.05$) and they both cross the exact site of the platform with similar frequencies (*right panel;* ANOVA $p<0.05$). (t) Training quadrant; (ar) adjacent quadrant to the right of t; (al) adjacent left; (op) opposite.

of *ras* could also affect spatial learning. We chose to study first mice lacking N-*ras* because studies of these mice showed that this mutation did not affect viability, and it seemed not to result in gross behavioral abnormalities. Our studies showed that the N-*ras* deletion does not have a general impact on the behavior of these mice but that it does have a profound effect on their performance in the spatial version of the Morris water maze. Recently, we have also found evidence that mice mutant for Ki-*ras* have a similar phenotype.

To further the analysis of the involvement of the *ras* pathway in learning and memory, we have recently generated mutants for a brain-specific exchange factor called GRF. This protein is thought to be activated by calcium/calmodulin, and it is therefore an important link between the calcium-mediated second-messenger pathways and *ras*-dependent pathways. The null mutants should result in less active *ras* in response to increases in neuronal calcium. We are currently analyzing the behavioral and electrophysiological effects of this mutation.

THE GENERATION OF A NULL MUTANT FOR THE β-1 SUBUNIT OF POTASSIUM CHANNELS

Most studies of the cellular basis of learning and memory have focused on synaptic plasticity. However, cells express a complex variety of electrophysiological properties that may also be involved in information storage and retrieval. For example, hippocampal neurons have a highly complex array of ion channels that control their firing properties. These neurons can show surprisingly intricate rhythmic behaviors that may be involved in coordinating the responses of neuronal ensembles. Thus, the modulation of intrinsic ion channels might be important for learning/memory.

Potassium channels show the highest diversity among ion channels, and there is indirect evidence that their modulation is crucial for cognitive processes. Thus, we decided to generate a mouse lacking the modulatory potassium channel subunit Kvβ1. Kvβ1 associates with Shaker-related potassium channel subunits, which form voltage-gated potassium channels (A channels or delayed rectifiers). The presence of Kvβ1 leads to an A-type inactivation and to a decrease in recovery time of these channels. Kvβ1 is expressed at high levels in the caudate putamen and in the hippocampal CA1 region. We obtained homozygous null mutants in a Mendelian ratio and could not detect any overt phenotype. We tested the mutants in several behavioral paradigms and found a very specific phenotype: The mutants behaved normally in an open field, showed no abnormalities in contextual conditioning (24 hr or 11 days after training), and were not affected in spatial learning in the Morris water maze. However, the mutants were impaired in reversal learning in the Morris water maze, as if acquired information is less flexible in the absence of this channel subunit. Initial electrophysiological studies show that CA1 neurons in hippocampal slices have decreased spike accommodation and there are also changes in hyperpolarization.

Altogether, the results summarized here, as well as results from previous years and from other laboratories, demonstrate that genetics is an effective approach in the search for the cellular mechanisms underlying learning and memory.

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NEUROTROPHIC FACTORS IN NEURAL PLASTICITY AND DEVELOPMENT

H. Nawa K. Mizuno
R. Lundsten
M. Waga

A large variety of neurotrophic factors have been identified in the central nervous system (CNS) and are now being extensively studied with respect to their application to neurological deseases. Many of these polypeptide factors were initially identified by monitoring their activity to promote neuronal survival and are thus called "neurotrophic factors." However, neurotrophic factors have other activities that influence synaptic transmission, organization, and plasticity. In addition, many growth factors and various cytokines can also exert similar activities in the CNS. Such polypeptide factors must therefore be understood as general signal mediators in all types of intercellular responses in the CNS. The number of neurotrophic factors, cytokines, and growth factors found in the brain totals more than 30. These factors have been proven to influence synaptic plasticity and development and contribute to learning and memory processes in the brain. Some of the factors act directly on the synapse and quickly alter amounts of neurotransmitter release, whereas other factors change neurotransmitter and its receptor synthesis by regulating their gene expression.

Our latest studies have shown that brain-derived neurotrophic factor (BDNF), which belongs to the nerve growth factor family (i.e., neurotrophin), elevates expression of inhibitory neuropeptides and glutamate receptors in cultured forebrain neurons. In contrast, activin, which is a member of the transforming growth factor (TGF) family, suppresses the expression of the receptors as well as various protein kinases. Our findings suggest that such polypeptide factors in the brain positively and negatively regulate production of synaptic molecules and are involved in synaptic development and plasticity.

Our current efforts have been focused on the three projects: (1) molecular mechanism of neurotrophins' activities in synaptic functions, (2) physiological significance of neurotrophins' activities in vivo, and (3) regulation of synaptic development by neurotrophic factors. These studies will elucidate how such neurotrophic factors contribute to neural plasticity such as learning and memory processes.

Effects of BDNF on Hippocampal Kindling

H. Nawa [in collaboration with D. Antoine, University Louis Pasteur, France]

It has been well demonstrated that production of BDNF is regulated by neuronal activity, i.e., stimulation of glutamate receptors increases BDNF mRNA in neurons from various parts of the brain as do seizures and intense bursts of high-frequency afferent activity. These observations raise the possibility that BDNF might mediate various activity-dependent processes, including synaptic plasticity and neuronal circuit reorganization. We have previously shown that one of the remarkable activities of BDNF on forebrain neurons is to regulate expression of the putative inhibitory neuropeptide, neuropeptide Y, and somatostatin.

To investigate the physiological consequences of the BDNF increase after seizures, chronic intrahippocampal perfusion of BDNF was carried out in the dorsal hippocampus kindling model in the rat. A 7-day perfusion of BDNF, in the region of the stimulating electrode, blocked the development of kindling during the perfusion period and for the following 15 days. Timm's staining revealed that the induction of axonal sprouting in the dentate gyrus was observed in kindled animals but not in the BDNF-treated animals or nonkindled controls. These observations suggest that the neurotrophic factor is involved in prevention of kindling epileptogenesis presumably by regulating neurochemical features of hippocampal synapses.

Downstream Genes of BDNF in Synaptic Organization

K. Mizuno, M. Waga [in collaboration with K. Okamoto, Cold Spring Harbor Laboratory]

BDNF can influence the pattern of thalamocortical projection and may be involved in the synaptic orga-

nization or stabilization in the developing neocortex. Although such phenomena have been well studied at a cellular level, the molecular mechanism of the BDNF function remains to be determined. To address the question, we are characterizing downstream genes of BDNF by using the differential display polymerase chain reaction (PCR) method. Cultured neocortical neurons prepared from rats at late embryonic stages were treated with BDNF, as well as another neurotrophin, NGF, and mRNA was extracted from these cultures. After displaying the PCR products from the mRNA, we have identified more than ten bands of cDNA that were more efficiently amplified from mRNA extracted from the BDNF-treated neurons than from mRNA extracted from the NGF-treated or untreated neurons. Northern analysis revealed that mRNA from clones CB4 and CB15 was most markedly induced by the treatment of BDNF among all clones isolated. CB4 mRNA is expressed preferentially in the brain and the muscle, whereas CB15 mRNA is widely distributed in almost all the tissues. Currently, we are trying to isolate their full-length cDNA clones to elucidate the entire nucleotide sequences.

Activin Regulates Development of Cortical Pyramidal Neurons

R. Lundsten, H. Nawa

Embryonic development is known to be regulated by a variety of polypeptide factors. Among them, members of the TGF family are extensively studied on germ-layer differentiation. We have examined effects of the family on immature neocortical neurons. In cultured neocortical neurons prepared from rat embryos, activin, but not TGF-β nor bone morphogenic proteins (BMPs), decreased protein levels of calmodulin-dependent protein kinase II, AMPA receptors, and glutaminase, which are all expressed predominantly by cortical pyramidal neurons. Expression of activin itself is regulated in the neocortex during development. The highest expression of activin mRNA was found in neonatal rats, and its level decreases sharply in the postnatal period. Moreover, activin receptor-like immunoreactivity was found in pyramidal neurons in cortical layers II-III. These results therefore suggest that activin might play a part as a negative regulator for coordinate development of immature neocortical neurons.

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TRANSMISSION AND PLASTICITY IN MAMMALIAN CENTRAL SYNAPSES

R. Malinow N. Dawkins T. Koothan M. Maletic-Savatic
B. Frenguelli D. Liao A. Shirke
H. Hinds Z. Mainen A. Weissmann

We address issues directed toward an understanding of learning and memory by studying the physiology of synapses. We study synaptic transmission in rat brain slices, which is complex enough to show glimpses of emergent properties as well as simple enough to allow hard-nosed biophysical scrutiny. It is our philosophy that synapses have some key properties whose understanding is possible and that such an understanding will provide insight into phenomena at higher levels of complexity.

The skeleton of synaptic transmission and plasticity involves (1) 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, (4) postsynaptic receptor binding of transmitter and channel opening, (5) entry of calcium with activation of calcium-activated enzymes if certain postsynaptic channels are opened, and (6) long-lasting changes at these synapses triggered by these enzymes. At each of these steps, there is the possibility for modulation, and thus we want to understand each step completely.

Our second year here at the Laboratory has been exhilarating. One postdoc left (Bruno Frenguelli) for a job as assistant professor at the University of Dundee, Scotland. One postdoc arrived (Zach Mainen) from the University of California at San Diego.

Dendritic Exocytosis Is Mediated by Activation of αCaMKII

M. Maletic-Savatic, R. Malinow

We have described a novel cell biological process in neurons using the fluorescent dye FM1-43. This dye is applied in the medium and taken into cultured neurons by endocytosis. If neurons are exposed to this dye for more than 16 hours, most (>95%) of the internalized dye ends up in intracellular compartments in the dendrites and cell bodies. We have found that a rise in intracellular calcium (produced by application of a calcium ionophore, A23187) causes fusion of these intracellular compartments with the plasma membrane and exocytosis of the internalized dye. We call this process calcium-evoked dendritic exocytosis (CEDE). We know that CEDE is exocytosis because it is blocked by tetanus toxin, a selective inihibitor of exocytosis.

CEDE is developmentally regulated: Only neurons cultured 9 days (9 DIV) or longer show CEDE; younger neurons will take up FM1-43 but will not release it when exposed to calcium ionophore. We have sought to find what factors allow 9 DIV neurons to show CEDE and believe that this may be due to the presence of the enzyme αCaMKII. We find that αCaMKII is expressed in dendrites only when CEDE is present. CEDE can be blocked by KN-62, an inhibitor of αCaMKII. The most conclusive experiment is one in which we take neurons that are 7 DIV, which neither have αCaMKII nor show CEDE, and infect them with a recombinant vaccinia virus that makes αCaMKII. These infected neurons now show CEDE. What is the role of CEDE? See below.

Is CEDE Involved In Long-term Potentiation?

A. Shirke, R. Malinow

CEDE is triggered by postsynaptic calcium and mediated by αCaMKII. Similarly, long-term synaptic potentiation (LTP) also requires a rise in postsynaptic calcium, and αCaMKII is thought to be both necessary and sufficient to generate this synaptic plasticity. To test directly if CEDE is necessary for LTP, we have conducted a series of experiments using several well-characterized toxins. We are currently determining the exact role of CEDE in synaptic plasticity.

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 asked if the rise in presynaptic calcium produced by an action potential is always the same or varies 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 can assess the role of the variability in calcium rise on transmitter release. 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 ac-

tion potential. It will be interesting to determine if this fluctuation in calcium rise can be modulated by activity or pharmacological agents.

"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 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; NMDA-type receptors require both glutamate and membrane depolarization. This year, we published 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, indicates that there are synapses with only NMDA receptors. We recently published several lines of evidence that pure NMDA synapses add AMPA receptors during LTP.

More recently, we have looked at the developmental progression of these pure NMDA synapses. We find that earlier in development in the hippocampus, there are more synapses that are pure NMDA. 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 (which have AMPA receptors). If the pure NMDA-receptor synapses are potentiated by LTP, they will now have AMPA receptors added to them and will be able to transmit information without the need for coactivation with other synapses.

Is Synaptic Transmission in the Brain a Point-to-Point Process?

Z. Mainen, R. Malinow

Although the traditional view of a synapse is that each presynaptic terminal releases transmitter that affects receptors on a single postsynaptic spine, there have been recent suggestions that this may not be so. It could be that transmitter released from one presynaptic terminal affects receptors on many nearby synapses. This is made plausible by the observation that some glutamate receptors (NMDA-type) have a 100-fold greater affinity for glutamate than other receptors (AMPA-type). Thus, any spillover of glutamate from one synapse could activate NMDA receptors on nearby synapses. This would have several important implications regarding information transfer and mechanisms of plasticity (e.g., since NMDA receptors need to be activated to induce LTP). Furthermore, this view would allow us to reinterpret the findings regarding "silent synapses" described above. We have begun to address this and several other related issues concerning basic mechanisms of synaptic function using a novel compound, caged glutamate. This synthetic compound is inert to neurons in its native form. When exposed to UV light, a bond is broken and glutamate is instantly released. By exposing neurons to caged glutamate and flashing UV light, known amounts of transmitter can be delivered to synapses in a manner that mimics presynaptic release of transmitter. We have evidence that the kinetics of an NMDA response depends on the concentration of transmitter to which it is exposed. We can thus determine the concentration of "flashed" transmitter that mimics the synaptic response. We have preliminary evidence that (1) pure NMDA synaptic responses exist because there are only functional NMDA receptors at some synapses and not because of spillover and resulting lower concentrations of transmitter at nearby synapses; (2) transmitter concentration during presynaptic release may be considerably lower than previously thought (by 100-fold); and (3) increases in probability of release produces release of more than one vesicle per synapse. With this handle on synapses, several related issues, including the locus of change during LTP, may be amenable to direct observation.

Use of Recombinant Viruses to Elucidate Mechanisms in LTP

T. Koothan, H. Hinds, R. Malinow

We continue to use recombinant vaccinia virus to test specific hypotheses regarding synaptic function and plasticity. In particular, we are generating constructs that will allow us to test whether AMPA receptors are rapidly delivered to the postsynaptic membrane during the generation of LTP. We will make an AMPA receptor with green fluorescent protein (GFP) on the extracellular part of the receptor. Similar fusion proteins have been made successfully by others. In addition, we will insert a thrombin cleavage site between the receptor and the GFP. Thus, if this construct is exposed to the extracellular membrane, it can be cleaved by thrombin and GFP will be released. We therefore plan to express this construct in neurons and expose them to thrombin and will then monitor fluorescence during the induction of LTP. A decrease in fluorescence will indicate that this construct was delivered to the plasma membrane (exposing it to extracellular thrombin).

We have begun to see if we can rescue LTP in slices from CaMKII knock-out animals by introducing CaMKII in slices with a recombinant vaccinia virus.

Chimeric Constructs to Study the Role of CaMKII in Neuronal Function

A. Weissmann, R. Malinow [in collaboration with Grigori 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 and expect 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.

CaMKII Promotes Maturation and AMPAfication of Developing Synapses

R. Malinow [in collaboration with Gang-Yi Wu and Hollis Cline, Cold Spring Harbor Laboratory]

The tadpole optic tectum provides several opportunities to analyze the physiological development of synapses. These synapses resemble hippocampal synapses (same transmitter and receptors). However, the tectal cells are much more compact and thus afford considerably better signal-to-noise resolution in electrophysiological measurements. In addition, the cell bodies are conveniently placed in a developmental pattern: Older cells are more rostral.

We find that initial synapses appear to be pure NMDA and that AMPA responses appear during development. This developmental process appears to mimic plasticity in hippocampus: LTP causes the addition of AMPA receptors to pure NMDA synapses. Furthermore, we find in the tectum that expression of a constitutive CaMKII construct in postsynaptic cells promotes physiological maturation: Young cells expressing constitutive CaMKII show AMPA responses. The ability to make detailed recordings and to perturb the system with molecular precision should allow a comprehensive understanding of the physiological maturation of synaptic transmission.

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 AND NEURONAL DIFFERENTIATION

G. Enikolopov J. Chubb B. Kuzin
N. Hellman N. Peunova
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P. Krasnov A. Weissmann

In our laboratory, we are interested in the molecular mechanisms responsible for linking the activity and differentiation of neuronal cells. In particular, we are studying signal transduction by diffusible messenger molecules to determine whether they provide such a link. We have shown that one of these molecules, nitric oxide (NO), mediates the survival functions of growth factors in neuronal cells and that it participates in the normal development of *Drosophila*. This is the first indication that gaseous messengers are important for development.

We are also developing new tools to dissect and manipulate complex signaling pathways in neurons. Our particular focus is on the role of protein kinases in regulatory cascades in neurons. We are trying to design conditionally active forms of proteins and to target chimeric proteins to the terminals of neuronal cells. We have found protein motifs that are necessary and sufficient for transporting proteins to nerve terminals and are using them now to uncouple signaling cascades at the terminal from the rest of the neuronal cell.

Nitric Oxide Mediates Survival of Neuronal Cells

N. Peunova, S. John, G. Enikolopov

Programmed cell death by apoptosis regulates normal development and tissue homeostasis in eukaryotes. Differentiated neuronal cells are dependent on growth factors for survival. Nerve growth factor (NGF) is a survival factor for sympathetic neurons and for PC12 cells, and when NGF-differentiated PC12 cells are deprived of growth factors, they undergo apoptosis. We have recently shown that NO mediates the NGF-driven neuronal differentiation of PC12 cells by initiating growth arrest during the early stages of NGF action. Now we have found that during the later stages of NGF action, in fully differentiated PC12 cells, NO also mediates the survival functions of NGF.

We have observed that NO, like growth factors, can inhibit apoptosis of differentiated PC12 cells after NGF withdrawal. When NO-releasing compounds were added to fully differentiated cells before or immediately after withdrawal of NGF, cell death was markedly reduced, demonstrating that NO can partially substitute for NGF in preventing apoptosis. This suggests that endogenously produced NO, like that produced by NO donors, could contribute to cell survival. Indeed, inhibition of nitric oxide synthase (NOS) in fully differentiated cells led to massive death by apoptosis. Importantly, no apoptosis was observed when a similar treatment was applied to naive PC12 cells or to cells that had been treated with NGF for 2 days (and hence, had only just begun the differentiation process). Similarly, inhibition of NOS did not impair the survival of mutant U2 cells, which retain the early steps of the response to NGF but have lost the capacity to differentiate; they are also impaired in the ability to induce NOS expression in response to NGF, and their capacity to differentiate can be rescued by applying NO. Together, our results with differentiated, naive, and mutant cells indicate that NOS mediates the survival functions of NGF. Fully differentiated PC12 cells develop a dependence on NO production for their survival, so that inhibition of NOS activity leads to cell death.

A possible explanation of the role of NO in preventing apoptosis may involve its antiproliferative properties. NO may prevent cells from traversing the cell cycle, thereby keeping them from reaching a critical point where mitogenic signals contradict the established cytostatic condition of the cells, and prompting them to undergo apoptosis. Indeed, within 6–12 hours after the addition of the inhibitor and before any morphological signs of apoptosis are seen, a large fraction of cells (>30%) started to incorporate bromodeoxyuridine (BrdU). This represents a five- to sixfold increase compared with the results obtained with control NGF-treated cells, consistent with the idea that the antiproliferative properties of NO contribute to the repression of the cell cycle machinery in differentiated neuronal cells.

An additional mechanism for the inhibition of apoptosis by NO may involve direct activation of an anti-apoptotic program by NO, for example, by changing the levels of expression of proteins that prevent or promote apoptosis. We tested whether treatment of PC12 cells with NO changes the expression of genes involved in regulating programmed cell death and whether these changes paralleled those induced by NGF. Prolonged treatment of PC12 cells with NGF leads to an increase in the level of Bcl-$x/_L$, a product that, like Bcl-2, can prevent apoptosis in many cell types. At the same time, the amount of Bax, a protein that counters the ability of Bcl-2 and Bcl-$x/_L$ to repress apoptosis, decreased with a similar time course. In contrast, the levels of these proteins are unchanged in the mutant U2 cells. Thus, NGF-induced differentiation of PC12 cells changes the expression of genes involved in the cell death program in favor of products that prevent apoptosis. This may lead to a switch of the cell program toward prevention of cell death, in line with the known role of NGF as a survival factor.

To determine whether part of the effect of NGF on the apoptosis machinery can be mediated by NO, we subjected PC12 cells to sustained treatment with NO donors. The changes in expression of a panel of apoptosis-related proteins were very similar to the changes evoked by NGF treatment. When the same donors were added to U2 cells (NGF-treated and untreated), the changes observed were identical to those seen in wild-type PC12 cells. Taken together, the responses of mutant and wild-type cells to NGF and NO indicate that NO can prevent apoptosis both by contributing to the growth arrest of the cells and by actively inducing their anti-apoptotic defense, further supporting the hypothesis that NO mediates part of the survival functions of NGF.

In the brain, NO production is associated with the death of neurons by necrosis and apoptosis after an ischemic stroke and, perhaps, neurodegenerative diseases. Paradoxically, NOS-positive neurons in the brain are remarkably resistant to damage, and most of them are spared after exposure to strong insults and diseases that kill other cells. Our results suggest that preexposure to sustained NO production may be responsible for the increased resistance of NOS-positive neurons to damage. Although apoptosis can be initiated by numerous different stimuli, both the control mechanisms and the execution mechanisms of programmed cell death seem to be remarkably uniform and appear to involve a relatively limited set of genes. The ability of NO to induce an anti-apoptotic program and prevent programmed cell death after withdrawal of growth factor may reflect its physiological function in the brain during disease. We propose that the unusual resistance of NOS-positive neurons in the brain is related both to the role of NO in maintaining growth arrest and to its ability to fortify the cellular anti-apoptotic defense, which may later protect the cells from insults.

Nitric Oxide Is Essential for the Development of *Drosophila*

B. Kuzin, N. Peunova, Y. Stasiv, G. Enikolopov
[in collaboration with I. Roberts, Cambridge University]

We have recently found that NO can affect differentiation in neuronal cells by inducing growth arrest and by mediating the survival function of growth factors. Now we want to examine whether NO has a similar role during the normal development of organisms, acting as an essential antiproliferative agent during cell differentiation.

We have chosen *Drosophila* as a model organism for our developmental studies. We have found that at the late stages of larval development, in the third instar larvae, and during the early stages of metamorphosis, the imaginal disks and the brain both accumulate high levels of diaphorase activity, indicating the presence of NO synthase (NOS). The diaphorase staining starts at the beginning of the third instar and intensifies toward the end of the third instar and the early stages of pupation. The staining pattern gradually becomes more defined, with some segments of the developing appendages, eyes, and the brain acquiring a very characteristic pattern of staining.

The highest levels of diaphorase staining occur during the period of development when DNA synthesis and cell division in most of the cells of the imaginal disks and the brain slow down. DNA synthesis and cell division resume during early pupation and then stop again before the final stages of cell differentiation occur. A very specific pattern of NOS distribution suggests that perhaps NO has a role as an antiproliferative agent, inhibiting DNA synthesis and supporting temporary cytostasis during the switch to metamorphosis.

If NO indeed acts to suppress cell division during the late stages of fly development, then inhibition of NOS might trigger excessive growth of organs and tissues, whereas ectopic overexpression of the NOS

gene might lead to reduced growth of organs and tissues.

To test this hypothesis, we inhibited NOS activity in the developing larvae by injecting NOS inhibitors at the end of the third period, several hours before metamorphosis. The resulting adult flies had exhibited numerous changes, the most dramatic being enlargements of the appendages, especially in the third pair of legs. Certain segments of the legs increased three- to fourfold in diameter, reflecting an almost tenfold overall increase in size. The number of bristles also increased, confirming that hyperproliferation of the tissue had occurred. Identical changes were observed when we used two structurally unrelated inhibitors of NOS, thereby confirming the specificity of their action. The segments most affected were those whose primordia had the highest levels of NOS at the early pupal stages.

For the reciprocal approach, the ectopic upregulation of NOS activity, we used transgenic *Drosophila* lines that contain the mouse gene for NOS2 (inducible NOS) under the control of a heat shock promoter, which were generated by Dr. Ian Roberts (Cambridge University). When transgenic larvae were heat-shocked shortly before pupation (approximately at the time when the action of the inhibitor is most effective), some segments of the resulting imago flies (the legs in particular) were reduced in size. Remarkably, the segments most often affected by the overexpression of NO were those that were not affected by the NOS inhibitors and whose precursors in the larval and pupal stages demonstrated particularly low levels of diaphorase staining.

The distributions of the segments that increased in size when NOS activity was inhibited and the segments that decreased in size when NOS activity was ectopically up-regulated were complementary and corresponded to the distribution of NOS. Taken together, these results support the hypothesis that NOS acts as an antiproliferative agent during cell differentiation and normal development of the fly.

The manipulation of NOS activity in the developing fly led to numerous changes not only in appendages, but in various other organs of the imago as well. These changes were again consistent with the hypothesis that NO acts as an antiproliferative agent during development. We are particularly interested in the role of NO in differentiating neurons, and our future experiments will focus primarily on elucidating the role of NO in the developing brain.

In addition to genetic experiments, we are trying to understand the molecular mechanisms of NO action in fly development, in particular, which genes regulate the synthesis of NO during development and what the molecular targets of NO action are during development.

Protein Transport to the Nerve Terminals

P. Krasnov, J. Chubb, N. Hellman, G. Enikolopov

The main goal of this project is to find protein motifs that are necessary and sufficient for transporting proteins to the nerve terminals, including synapses and growth cones. We intend to use these motifs to target chimeric proteins to the terminals. Our particular interest is to deliver to the terminal those proteins that can directly modify signal transduction pathways, such as protein kinases, protein phosphatases, and their inhibitors. In this way, we want to modulate the signal transduction cascades at the nerve terminal, but at the same time, we want to uncouple these perturbations from the signaling in the rest of the neuronal cell.

We tested synaptic vesicle proteins by tagging them with the HA epitope, transfecting recombinant constructs into neuronally differentiated PC12 cells, and visualizing the distribution of the chimeric molecules using immunocytochemistry. We chose one of the synaptotagmin isoforms that was especially efficient at accumulating at the nerve terminal. The distribution pattern of the recombinant tagged protein did not differ from the distribution of endogenous synaptotagmin.

We prepared a large number of deletions covering the entire coding region of the synaptotagmin gene and compared their distribution in the cell by immunocytochemistry. We found two signals, at the transmembrane domain and at the carboxy-terminal domain of the protein, that are necessary for the correct trafficking of the recombinant synaptotagmin molecule to the neuronal cell terminal.

By testing a set of more subtle deletions at the carboxyl end of the synaptotagmin molecule, we identified the amino acid residues that are crucial for transport to the nerve terminal. Deletion of as few as eight amino acids within the carboxy-terminal domain was enough to prevent the tagged recombinant molecules from accumulating at the terminal.

To test whether the signals identified as critical for transport to the nerve terminal are also sufficient for such transport, we prepared a series of constructs in

which more than two thirds of the entire molecule was deleted, leaving only the putative signal sequences intact. The intracellular distribution of these constructs was similar to (if not indistinguishable from) the distribution of the original recombinant synaptotagmin molecule and the endogenous synaptotagmin. These results demonstrate that the two identified signals are not only necessary but also sufficient for the targeting of a recombinant chimeric protein molecule to the nerve terminal.

To determine which interactions make the carboxy-terminal sequences crucial for the accumulation of recombinant molecules at nerve terminals, we studied the distribution of subcellular particles containing recombinant synaptotagmin by centrifugation in sucrose and glycerol gradients and compared it with the distribution of endogenous components of the synaptic vesicles. There was little difference between recombinant synaptotagmin molecules and the endogenous synaptotagmin or synaptophysin, unless the deletions covered the transmembrane domain. This suggests that recombinant synaptotagmin molecules, even those that do not reach the nerve terminal, are incorporated into the correct organelles. A possible explanation of these data is that the synaptotagmin molecule itself is responsible for the transport of subcellular particles to the nerve terminals. Recent data indicate that synaptotagmin is transported to the synapse as a component of a specialized transport particle, which contains some, but not all, of the synaptic proteins. Such particles, whose components are later incorporated into complete synaptic vesicles, are driven to the terminal by specialized kinesin-like motor proteins. Synaptotagmin might play the part of a cargo for these proteins, taking some (but not all) components of the synaptic vesicle along. We are currently testing this hypothesis.

Synaptotagmin molecules also bind to neurexins, which are plasma membrane proteins preferentially localized in nerve terminals. The signal we identified in the carboxy-terminal domain of the synaptotagmin molecule overlaps with the region of neurexin binding. The interaction between the carboxy-terminal domain of synaptotagmin and neurexin might provide the means for retaining synaptotagmin, and perhaps the whole vesicle, in the nerve terminal. We are presently investigating whether synaptotagmin-neurexin interactions have a role in the accumulation of recombinant synaptotagmin molecules at the nerve terminal.

One of the goals of this project is to use the identified motifs to target chimeric proteins to the nerve terminals. We have prepared a set of constructs that contain protein kinases and recombinant protein kinase inhibitors connected to the protein domains responsible for targeting to the terminal. We are also testing whether the accumulation of such molecules at the nerve terminal restricts signal transduction cascades to this region of the nerve cell.

Chimeric Constructs to Study the Role of Protein Kinases in Neuronal Function

A. Weissmann, G. Enikolopov [in collaboration with R. Malinow, Cold Spring Harbor Laboratory]

We are designing a system for expressing conditionally active forms of proteins in mammalian cells. We are constructing a vaccinia virus vector that will allow proteins to be expressed as fusions with the ligand-binding domains of steroid hormone receptors. Our current experiments concentrate mostly on the *Drosophila* ecdysone receptor. The steroids that are agonists of the ecdysone receptor do not cross-react with the mammalian steroid receptors. We hope that this will make it possible to maintain low basal levels of functional activity of chimeric proteins in mammalian cells and to activate them by adding insect hormone. These vaccinia virus vectors will then be used to deliver chimeric proteins to the cells and tissues. We will use them primarily to study the role of protein kinases in long-term potentiation in the brain.

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NEURONAL GROWTH AND DIFFERENTIATION

D.R. Marshak E. Araya
L. Peña

This laboratory has conducted 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 postmitotic, 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 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. During this year, we completed our work on the Program Project grant from the National Institute on Aging to conduct studies on the mechanism of action of S100β, a polypeptide growth factor produced by glial cells in the brain. This is a multisite program grant that brings together anatomists, pharmacologists, pathologists, molecular biologists and biochemists that work on S100β. Our lab discovered a function of this molecule several years ago and have more recently discovered its abnormal levels in Alzheimer's disease. Our work is central to understanding the potential role of this factor in the pathology of neurodegenerative disease.

REGULATION OF S100β BY β-AMYLOID

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

L. Peña, D.R. Marshak [in collaboration with
A. Rossomando, West Haven, Connecticut]

We have continued our studies of protein kinase responses to growth factors in neuronal cells. A complete study of p34^{cdc2} kinase and MAP kinases in PC12 cells that respond to nerve growth factor (NGF) has been conducted by 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, which 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.

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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 these young scientists to work independently at the Laboratory for a period of up to 3 years on projects of their choice. Junior Fellows are provided with a salary, research support, and technical assistance so that they can accomplish their goals free from other distractions. The interaction among research groups at the Laboratory and the program of courses and meetings on diverse topics in molecular biology contribute to a research environment that is ideal for innovative science by these Fellows.

Two previous Cold Spring Harbor Fellows, Dr. Adrian Krainer (1987) and Dr. Carol Greider (1988), are currently members of the scientific staff at the Laboratory. Dr. David Barford, our Junior Fellow since 1991, left in 1994 to go to Oxford University (Laboratory of Molecular Biophysics) as a university lecturer. Dr. Ueli Grossniklaus joined us in 1994 from the Department of Cell Biology, University of Basel, Switzerland. Our newest Fellow, Dr. Scott W. Lowe, comes from the Center for Cancer Research at the Massachusetts Institute of Technology.

U. Grossniklaus
S.W. Lowe

Dissection of *Arabidopsis* Ovule and Gametophyte Development by Enhancer Detection

U. Grossniklaus, J. Moore, W. Gagliano

In flowering plants, the ovule is composed of tissues derived from both phases of the plant life cycle, the diploid sporophyte and the haploid gametophyte. 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. A single cell within an ovule primordium differentiates into a megaspore mother cell, undergoes meiosis, and produces four megaspores, three of which die. The fourth divides through three consecutive mitoses to form the embryo sac harboring eight nuclei in a syncytium. These eight nuclei are partitioned into seven cells: one oocyte, two synergids, three antipodals, and a binucleate central cell whose nuclei fuse prior to fertilization (Fig. 1A). After double fertilization of both the egg and the central cell, the ovule develops into a seed. The seven sister cells of the female gametophyte 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.

Very little is known about the control of ovule and gametophyte development in angiosperms. Recent genetic screens have identified sporophytically required female sterile mutations that primarily affect the ontogenesis of the integumental cell layers. A few of these mutations also affect the female gametophyte, but virtually nothing is known about the genetic and molecular events involved in pattern formation, cell specification of the cell types in the embryo sac, and the signaling events thought to be crucial for these processes.

The goal of our research is the identification and characterization of genes required for ovule and megagametophyte development. We are performing a large-scale enhancer detection/gene-trap screen in order to identify genes that are expressed during megasporogenesis and megagametogenesis in a temporally or spatially restricted fashion.

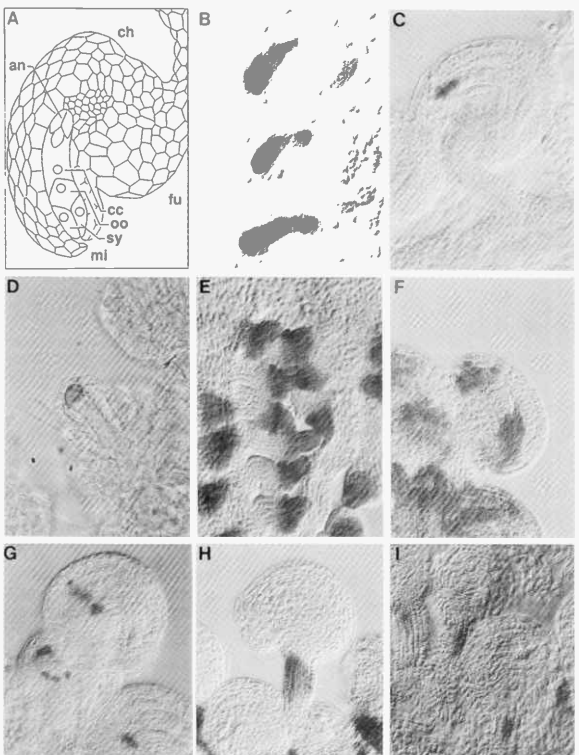

FIGURE 1 Selected GUS expression patterns in *Arabidopsis* ovules. (*A*) Schematic representation of a mature ovule. The central haploid gametophyte is surrounded by several layers of diploid sporophytic tissue, the endothelium, and the inner and outer integuments. The megagametophyte contains seven cells: three antipodals (an), two synergids (sy), an oocyte (oo), and a large central cell (cc). (fu) funiculus; (ch) chalazal pole; (mi) micropyle. (*B*) ET 760 shows expression in the entire gametophyte with the strongest level in the egg apparatus consisting of the two synergids and the oocyte. The reporter gene is first expressed during cellularization, and depending on how far this process has proceeded, we find GUS expression either restricted to the egg apparatus or in the entire gametophyte. (*C*) ET 179: reporter gene expression is restricted to the synergids. (*D*) ET 1117 shows expression early during ovule development in the subepidermal megaspore. (*E*,*F*) ET 252 is expressed throughout ovule development as soon as primordia arise. (*E*) At integument initiation, GUS expression is strong in the nucellus and the inner integument primordium, weaker in the outer integument primordium, and absent from the prospective funiculus. (*F*) In mature ovules, reporter gene expression has resolved into a bipolar mode of expression at the micropylar and chalazal pole. (*G*) ET 1131 shows expression in a ring of endothelial cells that undergo a last round of cell division. (*H*) ET 1111: GUS activity is restricted to the medial part of the funiculus. (*I*) ET 77: only a small patch of cells on the ventral surface of the funiculus expresses GUS.

ENHANCER DETECTOR/GENE-TRAP MUTAGENESIS

Enhancer detection and gene-trap systems are widely used in *Drosophila* and mouse developmental biology. Enhancer detection relies on a mobile genetic element carrying a reporter gene (in our case GUS) under the control of a weak constitutive promoter. If this promoter comes under the control of genomic *cis*-regulatory elements, the reporter gene is expressed in a specific temporal and spatial pattern. This pattern reflects the expression of a nearby gene controlled by the same regulatory elements and thus allows the identification of genes based on their pattern of expression, rather than on a mutant phenotype. Gene traps are a modification of this approach involving the generation of transcriptional fusions to the reporter gene. Enhancer/gene traps have been especially useful in identifying genes required both early and late during development and in processes characterized by high complexity and redundancy.

The plant group here at the laboratory has developed an enhancer/gene-trap system for *Arabidopsis thaliana* which is based on the *Ac/Ds* transposon of maize (Sundaresan et al., *Genes Dev. 9:* 1997 [1995]). 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. The enhancer detector/gene-trap mutagenesis system is a 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.

So far, we have focused on the generation of more than 3300 independent transposants carrying enhancer or gene-trap elements. This is a very laborious process and most of our effort in the past year was directed toward this goal (Table 1). In close to 2000 crosses, I generated approximately 50,000 F_1 seeds of which 20,000 were grown up individually to harvest their F_2 seeds. More than 15,000 of these F_2 families have been put through the positive/negative selection system to recover transposants. About one in four to five families yielded a new unlinked transposition event. To assure independence of events, usually only one transposant per family was isolated.

Table 1 Enhancer/Gene-Trap Mutagenesis

F_1 seeds generated	50,000
F_2 families harvested	20,037
F_2 families screened	15,392
Transposants isolated	3,302
enhancer traps	1,995
gene traps	1,307

EXPRESSION PATTERNS IN OVULES

In a pilot screen, we analyzed the expression of the GUS reporter gene in mature ovules of the first 1000 transposants that were collectively generated by the Cold Spring Harbor plant group. Carpels harboring mature ovules were dissected and stained for GUS activity. Stained carpels were mounted for compound microscopy in a lactate-based clearing solution. All transposants that showed staining in mature ovules were rescreened, and a developmental series was analyzed. Approximately 11% of the enhancer-traps (511) and 3% of the gene-traps (478) show a spatially restricted expression in mature ovules. Several lines show GUS expression patterns specific to individual cell types of the female gametophyte (oocyte, synergids, and antipodals; Fig. 1B,D), suggesting a role in cell specification and determination. Alternatively, the corresponding gene products may be involved in the specific functions performed by the different cell types of the female gametophyte. The expression patterns observed in enhancer transposants are summarized in Table 2. Almost two thirds of the lines staining in the gametophyte do so only after fertilization and also stain in the pollentube. GUS activity detected in the degenerating synergid after it has been penetrated by the pollentube could be paternally produced. These patterns may reflect male rather than female gametophyte-specific expression. The majority of expression patterns in the sporophytic tissues of the ovule show a highly polar distribution: Some of these are specific to the chalazal or micropylar pole, and a few patterns show differential expression along the dorsoventral axis (Fig. 1E–I). These patterns reflect regional gene expression and patterning in the developing ovule. The corresponding genes may be involved in establishing or maintaining the polarity of ovule and gametophyte or in localized signaling processes. Two lines are of particular interest since they express the reporter gene at a very early stage of ovule ontogenesis. ET 252 shows GUS expression in the distal half of emerging

Table 2 Spatially Restricted Expression Patterns (Enhancer Traps only)

Female gametophyte	14
entire gametophyte	2
egg apparatus	5
oocyte	2
synergids	3
megaspores	2
Sporophyte	13
ovule proper	5
funiculus	8
Sporophyte and Gametophyte	4
Degenerating synergid (after fertilization, pollentubes)	23

ovule primordia and could be involved in early processes of pattern formation. Its expression is maintained throughout ovule development in a dynamic pattern (Fig. 1E,F). ET 363 shows GUS activity restricted to a dorsal patch of cells in mature ovules. It starts to be expressed on one side of elongated finger-like ovule primordia in a small group of cells. Expression starts before any morphological difference between future dorsal and ventral sides of the ovule can be detected and represents the earliest marker for the prospective dorsal side. Among the sporophytic patterns, we found a surprisingly high number of lines staining in the funiculus, the structure that connects the ovule to the maternal tissue of the carpel (Fig. 1H,I). The funiculus is a structure of simple architecture and morphology, yet it appears to be highly patterned as judged from the array of expression patterns we found. We identified genes that are expressed in different domains along both the dorsoventral and proximodistal axis of the funiculus. The unexpected variety of expression patterns in the funiculus may be related to its complex interactions with the pollentube.

MOLECULAR CHARACTERIZATION OF SELECTED LINES

We have adapted a novel polymerase chain reaction (PCR)-based method, thermal asymmetric interlaced PCR (TAIL PCR) (Liu et al., *Plant Journal* **8**: 457 [1995]), to isolate genomic regions flanking the *Ds* insertions. We designed nested primers to isolate both 5′- and 3′-flanking regions that are used in combination with an arbitrary degenerate primer. Using a single arbitrary primer, we were able to isolate genomic fragments for 52 *Ds* insertions corresponding to a 75% success rate in a single attempt. It should be possible to recover the remaining fragments by using a different arbitrary primer. The fragment lengths range from 130 bp to 1200 bp with an average of 450 bp. In collaboration with Muhammad Lodhi and Dick McCombie here at the Laboratory, 35 fragments representing 23 loci were directly sequenced. A database search revealed that seven *Ds* insertions were close to or in a known *Arabidopsis* gene. Three of these show a spatially restricted expression within the ovule and have homology with the MADS box gene family and two *Arabidopsis* ESTs of unknown function, respectively. The remaining four belong to the class that is primarily expressed in the male gametophyte and the penetrated synergid. They are insertions into the U3C snRNA gene, the genes encoding S12 ribosomal protein, the basal transcription factor TFIIB, and an *Arabidopsis* EST of unknown function.

Ds-Induced Mutations Affecting Gametophyte Development and Function

J. Moore, W. Gagliano, U. Grossniklaus

We take advantage of the fact that *Ds* elements act as insertional mutagens and screen transposants for semisteriles and F_3 families for female sterile mutations. A mutation affecting megagametophyte development or function fails to transmit through the female gametophyte. Consequently, the ovule arrests and no seed develops. In a heterozygous plant, one half of the ovules will receive the mutant allele resulting in a semisterile phenotype. Fully female sterile mutations correspond to genes that show a sporophytic requirement for ovule development. Last year, we dedicated most of our growth space to the generation of transposants, and our screen for sterile mutations has not progressed very much. We have identified 10 male sterile mutations in the first 600 F_3 families but no female sterile mutant yet. In contrast, we have screened more than 4000 transposants for a semisterile phenotype and identified 150 candidates (Table 3). Since semisterility can result from poor environmental conditions, reciprocal translocations,

Table 3 Mutations Affecting Gametophyte Development or Function

Transposants screened for semisterility	4344
Semisterile candidates	153
Tested for segregation distortion (SD)	149
Lines with mild SD (Kan^S/Kan^r 0.6 to 0.8)	39
Lines with strong SD (Kan^S/Kan^r ≥0.8)	22

sporophytically required female sterile mutations with a low penetrance, or mutations affecting the female gametophyte, we subject all our candidates to a second test. If a mutation is not transmitted through the female gametophyte but normally through the male, the mutant allele should be segregating in one half of the progeny from a heterozygote. This deviation from a normal Mendelian segregation ratio (segregation distortion) is a hallmark of a mutation affecting the gametophyte. We can use the kanamycin resistance marker present on the *Ds* element to assay segregation distortion. This allows us to focus immediately on semisterile mutations that are linked to the *Ds* element. We have identified about 60 mutations displaying both a semisterile phenotype and distorted segregation. Based on the semisterility, they appear to affect the female gametophyte. To test whether the mutant allele also shows reduced transmission through the male or whether the defect is specific to the megagametophyte, we will perform reciprocal outcrosses to wild type and determine transmission frequencies through both gametophytes.

Characterization of *medea*, a Maternal Effect Mutation in *Arabidopsis*

U. Grossniklaus, W. Gagliano

One of our semisterile lines, GT 584, shows a unique phenotype where all ovules initiate seed development but then half of them abort during embryogenesis. These shriveled seeds contain embryos arrested at an early torpedo stage. The 1:1 segregation of normal to aborted seeds indicates a gametophytic control of the defect. If a heterozygous GT 584 plant is pollinated with wild-type pollen, the same phenotype is observed, whereas all seeds develop normally in the reciprocal cross. Thus, GT 584 displays a classical maternal effect embryo lethal phenotype. Moreover, this phenotype is controlled by the female gametophyte rather than sporophyte and can be termed a gametophytic maternal effect. The mutation cannot be rescued by an additional wild-type copy introduced through the male by using a tetraploid pollen donor. Since the genotype of the female gametophyte ("mother") determines whether the developing embryo will die or live, we named this mutation *medea* (*mea*) after the priestess Medea, daughter of the Greek king Aeëtes, who killed her own children (Euripides, 430 BC).

We have fortuitously isolated two alleles of *mea* from the same F_2 family. Both lines contain a *Ds* insertion that is tightly linked to the *mea* phenotype (<0.6 cM) but inserted at a different genomic location as judged from Southern blots. Both lines are derived from a single F_1 plant, suggesting that we are dealing with a tagged mutable *mea* allele carrying the *Ds* element at the locus and a stable derivative allele where the *Ds* reinserted close by, but the excision event did not restore *mea* function. The former should be revertible by reintroducing *Ac* transposase, and the latter should display a stable phenotype. Indeed, *mea-1* is mutable and we have isolated five independent revertants, whereas *mea-2* is a stable mutation. We isolated genomic DNA flanking the *Ds* insertion of *mea-1* by TAIL PCR and are currently characterizing the molecular organization of the gene.

The developing seed harbors both products of double fertilization, and it is difficult to distinguish whether a mutation primarily affects embryo or endosperm development. We have morphologically analyzed the developing seeds in *mea*. The endosperm appears to go through regular free nuclear division cycles and then cellularizes as in wild type. Embryos derived from *mea* eggs are indistinguishable from wild-type embryos until the time of arrest at the early torpedo stage. We can culture arrested embryos and have obtained homozygous *mea* plants producing siliques with 100% aborted seeds. Homozygous plants show a normal vegetative phase and produce wild-type flowers, indicating that *mea* is specifically required during embryogenesis. We speculate that *mea* is an imprinted gene that gets silenced in the male germ line. Thus, wild-type embryos are essentially hemizygous for *MEA*, the only active copy being transmitted through the female gametophyte. In *mea* mutants, this active copy is mutated, leading to arrest during embryogenesis.

Genetic Screen for *Arabidopsis* Mutants Displaying Apomictic Traits

U. Grossniklaus [in collaboration with R.E. Pruitt, Harvard University]

Seeds are generally considered to be the result of sexual reproduction. Their successful formation requires the coordinated development of embryo and endosperm, the products of double fertilization, and of the seed coat which is derived from the sporophytic tissues of the ovule. In more than 300 plant species, however, these events occur independent of fertilization in a process termed apomixis. Gametophytic apomixis closely resembles the sexual reproduction pathway, but meiosis is impaired or absent. Consequently, a diploid cell undergoes gametophyte development to produce a mature embryo sac. The diploid egg cell develops without fertilization into an embryo, and a viable seed is produced that is genetically identical to the mother plant. Differences between sexual and apomictic reproduction appear to be controlled by a single regulatory locus that controls a cascade of events leading to the formation of an unreduced oocyte and the initiation of the seed development program in the absence of fertilization. The transfer of apomixis into a sexual crop would allow clonal reproduction through seed and the immediate fixation of any desired heterozygous genotype. This would completely revolutionize breeding strategies and agriculture.

To identify genes that are relevant to apomictic reproduction, we performed a pilot screen for mutants showing seed development in the absence of fertilization. We took advantage of a tight temperature-sensitive male sterile mutation that was isolated in Bob Pruitt's laboratory. Under restrictive conditions, homozygous plants do not produce any seeds and their seed pots do not elongate. Homozygous seeds were mutagenized and M1 plants were grown under the defined conditions in the growth chambers at Harvard. The plants were screened for sectors that showed silique elongation, indicating seed development under the restrictive temperature. Plants producing such sectors were shifted to the permissive temperature to allow self-fertilization and rescue of the mutation. Among the 1582 plants that we screened, 26 putative mutants were isolated. We expect to find two different classes of mutations: (1) mutations suppressing the male sterility defect allowing the production of functional pollen (these should give rise to viable seeds) and (2) mutations allowing full or partial seed development in the absence of fertilization. Such mutations are expected to show a gametophytic requirement. Upon self-fertilization, 21 of 26 putative mutants produced 50% wild-type and 50% aborted seeds, indicating a gametophytic control of the defect. We are currently analyzing the inheritance characteristics of these mutants. Since seeds produced under the restrictive temperature are not viable, the seed development program appears to be activated only partially in these mutants. What part(s) is initiated autonomously (embryo, endosperm, or seed coat development) can be determined by microscopic inspection of the seeds. Whereas mutations that allow part of the program to occur independently of fertilization are recovered at a high frequency (1.6% in our pilot screen), we expect fully apomictic or parthenogenetic mutants to be extremely rare. The powerful screen we have established will allow the rapid analysis of very large numbers of M1 plants such that the recovery of these rare mutations should be possible.

Apoptosis, Cancer, and Chemosensitivity

S.W. Lowe, M. McCurrach, J. Polyakova, T. Connor, A. Lin, A. Samuelson

It is now apparent that cell death can occur by a programmed process known as apoptosis. This knowledge has important implications for our understanding of developmental biology and tissue homeostasis, for it implies that cell numbers can be regulated by genes that influence cell survival as well as those that control proliferation and differentiation. That apoptosis is regulated implies that cell death, like other metabolic or developmental programs, can be disrupted by mutation. Indeed, mutations that suppress apoptosis—for example, deregulated bcl-2 expression or inactivation of p53—can promote tumorigenesis.

As the objective of cancer therapy is to destroy malignant cells, factors that modulate cell death in tumors may also influence the efficacy of anticancer agents. Although the toxicity of anticancer agents has been attributed to their debilitating effects on proliferating cells, we now know that most anticancer agents induce apoptosis. This revelation has profound implications for cancer therapy, for it raises the possibility that agents with distinct primary targets

ultimately kill through similar effector mechanisms. Consequently, mutations in apoptotic programs may produce a general resistance to anticancer agents.

Our research is based on the premise that apoptosis provides a natural defense against tumor development and underlies the cytotoxicity of most current anticancer agents. Our laboratory investigates the molecular control of apoptosis in tumor cells, with the ultimate goal of identifying determinants of radiation and chemosensitivity and perhaps new targets for therapeutic intervention. Earlier studies focused on the p53 tumor suppressor gene and its anti-neoplastic activities. Using model systems, we identified p53 as an important regulator of apoptosis in tumor cells.

Role of p53 in Apoptosis

J. Polyakova, T. Connor, M. McCurrach, S. Lowe

In fibroblasts and in most normal tissues, p53 functions in a cell cycle checkpoint that limits proliferation following genomic damage induced by radiation (Kastan et al., *Cell 71:* 587 [1992]). In contrast, thymocytes undergo apoptosis following irradiation; in this setting, p53 is required for efficient cell death (Lowe et al., *Nature 362:* 847 [1993]). These observations suggest that the role of p53 in apoptosis can be tissue-specific. However, E1A-expressing fibroblasts undergo p53-dependent apoptosis following irradiation, indicating that E1A alters p53 function from facilitating growth arrest to promoting apoptosis (Lowe et al., *Cell 74:* 954 [1993]). Many cytotoxic agents also induce p53-dependent apoptosis in E1A/ras-transformed mouse embryo fibroblasts (Lowe et al., *Cell 74:* 954 [1993]), demonstrating that p53 is a determinant of radio- and chemosensitivity. Using transplanted tumors derived from oncogenically transformed fibroblasts expressing or lacking p53, we demonstrated that *p53* mutations can reduce the efficacy of anticancer agents in vivo (Lowe et al., *Science 266:* 807 [1994]).

If the murine model system described above accurately mimics the consequences of *p53* inactivation in human tumors, then *p53* mutations should contribute to radiation and drug resistance in human cancer. In the past 2 years, a considerable amount of clinical evidence associates *p53* mutations with shortened survival (for review, see Lowe 1995). For example, *p53* mutations are linked to drug resistance in breast cancer and several hematologic malignancies; patients harboring *p53* mutant tumors respond poorly to adjuvant therapy and die much sooner than patients with wild-type *p53*. The role of *p53* in apoptosis provides a compelling model to explain these correlations; conversely, the ability of the E1A model system to predict these associations supports its utility for identifying processes important in tumorigenesis and chemosensitivity. Therefore, we are currently using this model to identify other components of apoptotic programs.

p53 Uses Distinct Effector Genes in Promoting Growth Arrest or Apoptosis

M. McCurrach, S. Lowe [in collaboration with L. Attardi and T. Jacks, Massachusetts Institute of Technology]

Although p53 functions as a transcription factor, controversy exists as to whether this activity is important for apoptosis. We recently obtained strong evidence suggesting that apoptosis results from p53's transcriptional activity. Microinjection of wild-type p53 induces apoptosis in $p53^{-/-}$ populations expressing E1A, but p53 deletion mutants lacking the amino-terminal *trans*-activation domain are defective (Attardi et al. 1996). Most importantly, chimeric proteins consisting of a heterologous *trans*-activation domain (VP16) fused to the amino-terminal deletion mutant restores apoptosis. Therefore, this study provides definitive evidence that p53 promotes apoptosis by regulating transcription.

p53 facilitates cell cycle arrest, at least in part, by increasing transcription of the *p21/Cip1/WAF1* cyclin-dependent kinase inhibitor (see, e.g., Brugarolas et al., *Nature 377:* 552 [1995]). To test whether p21 might also function in apoptosis, we introduced E1A into wild-type, $p53^{-/-}$, and $p21^{-/-}$ mouse embryo fibroblasts (MEFs) by retroviral gene transfer. As expected, E1A-expressing $p53^{+/+}$ MEFs were susceptible to apoptosis in low serum or the chemotherapeutic drug adriamycin, whereas $p53^{-/-}$ populations were resistant. $p21^{-/-}$ populations expressing E1A were comparable to their wild-type counterparts. Thus, p53-dependent cell-cycle arrest and apoptosis utilize distinct effector genes (Attardi et al. 1996). We are currently assessing other candidate genes that might act downstream from p53 in apoptosis.

Oncogenic Changes Alter Chemosensitivity

A. Lin, A. Samuelson, M. McCurrach, S. Lowe

Anticancer agents are effective only when tumor cells are more readily killed than normal tissue. If the efficacy of anticancer agents is partly determined by their ability to induce apoptosis, then tumor cells must be *more susceptible* to apoptosis than the tissue from which they arose. Evidence from our laboratory and elsewhere is emerging to suggest that increases in cellular susceptibility to apoptosis are tightly linked to tumorigenesis itself. For example, the adenovirus E1A oncogene deregulates cellular proliferation and simultaneously promotes apoptosis in response to many physiologic and toxic agents (Lowe et al., *Cell 74:* 954 [1993]; Lowe et al., *Proc. Natl. Acad. Sci. 91:* 2026 [1994]). Since analogous changes are found only in neoplastic cells, their ability to enhance apoptosis may explain why tumor cells can be specifically killed by cytotoxic agents (Lowe 1995). In contrast, oncogenic mutations that suppress apoptosis (e.g., p53) promote resistance to anticancer agents—not by preventing the cellular damage produced by these agents, but rather the cellular response to this damage. These observations suggest that tumor cell chemosensitivity is determined, in part, by the combined effects of oncogenic mutations on apoptosis.

Our laboratory continues to use the E1A oncogene to mimic oncogenic changes occurring during spontaneous tumorigenesis and to modulate cellular susceptibility to apoptosis. We have recently optimized retrovirus-mediated gene transfer to introduce E1A into whole populations of primary cells for immediate analysis of cellular susceptibility and resistance to apoptosis. E1A-expressing populations

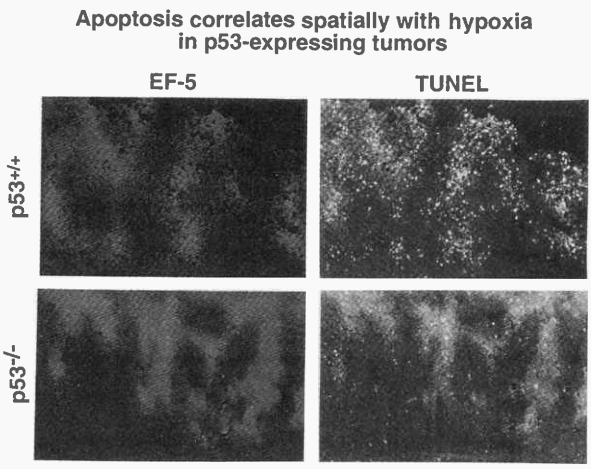

FIGURE 2 Nude mice were inoculated with p53-expressing ($p53^{+/+}$; *top*) and p53-deficient ($p53^{-/-}$; *bottom*) mouse embryo fibroblasts made tumorigenic by coexpression of the adenovirus E1A and activated *ras* oncogenes. Upon formation of tumors, animals were injected with the compound EF5, which is metabolically activated to bind macromolecules in a low-oxygen (hypoxic) environment. One hour after injection of EF5, tumors were isolated and prepared for histological analysis. Tumor sections were stained using both an anti-EF5 antibody to identify hypoxic regions (*left*; *light gray*) and TUNEL assay to identify apoptotic cells (*right*; *bright spots*). Apoptosis colocalizes with hypoxia in p53-expressing tumors but not in p53-deficient tumors.

rapidly become sensitive to the induction of p53-dependent apoptosis following serum depletion or treatment with radiation or chemotherapeutic agents. We anticipate that this system will facilitate the dissection of apoptotic programs active in transformed cells.

p53 Modulates Apoptosis in Response to Hypoxia

S. Lowe [in collaboration with T. Graeber and A. Giaccia, Stanford University]

Our previous work suggested that p53-dependent apoptosis might provide a brake on tumor growth (Lowe et al., *Proc. Natl. Acad. Sci. 91:* 2026 [1994]; Symonds et al., *Cell 78:* 703 [1994]). In a collaborative study that was completed following our arrival at Cold Spring Harbor, we asked whether oncogenes such as *E1A* or c-*myc* could promote apoptosis under physiological conditions prevalent during tumor development. Fibroblasts expressing c-*myc* or transformed by *E1A/ras* rapidly undergo apoptosis when placed in low oxygen (hypoxia), and Bcl-2 overexpression or p53 inactivation suppresses hypoxia-induced cell death (Graeber et al. 1996). Cells with defects in apoptosis survived hypoxia; thus, E1A/ras-transformed cells lacking p53 outgrow E1A/ras-transformed cells expressing p53 in a low-oxygen environment. Using a transplanted tumor model, we demonstrated that hypoxia and apoptosis correlate spatially in p53-expressing tumors but not in p53-deficient tumors (Fig. 2).

Hypoxia in the center of tumors has long been associated with necrosis. However, that low oxygen also induces apoptosis, a regulated form of cell death, implies that hypoxic stress may play a causal part in tumor evolution. As developing tumors outgrow their blood supply, they encounter hypoxia. Our results suggest that further tumor growth is limited by hypoxia-induced apoptosis. In this view, cells acquiring mutations that suppress this response have a survival advantage and begin clonal expansion within the tumor. Since p53 promotes hypoxia-induced apoptosis, p53 mutations enhance survival in a low-oxygen environment. Inactivation of p53 also reduces apoptosis induced by many cytotoxic agents; thus, selection for hypoxia-resistant variants may simultaneously select for drug-resistant tumors. Perhaps this explains why many solid tumors, which must overcome hypoxic stress, are inherently difficult to treat.

Future Perspectives

S.W. Lowe

Since p53 mutations occur in greater than 50% of human tumors, the effects of p53 loss on treatment efficacy may represent a significant obstacle in the treatment of human malignancy. However, several rational approaches can be envisioned to overcome the consequences of p53 loss. Among these are (1) identifying ways to restore the p53-dependent apoptotic program to p53 mutant cells and (2) identifying agents that are effective in the absence of p53 function. Practical application of these concepts will require a better understanding of p53-dependent and -independent apoptotic programs. To this end, we will continue to study the E1A oncogene as a model for understanding the molecular control of apoptosis in tumor cells. Preliminary studies suggest that apoptosis in E1A/ras-transformed cells can be induced by p53-dependent and -independent pathways, and the molecular characterization of these pathways is our primary objective in the coming year.

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COLD SPRING HARBOR MEETINGS AND COURSES

ACADEMIC AFFAIRS

The academic program at Cold Spring Harbor Laboratory comprises a wide-ranging series of postgraduate laboratory and lecture courses, workshops, large meetings, and a summer research program for undergraduates. The program now extends from a spring session of courses starting early in March through a fall session of courses ending in November. Thus, the academic year at the Laboratory now lasts as long as those at universities and colleges around the country. Each year, thousands of students, postdoctoral fellows, faculty, and staff scientists from around the world come to Cold Spring Harbor to participate in the various courses and meetings.

Several new courses and meetings were started this year and new facilities were extensively utilized. All of the neurobiology laboratory courses are now being held in the Howard Hughes Medical Institute (HHMI) teaching laboratories in the Beckman Neuroscience Center. These spacious and modern laboratories are now home to 15 molecular biology and neurobiology courses. A new course, Advanced Genome Sequence Analysis, was held in March in the HHMI teaching labs and was ably taught by Ellson Chen, Richard Gibbs, Dick McCombie, and Rick Wilson. This course, which will be held again next year, served to extend the academic year yet again and filled the teaching labs with large numbers of DNA sequencers (both human and automated). A new lecture course on brain mapping was taught by John Mazziotta and Arthur Toga and was held at the Banbury Center, along with the Laboratory's other neurobiology lecture courses. In all, the Laboratory held 24 courses this year, whose instructors, students, and lecturers are listed in the following pages.

The success of the courses is due in large part to instructors who work extensively, creatively, and for extremely long hours during their courses. This year, several scientists who have taught here for at least five years will be retiring, although we expect them to return to the Laboratory to give seminars and advice in the future. These include Jim Kadonaga and Dan Marshak, two of the original instructors of the course on Protein Purification and Characterization; Stan Malloy, Valley Stewart, and Ron Taylor who taught Advanced Bacterial Genetics; and Mark Learned, an instructor in the Advanced Molecular Cloning course.

The courses are supported by, and would not be possible without, a series of grants from federal and private sources. The summer molecular genetics courses have been supported for many years by grants from the National Institutes of Health and the National Science Foundation, and a grant from the National Institute of Mental Health has supported several of the neurobiology courses. However, it has been a large education grant (renewed again this year) from HHMI that has provided stable support for the neurobiology program and has allowed the Laboratory to begin to expand its series of spring and fall courses. The Laboratory also has an award from the Esther and Joseph A. Klingenstein Fund for the support of neurobiology courses. As has been the case for several years, the Grass Foundation provided funds for scholarships for students in neurobiology courses. Grants from the Departments of Energy and Agriculture have also helped in the funding of the courses in Macromolecular Crystallography and Molecular Markers for Plant Breeding. In addition, the Laboratory receives valuable support from many companies that donate supplies and lend equipment for the courses.

Although conferences at the Laboratory started with the Symposium some 62 years ago, they have grown to 15 meetings per year, covering a wide range of topics. This year's Symposium on Protein Kinesis provided an opportunity for scientists to present their latest work and synthesize ideas in this rapidly moving area of research. Special highlights of the year included a new meeting on Tyrosine Phosphorylation and Cell Signaling, organized by Nick Tonks and Ben Neel, as well as a meeting on Molecular Genetics of Bacteria and Phages which celebrated 50 years of phage at Cold Spring Harbor. This conference, arranged by Susan Gottesman, Carol Gross, Peter Model, Bill Reznikoff, and Jeff Roberts, brought together individuals ranging from graduate students to scientists who were instrumental in starting the field of molecular biology with their research on phage and bacteria.

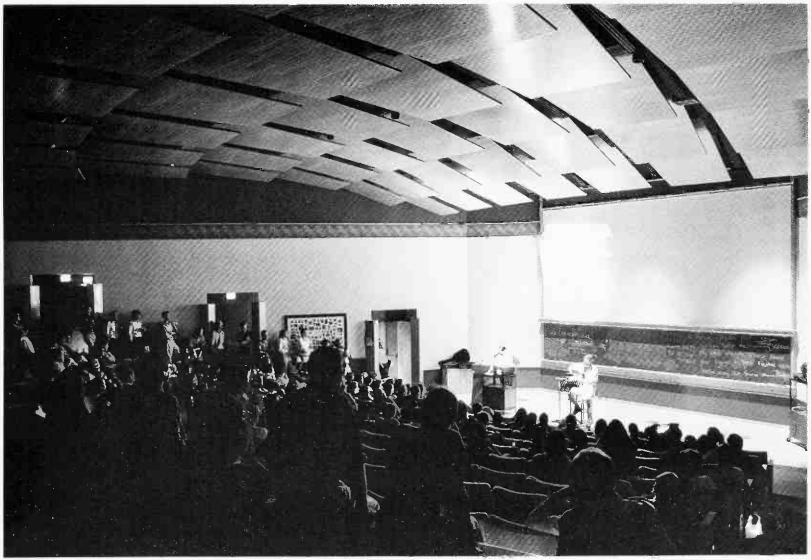
Other meetings covered a broad array of topics ranging from Eukaryotic DNA Replication to Neurobiology of *Drosophila*. Several of the meetings were in fact heavily subscribed, and Grace Auditorium was filled to capacity as more than 400 scientists met to discuss their research. These meetings included Genome Mapping and Sequencing, RNA Processing, Retroviruses, and the Cancer Cells Meeting on Regulation of Eukaryotic Transcription. The success of the meetings really depends on all of the scientists who serve as

organizers (listed in the following pages) and on the enthusiastic participation of all of the visiting scientists. As in past years, support from the Laboratory's Corporate Sponsor Program and from NIH, NSF, and the Department of Energy was important in helping scientists at all levels of their careers to attend the meetings. Contributors to the various meetings are listed in the pages that follow.

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, headed by David Stewart, Director of Meetings and Courses, coordinates the arrangements for all of the visiting scientists. This enormous job, which seems to grow every year, is carried out not only extremely efficiently, but most pleasantly as well. The staff, including Micki McBride, the Course Registrar, Diane Tighe, Marge Stellabotte, Andrea Stephenson, Nancy Weeks, Jim Koziol, Drew Mendelson, and Lesley Cook, as well as staff from several other departments, are crucial to the success of the meetings and courses. These include Herb Parsons, Ed Campodonico, and the audiovisual staff; Cliff Sutkevich and his staff who set up and maintain course equipment; Edie Kappenberg, the course coordinator; Mary Horton of the Grants Office; and Lee Martin of Cold Spring Harbor Laboratory Press.

Terri Grodzicker



Ira Herskowitz entertaining Symposium participants in Grace Auditorium.

60th COLD SPRING HARBOR SYMPOSIUM ON QUANTITATIVE BIOLOGY

Protein Kinesis: The Dynamics of Protein Trafficking and Stability

May 31–June 7 389 participants

ARRANGED BY **Bruce Stillman,** Cold Spring Harbor Laboratory

The way individual cells look and function follows directly from the program of cell-type-dependent gene expression and the nature of the proteins that are expressed. Once synthesized, however, the proteins themselves take on a life of their own that is determined by their amino acid sequence and their environment. Proteins have varied life times, cellular locations and interactions with other proteins, or cellular structures such as membranes, each of which can influence cell physiology directly. Thus, the life of proteins in the cell is an important topic that has deserved much attention. At the last Symposium that dealt with these issues, in 1981, the focus was on the structure of the cytoskeleton and general observations of protein trafficking within the cell, such as cell surface polarity and axonal transport. The science was dominated by microscopy and was primarily observational. The field was crying out for an influx of biochemistry and genetics to present a more detailed understanding of the mechanisms of protein stability, movement, and organization.

With remarkable results, the biochemists and geneticists heeded the call. During the last 14 years, we have witnessed a dramatic increase in knowledge about the mechanisms of protein movement within the cell. It was therefore prime time to dedicate the topic of the Symposium to the dynamic aspects of protein function. The title "Protein Kinesis" was invented to reflect the many aspects of protein modification, trafficking, stability, localization, and organization. Knowledge of the mechanisms of protein transport either through the endoplasmic reticulum and golgi to the cell surface or into the nucleus has progressed rapidly in recent years. The understanding is sufficient that we can now begin to appreciate links between these processes in different cell types and understand how protein kinesis influences human disease processes, a topic that will dominate future discussion and directions.

The program was arranged with the generous help of Günter Blobel, Jim Rothman, Randy Schekman, Mary-Jane Gething, Susan Gottesman, Ulrich Hartl, Ira Melman, and Ron Laskey. The formal program consisted of 87 talks and 189 poster presentations, and the meeting attracted a total of 389 participants. This year also saw the inaugural Reginald G. Harris Lecture, given by Randy Schekman. When Director of the Biological Laboratories at Cold Spring Harbor, Reginald Harris started the Symposia in 1933 to foster a quantitative approach to biology and promote interactions between scientists from diverse fields. The modern day success of these meetings stems directly from his foresight. Indeed, many Symposia have profoundly influenced the future of biology in general.

Essential funds that supported this meeting were obtained from the U.S. Department of Energy and the National Institutes of Health. Additional support came from our ever more important and needed Corporate Sponsors: American Cyanamid Company; Amgen Inc.; BASF Bioresearch Corporation; Beckman Instruments, Inc.; Becton Dickinson and Company; Bristol-Myers Squibb Company; Chugai Pharmaceutical Co., Ltd.; Chugai Research Institute for Molecular Medicine, Inc.; Diagnostic Products Corporation; The DuPont Merck Pharmaceutical Company; Forest Laboratories, Inc.; Genentech, Inc.; Glaxo; Hoffmann-La Roche Inc.; Human Genome Sciences, Inc.; Johnson & Johnson; Kyowa Hakko Kogyo Co., Ltd.; Life Technologies, Inc.; Marion Merrell Dow Inc.;

J. Beckwith

G. Blobel

G. Palade, B. Stillman

W. Neupert

R. Schekman

J. Rothman, T. Südhof

S. Schmid

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, Inc.; Sandoz Research Institute; Schering-Plough Corporation; SmithKline Beecham Pharmaceuticals; Sumitomo Pharmaceuticals Co., Ltd.; The Upjohn Company; The Wellcome Research Laboratories, Burroughs Wellcome Co.; Wyeth-Ayerst Research; Zeneca Group PLC.

PROGRAM

Welcoming Remarks: Bruce Stillman

Introduction
Chairperson: D. Sabatini, *New York University School of Medicine*

Protein Folding and Modification in the ER
Chairperson: H. Pelham, *Medical Research Council, Cambridge, United Kingdom*

Reginald G. Harris Lecture: "Protein Sorting and Vesicle Budding from the ER"
Speaker: R. Schekman, *Howard Hughes Medical Institute, Berkeley, California*

Protein Trafficking
Chairperson: A. Helenius, *Yale University School of Medicine*

Protein Trafficking at the Synapse
Chairperson: K. Simons, European Molecular Biology Laboratory, Heidelberg, Germany

Later Stages of Protein Trafficking
Chairperson: T. Südhof, *University of Texas Southwestern Medical Center*

Protein Translocation and Folding
Chairperson: S. Gottesman, *NCI, National Institutes of Health*

Protein Degradation
Chairperson: M.-J. Gething, *University of Melbourne, Australia*

Protein Trafficking at the Cell Surface
Chairperson: M. Farquhar, *University of California, San Diego*

Peptide and Protein Translocation
Chairperson: I. Mellman, *Yale University School of Medicine*

Dorcas Cummings Lecture: "How Proteins Find Their Addresses in the Cell"
Speaker: Gunter Blobel, *Howard Hughes Medical Institute, Rockefeller University*

Polarity
Chairperson: J. White, *University of Virginia, Charlottesville*

Nuclear Trafficking
Chairperson: G. Schatz, *Biozentrum, University of Basel, Switzerland*

Membrane Dynamics
Chairperson: S.L. Schmid, *Scripps Research Institute, La Jolla, California*

Protein Import into Organelles
Chairperson: J. Beckwith, *Harvard Medical School*

Molecular Motors
Chairperson: B. Stillman, *Cold Spring Harbor Laboratory*

Summary: Gecrge Palade, *University of California, San Diego*

MEETINGS

Molecular and Behavioral Biology of *Aplysia* and Related Molluscs

April 19–April 23 90 participants

ARRANGED BY **Leonard Kaczmarek,** Yale University School of Medicine
Eric Kandel, Columbia University College of Physicians & Surgeons
Richard Scheller, Stanford University and Howard Hughes Medical Institute

This fourth international meeting continued the trend of including presentations on data gleaned from work with identifiable neurons of Aplysia, Squid, and Lymnaea, as well as with some crustacean and annelid preparations. For the neurobiologist, the major advantage to using these preparations is the large size of their somata, which allows intracellular injection of enzymes and other reagents to probe synaptic transmission and other aspects of neuronal plasticity. This feature has allowed workers to gain numerous insights into the mechanisms of exocytosis of synaptic vesicles. In addition, the fact that the nerve cells of these animals are readily identifiable from preparation to preparation allows them to be used to determine the changes that occur in neuronal properties during learning and other prolonged changes in animal behavior.

This meeting was funded in part by the National Institute of Neurological Disorders and Stroke, a division of the National Institutes of Health, and the National Science Foundation.

PROGRAM

Plenary Lecture
Chairperson: R. Jahn, *Yale University, New Haven, Connecticut*

Synaptic Transmission
Chairperson: J. Kehoe, *Ecole Normale Superieure, Paris, France*

Modulation of Excitability and Synaptic Transmission
Chairperson: H. Gerschenfeld, *Ecole Normale Superieure, Paris, France*

Neuronal Circuitry and Behavior I
Chairperson: W. Geraerts, *Vrije Universiteit, Amsterdam, The Netherlands*

Peptides and Other Neurotransmitters
Chairperson: P. Haydon, *Iowa State University*

Neuronal Circuitry and Behavior II
Chairperson: P. Benjamin, *University of Sussex, United Kingdom*

Development
Chairperson: R. Scheller, *Stanford University, Howard Hughes Medical Institute*

Database Meeting

Learning
Chairperson: E. Mayeri, *University of California, San Francisco*

The Cytoskeleton and Cell Function

April 26–April 30 214 participants

ARRANGED BY **David Helfman,** Cold Spring Harbor Laboratory
Elizabeth Raff, Indiana University

The Cytoskeleton and Cell Function meeting brought together scientists working on the cytoskeleton in invertebrate and vertegrate systems. Although much information is now available concerning the structural and molecular bases for the various cytoskeletal components, it is only recently that the function and regulation of these components in cellular processes is beginning to be understood. The meeting highlighted topics in actin filament assembly and dynamics, cell motility, microtubule assembly and dynamics, cell division, intermediate filaments, molecular motors, intracellular trafficking, signal transduction, membrane-cytoskeleton interactions, and development and differentiation. Various experimental approaches were presented, including biochemistry, genetics, and molecular biology. The sessions encompassed the most recent advances in the field and made for an intense and stimulating exchange of information. Another meeting is planned for 1997.

This meeting was funded in part by the March of Dimes Birth Defects Foundation; The Council for Tobacco Research–U.S.A., Inc.; Department of the Navy, Office of Naval Research; and the National Science Foundation.

PROGRAM

Role of the Cytoskeleton in Development and Differentiation
Chairperson: L. Cooley, *Yale University Medical School*

Actin Filament Assembly and Dynamics
Chairperson: P.A. Rubenstein, *University of Iowa College of Medicine*

Microtubule Assembly and Dynamics
Chairperson: E.C. Raff, *Indiana University*

Molecular Motors
Chairperson: M. Porter, *University of Minnesota Medical School*

Cell Division
Chairperson: J.R. McIntosh, *University of Colorado, Boulder*

Motile Systems
Chairperson: G.M. Langford, *Dartmouth College*

Cytoskeletal Dynamics
Chairperson: U. Aebi, *University of Basel, Switzerland*

Signal Transduction: Membrane–Cytoskeleton Interactions
Chairperson: B. Geiger, *Weizmann Institute of Science, Rehovot, Israel*

C. Nokes, A. Weeds

L. Tilney, M. Tilney

Tyrosine Phosphorylation and Cell Signaling

May 3–May 7 338 participants

ARRANGED BY **Nick Tonks,** Cold Spring Harbor Laboratory
Ben Neel, Beth Israel Hospital

This was the first meeting on "Tyrosine Phosphorylation and Cell Signaling" at Cold Spring Harbor and it marked the fifteenth anniversary of the discovery of tyrosine phosphorylation by Tony Hunter, Bart Sefton, and colleagues. As such, it was particularly appropriate that Tony Hunter opened the meeting with a keynote address providing an overview of historical perspective, current progress, and future directions in the field. The format of the meeting provided a combined emphasis on the physiological roles of protein tyrosine kinases and phosphatases and how their actions are integrated to modulate signaling events in vivo. The sessions were based around physiological processes and cellular functions, rather than around particular categories of enzymes, so as to try to provide biological context to the data. The program included scientists from the United States, Europe, Far East, and South Pacific. Sixty three speakers presented their data in sessions that dealt with receptor protein tyrosine kinase signaling, cytokine-induced signaling, the cytoskeleton, signaling in lymphocytes, cell cycle and development, neuronal signaling and tyrosine phosphorylation and disease. A variety of systems were described with great progress reported in genetic and biochemical approaches to the characterization of physiological functions for tyrosine phosphorylation. The meeting was so successful that Tony Hunter and Bart Sefton have decided to organize a conference with the same format at The Salk Institute in 1996, with the aim of continuing with an annual meeting on "Tyrosine Phosphorylation" that alternates in venue between Cold Spring Harbor and The Salk.

This meeting was funded in part by the National Institute of Diabetes and Digestive and Kidney Diseases, National Cancer Institute, National Institute of General Medical Sciences (all divisions of the National Institutes of Health), and the National Science Foundation.

PROGRAM

Keynote Address: Tony Hunter, *Salk Institute*

Receptor PTK Signaling I
Chairperson: J. Brugge, *Ariad Pharmaceuticals, Cambridge, Massachusetts*

Cytokine Signaling
Chairperson: B. Neel, *Harvard Medical School, Boston, Massachusetts*

Cytoskeleton
Chairperson: T. Parsons, *University of Virginia, Charlottesville*

Lymphocyte Signaling
Chairperson: M. Thomas, *Washington University, St. Louis, Missouri*

Cell Cycle and Development
Chairperson: H. Piwnica-Worms, *Washington University, St. Louis, Missouri*

Neuronal Signaling
Chairperson: M. Greenberg, *Harvard Medical School, Boston, Massachusetts*

Tyrosine Phosphorylation and Disease
Chairperson: A.M. Pendergast, *Duke University Medical Center, Durham, North Carolina*

Receptor PTK Signaling II
Chairperson: S. Courtneidge, *EMBL, Heidelberg, Germany*

N. Tonks

B. Neel

Genome Mapping and Sequencing

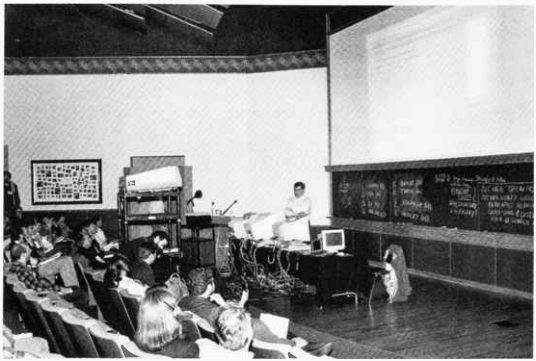
May 10–May 14 452 participants

ARRANGED BY **David Bentley,** Sanger Centre
Eric Green, National Institutes of Health
Robert Waterston, Washington University

More than 300 abstracts were presented at this eighth annual meeting, which covered a broad array of topics related to genome analysis of a number of organisms. The major advances in the field of genome analysis prompted the establishment of new approaches for presenting the wealth of incoming data and information at the meeting. For example, a poster symposium was once again held, in this case as the format for presenting the large amount of data being generated in the construction of physical maps of human chromosomes. This session included a highly interactive open forum among the presenters and meeting attendees that addressed a number of key issues relevant to the completion of human chromosome maps and the initiation of large-scale sequencing of the human genome. The rapidly expanding field of computational genomics creates a challenge in terms of devising meaningful ways of presenting the latest developments. New to this year's meeting was the use of projection-style computer demonstrations in Grace Auditorium (presented each afternoon), which allowed a highly interactive forum for learning about the informatics tools being developed for genome research.

Another major highlight of the meeting was the keynote session, where Maynard Olson and John Sulston summarized key aspects of genome mapping and sequencing, respectively. These talks focused on the central issues related to the construction of high-resolution physical maps of human DNA and the initiation of large-scale sequencing. These keynote talks, which were new to the 1995 meeting, were an excellent addition to the meeting and stimulated many further discussions among the attendees.

The overall tone of the meeting was highly optimistic. There seemed to be general consensus that the DNA sequences of a number of model organisms are nearing completion, that efforts to complete the physical maps of human and mouse chromosomes are on track, and that the time has arrived to initiate efforts to sequence the human genome. This meeting was funded in part by the National Center for Human Genome Research, a division of the National Institutes of Health. Additional support was provided by QuantumSoft, a division of General Atomics.



First interactive computer demonstration in Grace Auditorium.

PROGRAM

Gene Discovery and Transcript Mapping
Chairpersons: R. Gibbs, *Baylor College of Medicine*; B. Soares, *Columbia University*

Mapping Methods and Technologies
Chairpersons: E. Green, *National Institutes of Health*; C. Huxley, *St. Mary's Hospital*

Computer Demonstrations I
Moderator: T. Marr, *Cold Spring Harbor Laboratory*

Informatics
Chairpersons: M. Boehnke, *University of Michigan*; M. Boguski, *National Institutes of Health*

Human Chromosome Mapping Poster Symposium
Chairpersons: D. Page, *Whitehead Institution*; B. Trask, *University of Washington*

Computer Demonstrations II
Moderator: M. Boehnke, *University of Michigan*

ELSI Panel Discussion
Moderator: N. Wexler, *Columbia University*
Presentations: Lori Andrews, *Chicago-Kent College of Law*: Genetic Privacy
Neil Holtzman, *Johns Hopkins University*, Transition of Testing from Research to Practice
Troy Duster, *University of California, Berkeley*: Role of Bench Scientists in the Exploration of ELSI Issues

DNA Sequencing
Chairpersons: D. Bentley, *Sanger Centre*; R. Wilson, *Washington University*

Model Organisms: Mapping and Biology
Chairpersons: S. Brown, *St. Mary's Hospital*; P. Hieter, *Johns Hopkins University*

Computer Demonstrations III
Moderator: M. Boguski, *National Institutes of Health*

Keynote Addresses
Speakers: Maynard Olson, *University of Washington*; John Sulston, *Sanger Centre*

Human Genetics and Biology
Chairpersons: V. van Heyningen, *MRC Human Genetics Unit*; R. Waterston, *Washington University*

RNA Processing

May 17–May 21 449 participants

ARRANGED BY **Brenda Bass,** University of Utah School of Medicine
Iain Mattaj, EMBL, Germany
Michael Rosbash, Brandeis University and Howard Hughes Medical Institute
Jo Ann Wise, Case Western Reserve University

Exciting developments in several major areas of pre-mRNA splicing research were reported. First, protein factors that promote the transition between pre-splicing complexes and the mature spliceosome were discussed, including several new interactions involving the heterotrimeric U2 snRNP protein complex. A second focus of attention was the chemical requirements for splicing, with the identification of conditions under which synthetic half-introns react in *trans*. This approach represents a powerful new route to unraveling the contributions of individual functional groups. Third, there were several reports of the dynamic rearrangements involving small nuclear RNAs and their RNA or protein partners. Of particular interest was evidence that the excision of a new class of introns is mechanistically similar to the splicing of standard introns, but it involves a distinct set of snRNAs.

Recent research in the editing field suggests that a dsRNA adenosine deaminase is responsible for editing glutamate receptor mRNAs as well as the hepatitis delta virus antigenome. However, it is not known whether the dsRNA adenosine deaminase previously purified from mammals and frogs is the enzyme responsible for these editing events. Nor is it known how many additional factors may be required for editing in vivo.

Significant advances in 3′-end formation included an electron microscopic analysis of poly(A) binding protein (PABII) binding to poly(A) tails. Consistent with the idea that the number of PABII molecules in a polyadenylation complex determines poly(A) length, electron micrographs showed PABII forming spherical oligomeric particles that protect ~260 adenine residues, a length corresponding to that of newly synthesized poly(A) tails in vivo.

The beginnings of a definition of an RNA export pathway from the nucleus are emerging with the report of a cellular RNA-binding factor directly involved in RNA export. Also, there were the reports of signals in cellular and viral proteins implicated in RNA export. The viral protein was

M. Hollingsworth, L. Read, J. Wise, A. Krainer

B. Bass, I. Mattaj

also reported to interact with a protein resembling a nucleoporin. Taken together, these observations suggest an outline for some of the interactions required to move an RNA out of the nucleus.

The analogy between snRNAs and pre-mRNA splicing on the one hand and snoRNAs and pre-rRNA processing on the other was extended with the demonstration that base-pairing between two snoRNAs and the pre-rRNA is required for its processing. The number of different snoRNAs encoded in pre-mRNAs continues to rise. The fact that some of them are proposed to pair with important segments of mature rRNA predicts that these snoRNAs may not function to promote specific cleavage/processing steps but to prevent RNA misfolding during ribosome assembly.

The meeting concluded on Sunday morning with a session on RNA catalysis. Studies of ribozymes currently involve an emphasis on the tertiary interactions and conformational changes that appear required for certain catalytic events. Similar to protein enzymes, ribozymes achieve catalysis through substrate destabilization as well binding interactions that provide entropic fixation. There was also the exciting development of an in vitro system that reconstitutes the initial steps of intron mobility.

This meeting was funded in part by the National Science Foundation.

PROGRAM

Modification, Editing, and 3′ End Formation
Chairperson: S.L. Hajduk, *University of Alabama, Birmingham*

Mechanisms of Splicing
Chairperson: R. Reed, *Harvard Medical School, Boston, Massachusetts*

Workshop I: Straight Talk from NIH—Review of Postdoctoral Fellowships
Dr. Camilla Day, *Scientific Review, National Institutes of Health*

Transport, Localization, and Turnover
Chairperson: G. Dreyfuss, *University of Pennsylvania School of Medicine, Philadelphia*

Regulation of Splicing
Chairperson: P. Grabowski, *University of Pittsburgh, Pennsylvania*

Workshop II: Straight Talk from NIH—R01/R29 Navigation Basics
Dr. Camilla Day, *Scientific Review, National Institutes of Health*

RNA Structure and RNA-Protein Interactions
Chairperson: E. Wahle, *University of Basel, Switzerland*

Stable RNAs AND RNPs
Chairperson: D. Tollervey, *EMBL, Heidelberg, Germany*

RNA Catalysis
Chairperson: A.M. Pyle, *Columbia University, New York, New York*

Retroviruses

May 23–May 28 436 participants

ARRANGED BY **Hung Fan,** University of California, Irvine
Alan Rein, National Cancer Institute-Frederick Cancer Research Facility

This year's Retroviruses meeting covered many aspects of the replication of retroviruses and the mechanisms by which they cause disease in animals and man. One particularly important feature of the meeting is the interchange of information between researchers studying human retroviruses and those studying animal retroviruses.

Significant advances were reported in areas ranging from the three-dimensional molecular structure of viral proteins to the natural history of the AIDS virus in high-risk human populations. In particular, an X-ray crystallographic analysis of integrase, an essential enzyme in retrovirus replication, was reported for HIV-1, the causative agent of AIDS, and also for an avian retrovirus. This information should prove invaluable in the design of new classes of antiviral drugs. Other studies gave rise to new insights as to the mechanism by which HIV-1 and other retroviruses export some mRNA molecules to their cytoplasm without splicing, in contrast to the majority of cellular mRNAs. Several laboratories reported important advances in the experimental study of assembly of viral particles, including the apparent reproduction of this phenomenon in the test tube. Finally, a virus from a previously unknown retrovirus family has been isolated from fish.

Contributions from the Corporate Sponsors provided core support for this meeting.

PROGRAM

Pathogenesis
Chairpersons: J. Overbaugh, *University of Washington, Seattle*; K. Radke, *University of California, Davis*

Envelope/Receptors
Chairpersons: W.F. Anderson, *University of Southern California, Los Angeles*; P. Bates, *University of Pennsylvania, Philadelphia*

Reverse Transcriptase and Reverse Transcription
Chairpersons: S. LeGrice, *Case Western Reserve University*; J. Levin, *NICHD, National Institutes of Health*

Nuclear Import/Integration/Integrase
Chairpersons: F. Bushman, *Salk Institute for Biological Studies*; D. Grandgenett, *St. Louis University Medical Center*

Transcription/Tat/Tax
Chairpersons: K. Conklin, *University of Minnesota, Minneapolis*; N. Hernandez, *Cold Spring Harbor Laboratory*

J. Coffin, J. Staye

A. Rein, K. Beeman

F. Anderson, M. Januszeski, S. Goff

Posttranscriptional Events/Rev/Rex
Chairpersons: K. Beemon, *Johns Hopkins University*; M.-L. Hammarskjöld, *University of Virginia, Charlottesville*

Potpourri
Chairpersons: J. Dudley, *University of Texas, Austin*; J. Luban, *Columbia University, New York*

Assembly I
Chairpersons: E. Barklis, *Oregon Health Sciences University, Portland*; J. Wills, *Pennsylvania State University, Hershey*

Assembly II/Antivirals
Chairpersons: H. Göttlinger, *Dana-Farber Cancer Institute*; K.-T. Jeang, *NIAID, National Institutes of Health*

Other Accessory Genes
Chairpersons: N. Landau, *Aaron Diamond AIDS Research Center*; D. Trono, *Salk Institute for Biological Studies*

Yeast Cell Biology

August 15–August 20 383 participants

ARRANGED BY **Trisha Davis,** University of Washington
Mark Rose, Princeton University
Tom Stevens, University of Oregon

The conference on Yeast Cell Biology was the fifth biannual international meeting devoted to major aspects of cell biology in yeast. This conference is unique in that the study of all major areas of cell biology are integrated in a single simple eukaryotic organism, *Saccharomyces cerevisiae*. A common interest in one organism allowed for extensive cross-fertilization of ideas and methodologies. Important insights were further gained by studies in the fission yeast *Schizosaccharomyces pombe* as well as other yeasts. The impending completion of the sequencing of the *Saccharomyces* genome had a profound impact on the conference, greatly facilitating the identification of genes identified by their effects on critical cellular processes. One major area of interest included the functions of the actin cytoskeleton particularly with respect to cell polarity and organelle movement. A second area that was extensively described concerned the roles of microtubules and associated proteins and organizing structures in chromosome and nuclear movement. Third was targeting and sorting of proteins in the secretory, endocytotic and nuclear localization pathways.

One major discovery, described at the meeting, was the critical role of ubiquitination in the endocytosis of cell surface proteins and their eventual degradation by vacuolar, and not proteasomal, hydrolases. The integrated control of these various processes was described in several sessions devoted to the cell cycle, global regulatory networks, ion transport and signaling, and mating pathways. All in all it was a very exciting meeting with 383 scientists in attendance. Some 295 abstracts were presented, including 115 talks and 180 posters.

This meeting was funded in part by Anheuser-Busch Companies, Inc.

T. Davis

J. Becker, J. Warner, T. Fox

M. Rose

PROGRAM

The Actin Cytoskeleton and Cell Polarity
Chairperson: A. Bretscher, *Cornell University*

Mating Pathways and Sporulation
Chairperson: J. Thorner, *University of California, Berkeley*

Endocytosis and Protein Sorting
Chairperson: M. Cyert, *Stanford University*

Ions, Signaling, and Cell Wall
Chairperson: F. Solomon, *Massachusetts Institute of Technology*

Microtubules, Centromeres, Chromosome Segregation and Genome
Chairperson: D. Botstein, *Stanford University*

Functions of the Nuclear Envelope: Microtubule Organization and Nuclear Transport
Chairperson: J. Warner, *Albert Einstein College of Medicine*

Protein Folding/Degradation and Organelle Biogenesis
Chairperson: E. Jones, *Carnegie Mellon University*

Secretion
Chairperson: J.A. Wise, *Case Western Reserve University*

Global Regulatory Networks
Chairperson: J. Woolford, *Carnegie Mellon University*

Cell Cycle
Chairperson: D. Levin, *Johns Hopkins University*

Molecular Genetics of Bacteria and Phages

August 22–August 27 391 participants

ARRANGED BY **Susan Gottesman,** National Cancer Institute, National Institutes of Health
Carol Gross, University of California, San Francisco
Peter Model, Rockefeller University
William Resznikoff, University of Wisconsin, Madison
Jeff Roberts, Cornell University

This year's meeting on Molecular Genetics of Bacteria and Phages celebrated the roots of modern molecular biology and a long tradition of scientific discussion and experimentation on bacteriophage at Cold Spring Harbor—50 years since the first phage course in 1945, and nearly as long since the first CSH phage meeting. In addition to the presentation of over 300 abstracts in posters and short talks, each session was introduced by a scientist who has played an important part in the development of the field. A special historic session allowed longer talks by particular luminaries; François Jacob, Seymour Benzer, Frank Stahl, Gunther Stent, Rollin Hotchkiss, and Manny Delbruck. The subjects of these perspectives ranged from Evelyn Witkin's reminiscences of life at Cold Spring Harbor in 1945 to Frank Stahl's reconsideration of Edgar's discovery of amber suppressors in phage T4. Scientific sessions featured the customary summary of exciting progress in microbial molecular biology and two sessions on bacterial genome sequencing and analysis. The result was an exciting meeting that gave new generations of scientists a living introduction to the intellectual roots of molecular biology, and also celebrated the personalities and styles of the founders of the science.

This meeting was funded in part by National Institute of General Medical Sciences, National Science Foundation, U.S. Department of Energy, Genetics Computer Group, Inc., and Promega Corporation.

PROGRAM

Replication, Recombination, and Mutagenesis
Historical Introduction: G. Mosig, *Vanderbilt University*
Chairperson: M. Lieb, *University of Southern California Medical School*

RNA Polymerase
Historical Introduction: P. Geiduschek, *University of California, San Diego*
Chairperson: L. Rothman-Denes, *University of Chicago, Illinois*

Transcription Activation
Historical Introduction: B. Magasanik, *Massachusetts Institute of Technology*
Chairperson: A. Ullmann, *Institut Pasteur*

The Bacterial Genome
Historical Introduction: W. Szybalski, *University of Wisconsin Medical School*
Chairperson: J. Roth, *University of Utah*

F. Stahl

R. Hotchkiss, W. Szybalski

S. Benzer

G. Stent

F. Jacob, J.D. Watson

M. Delbrück

E. Witkin

J. Roberts, W. Resznikoff

S. Gottesman

Genome Software and Database Workshop
Chairperson: M. Berlyn, *Yale University*

Panel Discussion: "Community Needs, Interconnectivity, and Cooperative Efforts

Phage and Cell Surfaces
Historical Introduction: N. Zinder, *Rockefeller University*
Chairperson: M. Yarmolinsky, *National Institutes of Health*

DNA Structure
Historical Introduction: A. Campbell, *Stanford University*
Chairperson: N. Kleckner, *Harvard University*

Panel Discussion: Microbial Molecular Genetics—Past, Present, and Future
Panel: D. Botstein, J. Beckwith, J. Roth, L. Rothman-Denes

Global Control
Historical Introduction: E. Witkin, *Rutgers University*
Chairperson: T. Silhavy, Princeton University

Posttranscriptional Control
Historical Introduction: D. Kaiser, *Stanford University*
Chairperson: J. Beckwith, *Harvard Medical School*

Elongation, Termination, and Antitermination
Historical Introduction: C. Yanofsky, *Stanford University*
Chairperson: J. Miller, *University of California, Los Angeles*

Historic Session: Reflections on Fifty Years of Phage Research In and Out of Cold Spring Harbor
Chairperson: J.D. Watson, *Cold Spring Harbor Laboratory*

Speakers
R. Hotchkiss, *Rockefeller University*
S. Benzer, *California Institute of Technology*
F. Jacob, *Pasteur Institute*
F.W. Stahl, *Institute of Molecular Biology, University of Oregon*

Mechanisms of Eukaryotic Transcription

August 30–September 3 432 participants

ARRANGED BY **Winship Herr,** Cold Spring Harbor Laboratory
Robert Tjian, University of California, Berkeley
Keith Yamamoto, University of California, San Francisco

The 1995 Cancer Cells meeting, Mechanisms of Eukaryotic Transcription, was the fourth biennial meeting devoted to mechanisms of transcriptional regulation in eukaryotes. This conference, which benefits from the commonality of transcriptional regulatory mechanisms in eukaryotic species, attracted scientists from around the world studying transcription in species as diverse as yeast, plants, and invertebrate and vertebrate animals. Although there are many common mechanistic themes, the diversity of approaches to uncover these mechanisms is great, including structural biology, molecular genetics, biochemistry, and cellular biology. Important advances in our understanding of transcriptional regulation were presented, including the structure of the core region of the basal transcription factor TFIIB either alone or in complex with TBP and DNA; the roles of chromatin in transcriptional regulation; the relationship of the mammalian elongation factor elongin and the Von Hippel-Lindau tumor suppressor protein; detailed topographical studies of the regions of TBP important for interaction with RNA polymerase and other basal factors; and the structure of the a1/α2 regulatory complex to list only a few. The meeting was elegantly summarized by Steven McKnight. The meeting was funded in part by National Institute of General Medical Sciences and National Institute of Child Health and Human Development, both branches of National Institutes of Health.

PROGRAM

Basal Promoter Apparatus
Chairperson: N. Hernandez, *Cold Spring Harbor Laboratory*

Regulation of RNA Polymerase Activity
Chairperson: T. Maniatis, *Harvard University*

Coupling of Activators and the Initiation Machinery
Chairperson: R. Roeder, *Rockefeller University*

Induction and Repression
Chairperson: B. Graves, *University of Utah*

Higher Order Template Structure and Function
Chairperson: E. O'Shea, *University of California, San Francisco*

Site-specific Regulatory Complexes
Chairperson: A. Berk, *University of California, Los Angeles*

Noncovalent Modulation of Transcription Factors
Chairperson: M. Ptashne, *Harvard University*

Covalent Modification of Transcription Factors
Chairperson: M. Levine, *University of California, San Diego*

R. Ebright, K. Yamamoto

I. Grummt, D. Bohmann, N. Hernandez

Eukaryotic DNA Replication

September 6–September 10 349 participants

ARRANGED BY **Thomas Kelly,** Johns Hopkins University School of Medicine
Bruce Stillman, Cold Spring Harbor Laboratory

This was the fifth biannual meeting on Eukaryotic DNA Replication held at Cold Spring Harbor Laboratory. The field of eukaryotic DNA replication is undergoing a renaissance, and this has become the premier meeting on this subject. Particularly obvious at this meeting was the strong focus on the mechanisms of initiation of cellular DNA replication and the links between this process and the control of the cell cycle. These also were the beginnings of studies on the identification of the DNA sequences that determine the initiation of DNA replication in vertebrate cells, including human, and the identification of the proteins that potentially interact with these sequences. A mainstay of the field has been the study of the replication of viral genomes, and this year significant progress was reported in this area. Equally important was the characterization of chromosomal elements such as telomeres and the enzyme telomerase that maintains the integrity of the chromosome ends. A potentially interesting link of this enzyme to cancer progression was discussed. The meeting saw a record 349 participants and 262 presented abstracts. This reflects the strength of the meeting and the interest in this field at the moment.

Essential funding, increasingly to support Graduate Students, was from the National Institute of Child Health and Human Development, the National Institute of General Medical Sciences, and the National Cancer Institute (all within the National Institutes of Health), as well as from the National Science Foundation.

PROGRAM

Initiation of Cellular DNA Replication
Chairperson: S. Gerbi, *Brown University*

Replication Proteins
Chairperson: M. Wold, *University of Iowa*

Telomeres and Repair
Chairperson: C. Newlon, *UMD-New Jersey Medical School*

Control of DNA Replication
Chairperson: J. Li, *University of California, San Francisco*

Chromosome Replication
Chairperson: U. Hübscher, *University of Zürich, Switzerland*

Regulation
Chairperson: J. Hamlin, *University of Virginia School of Medicine, Charlottesville*

Replication Protein Mechanisms
Chairperson: P. Burgers, *Washington University, St. Louis*

Viruses and Organelles
Chairperson: S. Weller, *University of Connecticut Health Center, Farmington*

R. Laskey

C. Newlon, J. Campbell, E. Fanning

Molecular Approaches to the Control of Infectious Diseases

September 13–September 17 160 participants

ARRANGED BY **Fred Brown,** USDA, Plum Island Animal Disease Center
Dennis Burton, Scripps Research Institute
John Mekalanos, Harvard Medical School
Erling Norrby, Karolinska Institute, Sweden

The annual meeting on Molecular Approaches to the Control of Infectious Diseases highlighted recent developments in a number of related areas. Infectious disease has once more become a major health concern. AIDS is now the prime cause of death in the 25–44 year old age group in the United States, Lyme disease is widespread, new viruses such as the Sin Nombre hanta virus have emerged, older viruses such as Ebola have re-emerged, and antibiotic-resistant bacteria are seen ever more frequently. The challenge to basic and more applied science is being taken up vigorously as shown at this meeting.

There is ever-increasing awareness of the importance of both humoral and cellular responses to many pathogens. A session on immunity to viruses revealed some of the essential features of immune memory in the T-cell compartment. This theme was complemented in a session on HIV which suggested that long-term progression in some HIV-seropositive individuals was associated with strong cytotoxic T cell response to the virus. A session on antibodies was focused on the generation, in vitro evolution, and evaluation of recombinant antibodies using phage display technology. Recombinant human antibodies are close to immunoprophylactic evaluation in the clinic. Structural studies revealed the interaction of antibodies with rhinovirus at the molecular level. Vaccination with naked DNA has seen such an explosion of activity since its first presentation at this meeting in 1992 that two sessions were devoted to the approach. As feasibility has been established, several groups are now searching for understanding. Individual sessions were devoted to bacterial and parasite vaccines. Ingenious strategies were described in both areas which could have major impact.

Many important infections take place via mucosal surfaces and several papers described studies on mucosal immunity and the generation of vaccines designed to elicit mucosal immunity. Increasingly, the similarities between primary isolates of HIV and SIV are appreciated and a vigorous session dealt with vaccine protection against SIV and its implications for HIV. The full spectrum of infectious disease was covered with papers on the prion protein, the putative agent of diseases of protein conformation. This meeting was funded in part by American Cyanamid Company and Pharmacia LKB Biotechnology.

B. Stillman, W. Bessler, P. Doherty

R. Chanock, H. Sabin

L. Babiuk, E. Norrby

PROGRAM

Immunity and Viruses
Chairperson: P.C. Doherty, *St. Jude Children's Research Hospital*

DNA Immunization I
Chairperson: L.A. Babiuk, *Veterinary Infectious Disease Organization, Saskatoon, Canada*

DNA Immunization II
Chairperson: F. Brown, *USDA, Plum Island Animal Disease Center*

Bacterial Vaccines
Chairperson: J.J. Mekalanos, *Harvard Medical School*

Recombinant Antibodies
Chairperson: C.F. Barbas III, *Scripps Research Institute*
Keynote Speaker: T. Smith: Antigen-Antibody Interactions

Mucosal Immunity and Delivery Systems
Chairperson: J.R. McGhee, *University of Alabama, Birmingham*

SIV
Chairperson: R. Desrosiers, *New England Regional Primate Research Center, Harvard Medical School*

Parasite Vaccines
Chairperson: R.S. Nussenzweig, New York University Medical Center

HIV
Chairperson: B.D. Walker, *Massachusetts General Hospital and Harvard Medical School*

Albert B. Sabin Vaccine Foundation Award Ceremony and Memorial Lecture
Welcoming Remarks: Dr. James D. Watson, *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

1995 Albert B. Sabin Memorial Lecture
Dr. Robert M. Channock, *NIAID, National Institutes of Health*

Closing Remarks: Dr. James D. Watson, *Cold Spring Harbor Laboratory*

Programmed Cell Death

September 20–September 24 340 participants

ARRANGED BY **Stanley J. Korsmeyer,** Washington University School of Medicine
Eileen White, Rutgers University
H. Robert Horvitz, Massachusetts Institute of Technology

The field of programmed cell death (apoptosis) has blossomed over the last several years such that the timing was very appropriate for a large Cold Spring Harbor-style meeting. The meeting brought together diverse members of the field to present and critically discuss their findings. The highlights of the meeting included inroads into the function of the *Drosophila* Reaper gene product, which may act similarly to the death domain of Fas antigen in mammalian cells. The identification of genetic suppressors of *reaper* was reported. The Fas and TNF signaling of apoptosis was further elucidated with the identification of Fas death domain binding proteins. Downstream from Fas and TNF is the ICE family of cysteine proteases, first implicated in apoptosis through the discovery of sequence homology with the *C. elegans* Ced-3 apoptosis-promoting gene product. The poxvirus CrmA ICE inhibitor blocks apoptosis by Fas in other settings, and new evidence was reported that the baculovirus p35 gene product may have the same function. New Bcl-2-related proteins were reported, one of which has only a BH3 domain. The BH3 domain was found by several groups to be sufficient for interactions between Bcl-2 family members and a region slightly larger than BH3 of Bak was also sufficient for induction of cell death. The phenotype of the Bax knock-out mouse was reported to consist primarily of thymic hyperplasia and male sterility, suggesting redundancy in function among the Bcl-2-related apoptosis promoters. These and other discoveries, with discussion thereafter, produced a meeting of extraordinary intellectual interest and value to the field that we hope to recreate in 1997.

This meeting was funded in part by National Science Foundation; U.S. Department of Energy; and National Institute on Aging, National Institute of General Medical Sciences; and National Institute of Allergy & Infectious Diseases (all branches of the National Institutes of Health).

S. Nagata, P. Krammer

M.-C. Hsu, E. White

PROGRAM

Invertebrate Development
Chairperson: H. Steller, *Massachusetts Institute of Technology*

Immunology/Neurology
Chairperson: S. Nagata, *Osaka Bioscience Institute, Japan*

***bcl-2* Family**
Chairperson: S. Cory, *Walter and Eliza Hall Institute of Medicine, Australia*

Biochemistry
Chairperson: J. Yuan, *Massachusetts General Hospital East*

Viral
Chairperson: G. Evan, *Imperial Cancer Research Fund, London, United Kingdom*

Oncogenesis
Chairperson: A. Levine, *Princeton University*

Vertebrate Development
Chairperson: M. Raff, *University College, London, United Kingdom*

Disease
Chairperson: A. Wyllie, *University Medical School, Edinburgh, United Kingdom*

Signaling in Plant Development

September 27–October 1 174 participants

ARRANGED BY **Nam-Hai Chua,** Rockefeller University
June Nasrallah, Cornell University
Venkatesan Sundaresan, Cold Spring Harbor Laboratory

This meeting was organized to highlight recent advances in understanding the mechanisms by which external and internal signals can regulate plant development. The absence of mobility in higher plants has resulted in the evolution of elaborate developmental responses to environmental cues. Developmental programs in plants either require particular signals for initiation or are modulated by sets of signals that can evoke different types of developmental responses. This theme was stressed repeatedly during the course of the meeting, which covered every aspect of plant development regulated by signaling processes. Sessions were held on germination, photomorphogenesis, vegetative growth, flowering, pollination, and embryogenesis. In addition, plant responses to special conditions such as stress, and to pathogens and symbionts, were explored in separate sessions. Highlights of the meeting included announcements of the cloning of several regulatory loci controlling key signaling processes, such as genes that regulate responses to gibberellins, abscisic acid, light, cell-cell recognition and signaling, flowering time, embryo development, and pathogen recognition. Other highlights were descriptions of advances in technology, such as in vitro fertilization and patch clamping, that will permit more detailed investigation of these processes in the future.

V. Sundaresan, C. Dean, S. Long

R. Fischer, J. Nasrallah

The meeting was funded in part by grants from the Department of Energy, the National Science Foundation, and the United States Department of Agriculture. Contributions of financial support for graduate students were received from Ciba-Geigy Corporation, E.I. Du Pont de Nemours and Company, and Pioneer Hi-Bred International Inc.

PROGRAM

Germination
Chairperson: D. McCarty, *University of Florida, Gainesville*

Photomorphogenesis
Chairperson: X.-W. Deng, *Yale University*

Vegetative Growth I: Communications
Chairperson: J. Schroeder, *University of California, San Diego*

Vegetative Growth II: Shoots, Roots, and Leaves
Chairperson: K. Barton, *University of Wisconsin, Madison*

Flowering
Chairperson: C. Dean, *John Innes Centre, United Kingdom*

Pollination and Fertilization
Chairperson: D. Preuss, *University of Chicago*

Embryogenesis
Chairperson: B. Fischer, *University of California, Berkeley*

Altered Developmental Programs I: Environmental Effectors
Chairperson: R. Hangarter, *Indiana University, Bloomington*

Altered Developmental Programs II: Biological Effectors—Microbial Signals
Chairperson: J. Dangl, *University of North Carolina*

Altered Developmental Programs III: Biological Effectors—Host Responses
Chairperson: B. Baker, *PGEC-USDA, University of California, Berkeley*

Neurobiology of *Drosophila*

October 5–October 9 267 participants

ARRANGED BY **James Posakony,** University of California, San Diego
Kalpana White, Brandeis University

The primary goal of this year's meeting on *Drosophila* neurobiology was to provide a forum for exchange of ideas, techniques, and new discoveries within the field of *Drosophila* neurobiology. The meeting consisted of platform and poster presentations by 267 junior and established investigators. The platform sessions were arranged to reflect the exciting advances that have been made in understanding the molecular mechanisms underlying cell-cell communication, synaptic cell biology, ion channel function, signal transduction, and behavior. The work presented included genetic, cell biological, molecular, neurophysiological, and behavioral approaches to address questions that spanned nervous system development to nervous system function. The highlights of the meeting included presentation of exciting new developments in several areas,

J. Thomas, P. Taghert

M. Gonzales-Gatan, M. Gho

including biological rhythms, learning and memory, glial determination, plasticity, and synaptic cell biology. Also noteworthy were several new behavioral paradigms and technical advances in targeted cell ablation. The setting of the meeting provided for ample opportunities for informal discussions. The high quality of presentations and the novel findings in many areas made it amply clear that this format, which is inclusive of all aspects of neurobiology, is extremely useful to young and seasoned scientists, as it provides as ideal opportunity to cover all the different aspects of *Drosophila* neuroscience.

This meeting was funded in part by the National Science Foundation and the National Institute of Neurological Disorders and Stroke, a branch of the National Institutes of Health.

PROGRAM

Behavior
Chairperson: B.J.Taylor, *Oregon State University*

Sensory Transduction, Learning, and Memory
Chairperson: C. Zuker, *Howard Hughes Medical Institute, University of California, San Diego*

Channels, Receptors, and Synaptic Transmission
Chairperson: L.M. Hall, *State University of New York, Buffalo*

Cell-Cell Signaling in Development
Chairperson: M.W. Young, *Howard Hughes Medical Institute, Rockefeller University*

Cell-Cell Interaction: Axons and Glia
Chairperson: U. Heberlein, *University of California, San Francisco*

Transcriptional and Posttranscriptional Regulation
Chairperson: S. Crews, *University of North Carolina, Chapel Hill*

Cell Biology of the Neuron
Chairperson: H. Bellen, *Howard Hughes Medical Institute, Baylor College of Medicine*

H. Ellis, J. Posakony

POSTGRADUATE COURSES

The summer program of Postgraduate Courses at Cold Spring Harbor Laboratory is aimed at meeting the special need for training in interdisciplinary subjects that are either so new or so specialized that they are not adequately treated by universities. Our aim is to provide intensive study in the most recent developments and techniques in these subjects and to prepare students to enter directly into research in a particular area. To ensure up-to-date coverage of current research work, we bring together a workshop staff from many laboratories around the world and supplement this staff with a series of seminar speakers.

Advanced Genome Sequence Analysis

March 14–27

INSTRUCTORS

Chen, Ellson, Ph.D., Perkin Elmer Corporation
Gibbs, Richard, Ph.D., Baylor College of Medicine
McCombie, W. Richard, Ph.D, Cold Spring Harbor Laboratory
Wilson, Richard, Ph.D., Washington University School of Medicine

ASSISTANTS

Fitzgerald, Michael, Massachusetts General Hospital
Johnson, Arthur, Cold Spring Harbor Laboratory
Johnson, Douglas, Washington University School of Medicine
Kaplan, Nancy, Cold Spring Harbor Laboratory
Lodhi, Muhammad, Cold Spring Harbor Laboratory
Muzny, Donna, Baylor College of Medicine
Parson, Jeremy, Washington University School of Medicine
Wentland, Meredith, Baylor College of Medicine
Zho, Lin, Sequana Therapeutics

Recent advances in the automation of DNA sequencing have opened new possibilities for the analysis of complex genomes at the DNA sequence level. This 2-week course provided intensive training in this rapidly evolving field. The course emphasized techniques and strategies for using automated sequencers to sequence large, contiguous genomic regions. Students carried out all of the steps in the sequencing process from preparing cosmid DNA to computer analysis of the finished sequence.

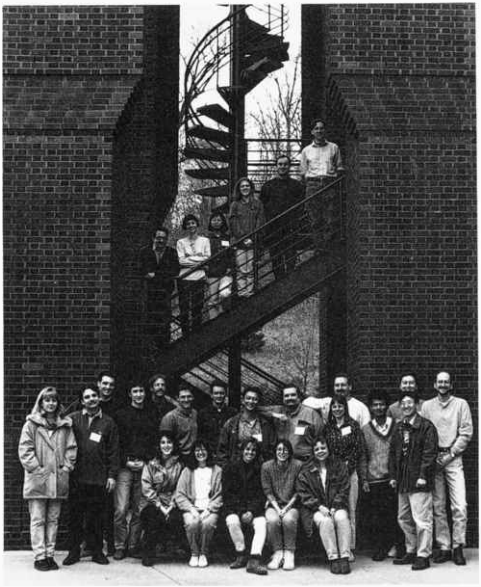
Topics included subclone library generation, large-scale template purification, sequencing reactions, gel analysis on automated sequencers, sequence assembly, gap filling, and conflict resolution. Students worked in groups to sequence a 44,913-base cosmid insert from fission yeast and through this process were trained in crucial project and data management techniques. A series of lecturers discussed their applications of these techniques as well as alternate strategies for high-speed automated DNA sequencing.

PARTICIPANTS

Ballard, W., Ph.D., CSIRO, Australia
Ben Asher, E., Ph.D., Weizmann Institute of Science, Israel
Gaudierei, S., Ph.D., University of Western Australia, Australia
James, C., Ph.D., University of Manchester, United Kingdom
Krall, J., B.S., Amersham Life Sciences, Inc., Cleveland, Ohio
Latinwo, L., Ph.D., Florida A&M University
Lau, C.H., B.S., M.S., National University of Singapore
McPherson, J., Ph.D., University of California, Irvine
Meyers, B., B.A., M.S., University of California, Davis
Myerson, J., B.S., M.S., Merck Research Laboratories
Paricio, N., Ph.D., University of Valencia, Spain
Schramm, S., B.S., GenPharm International, Mt. View, California
Silvey, M., B.S., University of East Anglia, United Kingdom
Singh, R., Ph.D., National Research Council of Canada, Canada
Tian, J., B.D., M.S., National Institute of Standards and Technology, Gaithersburg, Maryland
Young, A., Ph.D., Whitehead Institute for Biomedical Research, Cambridge, Massachusetts

SEMINARS

Caskey, T., Merck Research Laboratories, West Point, Pennsylvania. Triplet repeat diseases.
Dusterhoft, A., Qiagen GmbH., Germany. New developments in template purification for genome sequencing projects.
Marr, T., Cold Spring Harbor Laboratory. From pedigrees to sequences: Dissection of complex genetic diseases by computer.
Roe, B., University of Oklahoma. Human genomic sequencing: Strategies, results, analyses, and lessons learned from approximately one megabase.
Smith, D., Collaborative Research, Inc., Waltham, Massachusetts. Large-scale multiplex sequencing.
Smith, R., Baylor College of Medicine. Sequence alignment and database searching: A practical guide for molecular biologists.
Studier, W., Brookhaven National Laboratory, Upton, New York. DNA sequencing by primer walking with hexamer strings.
Watson, J., Cold Spring Harbor Laboratory. How the Human Genome Project got started.
Weiss, R., University of Utah. Automated multiplex sequencing: Technology development and applications.



Cloning and Analysis of Large DNA Molecules

March 30–April 12

INSTRUCTORS

Birren, Bruce, Ph.D., Whitehead Institute/MIT Center for Genome Research
Abderrahim, Hadi, M.D., Ph.D., Cell Genesys, Inc.
Vollrath, Douglas, M.D., Ph.D., Stanford University

ASSISTANTS

Dewar, Ken, Laval University, Canada
Friddle, Carl, Stanford University School of Medicine
Funke, Roel, University of Tennessee

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. Clones were produced and characterized by several approaches including library screening, contig assembly, long-range restriction mapping, and recovery of YAC ends. Lectures by outside speakers on topics of current interest supplemented the laboratory work.

PARTICIPANTS

Alexopoulou, L., B.S., Hellenic Pasteur Institute, Greece
Antoch, M., Ph.D., Northwestern University, Evanston, Illinois
Aragona, M., Ph.D., Instituto Sperimentale Patologia Vegetale, Italy
Bursell, C., B.S., University of Goteborg, Sweden
Boren, J., M.D., Ph.D., Gladstone Institute for Cardiovascular Research, San Francisco, California
Cappellano, C., Ph.D., Lepetit Research Center, Italy
Ferrario, S., Ph.D., University of Milan, Italy
Gayle, M., B.A.,M.S., Darwin Molecular Corporation, Bothell, Washington
Jongstra, J., Ph.D., University of Toronto, Canada
Koi, M., Ph.D., National Institutes of Health, Bethesda, Maryland
Leaves, N., B.Sc., Oxford Public Health Laboratory, United Kingdom
Liu, X., B.S., M.S., University of Connecticut
Ma, J., Ph.D., Harvard Medical School
Mendiola, J., Ph.D., University of Minnesota
Serratosa, J., M.D., Ph.D., University of California, Los Angeles
Terryn, N., Ph.D., University of Gent, Belgium

SEMINARS

Ecker, J., University of Pennsylvania. Physical and generic mapping of *Arabidopsis* genome.
Green, E., National Institutes of Health, Bethesda, Maryland. YAC-based physical mapping of human chromosomes.
Lai, E., University of North Carolina, Chapel Hill. BAC cloning of mammalian DNA, the true story.
Michelmore, R., University of California, Davis. Clusters of disease resistance genes in lettuce.
Shepherd, N., Glaxo Research Institute, Research Drive, North Carolina. Genetics and drug discovery.
Shizuya, H., California Institute of Technology. How to BAC.
Strauss, W., Beth Israel Hospital, Boston, Massachusetts. Transgenesis: The next generation.

Protein Purification and Characterization

March 30–April 12

INSTRUCTORS

Burgess, Richard, Ph.D., University of Wisconsin, Madison
Kadonaga, James, Ph.D., University of California, San Diego
Lin, Sue Hwa, Ph.D., University of Texas/M.D. Anderson Cancer Center
Marshak, Daniel, Ph.D., Cold Spring Harbor Laboratory

ASSISTANTS

Carpino, Nicholas, Cold Spring Harbor Laboratory
Earley, Karen, University of Texas
George, Catherine, University of California, San Diego
Burke, Thomas, University of California, San Diego
Grabski, Anthony, University of Wisconsin, Madison

This course was intended for scientists who were 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 pre-

cipitation 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 including immunological and biochemical assays, peptide mapping, amino acid analysis, protein sequencing, and mass spectrometry. Emphasis was placed on strategies of protein purification and characterization 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 were A. Courey, A. Kornberg, S.-H. Lin, N. Pace, G. Rose, B. Stillman, and N. Tonks.

PARTICIPANTS

Ansari, A., Ph.D., Harvard University
Burk, R., M.D., Albert Einstein College of Medicine, Bronx, New York
Cheung, A., Ph.D., USDA, National Animal Disease Center, Ames, Iowa
Dietmeier, K., Ph.D., University of Freiburg, Germany
Gong, M.C., Ph.D., University of Virginia
Gravel, A., B.S., M.S., CHUL Research Center, Canada
High, K., M.D., University of Pennsylvania School of Medicine
Hughes, T., Ph.D., Baylor College of Medicine, Houston, Texas
Hwang, L.-Y., Ph.D, University of Texas Southwestern Medical Center
Jain, R., Ph.D., Harvard Medical School
Jiang, R., B.S., Columbia University
Lukacs, G., Ph.D., University of Toronto, Canada
Michels, C., Ph.D., CUNY, Queens College
Miyoshi, E., B.S., University of California, Los Angeles
Strunnikov, A., Ph.D., Carnegie Institute of Washington
Wang, T., Ph.D., Massachusetts General Hospital

SEMINARS

Burgess, R., University of Wisconsin, Madison. Overview of protein purification methods.
Courey, A., University of California, Los Angeles. Transcription factors.
Kornberg, A., Stanford University School of Medicine. Enzyme purification.
Lin, S.-H., University of Texas, M.D. Anderson Cancer Center. Membrane protein purification.
Pace, N., Texas A&M University. Protein folding.
Rose, G., Johns Hopkins University Hospital. Protein structure.
Stillman, B., Cold Spring Harbor Laboratory. DNA replication protein.
Tonks, N., Cold Spring Harbor Laboratory. Protein tyrosine phosphatases.

Early Development of *Xenopus laevis*

April 4–13

INSTRUCTORS

Grainger, Robert, Ph.D., University of Virginia
Sive, Hazel, Ph.D., Whitehead Institute

ASSISTANTS

Amaya, Enrique, University of California, Berkeley
Gammill, Laura, Whitehead Institute
Papalopulu, Nancy, Salk Institute for Biological Studies

This course provided extensive laboratory exposure to the biology, manipulation, and use of embryos from the frog, *Xenopus laevis*. The course was suited both for investigators who have had no experience with *Xenopus* and for those who have worked with *Xenopus* and wished to expand their

repertoire of techniques. All students had current training in molecular biology and some knowledge of developmental biology. The course consisted of intensive laboratory sessions, supplemented by daily lectures and demonstrations from experts in both experimental and molecular embryology. Six areas were covered: (1) care and handling of adults and embryo isolation; (2) stages of embryonic development and anatomy; (3) whole mount in situ hybridization and immunocytochemistry; (4) microinjection of eggs and oocytes, including mRNA and antisense oligonucleotides; (5) micromanipulation of embryos, including induction and transplantation assays; and (6) preparation and use of cell cycle extracts. Lecturers and co-instructors included Enrique Amaya, Rick Elinson, Janet Heasman, John Gurdon, Richard Harland, Ray Keller, John Newport, and Nancy Papalopulu.

PARTICIPANTS

Andreazzoli, M., Ph.D., Ospedale San Raffaele, Italy
Antonelli, M., Ph.D., University of Chile, Chile
Aronheim, A., Ph.D., University of California, San Diego
Avis, J., Ph.D., University of Manchester, United Kingdom
Devchand, P., Ph.D., University of Lausanne, Switzerland
Dominguez, M.I., Ph.D., Harvard Medical School
Harnada, H., M.D., Ph.D., Cancer Institute, Japan
Hill, C., Ph.D., Imperial Cancer Research Fund, United Kingdom
Hoppler, S., Ph.D., University of Washington School of Medicine
Humbert-Lan, G., M.S., Georg August University, Argentina
Koster, R., M.D., MPI Biophysikolisch Chemie, German
Nastos, A., B.S., University of Essen, Germany
Sullivan, S., Ph.D., George Washington University
Sun, B., Ph.D., Whitehead Institute, Cambridge, Massachusetts
Suzuki, A., B.S., M.S., Hokkaido University, Japan
Zucker, S., B.S., Albert Einstein College of Medicine, Bronx, New York

SEMINARS

Elinson, R., University of Toronto, Canada. Cytoplasmic organization of the egg and dorsoventral polarity of the embryo.
Grainger, R., University of Virginia. Completing the body plan: Inductive mechanisms during organogenesis.
Gurdon, J., Cambridge University, United Kingdom. Induction of the mesoderm in *Xenopus*.
Harland, R., University of California, Berkeley. Molecular nature of Spemann's organizer: Role of peptide factors in *Xenopus* development.
Heasman, J., University of Minnesota. Maternal control of development of *Xenopus*.
Keller, R., University of California, Berkeley. Morphogenesis in the early *Xenopus* embryo.
Newport, J., University of California, San Diego. Cell cycle control mechanisms in *Xenopus*.
Sive, H., Whitehead Institute, Cambridge, Massachusetts. Anteroposterior patterning in *Xenopus*.

Molecular Embryology of the Mouse

June 9–29

INSTRUCTORS

Behringer, Richard, Ph.D., University of Texas, M.D. Anderson Cancer Center
Papaioannou, Virginia, Ph.D., Columbia University

CO-INSTRUCTORS

Koopman, Peter, Ph.D.,University of Queensland, Australia
Magnuson, Terry, Ph.D., Case Western Reserve University
Nagy, Andras, Ph.D., Mount Sinai Hospital, Canada

ASSISTANTS

Nichols, Jenny, University of Edinburgh, United Kingdom
Shawlot, William, University of Texas, M.D. Anderson Cancer Center

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 of embryos, immunohistochemistry, microinjection of DNA into pronuclei, and microinjection of embryonic stem cells into blastocysts. Last year's speakers were A. Bradley, M. Bronner-Fraser, N. Jenkins, T. Jessell, R. Lovell-Badge, L. Parada, A. McLaren, A. McMahon, J. Mann, G. Rinchik, L. Robertson, J. Rossant, P. Soriano, S. Strickland, P. Wassarman, and D. Wilkinson.

PARTICIPANTS

Biggs, W., B.A., Ph.D., University of California, San Diego
Collinge, J., B.S., M.D., St. Mary's Hospital Medical School, United Kingdom
Gow, A., B.S., M.S., Ph.D., Mt. Sinai School of Medicine, New York, New York
Incerti, B., M.D., Telethon Institute of Genetics & Medicine, Milan, Italy
Johnson, R., B.S., Ph.D., Harvard Medical School
Kasarskis, A., B.S., B.A., Ph.D., University of California, Berkeley
Larsson, N.-G., M.D., Ph.D., Stanford University School of Medicine
Luc, L., B.S., Ph.D., University of California, Los Angeles
McDonald, D., B.S., Ph.D., Wichita State University
Meagher, M., B.S., Ph.D. University of Washington, Seattle
Memet, S., M.S., Ph.D., Institute Pasteur, France
Obermayr, F., M.S., Imperial Cancer Research Fund, United Kingdom
Rauch, T., M.S., Hungarian Academy of Sciences, Hungary
Yueh, Y.G., Ph.D., Mayo Clinic, Scottsdale

SEMINARS

Bradley, A., Baylor College of Medicine, Houston, Texas. Mouse embryonic stem cells.
Mechanisms of homologous recombination.
Behringer, R., University of Texas, M.D. Anderson Cancer Center. Genetic factors that organize pattern in the mouse.
Bronner-Fraser, M., University of California, Irvine. Lineage studies of the neural crest.
Efstratiadis, A., Columbia University. The function of insulin-like growth factors during mouse embryogenesis.
Hogan, B., Vanderbilt University Medical School, HHMI, Nashville, Tennessee.
Preimplantation development and implantation.
Bone morphogenetic proteins and mouse development.
Jessell, T., Columbia University College of Physicians & Surgeons. Pattern formation in the vertebrate nervous system.
Joyner, A., New York University Medical Center. Genetic analysis of the vertebrate nervous system.
Koopman, P., University of Queensland, Australia. *Sox* genes and mouse development.
Lovell-Badge, R., National Institute for Medical Research, United Kingdom.
Sex determination in mammals.
Functional analysis of the *Sox* gene family.
Magnuson, T., Case Western Reserve University, Cleveland, Ohio. Mutations that alter germ layer formation in the mouse.
Mann, J., Beckman Research Institute, Duarte, California. Molecular analysis of genomic imprinting during mouse embryogenesis.
McMahon, A., Harvard University. Mechanisms of embryonic induction.
Nagy, A., Mount Sinai Hospital, Ontario, Canada. Genetic analysis of N-*mcy* during mouse organogenesis.
Papaioannou, V., Columbia University. Mouse chimeras in experimental embryology.
Rastan, S., Hammersmith Hospital, United Kingdom. X chromosome inactivation.
Solter, D., Max-Planck Institute of Immunology, Germany. Genomic imprinting.
Soriano, P., Fred Hutchinson Cancer Research Center, Seattle, Washington. Insertional mutagenesis in the mouse.
Tam, P., Children's Medical Research Institute, Australia. Postimplantation development.
Cell lineage analysis in early mouse embryogenesis.
Wilkinson, D., National Institutes of Medical Research, United Kingdom. Mechanisms of segmentation in the nervous system and paraxial mesoderm.
Woychik, R., Oak Ridge National Laboratory, Oak Ridge, Tennessee. Mouse models for human disease.

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

Darwin, Andrew, Ph.D., Cornell University
Skorupski, Karen, Ph.D., Darmouth Medical School
Zahrt, Thomas, M.S., University of Illinois, Urbana

The laboratory course demonstrated genetic approaches that can be used in diverse bacterial systems to analyze biological processes and their regulation, as well as detailed structure/function re-

lationships 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. Lecturers and discussions concentrated on the application of genetic analysis to contemporary questions in bacterial physiology, diversity, and pathogenesis.

Last year's guest lecturers were B. Bassler, S. Highlander, T. Silhavy, H. Shuman, M. Winkler, and J. Westpheling.

PARTICIPANTS

Barbieri, J., B.S., Ph.D., Medical College of Wisconsin
Darby, C., B.A., Ph.D., University of Washington, Seattle
Edwards, R., B.S., Ph.D., University of Pennsylvania
Galloway, D., B.S., Ph.D., Ohio State University
Gavigan, J.-A., B.A., Universidad de Zaragoza, Spain
Hondalus, M., D.V.M., Ph.D., Temple University of Medicine, Philadelphia, Pennsylvania
Hong, K., B.S., Ph.D., University of California, Los Angeles
Konkel, M., B.S., M.S., Ph.D., Washington State University
Mikulskis, A., M.S., Ph.D., Harvard Medical School
Miyagi, H., B.A., B.S., University of Illinois, Urbana
Moors, M., B.S., Ph.D., University of Pennsylvania
Norregaard-Madsen, M., M.S., Odense University, Denmark
Schmitz, R.A., B.S., Ph.D., University of California, Berkeley
Sullam, P., B.A., M.D., University of California, San Francisco
Widdel, F., B.S., Ph.D., MPI Fur Marine Microbiology, Germany
Zdych, E., B.A., B.S., University of Konstanz, Germany

SEMINARS

Bassler, B., Princeton University. Intercellular communication in luminous bacteria: The glow of social interaction.
Jacobs, W., Albert Einstein College of Medicine, HHMI, Bronx, New York. A 1914 penny, lotto, and tuberculosis control.
Manoil, C., University of Washington, Seattle. Tn*phoA*.
Roth, J., University of Utah. Recombination.
Silhavy, T., Princeton University. PRL suppressors and the mechanism of protein secretion in *E. coli*.
Trun, N., National Cancer Institute, Bethesda, Maryland. Counting chromosomes in *E. coli*.
Weinstock, G., University of Texas Health Sciences Center. Bacterial genometry.
Youderian, P., University of Idaho. Challenge phage.

Molecular Approaches to Ion Channel Structure, Expression, and Function

June 10–30

INSTRUCTORS

Liman, Emily, Ph.D., Harvard Medical School
Margulies, Jody, Ph.D., University of Hawaii
Ruben, Peter, Ph.D., Utah State University

ASSISTANT

Ono, Joyce, Ph.D., California State University

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 last year included M. Bennett, F. Bezanilla, C. Czajkowski, A. Goldin, R. MacKinnon, G. Mandel, J. Nerbonne, D. Papazian, F. Sigworth, and M. White.

PARTICIPANTS

Anegawa, N.J., B.A., University of Pennsylvania School of Medicine
Beck, C., B.S., Ph.D., Vanderbilt University, Nashville, Tennessee
Chaves, D., B.S., M.S., University of Guelph, Canada
Featherstone, D., M.S., B.S., University of Hawaii
Grichtchenko, I., M.S., Ph.D., New York University Medical Center
Saeki, M., B.A., Ph.D., Baylor College of Medicine, Houston, Texas
Shuey, D., B.S., Wyeth-Ayerst Research, Monmouth Junction, New Jersey
Stuart, A. B.A., Ph.D., University of North Carolina, Chapel Hill
Takahashi, M., B.S., University College London, United Kingdom
Tottene, A., B.S., University of Padova, Italy
Yang, W.-P., B.S., Ph.D., Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, New Jersey
Yuan, X.-J., M.D., Ph.D., University of Maryland School of Medicine

SEMINARS

Akabas, M., Columbia University. Structure of Ach and GABA receptor pore lining domains.
Bennett, P., Vanderbilt University Medical Center, Nashville, Tennessee. Structure-function and pharmacology of human sodium channel.
Bezanilla, F., University of California, Los Angeles. Voltage-dependent gating of channels.
MacKinnon, R., Harvard Medical School. Using mutant cycles to measure the pore of potassium channels.
Mandel, G., State University of New York, Stony Brook. Regulation of neuronal excitability through transcriptional silencing.
Papazian, D., University of California, Los Angeles. A strategy for determining the package of transmembrane segments in Shaker potassium channels.
Rayner, M., University of Hawaii. Modeling the gating mechanisms of voltage-gated ion channels.
Robertson, G., University of Wisconsin, Madison. The Eag family of K channels: Functional diversity and clinical relevance.
Role, L., Columbia University School of Medicine. Composition of neuronal nicotinic Ach receptor subunits.
Snutch, T., University of British Columbia, Canada. Molecular mechanisms of calcium channel modulation.

Brain Mapping

June 12–18

INSTRUCTORS

Mazziotta, John, M.D., Ph.D., University of California, Los Angeles
Toga, Arthur, Ph.D., University of California, Los Angeles

The aim of this lecture course was to describe the rapidly evolving developments in brain mapping that have been applied to the problem of mapping the structure and function of the brain, both to understand its normal function and to evaluate neurological, neurosurgical, and psychiatric disease states. This course described new methods as well as the application of traditional techniques to the study of brain structure and function. Methodologies that were discussed included magnetic resonance imaging (including functional, spectroscopic, and angiographic approaches), positron emission tomography, electrophysiological techniques, optical intrinsic signal imaging, digital approaches to conventional postmortem neuroanatomical investigations, data analysis, statistical analysis, statistical approaches, visualization, and stereotaxy. The course was not designed to simply describe methods, but rather to discuss how brain mapping strategies can be employed in combination with biological models for understanding the structure and function of the brain. Findings relevant to the function of the visual, motor, language, memory, and cognitive brain systems, as well as diseases that adversely affect them, were discussed. Specific hypotheses and experimental designs were developed by the students for mock execution, and as an actual experiment during a field trip to an imaging laboratory. Invited speakers included world leaders in each of the respective brain mapping subspecialties.

PARTICIPANTS

Chen, G., M.D., University of Minnesota
Doetsch, F., B.S., Ph.D., Rockfeller University, New York, New York
Haist, F., B.A., Ph.D., University of California, San Diego
Hanson, C., B.S., University of Minnesota
Kandel, A., B.A., Rutgers University, Newark, New Jersey
Larisch, R., M.D., Institute of Medicine and Research, Julich, Germany
Lewis, J., B.S., Washington University School of Medicine, St. Louis, Missouri
Moses, P., B.A., University of California, San Diego
Posse, S., B.S., Ph.D., Research Center Julich GmbH., Germany
Robertson, E., B.S., University of Oxford, United Kingdom
Sanders, I., B.S., M.D., Mt. Sinai Medical Center, New York, New York
Saron, C., B.A., M.S., Albert Einstein College of Medicine, Bronx, New York
Smith, H., B.A., M.D., University of North Carolina, Chapel Hill
Tommerdahl, M., B.S., M.S., Ph.D., University of North Carolina, Chapel Hill
Yeh, E., B.S., Case Western Reserve University, Cleveland, Ohio
Zimmermann, E., B.S., Ph.D., German Primate Center, Germany

SEMINARS

Cherry, S., University of California, Crump Institute, Los Angeles. PET methods. Uses and limits of PET studies.
Cohen, M., University of California Medical School, Los Angeles. MRI methods. Uses and limits of MRI studies.
Frackowiak, R., Hammersmith Hospital, United Kingdom. PET applications. Uses and limits of PET studies.
Friston, K., MRC Cyclotron, United Kingdom. Data Analysis, statistics.
Mazziotta, J., University of California School of Medicine, Los Angeles.
Brain mapping issues.
Time & space, in vivo imaging: PET, SPECT, MRI, CT.
Brain atlases.
Pascual-Leone, A., Universite de Neurobiologie, Spain. Transcranial magnetic stimulation.
Peterson, S., Washington University School of Medicine. Study design, hypothesis testing, cognitive studies.
Prichard, J., Yale University School of Medicine. MRI applications. Uses and limits of MRI studies.
Toga, A., University of California School of Medicine, Los Angeles.
General principles, microtechniques: OIS, histology, cryomacrotome.
Reconstruction, registration, segmentation, display.
Brain atlases.
Tootell, R., Massachusetts General Hospital, NMR. Micro/macro brain mapping, visual system.
Woolsey T., Washington University School of Medicine. Micro mapping, vascular mapping.
Zeffiro, T., Sensor Systems, Inc., Sterling, Virginia. Analysis, software.

Structure, Function, and Development of the Visual System

June 21–July 4

INSTRUCTORS

Bonhoeffer, Tobias, Ph.D., Max-Planck Institute, Germany
Fitzpatrick, David, Ph.D., Duke University

This lecture/discussion course explored the functional organization and development of the visual system as revealed by the use of a variety of anatomical, physiological, and behavioral methods. It was designed for graduate students and more advanced researchers who wished to gain a basic understanding of the biological basis for vision and to share in the excitement of the latest developments in this field. Topics included phototransduction and neural processing in the retina; functional architecture of striate cortex; cellular basis of cortical receptive field properties; the anatomy, physiology, and perceptual significance of parallel pathways; functional parcellation of extrastriate cortex; sensory-motor integration in the superior colliculus; and role of patterned neuronal activity in

the development of central visual pathways. Past lecturers included B. Chapman, D. Dacey, C, Gilbert, C. Gray, D. Hubel, L. Katz, K. Martin, J. Maunsell, T. Movshon, K. Nakayama, C. Shatz, M. Stryker, A. Thomson, D. Van Essen, and R. Wong.

PARTICIPANTS

Abel, P., B.S., University of Washington, Seattle
Baharloo, S., B.S., M.S., Ph.D., University of California, San Francisco
Bosking, W., B.A., Duke University, Durham, North Carolina
Brecht, M., B.S., Max-Planck Institute, Germany
Burrows, A., B.A., M.D., Ph.D., University of Texas
Dan, Y., B.S., Ph.D., Rockefeller University
Joris, P., B.S., M.D., University of Wisconsin, Madison
Platt, M., B.A., Ph.D., New York University
Rachel, R., B.S., Ph.D., Columbia University
Samantha R., Deborah, B.A., Yale University
Sax, C., B.S., Ph.D., National Institutes of Health, Bethesda, Maryland
Shmuel, A., B.S., M.S., Weizmann Institute of Science, Israel
Zhou, Y.-X., M.S, Ph.D., New York University

SEMINARS

Bonhoeffer, T., Max-Planck Institute, Germany. Development of functional architecture in visual cortex.
Chapman, B., University of California, Davis. How visual cortex got its stripes.
Dacey, D., University of Washington, Seattle. The primate retina: Cell types, circuits, and color coding.
Fitzpatrick, D., Duke University Medical Center. Local circuits in visual cortex: Relating patterns of connectivity to functional maps.
Gilbert, C., Rockefeller University, New York, New York. Spatial integration and cortical dynamics.
Gray, C., University of California, Davis. Response synchronization in visual cortex and its mechanism of generation.
Hubel, D., Harvard Medical School. Form, color, and stereopsis in visual cortex.
Katz, L., Duke University Medical Center. Constructing columns and circuits in the visual cortex.
Martin, K., MRC Anatomical Neuropharmacology Unit, United Kingdom. Making sense of cortical microcircuitry.
Maunsell, J., Baylor College of Medicine, Houston, Texas. Representing image and target in primate visual cortex.
Movshon, T., New York University. Neural foundations of visual perceptual judgment.
Nakayama, K., Harvard University. Visual surface representation: A critical link between lower-level and higher-level vision.
Shatz, C., University of California, Berkeley. Constructing the visual system from scratch: Early steps in brain wiring.
Thomson, A., Royal Fee Hospital School of Medicine, United Kingdom. Temporal and spatial properties of local circuits in neocortex.
Van Essen, D., Washington University School of Medicine. Organization and function of primate visual cortex.
Wong, R., Washington University School of Medicine. Retinal development: From cell birth to making connections.

Arabidopsis Molecular Genetics

July 3–23

INSTRUCTORS

Deng, Xing-Wang, Ph.D., Yale University
Last, Robert, Ph.D., Boyce Thompson Institute, Cornell University
Preuss, Daphne, Ph.D., University of Chicago

ASSISTANTS

Von Arnim, Albrecht, Yale University
Wilhelmi, Laura, University of Chicago
Zhao, Jianmin, Boyce Thompson Institute, Cornell University

This course provided an intensive overview of topics in plant growth and development, focusing on molecular genetic approaches to understanding plant biology. It emphasized recent results from *Arabidopsis thaliana* and other model plants and provided an introduction to current methods used in *Arabidopsis* research. The course also demonstrated the use of microbial systems in plant research, including *Agrobacterium*, *Escherichia coli*, *Saccharomyces cerevisiae*, and plant pathogens. It was designed for scientists with experience in molecular techniques or in plant biology who wish to work with *Arabidopsis*. The course consisted of a vigorous lecture series, a hands-on laboratory, and informal discussions. Speakers provided both an in-depth discussion of their own work and a review of their specialty.

Discussions of important topics in plant research were presented by the instructors and by invited speakers. These seminars reviewed plant anatomy; plant development (including development of flowers, roots, meristems, embryos, and the epidermis); perception of light and photomorphogenesis; responses to a variety of environmental stresses including pathogens, UV-B and ozone; synthesis and function of secondary metabolites and hormones; nitrogen assimilation; unique aspects of plant cell biology; the importance of transposons and *Agrobacterium* for manipulating plant genomes; and current approaches to genome analysis.

The laboratory sessions provided an introduction to important techniques currently used in *Arabidopsis* research. These included studies of *Arabidopsis* development, mutant analysis, studies of epidermal features, in situ detection of RNA, subcellular localization of proteins, analysis

of secondary metabolites, transformation with *Agrobacterium*, transient gene expression in plant cells, expression of plant proteins in microorganisms, detection and analysis of plant pathogens, and techniques commonly used in genetic and physical mapping.

Past speakers included J. Bender, A. Britt, T., C. Chapple, J. Chory, G. Drews, J. Ecker, S.Y. He, A. Lloyd, H. Ma, R. Martienssen, J. Medford, T. Mitchell-Olds, P. Quail, J. Schiefelbein, R. Scholl, B. Staskawicz, D. Stern, I. V. Sundaresan, Sussex, T. Voelker, V. Walbot, and P. Zambryski.

PARTICIPANTS

Agarwal, A., B.S., Ph.D., The Monsanto Company, Chesterfield, Missouri
Curtis, M., B.S., Ph.D., University of Queensland, Australia
Dodgson, S., B.S., Ph.D., University of Pennsylvania School of Medicine
Heard, J., B.S., Ph.D., Boston College
Hooks, M., B.A., Ph.D., University of Glasgow, Scotland
Mishkind, M., B.A., Ph.D., Bennington College, Bennington, Vermont
Oyama, T., B.S., M.S., Kyoto University, Japan
Preuss, S., B.A., Ph.D., University of California, Davis
Ramonell, K., B.S., Ph.D., Louisiana State University
Richeson, A., B.S., National Science Foundation
Shukla, V., B.S., Ph.D., Washington University
Strayer, C., B.S., University of Virginia
Ticknor, C., B.A., Yale University
Winge, P., M.S., University of Trondheim, Norway
Ye, Q., B.S., M.D., Dartmouth College
Zhu, X.-Z., B.S., M.S., Weizmann Institute of Science, Israel

SEMINARS

Bender, J., Whitehead Institute/Massachusetts Institute of Technology, Cambridge. Yeast methods for cloning genes.
Britt, A., University of California, Davis. DNA damage and repair.
Chapple, C., Purdue University. Secondary metabolites.
Chory, J., Salk Institute, La Jolla, California. Nucleus-organelle communication.
Colasanti, J., Cole Spring Harbor Laboratory. Floral determination in maize.
Deng, X.-W., Yale University. Photomorphogenesis.
Drews, G., University of Utah. Female gametophyte development and embryo development.
Ecker, J., University of Pennsylvania. Hormones.
He, S.-Y., University of Kentucky. Plant pathogens.
Last, R., Boyce Thompson Institute at Cornell University. Plant stress responses.
Amino acid biosynthesis.
Lloyd, A., Stanford University. The epidermis.
Ma, H., Cold Spring Harbor Laboratory. Floral development.
Martienssen, R., Cold Spring Harbor Laboratory. Enhancer traps in *Arabidopsis*.
Medford, J., Pennsylvania State University. Shoot meristems.
Mitchell-Olds, T., University of Montana.
QTLs and natural variation.
Mapping complex phenotypes.
Preuss, D., University of Chicago. Pollen development and fertilization.
Quail, P., USDA, Albany, New York. Light perception.
Schiefelbein, J., University of Michigan. Root development.
Scholl, R., Ohio State University. *Arabidopsis* resources.
Staskawicz, B., University of California, Berkeley. Plant defenses.
Stern, D., Cornell University. Plastid genomes and gene expression.
Sundaresan, V., Cold Spring Harbor Laboratory. The cell cycle.
Sussex, I., University of California, Berkeley. Plant anatomy.
Voelker, T., Calgene Inc. Fatty acids and genetic engineering in plants.
Walbot, V., Stanford University. Transposons.
Zambryski, P., University of California, Berkeley. *Agrobacterium*.

Molecular Cloning of Neural Genes

July 3–23

INSTRUCTORS

Boulter, Jim, Ph.D., Salk Institute for Biological Studies
Chao, Moses, Ph.D., Cornell University Medical College
Edwards, Robert, Ph.D., University of California, San Francisco
Julius, David, Ph.D., University of California, San Francisco
Lai, Cary, Ph.D., Scripps Research Institute

ASSISTANTS

Elgoyhen, Ana Belen, Instituto de Investigaciones Farmacologicas, Argentina
Kong, Haeyoung, University of Pennsylvania

This intensive laboratory and lecture course was intended to provide neuroscientists at all levels with an introduction to the techniques of molecular biology. The course consisted of daily laboratory exercises, morning and afternoon discussions on the practical aspects of molecular biology, and a series of evening research seminars presented by invited speakers. This lecture series emphasized the variety of ways in which recombinant DNA methodologies have been successfully applied to the study of invertebrate and vertebrate nervous systems.

The laboratory portion of the course included isolation and characterization of poly(A)$^+$ RNA; isolation and characterization of genomic, plasmid, phagemid, and viral DNAs; synthesis of complementary DNA and construction, plating and screening of expression libraries; gel electrophoresis of nucleic acids; use of DNA modification and restriction enzymes; Southern and Northern blotting; hybridization of RNA and DNA using radioactively labeled nucleic acid probes; plasmid and genomic DNA mapping; ligations, transformations, and subcloning; oligonucleotide design, synthesis, purification, and utilization; DNA sequencing and computer-assisted sequence analysis; polymerase chain reaction methods and applications; in vitro transcription for RNase protection, in situ hybridization and *Xenopus* oocyte injections; evaluation of subtractive hybridization protocols; differential display of cellular RNAs; and in vitro transfection of cultured mammalian cells. Sleeping was optional. Last year's guest lectures were presented by M.M. Poo, H. Cline, J. Morgan, J. Eberwine, A.M. Quinn, L. Niswander, K. Zinn, G. Lemke, B. Barres, H. Ingraham, G. Weinmaster, and K. O'Malley.

PARTICIPANTS

Bezprozvanny, I., M.S., Ph.D., Stanford University Medical Center
Burd, G., B.A., Ph.D., University of Arizona
Chang, W., B.A., Ph.D., University of California, San Diego
Chiu, A., B.A., Ph.D., City of Hope National Medical Center, Duarte, California
Cline, H., B.A., Ph.D., Cold Spring Harbor Laboratory
Firestein, S., B.S., Ph.D., Columbia University
Grice, D., M.D., Yale University Child Study Center
Hammonds-Odie, L., B.S., University of Alabama, Birmingham
Hay, M., B.A., Ph.D., University of Texas Health Science Center
Livesey, F., B.S., Medical Research Council, United Kingdom
Marcus, R., B.A., Ph.D., Columbia University College of Physicians & Surgeons, New York, New York
Messersmith, E., B.S., M.A., Ph.D., University of California, Berkeley/HHMI
Popov, S., M.S., Ph.D., University of Illinois at Chicago
Richards, L., B.S., Ph.D., Salk Institute, La Jolla, California
Shrager, P., B.A., B.S., Ph.D., University of Rochester Medical Center
Woo, K., B.A., M.S., California Institute of Technology

SEMINARS

Burden, S., New York University Medical Center. Signaling at neuromuscular junctions.
Craig, A.M., University of Illinois, Urbana. Heterologous gene expression in primary cultured neurons.
Darnell, R., Rockefeller University, New York, New York. Expression cloning of neural disease antigens.
Fields, S., State University of New York, Stony Brook. Yeast hybrid methods.
Hoffman, B., National Institutes of Mental Health. Expression cloning and molecular studies of neurotransmitter transporters.
Lemke, G., Salk Institute, La Jolla, California. Receptor tyrosine kinases and neural development.
Mandel, G., State University of New York, Stony Brook. Regulation of a neuronal sodium channel through transcriptional repression.
Mombaerts, P., Columbia University. Targeting olfaction.
Quinn, A.M., Yale University Medical School. Strategies for searching gene databases on the World Wide Web.
Worley, P., Johns Hopkins University School of Medicine. Identification of novel brain immediate early genes using subtractive hybridization and differential cloning strategies.

Neurobiology of *Drosophila*

July 3–July 23

INSTRUCTORS

Bieber, Alan, Ph.D., Purdue University
Taghert, Paul, Ph.D., Washington University
Hardie, Roger, Ph.D., Cambridge University, United Kingdom

ASSISTANT

Schaefer, Anneliese, Washington 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.

In last year's course, physiological emphasis was given to the 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.

Last year's guest lecturers included J. Campos-Ortega, J. Carlson, M. Dickinson, R. ffrench-Constant, V. Hartenstein, L. Iverson, R. Jackson, H. Keshishian, N. Patel, L. Restifo, M. Sokolowski, N. Strausfeld, B. Taylor, J. Truman, T. Tully, V. Venkatesh, C. F. Wu, and Y. Zhong.

PARTICIPANTS

Bhat, M., M.S., Ph.D., Baylor College of Medicine
Bohm, R., B.S., University of Texas at Austin
Callaerts, P., B.S., Ph.D., Biozentrum, Switzerland
Coleman, M., B.S., University of Pennsylvania School of Medicine
Dormand, E.-L., B.A., University of Cambridge, United Kingdom
Hardiman, K., B.A., University of Michigan, Ann Arbor
Levine, J., B.A., Ph.D., Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts
Marcolla Araujo, H., B.S., Ph.D., Federal University of Rio Je Janeiro, Brazil
McCabe, B., B.A., University of Cambridge, United Kingdom
Raghu, P., M.B., B.S., National Centre for Biological Sciences, India
Wurmbach, E., B.S., Universitat Hohenheim, Germany
Xu, H., B.S., M.S., Queen's University, Canada

SEMINARS

Bieber, A., Purdue University. Axon pathfinding.
Blochlinger, K., Fred Hutchinson Cancer Research, Seattle, Washington. Histogenesis of the nervous system.
Cagan, R., Washington University School of Medicine. Eye development.
Dickinson, M., University of Chicago. Flight behavior.
Ganetsky, B., University of Wisconsin, Madison. Introductory physiology, ion channels.
Hardie, R., Cambridge University, United Kingdom. Visual system.
Hall, J., Brandeis University. Circadian rhythms.
Hartenstein, V., University of California, Los Angeles. Embryonic development.
Kaiser, K., University of Glasgow, Scotland. The adult brain II.
Kernan, M., State University of New York, Stony Brook. Mechanosensory systems.
Keshishian, H., Yale University. Synaptogenesis.
Laurent, G., California Institute of Technology. Neural integration.
Meinertzhagen, I., Dalhousie University, Nova Scotia, Canada. The adult brain I.
Restifo, L., University of Arizona. Metamorphosis of the nervous system.
Taylor, B., Oregon State University. Sex determination and behavior.
Taghert, P., Washington University Medical School. Neurotransmitters/neuromodulators.
Tully, T., Cold Spring Harbor Laboratory. Learning and memory.
Yin, J., Cold Srping Harbor Laboratory. CREB and long-term memory formation in *Drosophila*.
Wu, C.-F., University of Iowa. Motor systems.
Zhong, Y., Cold Spring Harbor Laboratory. Neuromechanisms underlying *Drosophila* learning and memory.

The Biology of Memory: From Molecules to Behavior

July 7–20

INSTRUCTORS

Bryne, Jack, Ph.D., University of Texas-Houston Medical School
Mauk, Michael, Ph.D., University of Texas-Houston Medical School
Pearson, Keir, Ph.D., University of Alberta, Canada
Squire, Larry, Ph.D., University of California, San Diego

This lecture course provided an introduction to cellular, molecular, and systems approaches to learning and memory. It was suited for graduate students in molecular biology, neurobiology, and psychology as well as research workers who are interested in an introduction to this field. The course covered topics ranging from behavioral considerations of learning and memory to gene regulation in the nervous system. The lectures provided an intensive coverage of six selected areas: (1) an introduction to modern behavioral studies of learning and memory; (2) an overview of the cell biology of neuronal plasticity and second messenger systems; (3) the regulation of gene expression; (4) cellular and molecular mechanisms of simple forms of learning and memory in invertebrates and vertebrates; (5) cellular and molecular mechanisms of long-term potentiation and depression in various regions of the mammalian brain; and (6) systems approaches to learning in vertebrates and humans. Lecturers included G. Ball, M. Baer, A. Doupe, H. Eichenbaum, C. Gilbert, W. Greenough, P. Holland, E. Kandel, P. Pfaffinger, M. Raichle, C. Rankin, H. Schulman, J. Steinmetz, and T. Tully.

PARTICIPANTS

Buffalo, E., B.A., University of California, San Diego
Christensen, J., B.S., Cold Spring Harbor Laboratory
Cobain, M., B.S., Wyeth Research Ltd., United Kingdom
Connolly, C., B.S., Ph.D., University College London, United Kingdom
Garren, J., B.A., Brown University
Gusev, P., B.S., N.V. Lomonosov State University, Russia
Hittner, J., B.A., University of California, Irvine
Hudmon, A., B.S., M.S., University of Texas Health Science Center
Kabotyanski, E., Ph.D., University of Texas-Houston Medical School
Murphy, M., B.S., Ph.D., Walter & Eliza Hall Institute, Australia
Plasterk, R., Ph.D., Netherlands Cancer Institute, The Netherlands
Rogove, A., B.S., State University of New York, Stony Brook
Setlow, B., B.A., University of California, Irvine
Stubbs, L., B.S., Ph.D., Oak Ridge National Laboratory
Yamakawa, K., B.S., Ph.D., Cedars-Sinai Medical Center, Los Angeles, California

SEMINARS

Ball, G., Johns Hopkins University. Ethological approaches to learning.

Byrne, J., University of Texas-Houston Medical School.
Introduction to the cellular study of learning.
Overview of membranes and synaptic transmission I.
Overview of membranes and synaptic transmission II.
Classical conditioning in *Aplysia*.

Bear, M., Brown University. Mechanisms of long-term depression.

Doupe, A., University of California, San Francisco. Bird-song learning.

Eichenbaum, H., State University of New York, Stony Brook. Role of the hippocampus and hippocampal long-term potentiation in learning.

Gilbert, C., Rockefeller University. Functional reorganization in visual cortex.

Greenough, W., University of Illinois, Urbana. Morphological correlates of learning and experience.

Holland, P., Duke University.
Introduction to learning theory I.
Introduction to learning theory II.

Kandel, E., Columbia University.
Nonassociative learning in *Aplysia* I.
Nonassociative learning in *Aplysia* II.
Long-term potentiation I.
Long-term potentiation II.

Mauk, M., University of Texas-Houston Medical School.
The structure and function of ion channels.
Plasticity in the vestibulo-ocular reflex.

Pearson, K., University of Alberta, Canada. Mechanisms of motor learning.

Pfaffinger, P., Baylor College of Medicine.
Cloning of genes important to learning I.
Cloning of genes important to learning II.

Raichle, M., Washington University School of Medicine, St. Louis. Neuropsychology of cognition.

Rankin, C., University of British Columbia, Canada. Genes and behavior of *C. elegans*.

Steinmetz, J., Indiana University. Classical conditional of the nictatating membrane.

Squire, L., University of California, San Diego.
Memory in nonhuman primates.
Human memory and disorders of memory.

Tully, T., Cold Spring Harbor Laboratory. Genetic approaches to study associative learning in *Drosophila*.

Schulman, H., Stanford University. Overview of second messenger systems and their role in learning and memory.

Developmental Neurobiology

July 24–August 6

INSTRUCTORS

Lemke, Greg, Ph.D., The Salk Institute
O'Leary, Dennis, Ph.D., The Salk Institute

The aim of this lecture course was to discuss established principles and recent advances in developmental neurobiology. Major topics considered were proliferation, migration, and aggregation of

neurons; determination and differentiation of neural cells; trophic interactions in neural development; patterns; gradients and compartments; genetic programs for development; the guidance of axons to targets; and the formation of synaptic connections. These topics were considered within the context of the development of both invertebrate and vertebrate neural systems. Students had a background in neurobiology or molecular biology. Speakers included Y. Barde, C. Goodman, M.E. Hatten, N. Heinz, T. Jessell, C. Kintner, G. Lemke, D. O'Leary, J. Rubenstein, J. Sanes, C. Shatz, and L. Zipursky.

PARTICIPANTS

Ahlgren, S., B.A., Ph.D., University College London, United Kingdom
Araujo, H.M., M.S., Ph.D., Brazilian Federation for Experimental Biology, Brazil
Arber, S., B.S., Friedrich Miescher Institute, Germany
Belliveau, M., B.S., Harvard Medical School
Collignon, J., Ph.D., Harvard University
Craig, C., B.S., Ph.D., University of Toronto, Canada
Dale, J., B.S., Ph.D., National Institute for Medical Research, United Kingdom
Delaney, C., B.S., University of Wisconsin, Madison
Dokucu, M., M.D., Washington University School of Medicine
Episkipou, V., B.S., Ph.D., MRC Clinical Sciences Center, United Kingdom
Forjanic, J.P., B.S., Max-Planck Institute, Germany
Frade, J., B.S., Ph.D., Cajal Institute, Spain
Lustig, M., B.A., New York University Medical Center
Meijer, D., M.D., Erasmus University, The Netherlands
Mombaerts, P., M.D., Ph.D., Columbia University
Olsson, M., B.S., University of Lund, Sweden
Patapoutian, A., B.S., Ph.D., California Institute of Technology
Peles, E., M.S., Ph.D., Sugen Inc., Redwood City, California
Pierani, A., Ph.D., Institut Curie, France
Wallace, V., B.S., Ph.D., Medical Research Council, United Kingdom

SEMINARS

Barde, Y., Max-Planck Institute, Germany. Neurotrophins and their receptors in development.
Goodman, C., University of California, Berkeley. Establishing the specificity of the neuronal connections.
Hatten, M. and N. Heinz, The Rockefeller University. Cellular interactions in cortical and cerebellar development.
Jessell, T., Columbia University. Induction and cell patterning in the vertebrate nervous system.
Kinter, C., The Salk Institute, La Jolla, California. Neural induction and early A-P patterning of the nervous system.
Lemke, G., The Salk Institute. Myelin and myelination.
O'Leary, D., The Salk Institute. Systems development: Development of the cerebral cortex.
Rubenstein, J., University of California, San Francisco. Segmentation of the nervous system.
Sanes, J., Washington University School of Medicine. Development of neuromolecular junction.
Shatz, C., University of California, Berkeley. The generation and maintenance of synaptic connections.
Zipursky, S.L., University of California, Los Angeles. Cell fate choice and specification of the *Drosophila* eye.

Advanced Molecular Cloning and Expression of Eukaryotic Genes

July 25–August 14

INSTRUCTORS

Burtis, Kenneth, Ph.D., University of California, Davis
Learned, Marc, Ph.D., University of California, Davis
Smale, Stephen, Ph.D., University of California, Los Angeles

ASSISTANTS

Connolly, Erin, University of California, Davis
Kaelin, Christopher, University of California, Davis

This course focused on both the cloning and characterization of eukaryotic genes to probe their structure, function, and expression. As a model system, the students 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 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, including mobility shift assays, DNase I footprinting, and methylation interference. Regulation of gene expression by nuclear proteins was examined both by in vitro transcription assays using cell-free extracts and by transfection of cloned DNA into mammalian tissue culture cells. Analytical techniques for these expression studies included primer extension, nuclease protection, and enzymatic assays for reporter proteins.

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 present current problems in eukaryotic molecular biology as well as technical approaches to their solution. Experience with basic recombinant DNA techniques was a prerequisite for admission to this course. Seminar speakers included S. Bell, S. Burley, M. Carey, V. Chandler, L. Freedman, C. Greider, D. Gilmour, N. Hernandez, R. Sen, and K. Struhl.

PARTICIPANTS

April, C., B.S., University Cape Town, South Africa
Bazhenova, O., B.S., Ph.D., New England Deaconess Hospital
Bernstein, L., B.A., Ph.D., National Cancer Institute
Brunson, D., B.S., M.S., D.V.M., University of Wisconsin School of Veterinary Medicine
Buxbaum, J., B.S., M.S, Ph.D., Rockefeller University
Carpino, N., B.A., Cold Spring Harbor Laboratory
Crosby, J., B.S., University of Chile, Chile
Ekengren, S., B.S., Stockholm University, Sweden
Gallicano, I., B.S., Ph.D., Arizona State University
Greenberg, A., B.A., M.D., Tufts University
Keinanen, R., M.S., Ph.D., University of Kuopio, Finland
Kontoyiannis, D., M.D., Harvard Medical School
Losordo, D., B.A., M.D., Tufts University
Mahal, S., B.S., St. Mary's Hospital Medical School, United Kingdom
Watts, R., B.S., National University of Singapore, Singapore
Zander, C., B.S., Karolinska Hospital, Sweden

SEMINARS

Bell, S., Massachusetts Institute of Technology. Initiation of DNA replication in yeast.

Burley, S., The Rockefeller University. X-ray crystallographic studies of eukaryotic transcription factors.

Carey, M., University of California, Los Angeles. A mechanism for eukaryotic gene activation and synergy.

Chandler, V., University of Oregon. Multiple levels of regulation of the maize anthocyanin pathway.

Freedman, L., Memorial Sloan-Kettering Institute, New York, New York. Modulation of nuclear receptor target gene selectivity by receptor dimerization.

Greider, C., Cold Spring Harbor Laboratory. Isolation and characterization of telomerase genes.

Gilmour, D., Pennsylvania State University. In vivo and in vitro analyses of the hsp70 promoter reveal interactions involved in establishing transcriptional potential.

Hernandez, N., Cold Spring Harbor Laboratory. TBP-containing complexes in transcription by RNA polmerases II and III.

Sen, R., Brandeis University. Factors regulating Ig gene expression.

Struhl, K., Harvard Medical School. Molecular mechanisms of transcriptional regulation in yeast.

Imaging Structure and Function in the Nervous System

July 25–August 14

INSTRUCTORS

Augustine, George, Ph.D., Duke University Medical Center
Lichtman, Jeff, Ph.D., Washington University School of Medicine, St. Louis
Smith, Stephen, Ph.D., Stanford University

ASSISTANTS

Culican, Susan, Washington University School of Medicine, St. Louis
Eilers, Jens, University of Saarlandes, Germany
Finch, Beth, Duke University Medical Center
Handran, Shawn, Washington University School of Medicine, St. Louis

Advances in optical microscopy, digital image processing, and the development of a variety of powerful fluorescent probes presented 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, and the application of different kinds of video cameras and digital image processing to enhance microscopic images. To learn the principles of light microscopy, students built and operated an optical bench microscope. In other laboratory exercises, students used calcium-sensitive probes (e.g., fura-2), "caged" compounds, exocytost's tracers, whole-cell patch clamp methods to microinject fluorescent indicators into single cells, and other methods to explore neuronal function. A variety of neural systems, including living animals, brain slices, peripheral synapse preparations, acutely dissociated neurons, and cultured cells, were used in our experiments.

Lecturers included W. Betz, J. Connor, S. Fraser, F. Fay, L. Cohen, G. Ellis-Davies, J. Heuser, T. Inoue, L. Katz, A. Konnerth, F. Lanni, R. Lewis, M. Minsky, J. Nerbonne, J. Swedlow, R. Y. Tsien, W. Webb, and J. White.

PARTICIPANTS

Cohen, U., B.A., National Institutes of Health, HHMI
Deisseroth, K., B.S., Stanford University
Dresbach, T., B.S., Max-Planck Institute for Brain Research. Germany
Gan, W., B.E., Ph.D., Columbia University
Martin, K., B.A., M.D., Ph.D., Columbia University, HHMI
Mintz, I., Ph.D., Boston University School of Medicine
Mochida, S., M.A., Ph.D., Tokyo Medical College, Japan
Murthy, V., B.T., M.S., Ph.D., Salk Institute, La Jolla, California
Otis, T., B.S., M.S., Ph.D., University of Wisconsin Medical School
Pedarzani, P., M.D., Ph.D., University of Oslo, Norway
Sinha, S., B.S., Baylor College of Medicine
Uchitel, O., M.D., University of Buenos Aires, Argentina

SEMINARS

Augustine, G., Duke University Medical Center.
Calcium indicators. Calibration of fluorescent Ca indicator dyes.
Exploring synaptic function with optical methods.
Laser principles.
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, Nutley, New Jersey. Calcium imaging in brain slices.
Ellis-Davis, G., Oregon Health Sciences University. Design of caged compounds.
Heuser, J., Washington University School of Medicine. Interference reflection microscopy.
Inoue, T., Universal Imaging Corporation, West Chester, Pennsylvania. Image analysis of software.
Katz, L., Duke University Medical Center. Flash photolysis in brain slices.
Konnerth, A., Physiologisches Institut, Germany. Patch clamp measurements from CNS neurons in slices.
Lanni, F., Center for Light Microscope Imaging.
Standing wave fluorescence microscopy.
Near-field optical scanning.
Evanescent wave microscopy.
Lewis, R., Stanford University School of Medicine. Calcium signaling pathways.
Lichtman, J., Washington University.
Optical principles.
The light microscope.
Resolution.
Fluorescence principles.
Fluorescence microscopy and phototoxicity.
In vivo imaging.
Introduction to confocal microscopy.
Minsky, M., Massachusetts Institute of Technology. Development of the confocal microscope.
Oshiro, M., Hamamatsu Photonic Systems, Inc., Bridgewater, New Jersey. Low-light-level cameras.
Smith, S., Stanford University.
The measurement of light.
Video signals and standards.
The digitized image.
Polarized light.
Contrast from transparent specimen.
Life-support systems.
Multi-site time lapse.
Laser confocal microscopy.
Imaging studies of dendrite growth and synaptogenesis.
Swedlow, J., University of California, San Francisco.
Digital image restoration.
Volume rendering.
Tsien, R., Howard Hughes Medical Institute. Design and application of indicator dyes.
Webb, W., Cornell University. 2-photon microscopy principles.
White, J., Integrated Microscopy Resources, Madison, Wisconsin. 2-photon microscopy applications.

Yeast Genetics

July 25–August 14

INSTRUCTORS

Adams, Alison, Ph.D., University of Arizona
Kaiser, Chris, Ph.D., Massachusetts Institute of Technology
Gottschling, Daniel, Ph.D., University of Chicago

ASSISTANTS

Bickle, Steve, Massachusetts Institute of Technology
Davis, Dana, University of Arizona
Diede, Scott, University of Chicago

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. Micromanipulations used in tetrad analysis were carried out by all students. Molecular genetic techniques, including yeast transformation, gene replacement, and analysis of gene fusions, 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

Bauer, M., M.D.,. Universitat Munchen, Germany
Bentley, D., Ph.D., University of Toronto, Canada
Folkerts, O., B.S., M.S., Ph.D., DowElanco, Indianapolis, Indiana
Foskel, D., B.A., Ph.D., University of California, Irvine
Heinrich, J., B.A., Ph.D., American Cyanamid Co., Princeton, New Jersey
Holkeri, H., M.S., University of Helsinki, Finland
Li, Q., M.S., M.D., Karolinska Institute, Sweden
Pember, S., B.S., Ph.D., E.I. Dupont Company
Shimizu, K., B.S,. Ph.D., Osaka University, Japan
Stahl, G., Ph.D., CNRS-URA 1354, France
Stafford, G., B.A., Ph.D., State of New York Department of Health
Sun, Z., B.S., M.S., Yale University
Vasconcelles, M., B.A., M.D., Harvard Medical School
Weissman, J., B.A., Ph.D., Yale University/HHMI
White, T., B.S., Ph.D., University of California, San Francisco
Zuk, D., M.S., Ph.D, University of Massachusetts Medical School

SEMINARS

Adams, A., University of Arizona. Genetic analysis of the actin cytoskeleton.
Cherry, M., Stanford University. The *Saccharomyces* genome database.
Fields, S., State University of New York, Stony Brook. Yeast hybrid methods to analyze protein-protein, peptide-peptide, and protein RNA interactions.
Fink, G., Whitehead Institute, Cambridge, Massachusetts. The information superhighway: How they get from here to there.
Futcher, B., Cold Spring Harbor Laboratory. The yeast cell cycle engine.
Gottschling, D., University of Chicago. The secrets of silence.
Heiter, P., Johns Hopkins University. Determinants of chromosome transmission in yeast and humans.
Herskowitz, I., University of California, San Francisco. Control of cell specialization in yeast.
Kaiser, C., Massachusetts Institute of Technology. Life, secs, and then you die!
Michaelis, S., Johns Hopkins School of Medicine. Biogenesis of the A-factor mating pheromone.
Mitchell, A., Columbia University. Regulators that govern meiosis in yeast.
Petes, T., University of North Carolina, Chapel Hill. Macro- and micro-rearrangements of the yeast genome.
Rose, M., Princeton University. Nuclear fusion in yeast.
Sherman, F., University of Rochester School of Medicine. Cytochrome c, the complete story.
Struhl, K., Harvard Medical School. Molecular mechanisms of transcriptional regulation in yeast.
Weinert, T., University of Arizona. Checkpoints in yeast.
Winston, F., Harvard Medical School. Analysis of histones, TATA-binding protein, and other transcription factors of yeast.

Neurobiology of Human Neurological Disease: Mechanisms of Neurodegeneration

August 8–14

INSTRUCTORS

Choi, Dennis, M.D., Ph.D., Washington University School of Medicine
Mobley, William, M.D., Ph.D., 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,

amyotrophic lateral sclerosis, 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, so course participants did not need to be familiar with neurological diseases. The course focused on the specific hypotheses and approaches driving current research, and emphasized 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. Last year's faculty were D. Bredesen, M. Chalfie, V. Dawson, K. Fischbeck, E. Johnson, D. Landis, J. McNamara, H. Monyer, D. Price, S. Prusiner, and D. Selkoe.

PARTICIPANTS

Adams, A., B.S., Vanderbilt University, Nashville, Tennessee
Ahn, Y.S., M.D., Ph.D., Yonsei University College of Medicine, Korea
Arnold, C.S., B.S., University of Alabama, Birmingham
Bertorelli, R., B.S., Shering Plough Corporation, Milan, Italy
Burns, C., B.S., Vanderbilt University
Campos, L., M.D., MRC Cambridge, United Kingdom
Cheung, V., B.S., M.D., University of California, Los Angeles
Filburn, C., B.A., Ph.D., National Institutes of Health
Herdegen, T., M.D., Ph.D., University of Heidelberg, Germany
Krause, B.J., M.D., Research Center Julich, Germany
Maciel, P., B.S., University of Porto, Portugal
Mahanthappa, N., B.A., Ph.D., Cambridge Neuroscience, Inc., Cambridge, Massachusetts
Majeed, A.B. Abdul, Ph.D., University Sains, Malaysia
Marraccino, R., B.S., Ph.D., Roche Institute of Molecular Biology, Nutley, New Jersey
Meldahl, A.-S., Ph.D., University of Oxford, United Kingdom
Moechars, D., M.S., Katholieke University van Leuven, Belgium
Schmitt, B., M.D., University Zurich, Switzerland
Schneider, H., B.S., Ph.D., Schering AG, Berlin, Germany
Shirsat, N., M.S., Ph.D., Tata Institute, India
Tekirian, T., B.S., University of Kentucky
Tompkins, M., B.S., Medial College of Georgia
Yan, H., B.S., Ph.D., Amgen, Inc., Thousand Oaks, California
Zacharias, D., B.S., M.S., Mayo Graduate School, Rochester, Minnesota

SEMINARS

Bredesen, D., La Jolla Cancer Research Foundation, La Jolla, California. Control of neural apoptosis.
Chalfie, M., Columbia University. Genetically determined neurodegeneration.
Choi, D., Washington University School of Medicine, St. Louis. Excitotoxicity.
Dawson, V., John Hopkins University School of Medicine. Nitric oxide and neurodegeneration.
Fischbeck, K., University of Pennsylvania School of Medicine. Molecular genetics of neurological diseases.
Johnson, E., Washington University School of Medicine, St. Louis. Programmed cell death.
Landis, D., Case Western Reserve School of Medicine, Cleveland, Ohio. Role of glia in CNS injury.
McNamara, J., Duke University Medical Center. Epilepsy and ammon's sclerosis: Chicken and egg.
Mobley, W., University of California School of Medicine, San Francisco. Neuronal growth factors.
Monyer, H., University of Heidelberg, Germany. Glutamate receptors in health and disease.
Price, D., Johns Hopkins University School of Medicine. Motor neuron disease and Alzheimer's disease.
Prusiner, S., University of California School of Medicine, San Francisco. Prions.
Selkoe, D., Brigham and Womens's Hospital, Boston, Massachusetts. Molecular pathogenesis of Alzheimer's disease.

Advanced In Situ Hybridization and Immunocytochemistry

October 12–25

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

Howard, Tamara, Cold Spring Harbor Laboratory
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 is in need of microscopic approaches and for the cell biologist who is not familiar with the practical application of the advanced techniques presented in the course. Among the methods presented was the preparation of tagged nucleic acid probes, fixation methods, detection of multiple DNA sequences in single nuclei or chromosome spreads, comparative genomic hybridization, use of a variety of reporter molecules and nonantibody fluorescent tags, and indirect antibody labeling 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

Cass, D., A.B., M.D., Children's Hospital of Pennsylvania
Jin, Y., M.D., Ph.D., University of Texas Southwestern Medical Center, Dallas
Kerr, J., B.A., Ph.D., DuPont Merck Pharmaceutical Company, Wilmington, Delaware
Lightfoote, M., B.A., Ph.D., Food and Drug Administration
Lanoil, B., B.A., Oregon State University
Miotke, J., B.S., University of California, Irvine
Nepveu, A., B.S., M.S., Ph.D., McGill University, Canada
O'Driscoll, K., B.S., Ph.D., Columbia University
Ogryzko, V., M.S., Ph.D., National Institutes of Health
Ohls, R., B.A., M.D., University of Florida College of Medicine
Platt, K., B.S., M.S., Ph.D., University of California, Riverside
Salin, T., M.S., Karolinska Institute, Sweden
Shelbourne, P., B.S., Ph.D., Stanford University
Shmueli, O., B.S., M.S., Weizmann Institute, Israel
Solovei, I., M.S., Ph.D., University of St. Petersburg, Russia
Wolda, S., B.S., Ph.D., ICOS Corporation, Bothell, Washington

SEMINARS

Bagasra, O., Thomas Jefferson University, Philadelphia, Pennsylvania. In situ PCR and its applications in research and diagnosis.
Brinkley, W., Baylor College of Medicine. 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 video microscopy. Fluorescence microscopy and low-light-level cameras.
Murray, J., University of Pennsylvania. Principles of confocal microscopy and deconvolution techniques.
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. Immunocytochemistry. An integrated microscopic approach to examining nuclear organization.
Waggoner, A., Biological Detection, Inc., Pittsburgh, Pennsylvania. Development of fluorochromes and filters for fluorescence microscopy.
White, J., University of Wisconsin, Madison. 2-photon microscopy.

Macromolecular Crystallography

October 12–25

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

Kjeldgaard, Morten, Aarhus University, Denmark

Crystallography and X-ray diffraction yield a wealth of structural information unobtainable through other methods. This "intensive" laboratory/computational course focused on the major techniques used to determine the three-dimensional structures of macromolecules. It was designed for scientists with a working knowledge of protein structure and function, but who were new to macromolecular crystallography. Topics that were covered included crystallization (proteins, nucleic acids, and complexes), crystal characterization, X-ray sources and optics, crystal freezing, data collection, data reduction, anomalous dispersion, multiple isomorphous replacement, phase determination, solvent, flattening, molecular replacement, and averaging, electron density interpretation, structure refinement, and molecular graphics. Participants learned through extensive hands-on experiments where they crystallized and determined a protein structure, along with lectures and informal discussions on the theory behind the techniques.

PARTICIPANTS

Batchelor, A., Ph.D., Johns Hopkins Medical School
Bordo, D., Ph.D., University of Groningen, The Netherlands
Briggs, S., B.S., Eli Lilly and Company, Indianapolis, Indiana
Eakin, A., B.A., Ph.D., University of Puerto Rico School of Medicine
Gonnella, N., A.B., Ph.D., Ciba-Geigy Corporation, Summit, New Jersey
Grabarek, Z., M.S., Ph.D., Boston Biomedical Research Institute, Boston, Massachusetts
Hall, T., B.S., Ph.D., Johns Hopkins University School of Medicine
Horwich, A., A.B., M.D., Yale University School of Medicine
Kanellopoulos, P., Ph.D., EMBL, Germany
Lehtonen, J., M.S., University of Turku, Finland
MacKinnon, R., B.A., M.D., Harvard Medical School
Rabijns, A., Ph.D., Catholic University of Leuven, Belgium
Rizvi, S., B.S., M.S., Ph.D., University of Pennsylvania School of Medicine
Sayre, P., M.D., Ph.D., University of California, San Francisco
Song, Y.-H., B.S., Ph.D., University of Toronto, Canada
Weiss, M., A.B., Ph.D., University of Chicago

SEMINARS

Brunger, A., Yale University. Protein solvation and discrete disorder observed at high resolution using experimental phases.
Cheng, X., Cold Spring Harbor Laboratory. DNA modification by methyltransferases.
Clore, G.M., National Institutes of Health. Molecular basis of 46 XY sex reversal from the structure of the SRY-DNA complex.
Edwards, A., McMaster University, Ontario, Canada. Crystal structure of the origin binding protein EBNA1 bound to DNA suggests a mechanism for replication origin unwinding.
Freer, A., University of Glasgow, Scotland. Membrane proteins in bacterial photosynthesis: A circular arrangement.
Gilliland, G., National Institute of Standards & Technology, Rockville, Maryland. Engineering of subtilisin BPN and its foldase prosegment for increased stability, calcium independence, and efficient folding.
Newcomer, M., Vanderbilt University. Determination of the quaternary structure of the transthyrein/retinol-binding protein complex.
Sweet, R., Brookhaven National Laboratory, Upton, New York. Laue diffraction as a tool for study of dynamic effects in protein crystals.
Ramakrishnan, V., University of Utah School of Medicine. Toward the structure of the ribosome: Structure of the ribosomal proteins and initiation factor 3.

YACs in Structural and Biological Genome Analysis

October 12–25

INSTRUCTORS

Huxley, Clare, Ph.D., St. Mary's Hospital Medical School, United Kingdom
Lovett, Michael, Ph.D., University of Texas Southwestern Medical Center
Reeves, Roger, Ph.D., Johns Hopkins University

ASSISTANTS

Cabin, Deborah, Johns Hopkins University
Clines, Greg, University of Texas Southwestern Medical Center
Simpson, Kaetrin, St. Mary's Hospital Medical School, United Kingdom

Yeast artificial chromosomes (YACs) are an essential tool in genome analysis involving physical mapping, contig building, gene isolation, and functional analysis of cloned DNA. This lab-based course covered techniques used in physical mapping, including the basics of growth and storage of YACs, analysis of YACs by pulsed-field gel electrophoresis, creation of nested derivatives by fragmentation at repeat sequences, contig building by STS content mapping, and end-clone rescue. Gene identification was carried out by direct cDNA selection from purified cosmid, YAC, and total chromosomal DNA, and exon trapping was covered in lectures. Techniques for functional analysis of YAC DNA included introduction of YACs into mouse cells by fusion with yeast spheroplasts, lipofection into mouse cells with gel-purified DNA, and preparation of YAC DNA for pronuclear injection. YACs were also manipulated by homologous recombination for the introduction of specific modifications. Lectures on complementary topics were given by invited speakers who are prominent researchers in the field and who then spent time talking individually to the participants. There was also structured discussion of how to apply the current technology to the specific research projects of the participants.

PARTICIPANTS

Albarosa, R., Ph.D., Instituto Nazionale Neurologico "C. Besta," Italy
Black, D., B.S., Zeneca Pharmaceuticals, United Kingdom
Engels, P., B.S., Ph.D., Sandoz Pharma Ltd., Switzerland
Guillemot, F., B.S., CNRS, UPR 420, France
Hiraga, A., M.D., Tohokku University, Japan
Kelavkar, U., M.S., Ph.D., Clark Atlanta University
Leister, D., M.S, Max-Planck Institute, Germany
Lengeling, A., B.S., University of Bielefeld, Germany
Mjumder, K., B.S., M.S., Ph.D., Baylor College of Medicine
Maurici, D., B.S., Brigham and Women's Hospital
Peltz, G., B.S., M.D., Ph.D., Roche Bioscience, Palo Alto, California
Queimado, L., M.S., M.D., New University of Lisbon, Portugal
Rayl, E., B.S., Ph.D., Yale University
Richter, A., B.S., Ph.D., University of Montreal, Canada
Sidjanin, D., B.S., Ph.D., University of Pennsylvania
Wang, G., B.S., Ph.D., Loma Linda University

SEMINARS

Buckler, A., Massachusetts General Hospital. Physical and transcription mapping with exons.

Green, E., National Institutes of Health. Mapping human chromosomes with YACs.

Huxley, C., St. Mary's Hospital Medical School, United Kingdom. Transfer of YAC DNA into mammalian cells and transgenic mice.

Lovett, M., University of Texas Southwestern Medical Center. Gene isolation by cDNA selection.

Reeves, R., Johns Hopkins University. Functional analysis of YAC DNA in mammalian cells.

Riethman, H., The Wistar Institute, Philadelphia, Pennsylvania. RARE cleavage and long-PCR-based analysis of subtelomeric DNA.

Rothstein, R., Columbia University. Yeast genetics.

Trask, B., University of Washington, Seattle. Chromosomal mapping of YACs.

Wilson, R., Washington University School of Medicine. Genome analysis by means of large-scale DNA sequencing.

Computational Genomics

November 1–6

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 provided a comprehensive overview of the methods used to identify genes and to infer their function and evolutionary history. Today, the most powerful technique for characterizing a new DNA or protein sequence is by similarity searching for homologous proteins; the course presented the theory and practice for similarity searching using the BLASTP, FASTA, and Smith-Waterman algorithms. Students were able to see the structural implications of protein sequence homology by viewing homologous proteins in three dimensions on state-of-the-art UNIX workstations. Talks on protein evolution and the computer programs used to discover homologous proteins were extended with lectures on the statistics of sequence similarity scores, multiple alignment techniques, and phylogenetic methods. The course was expanded this year to include discussions of recent advances in statistical techniques for multiple sequence alignment and consensus sequence identification. In addition, a lecture and workshop focused on the identification of protein coding genes

in anonymous DNA sequences. This year, the Computational Genomics course provided additional "hands-on" problems by splitting the group into five teams and providing each team with a different and challenging protein identification problem. This course was intended for people with a solid fundamental knowledge of UNIX who wanted to acquire advanced skills in DNA and protein sequence analysis. This course provided both practical examples and problems in sequence analysis using methods that are available today and a foundation in the algorithmic approaches that are likely to produce new methods in the future. The course was ideal for computer core directors and staff, for molecular biology and genetics resources, for biologists who wished to acquire advanced skills in genome analysis, and for computer scientists who wanted an overview of the state of the art in this area.

PARTICIPANTS

Aman, P., Ph.D., University of Lund, Sweden
Atrian, S., B.S., Ph.D., University of Barcelona, Spain
Bergstrom, T., B.S., University of Uppsala, Sweden
Brefort, G., M.D., Ph.D., Rhone-Poulenc Rorer, France
Bringas, R., M.S., Center for Genetic Engineering and Biotechnology, Cuba
Caruso, A., B.S., Genetics Institute, Cambridge, Massachusetts
Cueto, M., B.A., Ph.D., Sandoz Research Institute, East Hanover, New Jersey
Kawai, T., M.S., Ph.D., ImmunoPharmaceutics, Inc., San Diego, California
Leshkowitz, D., Ph.D., Weizmann Institute of Science, Israel
Lieb, M., B.A, M.D., Mount Sinai School of Medicine
Lindqvist, A.-K., M.S., University of Uppsala, Sweden
Nel, L., B.S., Ph.D., University of Pretoria, South Africa
Prasad, R., M.S., Ph.D., Thomas Jefferson University, Philadelphia, Pennsylvania
Vallejo, R., B.S., M.S., Ph.D., United States Department of Agriculture
Van Wiemeersch, L., B.S., University of Gent, Belgium
Vass, K., B.S., Ph.D., Glasgow University, United Kingdom
Viguera, E., B.S., Ph.D., Centre de Investigaciones Biologicas, Spain
Zou, J., M.S., Ph.D., Hoffman-La Roche, Inc., Nutley, New Jersey

SEMINARS

Altschul, S., National Library of Medicine. Statistics of similarity scores.
Davison, D., University of Houston. Approaches to phylogeny reconstruction.
Kececioglu, J., University of Georgia. Multiple sequence alignment: Optimal approaches.
Lawrence, C., Wadsworth Center, New York Department of Health, Albany, New York.
Statistical approaches to multiple sequence analysis.
Workshop: Sequence comparison. Searching with different programs, comparison of results
Marr, T., Cold Spring Harbor Laboratory. Integration of biological information, from sequences to maps to function.
Pearson, W., University of Virginia.
Introduction and overview.
Protein evolution: Biology.
Biological sequence comparison: Algorithms.
Practical issues in protein sequence searching.
Workshop: Getting sequences, searching with sequences, identifying distant homologs.
Smith, R., Baylor College of Medicine.
Hierarchical multiple alignment approaches.
Workshop: Multiple sequence alignment using both MSA2.1, ClustalV and PIMA approaches.
Stormo, G., University of Washington, Seattle.
Finding patterns in unaligned sequences.
Workshop: Finding promoters, regulatory sites, and protein motifs. Gene parsing.
Workshop: Multiple sequence alignment/HMM.
Workshop: Gene identification.

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
Tingey, Scott, Ph.D., DuPont Experimental Station

ASSISTANT
Jung, Mark, DuPont Company

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 lectures, 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 included DNA amplification-based, 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. Last year's lecturers included W. Beavis, M. Clegg, D. Duvick, M. Gale, B. Mazur, R. Michelmore, A. Rafalski, J. Romero-Severson, R. Sederoff, S. Tanksley, and J. Wendel.

PARTICIPANTS

Brule-Babel, A., B.S., Ph.D., University of Manitoba, Canada
Byrum, J., B.S., M.S., Asgrow Seed Company, Ames, Iowa
Chin, D., B.S., M.S., University of California, Davis
Crouch, J., B.S., Ph.D., International Institute Tropical Agriculture, United Kingdom
Elliott, P. (Beth), M.S., Ph.S., Cold Spring Harbor Laboratory
Hobart, B., A.S., Pioneer Hi-Bred International, Inc., Johnston, Iowa
Hunt, M., B.S., Ph.D., Ciba Agricultural Biotechnology, Research Triangle Park, North Carolina
Johnston, S., B.S., Rogers Seed Company, Gilroy, California
Krone, T., B.S., M.S., Ph.D., Asgrow Seed Company, Ames, Iowa
Lawson, D., B.S., M.S., Ph.D., Cornell University
Phillips, D., B.S., Pioneer Hi-Bred International, Inc.
Piper, T., B.S., Ph.D., Pioneer Hi-Bred International, Inc
Sebastian, S., Ph.D., E.I. DuPont de Nemours Company, Newark, Delaware
Shimosaka, E., B.A., Hokkaido National Agriculture Experimental Station, Japan
Van Gysel, A., B.S., Ph.D., University of Gent, Belgium
Vogelaar, A., M.S., Riik Zwaan. The Netherlands

SEMINARS

Ausubel, F., Harvard Medical School. Positional cloning of defense-related genes in *Arabidopsis*.

Beavis, W., Pioneer Hi-Bred International, Inc. QTL mapping in plant breeding populations: Lessons from experimental populations.

Briggs, S., Pioneer Hi-Bred International, Inc. Genetics of plant-pathogen interactions.

Dooner, H., Rutgers University. Use of the maize transposon activator to tag genes in other species.

Kochert, G., University of Georgia. Germplasm variation and utilization in peanuts.

Mazur, B., DuPont Company. Commercializing the products of plant biotechnology.

Michelmore, R., University of California, Davis. Molecular markers and the manipulation of disease resistance.

Rafalski, A., DuPont Company. Comparative analysis of marker systems in mapping and germplasm characterization applications.

Sederoff, R., North Carolina State University. Use of molecular markers in genetics of forest trees.

Vogel, J., DuPont Company. Multiplexed polymorphism detection: AFLP and SAMPL.

Phage Display of Combinatorial Antibody Libraries

November 8–21

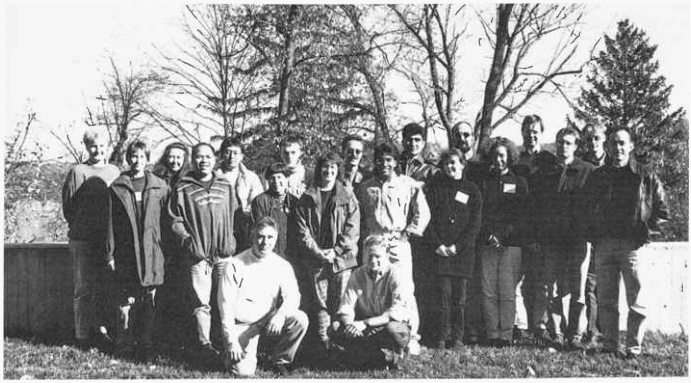
INSTRUCTORS

Barbas, Carlos, Ph.D., Scripps Research Institute
Burton, Dennis, Ph.D., Scripps Research Institute
Silverman, Gregg, M.D., University of California, San Diego

ASSISTANTS

Nayak, Jayakar, University of California, San Diego
Pilkington, Glenn, Intracel Corporation, Boston

Recent advances in the generation and selection of antibodies from combinatorial libraries allow for the rapid production of antibodies from immune and nonimmune sources. This laboratory/lecture course focused on the construction of combinatorial antibody libraries expressed on the surface of phage and selection of desired antibodies from the library. Students learned the theoretical and practical aspects of constructing combinatorial antibody libraries as well as the construction of synthetic antibody libraries. Antibodies were selected from the library by panning. Production, purification, and characterization of Fab fragments expressed in *E. coli* were also covered. The lec-



ture series presented by a number of invited speakers focused on PCR of immunoglobulin genes, the biology of filamentous phage and the utility of surface expression libraries, expression of antibodies in *E. coli* and mammalian cells, antibody structure and function, catalytic antibodies, the whole biology and molecular genetics of antibody activity, and recent results on the use of antibodies in therapy.

PARTICIPANTS

Ansari, K., B.S., University of Toronto, Canada
Bieboer, S., B.S., Utrecht University, The Netherlands
Dreier, B., B.S., University of Erlangen, Germany
Hunt, A., B.S., M.S., National Center for Infectious Diseases
Jung, S., B.S., University of Zurich, Switzerland
Leelayuwat, C., B.S., Ph.D., University of Western Australia, Australia
Mamalaki, A., B.S., Hellenic Pasteur Institute, Greece
Mobini, R., B.S., Goteborgs University, Sweden
Olsson, J., B.S., University of Lund, Sweden
Rondon, I., B.S., Ph.D., Harvard Medical School
Sleister, H., B.S., Ph.D., Pioneer Hi-Bred International, Inc., Johnston, Iowa
Turksen, K., B.S., Ph.D., Ottawa Civic Hospital, Canada
Valvatne, H., B.S., M.S., University of Bergen, Norway
Watzka, H., B.S., Ph.D., University of Stuttgart, Germany
Yamaguchi, S., D.D.S., Ph.D., Japanese Foundation for Cancer Research, Japan
Du Putlitz, J., M.D., Harvard Medical School

SEMINARS

Carter, P., Genentech, Inc. Expression of antibodies and antibody fragments.
Marquis, D., La Jolla Pharmaceutical. Phage display.
Model, P., Rockefeller University. Phage biology.
Nessler, P., Cold Spring Harbor Laboratory. Combinatorial chemistry.
Persson, M., Karolinska Hospital, Sweden. Combinatorial libraries from macaques.
Sanz, I., University of Texas Health Science Center. Generation and features of antibody recognition.
Scanlon, T., University of California, San Francisco. Catalytic antibodies.
Wilson, I., 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.
Ambion, Inc.
Amersham Corporation
AMRESCO Inc.
Applied Biosystems
Applied Precision
Axon Instruments
Baxter Scientific
Beckman Instruments
Becton Dickinson
Bio 101 Inc.
Bio Metra
Bio-Rad Laboratories
Boehringer-Mannheim Biochemicals
Brinkmann Instruments
Carl Zeiss, Inc.
CBS Scientific, Co., Inc.
ChromaTechnology
Corning/Costar Corp.
Dage-MTI, Inc.
Drummond Scientific Co.
DuPont/New England Nuclear
Epicentre Technologies
FMC Bioproducts
Gatan Inc.
Gelman Sciences Inc.
General Valve Corporation
Genetix
GIBCO Life Technologies
Grass Instrument Co.
Hamamatsu Phonic Systems
Hampton Research
Hewlett-Packard Co.
Hitachi
Hoefer Scientific Instruments
Institute Genomics
Instrutech
Invitrogen Corp.
Kodak/IBI
Leica
Leitz/Leica
LI-COR Inc.
Life Technologies, Inc.
Ludl Electronic Products
Matrix Technologies Corp.
MJ Research
Molecular Probes Inc.
Molecular Research Center Inc.
Morrell Instrument Co. Inc.
Nalge Co.
Narishige USA Inc.
New England Biolabs, Inc.
Nikon Inc.
NORAN Instruments Inc.
Novagen Inc.
Olympus America Inc.
Omega Optical Inc.
Optronics Engineering
Owl Scientific Inc.
PCR Inc.
Perkin-Elmer
PerSeptive Biosystems
Pharmacia Biotech Inc.
Promega Corp.
Protein Solutions Inc.
Qiagen Inc.
Replicatech
Research Genetics
Robbins Scientific Corp.
Savant Instruments Inc.
SGI
Sigma Chemical Co.
Stoelting Co.
Stratagene Cloning Systems
Sutter Instrument Co.,
Universal Imaging Corp.
University of California, San Francisco
USA/Scientific Plastics Inc.
Vector Laboratories Inc.
VWR Scientific
Vysis
Wallac Inc.
Warner Instrument Corp.

SEMINARS

Invited Speaker Program

Each year, Cold Spring Harbor Laboratory invites speakers from outside the institution to present their latest findings on a weekly basis. These seminars keep the CSHL staff current on the latest developments and broaden their perspectives. Graduate students and postdoctoral fellows meet with the seminar speakers for lunch immediately after the seminar, allowing an opportunity for the exchange of ideas in an informal setting.

1995

January

Jim Ihle, St. Jude's Children Hospital, Memphis. JAKs and STATs in cytokine signaling. (Host: Henry Sadowski)

Leon Avery, University of Texas, Southwestern. Genetics of neurotransmission in the *C. elegans* pharynx. (Host: Michael Hengartner)

Stephen Doxsey, University of Massachusetts. Molecular function of centrosomes: The role of pericentrin. (Host: Michael Wigler)

George Rose, Johns Hopkins University Medical School. The protein folding problem: New results. (Host: Winship Herr)

February

Charles Gilbert, Rockefeller University. Spatial integration and cortical dynamics. (Host: Holly Cline)

Bruce McNaughton, University of Arizona. Ensemble coding for spatial memory: The stuff dreams are made of. (Host: Alcino Silva)

John Lisman, Brandeis University. Synaptic plasticity is greatly enhanced during cholinergic brain oscillations. (Host: Robert Malinow)

Mark Estelle, Indiana University. Genetic studies of auxin action in *Arabidopsis*. (Host: Hong Ma)

March

Daniel Storm, University of Washington, Seattle. Calcium regulated adenylyl cyclases: Their role in neuroplasticity. (Host: Grisha Enikolopov)

Bruce Paterson, Laboratory of Biochemistry, NCI, NIH. Studies on the dimerization specificity of the myogenic bHLH proteins. (Host: Ueli Grossniklaus)

Yuy-Ii Wang, Worcester Foundation. Regulation of cortical dynamics during cytokinesis. (Host: David Helfman)

Francine Perler, New England Biolabs, Inc. Protein splicing: Control, mechanism, and prospects. (Host: Adrian Krainer)

Charles Weissmann, University of Zurich. The role of PrP in susceptibility to scrapie. (Host: Adrian Krainer)

April

Xing-Wang Deng, Yale University. Mechanism of light-mediated development switch (Host: Hong Ma)

October

Nam-Hai Chua, Rockefeller University. Genetic and biochemical dissection of the phytochrome phototransduction pathway (Host: Lavina Faleiro)

David Goeddel, Tularik Inc., San Francisco. TNF receptor signal transduction. (Host: Bruce Stillman)

November

Frederick Ausubel, Harvard Medical School. A multi-host pathogen (*Pseudomonas aeruginosa*) that infects plants, nematodes, and mice (Host: Hong Ma)

Gerald Rubin, University of California, Berkeley. Signal transduction during fly eye development. (Host: Hong Ma)

December

Robin Lovell-Badge, MRC, London. The SRY gene family, sex determination and neural development. (Host: Winship Herr)

Kim Nasmyth, IMP, Research Institute Molecular Pathology, Vienna. Determinants of mother-cell-specific mating-type switching in the budding yeast *S. cerevisiae*. (Host: Bruce Stillman)

In-House Seminar Program

Cold Spring Harbor In-House Seminars were initiated to provide a semiformal avenue for communication between the various research groups at the Laboratory. They are particularly useful for research personnel who have recently joined the Laboratory. The seminars also afford a necessary opportunity for the graduate students and postgraduate staff to develop their skills in organizing, presenting, and defending their research.

1995

January

Konstantin Galactionov, Cold Spring Harbor Laboratory. A direct link between cell cycle machinery and signal transduction.

Eric Chang, Cold Spring Harbor Laboratory. Cooperative interaction of *S. pombe* proteins required for mating and morphogenesis.

Sui Huang, Cold Spring Harbor Laboratory. Functional organization of RNAs in the mammalian cell nucleus.

Erich Grotewold, Cold Spring Harbor Laboratory. Maize Myb-domain proteins: Transcription "in color."

February

Peter Barker, Cold Spring Harbor Laboratory. Detection of gene amplification in human breast cancer.

Bruce Futcher, Cold Spring Harbor Laboratory. The yeast cell cycle engine.

Harriet Feilotter, Cold Spring Harbor Laboratory. The search for genes associated with manic depressive illness using linkage to physical markers through the human genome.

Dick McCombie, Cold Spring Harbor Laboratory. Technology development and *S. pombe* genome sequencing.

March

Ariel Avilion, Cold Spring Harbor Laboratory. Talking about telomerase for the 13th time.

Bill Henry, Cold Spring Harbor Laboratory. SNAPc: A protein complex required for transcription of human snRNA genes.

Masafumi Tanaka, Cold Spring Harbor Laboratory. Unexpected determinants of the binding of transcriptional activator proteins to their target DNA in vivo.

Shobha Gunnery, Cold Spring Harbor Laboratory. Functional mRNA can be generated by RNA polymerase III.

Joe Colasanti, Cold Spring Harbor Laboratory. In search of "florigen": The maize *indeterminate* gene and the transition to flowering.

April

Andrea Doseff, Cold Spring Harbor Laboratory. LAS1, a nuclear protein involved in bud formation.

Tim Tully, Cold Spring Harbor Laboratory. Fly fishing for the engram.

October

David Horowitz, Cold Spring Harbor Laboratory. A new human protein required for the second catalytic reaction of pre-mRNA splicing.

Peter Nestler, Cold Spring Harbor Laboratory. Ex Pluribus Unum—The combinatorial quest for the optimal receptor.

Mario Gimona, Cold Spring Harbor Laboratory. How to zip and unzip tropomyosin: The selectivity of dimer formation, and interaction with S100-like CA^{++}-binding proteins.

November

Frank Pessler, Cold Spring Harbor Laboratory. The long and the short of HIV-2 transcription.

David Beach, Cold Spring Harbor Laboratory. Oncogene cooperation.

December

May Luke, Cold Spring Harbor Laboratory. A family of SIT4-associated proteins: The SAPs are required for the in vivo functions of the SIT4 phosphatase.

Chantal Autexier, Cold Spring Harbor Laboratory. Functional analysis of *Tetrahymena* and human telomerase RNAs.

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, 440 students have participated in the course, and many have gone on to productive careers in biological science.

The objectives of the program are to provide (1) a greater understanding of the fundamental principles of biology; (2) an increased awareness of experimental approaches to science; (3) a deeper understanding of the major issues in the fields of biochemistry and genetics, and molecular and cellular biology; and (4) a personal acquaintance with research, research workers, and centers for study.

The following students, selected from over 280 applicants, took part in the program, which was supported by 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.

Jennifer Ames, University of Pittsburgh
Advisor: **David Beach**
Sponsor: Burroughs-Wellcome Fund
Cell immobilization.

Neeraj Arora, Cambridge University
Advisor: **Timothy Tully**
Sponsor: Robert P. Olney Memorial Cancer Fund
Planimetric analysis of brain structures in the *Drosophila* learning mutants *latheo* and *linotte*.

Rebecca Blankenburg, California Institute of Technology
Advisor: **Yi Zhong**
Sponsor: Jephson Education Trust
Ras signal transduction pathway involvement in *Drosophila* synaptic plasticity and cell patterning.

Tanita Casci, University of Glasgow
Advisor: **Michael Hengartner**
Sponsor: Federica von Stade Internship
Programmed cell death in *C. elegans.*

Michelle DaCosta, Yale University
Advisor: **Arne Stenlund**
Sponsor: National Science Foundation
Examining the interaction of the BPV E2 hinge with the E1 protein.

Katharine Eklof, Rice University
Advisor: **Richard McCombie**
Sponsor: Burroughs-Wellcome Fund
Analysis of open reading frame expression in *S. pombe.*

Rebecca Farkas, Yale University
Advisor: **Hong Ma**
Sponsors: Bliss Memorial Fund
Phillips Petroleum Foundation, Inc.
FON1 and floral developmental genetics.

Christine Ford, Bellarmine College.
Advisor: **Erich Grotewold**
Sponsor: National Science Foundation
Characterization of proteins in the flavonoid biosynthetic pathway.

Nathan Hellman, Yale University
Advisor: **Grigori (Grisha) Enikolopov**
Sponsor: Burroughs-Wellcome Fund
Targeting synaptotagmin II to synaptic vesicles.

Brian D. Hoerneman, University of Wisconsin-Madison
Advisor: **Bruce Stillman**
Sponsor: Burroughs-Wellcome Fund
Construction and utilization of multicopy libraries to screen for suppressors of a conditional mutation in the 58-kD subunit of Pol-α/primase.

Emmitt R. Jolly, Tuskegee University
Advisor: **David Helfman**
Sponsor: National Science Foundation
Comparisons of protein factors in muscle and nonmuscle cell types that may regulate alternative RNA splicing.

John Kehoe, Northwestern University
Advisor: **Ryuji Kobayashi**
Sponsor: Burroughs-Wellcome Fund
A chemiluminescent approach to protein sequencing.

George Laszlo, Oberlin College
Advisor: **Alcino J. Silva**
Sponsor: National Science Foundation
Learning and memory in NF1-deficient mice.

Miro Pastrnak, Wabash College
Advisor: **Adrian R. Krainer**
Sponsor: Cold Spring Harbor Laboratory
Expression of human SR proteins in yeast.

Loren del Mar Peña, Duke University
Advisor: **Winship Herr**
Sponsor: National Science Foundation
Cloning the *C. elegans* HCF gene.

Elizabeth Pinches, King's College, Cambridge
Advisor: **Holly Cline**
Sponsor: William Shakespeare Internship
Synapse distribution on the retinotectal projection of *Xenopus.*

Cynthia Snyder, Colorado State University
Advisor: **Carol Greider**
Sponsor: National Science Foundation
Human telomerase RNA.

Hana Sugimoto, Wellesley College
Advisor: **Kim L. Arndt**
Sponsor: Libby Internship
Sequencing of SAP4 and isolation of SAP190 ts mutants.

Pei Lin Tan, Mount Holyoke College
Advisor: **Shobha Gunnery**
Sponsor: The Garfield Internship
Termination signal of RNA polymerase III (Pol III) transcription.

Rachel Ventura, Harvard University
Advisor: **Xiaodong Cheng**
Sponsor: National Science Foundation
Toward solving the three-dimensional structure of p16: Crystallization trials.

Kevin Wang, Stanford University
Advisor: **Venkatesan Sundaresan**
Sponsor: Burroughs-Wellcome Fund
Isolation and characterization of embryo-specific genes in *Arabidopsis* using insertional trap transposons.

Audrey Wells, University of New Mexico
Advisor: **Rob Martienssen**
Sponsor: National Science Foundation
Characterization of the *Arabidopsis* genome.

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 1995, a total of 426 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, two-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

Kimberly Bennett, M.S., Science Teacher, Locust Valley School District
Michelle Malich, B.A., Elementary School Candidate
Marjorie Medwed, M.S., Science Teacher, Locust Valley School District
Donna Pandaliano, M.S., Science Teacher, Valley Stream School District
Linda Payoski, M.S., Science Teacher, Uniondale School District
Brian Withers, Fine Arts Teacher, New York City School System.

COURSES

Nature Bugs
Nature Detectives
Nature Discovery
Ecology Explorers
Frogs, Flippers, and Fins
Pebble Pups
Freshwater Life
Seashore Life
Marine Biology
Nature Photography
Adventure Education


BANBURY
CENTER

BANBURY CENTER DIRECTOR'S REPORT

During the 18 years of the Banbury Center's existence, there has been an extraordinary increase in the ways scientists communicate with each other. The number of journals continues to rise without end in sight; there are meetings on every topic; and the Internet and World Wide Web are now providing an instant, global means of exchanging information. Paradoxically, Banbury Center's role—promoting research by providing a quiet haven where small groups of scientists can meet—has become even more important in this period of overwhelming information dissemination.

Meetings and Participants

The year 1995 must have been a record year for the use of Banbury Center. We held no fewer than 19 science-related meetings with a proportionate number of participants—almost 600—who came from all over the world. Almost 100 came from outside the United States, principally from the United Kingdom (42), Germany (16), and France (12). In addition, the Center was used on 11 other occasions and for 5 neurobiology courses during the summer.

Human Genetics

In 1989, Banbury Center was the site of a discussion meeting that was important in the early development of the Human Genome Project. It was exciting that in 1995, the Human Genome Organization (HUGO) came to Banbury, just prior to the main Genome Mapping and Sequencing meeting, for the **Human Gene Map Workshop II.** The workshop presented an up-to-the-minute snapshot of progress in genome centers in the United States and in Europe.

Scientists hunting human disease genes are facing two major hurdles, each of which was the topic of a meeting this year. Localizing genes by means of genetic linkage is now relatively straightforward (although still difficult in practice) for disorders believed to arise from mutations in one gene. What is not clear, however, is the most effective strategy to be followed when dealing with complex disorders in which mutations in several genes contribute to the phenotype. **Looking to the Next Generation of Genetic Analysis** (funded by the Charles A. Dana Foundation) attempted to gaze into the crystal ball to see how technical developments in genetics analysis (mathematical modeling) and genomic technologies (analyzing DNA) are going to affect the ways genes are localized.

Finding Genes: Experimental and Computational Methods was concerned also with genetic analysis strategies, in this case with how to find a gene once genetic linkage methods have localized it to a region of a chromosome. These regions are typically on the order of 2 million base pairs in length and finding a gene within such a region is not trivial. The meeting reviewed experimental strategies (selecting cDNAs, trapping) and computational methods (identifying genes in DNA sequence). It is likely that computational methods will become more important as it becomes easier to sequence large amounts of DNA, and this in turn will lead to more accurate recognition of sequence motifs that characterize genes.

Cancer

Two meetings dealt specifically with cancer. **DNA Repair and Genetic Instability in Cancer** reviewed the exciting findings that human cancers are caused in part by mutations in the enzymes and other proteins involved in the repair of DNA damage and by alterations in sequences, such as trinucleotide repeats, that are unstable. It was a special pleasure to have as participants two indi-

N. Freimer, L. Kruglyak (Genetic Analysis)

viduals who had significant roles in the life of Cold Spring Harbor Laboratory—John Cairns and Joe Sambrook.

Banbury Center held an important meeting on neurofibromatosis in 1990, the NF1 gene having just been cloned and its relationship to the ras pathway recognized. Now, 5 years later, in **Neurofibromatosis: How to Develop Therapies**, we examined what progress has been made in elucidating the causes of neurofibromatosis with a special emphasis on what these new data mean for developing treatments. The focus of the meeting was to determine what needs to be done to translate research findings into therapies. This requires not only continued research, but also, perhaps, new funding strategies to encourage interdisciplinary collaborations. Bruce Stillman played a critical part in the last session, forcing participants to face up to this task.

Protein Degradation

At one time, it was thought that protein degradation occurred by some nonspecific breakdown process, but about 10 years ago, it was realized that careful regulation of protein degradation was just as important as regulation of protein synthesis. In 1988, Banbury Center held a meeting on the ubiquitin system and this year we held a follow-up: **The Targets and Regulation of the Ubiquitin System.** The most notable change has been the realization of ubiquitin's role in key areas of cell function. For example, a session was devoted to the part played by ubiquitin in control of cell cycle control proteins like the cyclins.

Infectious Diseases

Two very different topics come under this heading. In March, we held a meeting in our continuing series on Lyme disease, **Lyme Disease: Molecular and Immunologic Aspects of Detection and Vaccine Development.** Each meeting is a marker of the increasing knowledge that we have of the genetics and immunology of the *Burgdorferi* spirochaete, knowledge that we hope will be used to develop vaccines. This meeting still covered a broad range of topics, but the time has now come to look in detail at just one or two topics.

The subject of our second meeting is still enigmatic. Prions, infectious protein particles devoid of nucleic acid, are believed to be the causative agents of diseases such as Kuru in human beings and scrapie in sheep, and in the outbreak of bovine spongiform encephalopathy in the United

Kingdom. The latter has raised public health concerns about this type of infectious agent on a new and dramatic scale. The **Molecular Biology of Prions and Pathology of Prion Diseases** meeting was held at a most exciting time for the field when data from transgenic models are becoming available and intensive investigations are under way of prion-like proteins in yeast.

Plant Molecular Biology

Banbury Center has held several meetings on plant molecular biology but not enough to reflect the importance and intrinsic interest of plant science. The potential benefits of genetic manipulations of crop plants is enormous, and **Molecular Biology of Disease Resistance Genes in Plants** was an example of that potential. The topic was suggested to me by Richard Michelmore when we met at the International Genetics Conference in Birmingham, United Kingdom, in 1993. Fortuitously, the delay in holding the meeting worked to our advantage as the first of these resistance genes was cloned in the period leading up to the meeting. Beginning in 1996, we shall try to rectify our comparative neglect of plant science. A small group of key companies has contributed to the Plant Corporate Associates Program that will fund at least one meeting each year on plant science.

Meetings in Neurobiology

Within the broad field of "neurobiology," we held two meetings that could hardly have been further apart and yet one, **CREB and Memory**, points the way in which basic research is beginning to tackle topics like that of the second meeting, **Genetics of Human Behavior.** The premise of the latter meeting was that we need to understand the scientific basis of various claims for the role of genetics in influencing, or even determining, human behavior, in order to assess those claims. We brought together behavoral scientists, psychologists, geneticists, and sociologists, both sceptics and supporters of this research.

Another approach to studying the functioning of our brains uses the tools of recombinant DNA to probe molecular systems that may underlie such functions as learning and memory. This approach is developing rapidly. In particular, the CREB transcription appears to have a key role in the development of memory, and this meeting reviewed the data on CREB and learning in a variety of organisms.

Cocktails during CREB and CREM meeting.

Visualizing the Working Body

In vivo imaging is a technique that is becoming increasingly important for scientists wishing to examine the working human brain. It is now possible to see how different areas of the brain change their metabolism as the subject performs some task. However, as our meeting **Advances in Imaging and Their Application** showed, this is only a small part of the imaging picture. These techniques are also used extensively in cancer and cardiovascular studies. Most of the meeting was taken up with discussions of future developments that must take place if imaging is to be used even more widely. In particular, increased resolution and decreased response time are going to be essential if living processes are to be analyzed in real time.

Educational Activities

Breast cancer is a major concern throughout the nation and especially on Long Island, which has one of the nation's higher rates. The 1 in 9 Breast Cancer Group provides support and promotes research on breast cancer, some of which is done at the Laboratory. Because of the intense interest aroused by the cloning and characterization of the *BRCA1* and *BRCA2* genes, Banbury Center and the DNA Learning Center collaborated in holding a 1-day **Workshop on Genetics** for members of the Group.Twenty-three members of 1 in 9 attended, and in addition to learning some of the fundamentals of human genetics, they fingerprinted their own DNA using PCR and heard of the latest research from Elizabeth Claus from Yale.

We could not have held the workshop for 1 in 9 members without the experience we have gained with our series of **Human Molecular Genetics** workshops, supported by the Ethical, Legal, and Social Issues Program of the Department of Energy's Human Genome Project. These workshops have brought information about genetics to people from a remarkable range of backgrounds. Participants have included print and broadcast journalists, Congressional staff, bioethicists, patient groups, lawyers, philosophers, sociologists, high school teachers, and physicians from primary care facilities. Despite the success of the workshops, this, the sixth in the series, was the last to be funded from this grant. For the second time, we concentrated on physicians who come from hospitals and institutions that do not have ready access to the latest advances in genetics, and we went nationwide, with participants coming from as far afield as Louisiana and North Dakota. This is an opportune time to thank all our invited speakers who made these workshops especially memorable.

Science and Policy Meetings

The first Banbury Center meetings on environmental hazards examined the scientific basis of issues that had important policy implications and such hybrid meetings have continued to the present. Four such meetings were held in 1995. Three of these dealt specifically with how the fruits of modern biomedical research are going to be implemented in a health care system that is undergoing a radical restructuring.

The first of these was **HIV and the Pathogenesis of AIDS.** This was a remarkable meeting that set out to analyze the current status of eight areas of research, to propose the key questions that have to be answered in each area, and to suggest the specific experiments that need to be done to provide the answers. The meeting was structured so that only one third of the time was given over to formal presentations, of which there were only two for each topic. The participants included key scientists from Europe as well as North America, but in the end, it was not clear how to implement the radical restructuring and rethinking that the field requires.

There is increasing public interest in the uses of modern human genetics in medicine. In part, this increased awareness is due to the realization that genetic information may be used by HMOs and insurance companies in making decisions about care. The Robert Wood Johnson Foundation, the leading foundation in heath care area, funded two meetings at Banbury Center to examine these issues. The first meeting, held in 1994, concluded that many potential problems could be

avoided or alleviated if the quality of genetics education was improved in medical and nursing schools. The second meeting, **Incorporating Genetics into Medicine and Nursing Education and Practice** held in April, 1995, examined this issue in more detail. We reviewed the current state of genetics education and how it should be improved for both physicians and nurses. It was an outstanding meeting, which included representatives of the main professional organizations which set the curricula in medical and nursing schools.

One genetic topic that has received much public attention is breast cancer. With the cloning and sequencing of two genes (*BRCA1* and *BRCA2*) involved in familial breast cancer, there is a strong movement to applying these findings in diagnostic tests. However, this is highly controversial because of concerns that results are difficult to interpret and treatments are few and ineffective. The **Molecular Diagnosis of Inherited Breast Cancer** meeting held in October covered topics ranging from the mutations in these genes to, once again, how to use this information in health care. It was particularly noteworthy for the strong representation from the United Kingdom where I like to think these issues are dealt with in a particularly rational manner.

Our final meeting in this science-policy group was supported by the Albert B. Sabin Vaccine Foundation. In 1994, Banbury Center held a meeting that reviewed the current status of vaccine production, examining all those factors that determine how a vaccine is developed, produced, and distributed. The 1995 meeting, **Vaccine Development and Delivery in the Era of Managed Care**, faced up to the fact that the rapidly changing nature of health care provision in the United States is having a profound effect on the ways in which long-term, preventative strategies like vaccination are going to be implemented and paid for.

The Executives' Conference

J.P. Morgan honored us by sponsoring **Infectious Diseases: Ancient Plagues, New Epidemics**, the tenth in this series of meetings. It was a particularly timely meeting as evident from the successes of *The Hot Zone* and *The Coming Plague*, and *Outbreak* starring Dustin Hoffman! Our meeting was also of star quality, covering a wide range of topics, from Richard Horton's historical perspective to Don Wiley's X-ray crystallography via Ham Smith's genome sequencing. Pursuing the film theme, Don Ganem was able to weave a scene from *Beverly Hills Cop* into his review of herpesviruses!

Other Meetings

Once again the local community made use of the Center. Of the two Lloyd Harbor Seminars, the Laboratory contributed one of these when Jerry Latter (Director, Computer Center), John Inglis (Executive Director, Cold Spring Harbor Laboratory Press), and I did a presentation on the Internet, including a "live" demonstration of net surfing. In addition, the Lloyd Harbor Conservation Board, Heckscher Museum, Huntington Hospital, the Cold Spring Harbor School District, and West Side School used the facility.

Funding

Support for our 1995 program was strong and drawn from many quarters, demonstrating the wide recognition of Banbury Center as the preeminent small meeting place for molecular biology and genetics. The Cold Spring Harbor Laboratory Corporate Sponsor Program again provided the funding for six Banbury Center meetings (for a full listing of the members of the Program, see the Financial Support Section). Suffice to write here that without the generosity of these companies, the Banbury Center program would be very much the poorer and the scientific world deprived of a unique resource.

Some of these same companies (Glaxo, Pfizer, SmithKline Beecham, Zeneca), together with Merck and Sequana, made contributions to the HUGO **Human Gene Map Workshop II.** This meeting was also supported by funds from the Department of Energy and the National Institutes of Health, and from Europe, the European Commission, the Wellcome Trust, and the Medical Research Council.

A similar combination of federal and private enterprise support funded the meeting on **Lyme Disease.** The former included the Centers for Disease Control, Food and Drug Administration, Med Immune, and the National Institute of Allergy and Infectious Disease, whereas the latter included Connaught Laboratories, Fort Dodge Laboratories, and SmithKline Beecham Clinical Laboratories.

The meeting on **HIV and the Pathogenesis of AIDS** was supported by contributions from Mothers' Voices, the Office of AIDS Research (NIH), the Albert B. Sabin Foundation, Pediatric AIDS Foundation, and Viaticus, Inc. These groups not only were generous in their support, but also committed the funds at very short notice.

The Department of Energy also funded the last in the series of **Genetics Workshops** for nonscientists. This was a very successful venture that provided a grounding in genetics to a very diverse range of individuals.

Foundations had a prominent role in the year. The Robert Wood Johnson Foundation, The William Stamps Farish Fund, the Charles A. Dana Foundation, and the Albert B. Sabin Foundation each funded a meeting in its entirety. The very successful meeting on **Neurofibromatosis** was supported by the National Neurofibromatosis Foundation and the Wilson Foundation, together with private contributions.

Robertson House provides dining and housing accommodations at Banbury Center.

Sammis Hall

J.P. Morgan generously funded the Executives' Conference. Two other meetings were funded by individual companies. **DNA Repair and Genetic Instability** was supported by OncorMed, Inc., and **Advances in Imaging and Their Applications** was funded by the Finisterre Fund. These meetings are, in all senses, Banbury Center meetings, with the same rigorous standards of participants' selection and program design applied to them. It may be that we will increase the numbers of such meetings as a means of being able to hold first-class meetings on a greater variety of topics.

Acknowledgments

This sustained level of activities could not be maintained without the dedicated help of many people. All the organizers worked hard, sometimes at very short notice, to make sure their meetings were a success; Bea Toliver, Ellie Sidorenko, and Katya Davey kept the Center running smoothly; Chris McEvoy and Bill Bishop kept the grounds looking beautiful; and Jim Hope (Catering) and Art Brings (Buildings & Grounds) and their staff coped with a very tight schedule.

Looking Forward to 1996

We have some very exciting meetings planned for 1996, including ones on DNA base "flipping," plant reproductive biology, and triplet repeat and polyglutamine tract diseases. Although the framework of the 1996 program is in place, we continue to add meetings. One of the great advantages that Banbury Center has over other meetings venues is its flexibility. If there are rapid developments in a subject that require critical analysis, we can organize a discussion meeting at very short notice. This can be done only because of the support provided by the scientists of the Laboratory, and I would like to acknowledge that support, on behalf of myself and all the scientists who attend our meetings.

Jan Witkowski

MEETINGS

1-in-9 Breast Cancer Group: A Genetics Workshop

January 28

FUNDED BY

Cold Spring Harbor Laboratory

ARRANGED BY

M. Bloom, DNA Learning Center, Cold Spring Harbor Laboratory
D. Micklos, DNA Learning Center, Cold Spring Harbor Laboratory
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1

M. Bloom and D. Micklos, DNA Learning Center, and J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory: Laboratory: Fingerprinting your own DNA.

SESSION 2

D. Micklos, DNA Learning Center, Cold Spring Harbor Laboratory: Mendelian genetics and why you aren't your parents.
M. Bloom, DNA Learning Center, Cold Spring Harbor Laboratory: What is a gene?
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory: Diagnosis using DNA.

SESSION 3

E.B. Claus, Yale University, New Haven, Connecticut: Breast cancer genetics.
M. Bloom and D. Micklos, DNA Learning Center, and J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory: Laboratory: Results of DNA-fingerprinting experiment.

B. Hummel-Rossi, L. Levine, R. Schaefer

I. Kritchek

DNA Repair and Genetic Instability in Cancer

January 29-January 31

FUNDED BY

OncorMed, Inc.

ARRANGED BY

D. Dolginow, OncorMed, Inc., Gaithersburg, Maryland
D. Sidransky, Johns Hopkins University, Baltimore, Maryland

SESSION 1
Chairperson: D. Sidransky, Johns Hopkins University, Baltimore, Maryland

J. Cairns, Radcliffe Infirmary, Oxford, United Kingdom: Mutation, promotion, and the kinetics of carcinogenesis.

SESSION 2
Chairperson: P. Modrich, Howard Hughes Medical Institute, Duke University Medical Center, Durham, North Carolina

B. Vogelstein, The Johns Hopkins Oncology Center, Baltimore, Maryland: DNA-repair and genetic instability in colorectal cancer.

L.A. Loeb, University of Washington, Seattle: Multiple mutations in cancer.

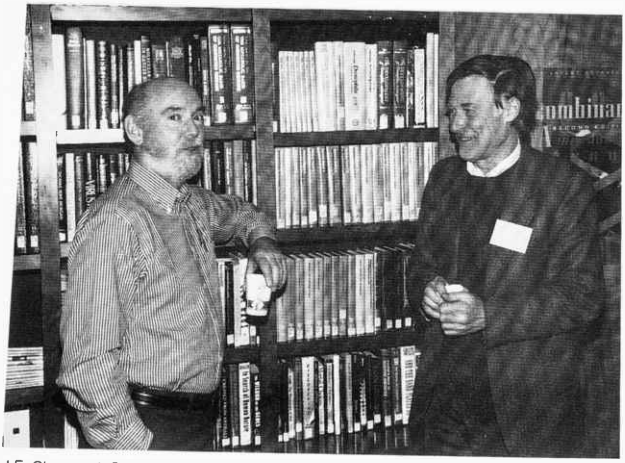
S.N. Thibodeau, Mayo Clinic, Rochester, Minnesota: Clinical significance of microsatellite instability.

T.D. Petes, University of North Carolina, Chapel Hill: Genetic control of genetic instability in yeast.

P. Karran, Imperial Cancer Research Fund, Herts, United Kingdom: Mismatch repair, drug resistance, and colon cancer.

A.M. Carr, Sussex University, Falmer, United Kingdom: Cell cycle checkpoints in yeast and response to DNA damage.

T.A. Kunkel, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Studies of DNA replication fidelity and mismatch repair in human cell extracts.



J.E. Cleaver, J. Cairns

SESSION 3
Chairperson: J.E. Cleaver, University of California, San Francisco

P. Modrich, Howard Hughes Medical Institute, Duke University Medical Center, Durham, North Carolina: Mismatch repair deficiency and genetic destabilization in tumor cells.
G. Bedi, Johns Hopkins University School of Medicine, Baltimore, Maryland: Microsatellite instability in AIDS-related neoplasms.
D. Monckton, Baylor College of Medicine, Houston, Texas: Triplet repeat instability in myotonic dystrophy.
D. Toczyski, University of Washington, Seattle: Adaptation to the RAD9-dependent checkpoint in the presence of an irreparable dsDNA break.
B.A. Donahue, Stanford University, California: Role of transcription in nucleotide excision repair.
P. Peltomaki, University of Helsinki, Finland: Microsatellite instability in colorectal adenomas and carcinomas.
J.H. Miller, University of California, Los Angeles: Mechanisms of base-change damage in mutagenesis.

SESSION 4
Chairperson: B. Vogelstein, The Johns Hopkins Oncology Center, Baltimore, Maryland

J.E. Cleaver, University of California, San Francisco: DNA repair and gene expression studies involving the XPA photoproduct-specific DNA-binding protein.
N. Arnheim, University of Southern California, Los Angeles: Germ-line trinucleotide repeat instability.
P.L. Foster, Boston University School of Medicine, Massachusetts: Mechanisms of adaptive mutation in *Escherichia coli.*
D. Sidransky, Johns Hopkins University, Baltimore, Maryland: Tumor instability in cancer detection.
J. Jiricny, IRBM P. Angeletti, Pomezia, Italy: G-T mismatch binding activities present in HELA cell extracts.
R.M. Liskay, Oregon Health Sciences University, Portland, Oregon: Mutation and cancer avoidance.
L.A. Hedrick, Johns Hopkins University, Baltimore, Maryland: Mutational analysis of repair genes in endometrial carcinoma.

SESSION 5
Chairperson: J. Cairns, Radcliffe Infirmary, Oxford, United Kingdom

L.H. Thompson, Lawrence Livermore National Laboratory, California: Characterization of human repair genes in terms of genetic instability.
N.G.J. Jaspers, Erasmus University, Rotterdam, The Netherlands: Nucleotide excision-repair deficiency in man.
E. Alani, Dana-Farber Cancer Institute, Boston, Massachusetts: Yeast and human mutators genes.

The Targets and Regulation of the Ubiquitin System

February 12–February 15

FUNDED BY

Cold Spring Harbor Laboratory Corporate Sponsor Program

ARRANGED BY

P. Howley, Harvard Medical School, Boston, Massachusetts
A. Varshavsky, California Institute of Technology, Pasadena

SESSION 1: UBCs and Cell Cycle Control
Chairperson: P. Howley, Harvard Medical School, Boston, Massachusetts

S. Jentsch, Heidelberg University, Germany: A ubiquitin-conjugating enzyme involved in the degradation of both S- and M-phase cyclins.
D. Finley, Harvard Medical School, Boston, Massachusetts: Induction of ubiquitination in erythroid differentiation.
F. Cross, The Rockefeller University, New York, New York: Degradation of the CDC28 inhibitor FAR1.
A. Murray, University of California, San Francisco: The spindle assembly checkpoint and cyclin proteolysis.
A. Hershko, Technion-Israel Institute of Technology, Haifa: Mechanisms and regulation of cyclin degradation.
M. Ellison, University of Alberta, Edmonton, Canada: Placing the mechanism of protein ubiquitination within a structural context.

SESSION 2: Targets of Ubiquitination
Chairperson: A. Hershko, Technion-Israel Institute of Technology, Haifa

D. Bohmann, European Molecular Biology Laboratory, Heidelberg, Germany: Ubiquitin-dependent degradation of Jun.

P.A. Baeuerle, Albert-Ludwigs-University of Freiburg, Germany: Role of proteolysis in the activation of transcription factor NF-κb.

T. Maniatis, Harvard University, Cambridge, Massachusetts: Ubiquitin and proteasome-dependent activation of NF-κb.

M. Hochstrasser, University of Chicago, Illinois: Ubiquitin and proteasome-mediated degradation of the yeast MATα2 transcriptional regulator.

M. Scheffner, Deutsches Krebsforschungszentrum, Heidelberg, Germany: Regulation of p53 stability.

K. Madura, Robert Wood Johnson Medical School, UMDNJ, Piscataway: Ubiquitin-dependent degradation of yeast G-α.

SESSION 3: Regulation of Ubiquitination
Chairperson: C.M. Pickart, State University of New York, Buffalo

A. Varshavsky, California Institute of Technology, Pasadena: Substrates and functions of the N-end rule pathway.

J. Becker, University of Tennessee, Knoxville: Involvement of *UBR1* in the transport of peptides in yeast.

V. Chau, Wayne State University School of Medicine, Detroit, Michigan: Mechanistic analyses with a reconstituted N-end rule pathway.

J. Huibregtse, Harvard Medical School, Boston, Massachusetts: A class of proteins structurally and functionally related to the Eb-AP protein ligase.

A. Ciechanover, Technion-Israel Institute of Technology, Haifa: Novel ubiquitin-protein ligates (E3s).

R. Vierstra, University of Wisconsin-Madison: Targeted degradation: Potential new method for selective protein degradation.

SESSION 4: Ubiquitination Targets and Related Topics
Chairperson: A.L. Goldberg, Harvard Medical School, Boston, Massachusetts

K.L. Rock, Dana Farber Cancer Institute, Boston, Massachusetts: Role of ubiquitin-proteasome pathway in the degradation of cellular proteins and the generation of MHC class-I-presented peptides.

M. Rechsteiner, University of Utah, Salt Lake City: KEKE motifs, proteolysis, and antigen presentation.

A. Weissman, National Institutes of Health, Bethesda, Maryland: Ubiquitination and early events in T-cell activation.

L.A. Guarino, Texas A&M University, College Station: Phospholipid anchors ubiquitin to the membranes of viral particles.

A.L. Haas, Medical College of Wisconsin, Milwaukee: Ubiquitin cross-reactive protein (p15), an interferon-induced ubiquitin homolog.

P. Coffino, University of California, San Francisco: Ornithine decarboxylase: Regulating degradation without ubiquitin.

M. Rechsteiner, A. Hershko

SESSION 5: Protein Degradation
Chairperson: A. Varshavsky, California Institute of Technology, Pasadena

E. Craig, University of Wisconsin, Madison: Relationship between molecular chaperones and the ubiquitin system.
A.L. Goldberg, Harvard Medical School, Boston, Massachusetts: Protein degradation.
G.N. DeMartino, University of Texas Southwestern Medical Center, Dallas: Regulatory proteins of the proteasome.
C. Pickart, State University of New York, Buffalo: Mechanism of proteolytic targeting by multiubiquitin chains.
K.D. Wilkinson, Emory University School of Medicine, Atlanta, Georgia: Processing of polymeric ubiquitin: The *UCH* and *UBP* gene families.
C.A. Slaughter, Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas: High-molecular-weight modifiers of proteasome activity.

Genetics of Human Behavior

March 5-March 8

FUNDED BY

The William Stamps Farish Fund

ARRANGED BY

E. Balaban, The Neurosciences Institute, La Jolla, California
J. Beckwith, Harvard Medical School, Boston, Massachusetts
L.N. Geller, Harvard Medical School, Boston, Massachusetts
K.S. Kendler, Virginia Commonwealth University, Richmond
N.J. Risch, Yale University School of Medicine, New Haven, Connecticut
J.D. Watson, Cold Spring Harbor Laboratory

SESSION 1: Phenotypes
Moderators: E. Balaban, The Neurosciences Institute, La Jolla, California and
K. Merikangas, Yale Family Study Center, New Haven, Connecticut

K.S. Kendler, Virginia Commonwealth University, Richmond: Assessment of psychiatric disorders in a genetic context.
E. Ostrander, University of Seattle, Washington: Animal behavior.
E. Balaban, The Neurosciences Institute, La Jolla, California: Respondent.

J.S. Alper, J. Beckwith, P.R. Billings

N.J. Risch, I. Gottesman

SESSION 2: Estimating Genetic Contributions
Moderators: M. Feldman, Stanford University Medical School, California and **C.R. Cloninger,** Washington University School of Medicine, St. Louis, Missouri

T. Reich, Washington University School of Medicine, St. Louis, Missouri: Family studies.
N.G. Martin, Queensland Institute of Medical Research, Brisbane, Australia: Twin studies.
L.J. Eaves, Virginia Commonwealth University, Richmond: Twin-family studies.
D.W. Fulker, University of Colorado, Boulder: Colorado adoption study.
M. Feldman, Stanford University Medical School, Stanford: Respondent.

SESSION 3: Linkage, Association, etc.
Moderator: N.J. Risch, Yale University School of Medicine, New Haven, Connecticut

P. McGuffin, University of Wales College of Medicine, Cardiff, United Kingdom: Detecting and locating QTL and genes of small effect.
G. Ebers, University Hospital, London, Ontario, Canada: Sib-pair studies in MS and sexual orientation.
E.S. Gershon, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland: Positive results in complex inheritance traits.
J. Crabbe, Oregon Health Sciences University, Portland: QTL approaches with animal models.

SESSION 4: Presentation of Results to the Public
Moderators: G. Carey, University of Colorado, Boulder and **J. Beckwith,** Harvard Medical School, Boston, Massachusetts

D. Nelkin, New York University, New York: The gene as a cultural icon.
T. Duster, University of California, Berkeley: The public reception of the genetic revolution.
R.W. Cooke, Newsday, Inc., Melville, New York: A journalist's perspective.
I.I. Gottesman, University of Virginia, Charlottesville: Respondent.

HIV and the Pathogenesis of AIDS

March 19-March 22

FUNDED BY

Mothers' Voices, Office of AIDS Research, National Institutes of Health, Pediatric AIDS Foundation, The Albert B. Sabin Vaccine Foundation, and Viaticus, Inc.

ARRANGED BY

D.D. Ho, Aaron Diamond AIDS Research Center, New York, New York
S.M. Wolinsky, Northwestern University Medical School, Chicago, Illinois

Introductory remarks: D.D. Ho, Aaron Diamond AIDS Research Center, New York, New York, and **S.M. Wolinsky,** Northwestern University Medical School, Chicago, Illinois.

SESSION 1: Viral Dynamics
Chairperson: J.M. Coffin, Tufts University School of Medicine, Boston, Massachusetts

J.M. Coffin, Tufts University School of Medicine, Boston, Massachusetts: Introduction
G.M. Shaw, University of Alabama at Birmingham: Review.
General discussion and summary of key questions and experiments.

W.E. Paul, R.A. Weiss

SESSION 2: Mechanism of CD4 Destruction
Chairperson: S. Wain-Hobson, Institut Pasteur, Paris, France

S. Wain-Hobson, Institut Pasteur, Paris, France: Introduction.
M. Feinberg, Gladstone Institute of Virology/Immunology, San Francisco, California: Review.
General discussion and summary of key questions and experiments.

SESSION 3: CD4 Regeneration
Chairperson: W.E. Paul, AIDS Research, National Institutes of Health, Bethesda, Maryland

W.E. Paul, AIDS Research, National Institutes of Health, Bethesda, Maryland: Introduction.
B.F. Haynes, Duke University, Durham, North Carolina: Review.
General discussion and summary of key questions.

SESSION 4: Correlates of Protection/Host Resistance
Chairperson: A.S. Fauci, NIAID, National Institutes of Health, Bethesda, Maryland

A.S. Fauci, NIAID, National Institutes of Health, Bethesda, Maryland: Introduction.
R.A. Koup, Aaron Diamond AIDS Research Center, New York, New York: Review.
General discussion and summary of key questions and experiments.

SESSION 5: Issues of Vaccine Development
Chairperson: D.P. Bolognesi, Duke University Medical Center, Durham, North Carolina

D.P. Bolognesi, Duke University Medical Center, Durham, North Carolina: Introduction.
K.S. Steimer, Biocine-Chiron, Emeryville, California: Review.
General discussion and summary of key questions and experiments.

SESSION 6: Implication for/of Treatment Studies
Chairperson: R.T. Schooley, University of Colorado Health Sciences Center, Denver, Colorado

R.T. Schooley, University of Colorado Health Science Center, Denver: Introduction.
D.D. Richman, University of California, San Diego: Review.
General discussion and summary of key questions and experiments.

SESSION 7: Viral Compartments and Transmission
Chairperson: A.T. Haase, University of Minnesota, Minneapolis

A.T. Haase, University of Minnesota, Minneapolis: Introduction.
J.I. Mullins, University of Washington, Seattle: Review.
General discussion and summary of key questions and experiments.

SESSION 8: Antigenic Diversity and Viral Variation
Chairperson: M.A. Nowak, University of Oxford, United Kingdom

M.A. Nowak, University of Oxford, United Kingdom: Introduction.
B.T.M. Korber, Los Alamos National Laboratory, New Mexico: Review.
General discussion and summary of key questions and experiments.

SESSION 9: Conclusions and Future Prospects
Chairperson: R.A. Weiss, Institute of Cancer Research, London, United Kingdom

Summary

J.M. Coffin, A.T. Haase, D.D. Ho

Lyme Disease: Molecular and Immunologic Aspects of Detection and Vaccine Development

March 26-March 29

FUNDED BY

Centers for Disease Control, Connaught Laboratories, Inc., Food and Drug Administration, Fort Dodge Laboratories, MedImmune, Inc., National Institute of Allergy and Infectious Diseases, and SmithKline Beecham Clinical Laboratories

ARRANGED BY

J.J. Dunn, Brookhaven National Laboratory, Upton, New York
S.E. Schutzer, UMDNJ-New Jersey Medical School, Newark

SESSION 1: Molecular Biology
Chairperson: J.J. Weis, University of Utah School of Medicine, Salt Lake City

S. Casjens, University of Utah Medical School, Salt Lake City: Structure and diversity of the *B. burgdorferi* (*sensu lato*) genome.

C.J. Luke, University of Texas Health Science Center, San Antonio: Alternative candidate antigens for vaccine development.

B. Stevenson, Rocky Mountain Laboratories, NIH, Hamilton, Montana: Molecular mechanisms of Osp variation, regulation of expression.

E. Fikrig, Yale University School of Medicine, New Haven, Connecticut: Immunogenicity of new surface antigens.

SESSION 2: Immunology
Chairperson: P.K. Coyle, State University of New York, Stony Brook

B. Wilske, Max von Pettenkofer-Institut, Munich, Germany: Immune response to OspC.

S.E. Schutzer, UMDNJ-New Jersey Medical School, Newark: Immune responses to OspA in previously infected individuals.

T.G. Schwan, Rocky Mountain Laboratories, NIAID, NIH, Hamilton, Montana: Osp expression in ticks.

M.M. Simon, S.P. Nickell, E.S. Raveche, E. Fikrig

SESSION 3: Vaccine
Chairperson: J. Soreth, U.S. Food and Drug Administration, Rockville, Maryland

B. Johnson, Centers for Disease Control, Fort Collins, Colorado: Humoral response and protection to key epitopes of Bb proteins.
S.W. Barthold, Yale University School of Medicine, New Haven, Connecticut: Immunization against tick-borne Lyme borreliosis.
M. Hanson, MedImmune Inc., Gaithersburg, Maryland: Protective immunity via recombinant chimeric OspA BCG vaccine.
F. Meurice, SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania: SKB vaccine trial data.

SESSION 4: Special Discussion: Infection and Autoimmunity in Chronic Lyme Disease
Moderator: M.T. Philipp, Tulane Regional Primate Research Center, Covington, Louisiana
Commentaries: F.S. Kantor, Yale University School of Medicine, New Haven, Connecticut, and **J.J. Weis,** University of Utah School of Medicine, Salt Lake City

SESSION 5: Animal Models
Chairperson: E. Fikrig, Yale University School of Medicine, New Haven, Connecticut

R.H. Jacobson, Cornell University College of Veterinary Medicine, Ithaca, New York: Immune response and Bb-specific immune complexes in dogs.
J. Radolf, University of Texas Southwestern Medical Center, Dallas, Texas: The human Lyme disease vaccine, OspA, protects mice against tick transmission of infection with homologous but not heterologous strains of *B. burgdorferi.*
M.T. Philipp, Tulane Regional Primate Research Center, Covington, Louisiana: Lyme disease and immune response in rhesus monkey.
S.W. Barthold, Yale University School of Medicine, New Haven, Connecticut: Evolution of Bb antigens in the host.

SESSION 6: Pathogenesis I
Chairperson: F.S. Kantor, Yale University School of Medicine, New Haven, Connecticut

Y.-W. Chiang, Fort Dodge Laboratories, Iowa: Dog models of Lyme disease.
E.S. Raveche, UMDNJ-New Jersey Medical School, Newark: Bacterial induction of autoimmune response.
J.L. Benach, State University of New York, Stony Brook: Evasion mechanisms of Bb.
R.J. Dattwyler, State University of New York, Stony Brook: Influence of antimicrobials on the immune response.
L. Bochenstedt, Yale University School of Medicine, New Haven, Connecticut: Antigen variation.
P.K. Coyle, State University of New York, Stony Brook: Evidence for early and persistent infection in neurologic Lyme disease.
M.M. Simon, Max-Planck Institut fur Immunobiologie, Freiburg, Germany: Cellular interaction.
J.J. Weis, University of Utah School of Medicine, Salt Lake City: Mechanisms of Bb persistence.

SESSION 7: Special Discussion: Immune Response to Unique versus Many Proteins
Moderator: M.T. Philipp, Tulane Regional Primate Research Center, Covington, Louisiana
Commentary: B. Johnson, Centers for Disease Control, Fort Collins, Colorado

SESSION 8: Pathogenesis II/Recombinant Proteins
Chairperson: R.J. Dattwyler, State University of New York, Stony Brook

J.J. Dunn, Brookhaven National Laboratory, Upton, New York: Construction of Bb cosmid library.
D.H. Persing, Mayo Clinic, Rochester, Minnesota: Genetic stability of Bb in animal model.
B.J. Luft, State University of New York, Stony Brook: Chimeric molecules for detection and vaccination.
S.P. Nickell, Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland: T-cell response.

SESSION 9: Special Discussion: Entry and Endpoints for Antibiotic and Vaccine Trials
Moderator: M.T. Philipp, Tulane Regional Primate Research Center, Covington, Louisiana
Commentaries: J. Collins, Glaxo Inc. Research Institute, Research Triangle Park, North Carolina, and **R.J. Dattwyler,** State University of New York at Stony Brook

SESSION 10: Discussion and Future Directions and Chronic Lyme Disease
Chairperson: E. McSweegan, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland

Incorporating Genetics into Medicine and Nursing Education and Practice

April 3-April 6

FUNDED BY

The Robert Wood Johnson Foundation

ARRANGED BY

N.A. Holtzman, The Johns Hopkins Medical Institutions, Baltimore, Maryland

SESSION 1
Chairperson: N.A. Holtzman, The Johns Hopkins Medical Institutions, Baltimore, Maryland

N.A. Holtzman, The Johns Hopkins Medical Institutions, Baltimore, Maryland: Introductory remarks.
F.S. Collins, National Center for Human Genome Research, National Institutes of Health, Bethesda, Maryland: Current state of human genetics and genetics education.

SESSION 2: Report of Survey Findings
N.A. Holtzman, The Johns Hopkins Medical Institutions, Baltimore, Maryland: Analysis of survey. Discussion of implications and summary.

SESSION 3: Defining the Issues: Incorporating Genetics in Primary Care Practice (Panel Discussion)
Chairperson: S. Feetham, National Institute of Nursing Research, National Institutes of Health, Bethesda, Maryland

S. Feetham, National Institute of Nursing Research, National Institutes of Health, Bethesda, Maryland: Introductory remarks.
J.R. Allen, American Medical Association, Chicago, Illinois
R.E. Pyeritz, American College of Medical Genetics, Pittsburgh, Pennsylvania

J.G. Davis, N. Fisher

SESSION 4: Group discussions on Genetics in Primary Care Practice
Group 1: Chairperson: C. Scanlon, American Nurses Association, Washington, D.C.
Rapporteur: N.L. Fisher, Medical Genetic Services, Seattle, Washington
Group 2: Chairperson: W.L. Freeman, American Academy of Family Physicians, Albuquerque, New Mexico
Rapporteur: G. Anderson, Shriver Center for Mental Retardation, Waltham, Massachusetts

SESSION 5: Reports of Discussion Groups
Chairperson: N.A. Holtzman, The Johns Hopkins Medical Institutions, Baltimore, Maryland

Group 1: Rapporteur: N.L. Fisher, Medical Genetic Services, Seattle, Washington
Group 2: Rapporteur: G. Anderson, Shriver Center for Mental Retardation, Waltham, Massachusetts

SESSION 6: Needs and Implementation of Genetics Education (Panel Discussion)
Chairperson: N.A. Holtzman, The Johns Hopkins Medical Institutions, Baltimore, Maryland

M. Grey, Yale School of Nursing, New Haven, Connecticut
F. McCurdy, University of Nebraska Medical Center, Omaha
M. Genel, Yale University School of Medicine, New Haven, Connecticut
J.G. Davis, New York Hospital-Cornell University College of Medicine, New York

SESSION 7: Group Discussions of Genetics Education
Group 1: Chairperson: S. Feetham, National Institute of Nursing Research, National Institutes of Health, Bethesda, Maryland
Rapporteur: C.M. Hanson, American College of Nurse Practitioners, Statesboro, Georgia
Group 2: Chairperson: J.R. Allen, American Medical Association, Chicago, Illinois
Rapporteur: P. Rappo, American Academy of Pediatrics, North Easton, Massachusetts

SESSION 8: Reports of Discussion Groups
Chairperson: J.R. Allen, American Medical Association, Chicago, Illinois

Group 1: Rapporteur: C.M. Hanson, American College of Nurse Practitioners, Statesboro, Georgia
Group 2: Rapporteur: P. Rappo, American Academy of Pediatrics, North Easton, Massachusetts

SESSION 9: Setting Out Future Goals: Preliminary Discussion

SESSION 10: Future Goals: Defining Proposals
Chairperson: S. Feetham, National Institute of Nursing Research, National Institutes of Health, Bethesda, Maryland

N.A. Holtzman, The Johns Hopkins Medical Institutions, Baltimore, Maryland: Closing remarks.

N.A. Holtzman, J.R. Allen

Molecular Biology of Disease Resistance Genes in Plants

April 9-April 12

FUNDED BY

Cold Spring Harbor Laboratory Corporate Sponsor Program

ARRANGED BY

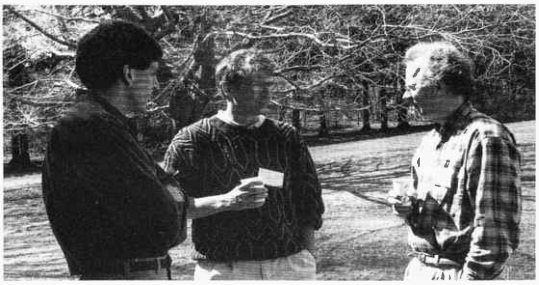
S.P. Briggs, Pioneer Hi-Bred International, Inc., Johnston, Iowa
R.W. Michelmore, University of California, Davis
B. Staskawicz, University of California, Berkeley

SESSION 1: Genetics and Evolution of Plant-Pathogen Interactions
Chairperson: S.P. Briggs, Pioneer Hi-Bred International, Inc., Johnston, Iowa

S.P. Briggs, Pioneer Hi-Bred International, Inc., Johnston, Iowa: Remarks.
I. Crute, Horticulture Research International, Warwick, United Kingdom: The genetics of pathogenic specificity to host species and higher-order host taxa.
R.W. Michelmore, University of California, Davis: Clusters of resistance genes of lettuce.
C. Gebhardt, Max-Planck-Institut fur Zuchtungsforschung, Kohn, Germany: Present state of map-based cloning of the nematode resistance gene G1 and the fungal resistance gene R1 in potato.
S. Hulbert, Kansas State University, Manhattan: Evolutionary events at the Rp1 complex.
P. Schulze-Lefert, Biologie I, Aaachen, Germany: Genes required for function of resistance genes to powdery mildew infection in barley.
P. Vos, Keygene N.V., Wageningen, The Netherlands: State of the art of positional gene isolation technology.
J. Howard, University of Cologne, Germany: Origin and maintenance of variation in class I genes of the vertebrate MHC.

SESSION 2: Receptor-related Disease Resistance Genes
Chairperson: R.W. Michelmore, University of California, Davis

G.B. Martin, Purdue University, West Lafayette, Indiana: Characterization of the Pto resistance gene family in tomato.
B.J. Baker, USDA/ARS, Albany, California: The product of the tobacco mosaic virus disease resistance gene N: Similarity to toll and the interleukin-1 receptor.
F.M. Ausubel, Massachusetts General Hospital, Boston: RPS2 and other *Arabidopsis* defense-related genes.
J.D.G. Jones, John Innes Centre, Norwich, United Kingdom: Characterization of the Cf9, Cf2, and RPP5 disease resistance genes.
B. Staskawicz, University of California, Berkeley: Signal transduction events specifying plant disease resistance.
J. Ellis, CSIRO Plant Industry, Canberra, Australia: The L and M rust resistance genes from flax.
P. Ronald, University of California, Davis: The rice Xa21 locus: Isolation of a multigene family with striking similarity to dicot disease resistance genes.



S. Hulbert, J. Ryals, D.C. Baulcombe

SESSION 3: Characterization of Additional Resistance Genes
Chairperson: B.J. Baker, USDA/ARS, Albany, California

B.J. Baker, USDA/ARS, Albany, California: Remarks.
J. Dangl, Max-Delbruck-Laboratorium in der MPG, Koln, Germany: *Arabidopsis* and *Pseudomonas syringae* loci controlling pathogen recognition and cell death.
R. Innes, Indiana University, Bloomington: Comparison of the *Arabidopsis RPS3* and soybean *RPG1* genes. Are they true homologs?
G.S. Johal, University of Missouri, Columbia: What art thou, o mimics?
S.P. Briggs, Pioneer Hi-Bred International, Inc., Johnston, Iowa: Inactivation of candidate defense genes.

SESSION 4: Pathogen Determinants of Avirulence
Chairperson: J. Dangl, Max-Delbruck-Laboratorium in der MPG, Koln, Germany

D.C. Baulcombe, The Sainsbury Laboratory, Norwich, United Kingdom: Virus-encoded elicitors of resistance.
P.J.D.M. de Wit, Wageningen Agricultural University, The Netherlands: Avirulence gene products of *Cladosporium fulvum* and their receptors in plants.
B. Valent, The Du Pont Company, Wilmington, Delaware: Gene-for-gene interactions in the rice blast system.

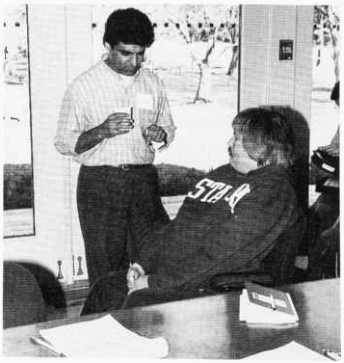
SESSION 5: The Oxidative Burst and Systemic Acquired Resistance
Chairperson: B. Staskawicz, University of California, Berkeley

U.G. Knaus, The Scripps Research Institute, La Jolla, California: Regulation of the oxidative burst in human leukocytes.
P.S. Low, Purdue University, West Lafayette, Indiana: Signal transduction pathways during the oxidative burst.
C.J. Lamb, Salk Institute for Biological Studies, San Diego, California: Mechanism and function of the oxidative burst in the hypersensitive response.
D. Shah, Monsanto Company, St. Louis, Missouri: Engineering disease resistance through manipulation of active oxygen in potato.
I. Raskin, Rutgers University, New Brunswick, New Jersey: Salicylic acid as a signal in disease resistance.
D.F. Klessig, Rutgers University, Piscataway, New Jersey: A mechanism of action of salicylic acid in plant disease resistance.
X. Dong, Duke University, Durham, North Carolina: Genetic dissection of systemic acquired resistance response using *Arabidopsis* mutants.
J. Ryals, Ciba Agricultural Biotechnology, Research Triangle Park, North Carolina: Signaling and signal transduction in acquired resistance.

SESSION 6: Disease Resistance Physiology and Signal Transduction
Chairperson: J.D.G. Jones, John Innes Centre, Norwich, United Kingdom

R. Fluhr, The Weizmann Institute of Science, Rehovot, Israel: The tomato 12 locus and pathways to the pathogenesis response.
G. De Lorenzo, Universita "La Sapienza", Roma, Italy: Polygalacturonase-inhibiting proteins (PGIPs): Plant proteins specialized for recognition of fungal pathogens.
C.A. Ryan, Washington State University, Pullman: Systemin: A mobile wound signal in plants.
J.C. Walker, University of Missouri, Columbia: Signal transduction in plants: The interaction of protein kinases and phosphatases.
J.R. Ecker, University of Pennsylvania, Philadelphia: Ethylene signal transduction.

General discussion: Signaling in disease resistance.



G.S. Johal, J. Dangl

Human Gene Map Workshop II

May 9-May 10

FUNDED BY

European Commission, Glaxo, Merck, Pfizer Inc., Sequana Therapeutics, Inc., SmithKline Beecham, Wellcome Trust, United Kingdom Medical Research Council, United States Department of Energy, United States National Institutes of Health, and Zeneca Pharmaceuticals

ARRANGED BY

R.H. Waterston, Washington University School of Medicine, St. Louis, Missouri

SESSION 1: EST Reports/Libraries

R. Wilson, Washington University School of Medicine, St. Louis, Missouri
M.B. Soares, Columbia University, New York, New York
K. Okubo, Osaka University, Japan
C. Auffray, CNRS, Villejuif, France
K. Gibson, HGMP Resource Center, Cambridge, United Kingdom
M. Adams, The Institute for Genomic Research, Gaithersburg, Maryland
M.H. Polymeropoulos, National Center for Human Genome Research, National Institutes of Health, Bethesda, Maryland
H. Shizuya, California Institute of Technology, Pasadena

SESSION 2: Reports on Mapping of ESTs

D.R. Cox, Stanford University School of Medicine, California: Introduction.
J. Weissenbach, Laboratoire des Maladies Genetiques Humaines, Evry, France
K. Schmitt, University of Cambridge, United Kingdom
D.R. Cox, Stanford University School of Medicine, California
T.J. Hudson, Whitehead Institute, Massachusetts Institute of Technology, Cambridge
D.R. Bentley, The Sanger Centre Hinxton Hall, Cambridgeshire, United Kingdom
M.R. James, The Wellcome Trust Centre for Human Genetics, Oxford, United Kingdom

SESSION 3: Informatics

M. Boguski, National Center for Biotechnology Information, Bethesda, Maryland: Introduction.
K.O. Elliston, Merck Research Laboratories, Rahway, New Jersey
M. Boguski, National Center for Biotechnology Information, Bethesda, Maryland
G. Cameron, The European Bioinformatics Institute, Cambridge, United Kingdom
Y. Tateno, DNA Research Center, National Institute of Genetics, Mishima, Japan
C.A. Fields, National Center for Genome Resources, Santa Fe, New Mexico
K.H. Buetow, Fox Chase Cancer Center, Philadelphia, Pennsylvania
K. Fasman, Genome Data Base, Baltimore, Maryland

SESSION 4: Conclusions/Future Plans

G. Lennon, Lawrence Livermore National Laboratory, California: IMAGE update.

Discussion:
Moderator: R.H. Waterston, Washington University School of Medicine, St. Louis, Missouri

D.R. Cox, M.S. Guyer, D.A. Smith

CREB and Memory

June 8-June 11

FUNDED BY

Cold Spring Harbor Laboratory Corporate Sponsor Program

ARRANGED BY

T. Tully, Cold Spring Harbor Laboratory
J. Yin, Cold Spring Harbor Laboratory

SESSION 1: Behavior I
Chairperson: G. Schutz, German Cancer Research Center, Heidelberg, Germany

D. Bartsch, HHMI, Columbia University, New York, New York: Long-term facilitation in *Aplysia* neurons requires coordinated transcriptional activation by Af-1 and derepression of ApCREB-2.
C. Bailey, Columbia University, New York, New York: Structural changes during long-term memory.
T.J. Carew, Yale University, New Haven, Connecticut: Parallel processing of short-term and long-term synaptic facilitation in *Aplysia*.
J. Yin, Cold Spring Harbor Laboratory: CREB and the formation of long-term memory in *Drosophila*.
E.J. Nestler, Yale University School of Medicine, New Haven, Connecticut: Regulation of CREB expression: In vivo evidence for a functional role in the brain.

SESSION 2: CREB/CREM
Chairperson: M.E. Greenberg, Children's Hospital, Boston, Massachusetts

J.F. Habener, HHMI, Harvard Medical School, Boston, Massachusetts: Alternative exon splicing generates alternative activator and repressor isoforms of CREB in the testis.
M. Montminy, The Salk Institute, La Jolla, California: Transcriptional regulation by cAMP.
G. Schutz, German Cancer Research Center, Heidelberg, Germany: Molecular genetic analysis of cAMP-dependent gene expression in development.
P. Sassone-Corsi, I.G.B.M.C., Strasbourg, France: Molecular and physiological aspects of the transcriptional response to cAMP.
N.S. Foulkes, I.G.B.M.C., Strasbourg, France: Regulation of CREM and circadian rhythms.
E. Lalli, I.G.B.M.C., Strasbourg, France: CREM in endocrine glands.

E. Kandel, D. Bartsch, D. Michael

R. McKay, T. Tully

SESSION 3: Downstream
Chairperson: J. Yin, Cold Spring Harbor Laboratory

J. Lundblad, Vollum Institute, Oregon Health Sciences University, Portland: The cAMP:CREB:CBP pathway: A target of DNA and RNA tumor viruses.
R. Kwok, Vollum Institute, Oregon Health Sciences University, Portland: Differential utilization of the CREB:CBP pathway allows specific activation of viral versus cellular promoters.
J.P. Hoeffler, University of Colorado Health Science Center, Denver: Elucidating in vivo promoter-binding specificity of CREB/ATF proteins.
T. Hai, Ohio State University, Columbus: ATF3 and ATF4: The ebb and flow of transcriptional regulation by ATF.

SESSION 4: Transduction
Chairperson: D.R. Storm, University of Washington School of Medicine, Seattle

D.D. Ginty, Johns Hopkins University School of Medicine, Baltimore, Maryland: CREB confers growth factor activation of gene expression.
G. Enikolopov, Cold Spring Harbor Laboratory: NO, CREB, and cAMP in short- and long-term signaling.
M.E. Greenberg, Children's Hospital, Boston, Massachusetts: CREB: A mediator of neurotrophin and neurotransmitter signaling.
C.J. Fiol, Indiana University School of Medicine, Indianapolis: Molecular mechanisms in hierarchal phosphorylations.
R. Maurer, Vollum Institute, Oregon Health Sciences University, Portland: Differential regulation of CREB by Ca^{++}/calmodulin-dependent protein kinases.

SESSION 5: PKA
Chairperson: M. Montminy, The Salk Institute, La Jolla, California

S.S. Taylor, University of California, San Diego: cAMP-dependent protein kinase: Structure and subcellular localization.
H. Bayley, Worcester Foundation, Shrewsbury, Massachusetts: Functional diversity of cAMP-dependent protein kinases in *Aplysia*.
R.L. Idzerda, University of Washington, Seattle: Targeted disruption of protein kinase A subunits.
J.D. Scott, Vollum Institute, Oregon Health Sciences University, Portland: Neuronal targeting of kinases and phosphatases: Their role in postsynaptic events.
R.Y. Tsien, HHMI, University of California, San Diego: Fluorescence imaging of cAMP and gene expression in single cells.
M. Bollen, Afdeling Biochemie, Campus Gasthuisberg, Leuven, Belgium: Nuclear Ser/Thr protein phosphatases and their substrates.

SESSION 6: General Discussion
Discussion Leaders:
T. Curran, Roche Institute of Molecular Biology, Nutley, New Jersey
E. R. Kandel, HHMI, Columbia University, New York, New York
T. Tully, Cold Spring Harbor Laboratory

SESSION 7: Behavior II
Chairperson: J. Yin, Cold Spring Harbor Laboratory

K. Deisseroth, Stanford University, Palo Alto, California: CREB phosphorylation during synaptic plasticity.
H. Bito, Stanford University, Palo Alto, California: CREB phosphorylation pathways in action-potential-driven hippocampal neurons.
D.R. Storm, University of Washington School of Medicine, Seattle: Role of cAMP and Ca^{++}-sensitive adenylyl cyclase for neuroplasticity.
A. Silva, Cold Spring Harbor Laboratory: CREB and long-term memory in mice.
E.R. Kandel, HHMI, Columbia University, New York, New York: CREB and its role in implicit and explicit memory storage.
R. McKay, LMB/NINDS, National Institutes of Health, Bethesda, Maryland: Constructing a chimeric hippocampus.

Molecular Diagnosis of Inherited Breast Cancer

October 10-October 13

FUNDED BY

Cold Spring Harbor Laboratory Corporate Sponsor Program

ARRANGED BY

B.J. Ponder, University of Cambridge, United Kingdom
C.S. Richards, Baylor College of Medicine, Houston, Texas
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Mutations in BRCA1
Chairperson: S.H. Friend, Fred Hutchinson Cancer Research Center, Seattle, Washington

B.J. Ponder, University of Cambridge, United Kingdom: Introduction.
D. Easton, Institute of Public Health, Cambridge, United Kingdom: Contribution of BRCA1, BRCA2, and other breast cancer genes to familial and nonfamilial breast cancer.
C. Szabo, University of Washington, Seattle: Mutations in BRCA1.
L.C. Brody, National Center for Human Genome Research, National Institutes of Health, Bethesda, Maryland: BRCA1 mutations in the Ashkenazi Jewish population.
B.J. Ponder, University of Cambridge, United Kingdom: Summary.
Discussion of BRCA mutations.

SESSION 2: Mutations in Other Genes
Chairperson: J.E. Garber, Dana Farber Cancer Institute, Boston, Massachusetts

B.J. Ponder, University of Cambridge, United Kingdom: Introduction.
D.A. Tagle, National Center for Human Genome Research, National Institutes of Health, Bethesda, Maryland: The AT gene: Its functions and contributions to breast cancer.
B.E. Henderson, University of Southern California School of Medicine, Los Angeles: Estrogen metabolism genes and BRCA1.
Discussion of genes other than BRCA1 and breast cancer.

SESSION 3: Populations in Screening
Chairperson: J.E. Garber, Dana Farber Cancer Institute, Boston, Massachusetts

E.B. Claus, Yale University School of Medicine, New Haven, Connecticut: Risk estimates/individuals at risk/identifying women at risk of BRCA1, etc.
W.W. Grody, University of California, Los Angeles, School of Medicine: Population carrier screening in a complex gene: The CF experience.
P.D. Murphy, OncorMed, Inc., Gaithersburg, Maryland: The approach OncorMed uses for BRCA1 testing.
Discussion of screening strategies.

SESSION 4: Technologies for Detecting Mutations
Chairperson: C.S. Richards, Baylor College of Medicine, Houston, Texas

J. Shumaker, Baylor College of Medicine, Houston, Texas: Comparative DNA sequencing by APEX.
R.A. Gibbs, Baylor College of Medicine, Houston, Texas: Improvements in DNA sequencing for mutational analysis.
A.-C. Syvanen, National Public Health Institute, Helsinki, Finland: Solid-phase minisequencing: A promising tool for large-scale DNA diagnostics.
Discussion of sequencing.
R.G.H. Cotton, Murdoch Institute, Melbourne, Victoria, Australia: Gene-specific databases: How do we ensure they are online and update?
J. Gordon, Abbott Labs, Abbott Park, Illinois: Simple rapid technologies for readout of complex mutations.
P. Devilee, University of Leiden, The Netherlands: Performance of the protein truncation test in screening out BRCA1 mutations: The Dutch experience.
Discussion of other tests.

B.B. Biesecker, J. Chamberlain, C.S. Richards, M.J.E. Kahn

SESSION 5
Chairperson: B.J. Ponder, University of Cambridge, United Kingdom

J.E. Garber, Dana Farber Cancer Institute, Boston, Massachusetts: Clinical Issues in BRCA1 testing.

M.J. Ellis Kahn, Richmond, Virginia: Making personal decisions.

B. Bowles Biesecker, National Center for Human Genome Research, National Institutes of Health, Bethesda, Maryland: Informed consent issues in BRCA1 testing.

M.S. Watson, Washington University School of Medicine, St. Louis, Missouri: Considerations in test transition into clinical service.

J. Chamberlain, Institute of Cancer Research, Sutton, Surrey, United Kingdom: What can be offered to premenopausal women with molecular diagnosis of inherited breast cancer?

C. Eng, Mount Sinai School of Medicine, New York, New York: Acceptance of genetic screening in the Ashkenazi Jewish population.

M. Bobrow, Addenbrooke's Hospital, Cambridge, United Kingdom: Implementing genetic screening and testing in the National Health Service.

P. Reilly, Shriver Center for Mental Retardation, Waltham, Massachusetts: DNA-based testing and managed care.

General Discussion:
Moderator: B.J. Ponder, Cambridge University, United Kingdom

Neurofibromatosis: How to Develop Therapies?

October 15-October 18

FUNDED BY

The National Neurofibromatosis Foundation, Inc., The Wilson Foundation, and private contributions

ARRANGED BY

F. McCormick, Onyx Pharmaceuticals, Richmond, California
B.R. Seizinger, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, New Jersey

SESSION 1: Clinical Clues for Intervention in NF1
Chairperson: B.R. Korf, Children's Hospital, Boston, Massachusetts

B.R. Korf, Children's Hospital, Boston, Massachusetts: Clinical clues that suggest opportunities for intervention in NF1.

A. Rubenstein, Mt. Sinai School of Medicine, New York, New York: Clinical clues to therapy in NF1: Data and data collection.

K.N. North, Children's Hospital, Boston, Massachusetts: Current understanding of cognitive dysfunction in patients with NF1: Implications for research and therapy.

P.C. Phillips, Children's Hospital of Philadelphia, Pennsylvania: NF1 clinical trials: Progress and problems.

SESSION 2: Molecular Genetic and Cell Biological Aspects of NF1 (Other Than Ras)
Chairperson: N.G. Copeland, National Cancer Institute-Frederick Cancer Research Center, Maryland

D.H. Gutmann, Washington University School of Medicine, St. Louis, Missouri: Tumor suppressor gene products and the cytoskeleton: Potential avenues for cancer therapy.
L.F. Parada, University of Texas Southwestern Medical Center, Dallas, Texas: Role of the NF1 gene in neuronal survival.
F. McCormick, Onyx Pharmaceuticals, Richmond, California: Regulators and effectors of Ras proteins.
B.R. Seizinger, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, New Jersey: Toward the development of a new generation of more specific anti-cancer drugs based on rational insights into the signaling pathways of tumor suppressor genes: p53.
K.M. Shannon, University of California, San Francisco: NF1 in myeloid growth control and leukemogenesis.
R.L. White, Howard Hughes Medical Institute, University of Utah Medical Center, Salt Lake City: Analysis of NF1 mutations in yeast and mammalian cells.

SESSION 3: NF1 Function and Ras Signaling: (A) Ras-Farnesylation Inhibitors
Chairperson: B.R. Seizinger, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, New Jersey

N. Ratner, University of Cincinnati, Ohio: In vitro systems to test therapeutic agents in NF1-deficient cells.
S. Sebti, University of Pittsburgh School of Medicine, Pennsylvania: Farnesyltransferase inhibitors induce cytoplasmic accumulation of inactive Ras/Raf complexes.
J.B. Gibbs, Merck Research Laboratories, West Point, Pennsylvania: Farnesyltransferase inhibitors as potential cancer chemotherapeutics.
D. Leopold, Parke-Davis Pharmaceutical Research, Ann Arbor, Michigan: Inhibitors of Ras-related signal transduction and challenges to evaluation of their therapeutic potential.

SESSION 4: NF1 Function and Ras Signaling: (B) Other Aspects
Chairperson: J.B. Gibbs, Merck Research Laboratories, West Point, Pennsylvania

F. McCormick, Onyx Pharmaceuticals, Richmond, California: A novel therapeutic approach for p53-defective cancers.
J. DeClue, National Cancer Institute, Bethesda, Maryland: Roles of neurofibromin and tuberin as tumor suppressor gene products and regulatory GAP proteins.
N. Wright, Cold Spring Harbor Laboratory: The Ras pathway in synaptic transmission.

SESSION 5: Animal Models for NF1
Chairperson: A. Rubenstein, Mt. Sinai School of Medicine, New York

A. Silva, Cold Spring Harbor Laboratory: Genetic, electrophysiological, and behavioral studies of NF1 mutant mice.
N.G. Copeland, National Cancer Institute-Frederick Cancer Research Center, Maryland: A mouse model for NF1-associated juvenile chronic myelogenous leukemia.
A. McClatchey, Massachusetts Institute of Technology, Cambridge: Toward a mouse model for NF1.
M. Henkemeyer, Mt. Sinai Hospital, Toronto, Ontario, Canada: Synergy of Gap and NF1 mutations in embryonic development and tumor formation.

F. McCormick, L.F. Parada, B.R. Seizinger

SESSION 6: NF2
Chairperson: B.R. Seizinger, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, New Jersey

N. Kley, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, New Jersey: Molecular genetic analysis of the NF gene.
V. Ramesh, Massachusetts General Hospital, Charlestown: Expression and cellular localization of the NF2 protein Merlin.
A. McClatchey, Massachusetts Institute of Technology, Cambridge: Consequences of a targeted mutation at the NF2 locus.

SESSION 7: Funding
Chairperson: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

B. Stillman, Cold Spring Harbor Laboratory
F. McCormick, Onyx Pharmaceuticals, Richmond, California

Advances in Imaging and Their Applications

October 22-October 24

FUNDED BY

Finisterre Fund

ARRANGED BY

P.P. Antich, University of Texas Southwestern Medical Center at Dallas
R.W. Parkey, University of Texas Southwestern Medical Center at Dallas
J.E. Smith, Kingston, New Hampshire

Keynote address: F.J. Bonte, University of Texas Southwestern Medical Center at Dallas

SESSION 1: Issues in Clinical Functional Imaging
Chairperson: R.W. Parkey, University of Texas Southwestern Medical Center at Dallas

C.L. Partain, Vanderbilt University Medical Center, Nashville, Tennessee: Functional imaging.
D.M. Wieland, University of Michigan Medical Center, Ann Arbor: Clinical applications of neuronal mapping with PET.
A. Alavi, University of Pennsylvania Hospital, Philadelphia: Role of modern imaging techniques in the management of patients with brain tumors.
H.W. Strauss, Stanford University, California: Cardiac infection imaging.
R.M. Peshock, University of Texas Southwestern Medical Center, Dallas: Integrated assessment of cardiac function with MRI.
R. Foster, University of Alabama at Birmingham: Applications of magnetic resonance to the cardiovascular system.
General Discussion: Impact of Functional Imaging on Future Patient Care
Moderator: F.J. Bonte, University of Texas Southwestern Medical Center at Dallas

SESSION 2: Issues in Functional Imaging: Modalities, Quantitation, Therapy
Chairperson: P.P. Antich, University of Texas Southwestern Medical Center at Dallas

J.G. McAfee, National Institutes of Health, Bethesda, Maryland: Peptides, growth factors, and cytokines in nuclear medicine.
B.C. Lentle, Vancouver Hospital Health Sciences Center, Canada: 511KeV SPECT with F-18 FDG.
E. Hahn, German Cancer Research Center, Heidelberg, Germany: Functional imaging in oncology.
N.A. Lassen, Bispebjerg Hospital, Copenhagen NV, Denmark: Co-registration of SPECT and MRI.
J. Fowler, Brookhaven National Laboratory, Upton, New York: PET and neuropharmacology.
H.F. Kung, University of Pennsylvania, Philadelphia: CNS receptor imaging with SPECT.
Panel: Functional Imaging in Basic Science Studies
Moderator: W.C. Eckelman, National Institutes of Health, Bethesda, Maryland

J.A. Barrett, DuPont Merck Radiopharmaceuticals, North Billerica, Massachusetts
P.P. Antich, University of Texas Southwestern Medical Center at Dallas
E.M. Stokely, University of Alabama at Birmingham
H.D. Burns, Merck Research Laboratories, West Point, Pennsylvania

L. Partain, H.F. Kung, F.J. Bonte, J.G. McAfee

SESSION 3: Issues in Functional Imaging: Contrast Agents and Radiopharmaceuticals
Chairperson: J.E. Smith, Kingston, New Hampshire

T.J. Brady, Massachusetts General Hospital-NMR Center, Charlestown: Target-specific MR contrast agents.
W.C. Eckelman, National Institutes of Health, Bethesda, Maryland: The uses of 18F and 99mTc radiopharmaceuticals as biochemical probes.
K. Linder, Bracco Diagnostics USA, Princeton, New Jersey: Imaging hypoxia with technetium-nitroimidazoles.
A. Davison, Massachusetts Institute of Technology, Cambridge: Technetium-based radiopharmaceuticals: Is it reasonable to expect further advances in the chemical design of imaging agents?
M.J. Welch, Mallinckrodt Institute of Radiology, St. Louis, Missouri: Receptors in oncology: Receptor ligands for therapy.

Panel: Biology, Physics, Chemistry: Inroads and Barriers
Moderator: J.G. McAfee, National Institutes of Health, Bethesda, Maryland
R.M. Peshock, University of Texas Southwestern Medical Center at Dallas
J.A. Barrett, DuPont Merck Radiopharmaceuticals, North Belterica, Massachusetts
H.D. Burns, Merck Research Laboratories, West Point, Pennsylvania

J.P. Morgan & Co. Incorporated/Cold Spring Harbor Laboratory Executive Conference on Infectious Diseases: Ancient Plagues, New Epidemics

October 28-October 30

ARRANGED BY

J.D. Watson, Cold Spring Harbor Laboratory
J.A. Witkowski, Cold Spring Harbor Laboratory

SESSION 1

R. Horton, *The Lancet*, London, United Kingdom: Plagues and epidemics in human society.

SESSION 2

R. Berkelman, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia: Ebola, Marburg, and other newly emerging viruses.
M.J. Blaser, Vanderbilt University School of Medicine, Nashville, Tennessee: Thinking about ulcers as an infectious disease.

H. Wendt, J. Karabellas, J.D. Watson

D. Ganem, HHMI, University of California, San Francisco: Herpesviruses old and new: Challenges and opportunities.
B. Bloom, Howard Hughes Medical Institute, Albert Einstein College of Medicine, Bronx, New York: The dangers of antibiotic-resistant bacteria.

SESSION 3

D. Micklos and M. Bloom, DNA Learning Center, Cold Spring Harbor Laboratory: Laboratory experiment: Making antibiotic-resistant bacteria.

SESSION 4

D.T. Jamison, University of California, Los Angeles: The economic impact of infectious diseases.
H.O. Smith, Johns Hopkins University School of Medicine, Baltimore, Maryland: Sequencing bacteria and viruses: A new approach to understanding infectious agents.
D.C. Wiley, Howard Hughes Medical Institute, Harvard University, Cambridge, Massachusetts: Atomic models: Providing clues about infectious and human immunity, and suggesting therapies.

Molecular Biology of Prions and Pathology of Prion Diseases

November 5-November 8

FUNDED BY

Cold Spring Harbor Laboratory Corporate Sponsor Program

ARRANGED BY

P. Gambetti, Case Western Reserve University, Cleveland, Ohio
S.B. Prusiner, University of California, San Francisco
R.B. Wickner, National Institutes of Health, Bethesda, Maryland

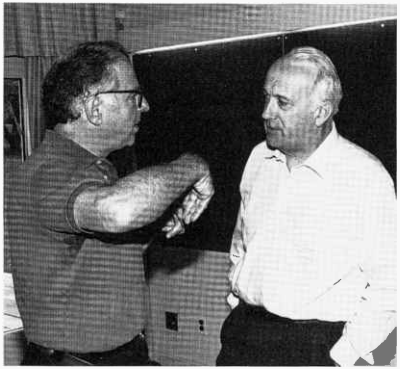
Opening remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory, and **S.B. Prusiner,** University of California, San Francisco, School of Medicine

SESSION 1: Human Genetics and Neuropathology
Chairperson: S.J. De Armond, University of California, San Francisco

B. Ghetti, Indiana University Medical Center, Indianapolis: Neuropathology of the PrP amyloidoses.
P. Gambetti, Case Western Reserve University, Cleveland, Ohio: Molecular pathology of FF1 and other prion diseases.
R. Gabizon, Hadassah University Hospital, Jerusalem, Israel: Biochemistry of the inherited prion disease E200K.
J.-L. Laplanche, Hospital St. Louis, Paris, France: Molecular basis of sheep susceptibility to natural scrapie in France.
G. Wells, Central Veterinary Laboratory, Surrey, United Kingdom: Neuropathology of BSE and oral transmission.
D. Dormont, Service De Neurovirologie, Fontenay-aux-Roses Cedex, France: Polyene antibiotics in prion diseases.

SESSION 2: Prion Protein Structures
Chairperson: C. Weissmann, University of Zurich, Switzerland

M.A. Baldwin, University of California, San Francisco: Covalent structure of PrP isoforms, spectroscopy of PrP peptides, and molecular models.
J. Safar, National Institutes of Health, Bethesda, Maryland: Conformational mechanisms in PrP^{Sc} formation and infectivity.
B. Caughey, National Institutes of Health Rocky Mountain Labs, Hamilton, Montana: In vitro protease-resistant PrP formation.
D. Riesner, Heinrich-Heine University of Dusseldorf, Germany: Conformation and solubility of PrP.
F. Tagliavini, Instituto Nazionale Neurologico "Carlo Besta," Milan, Italy: Aggregation and biological properties of PrP peptides.
T.L. James, University of California, San Francisco, School of Medicine: Conformational transitions of PrP and peptides studied by NMR.
P.T. Lansbury, Massachusetts Institute of Technology, Cambridge: Molecular mechanisms of amyloid formation.



C. Weissmann, B. Ghetti

P.T. Lansbury, D.A. Harris

SESSION 3: Prion Protein Structures
Chairperson: R.F. Marsh, University of Wisconsin, Madison

D. Wemmer, University of California, Berkeley: PrP peptide structure determination with solid-state NMR.

K. Kaneko, University of California, San Francisco: Conversion of PrP^{C} into a PrP^{Sc}-like molecule.

SESSION 4: Transgenic and Gene-targeted Mice
Chairperson: R.F. Marsh, University of Wisconsin, Madison

C. Weissmann, University of Zurich, Switzerland: Role of PrP in experimental scrapie.

J. Manson, Institute for Animal Health, Edinburgh, United Kingdom: PrP in gene-targeted mice.

G. Telling, University of California, San Francisco: Prion propagation and protein X in transgenic mice.

A. Aguzzi, Institute of Neuropathology, Zurich, Switzerland: PrP^{Sc} in brain grafts.

SESSION 5: Yeast Prions
Chairperson: R.B. Wickner, National Institutes of Health, Bethesda, Maryland

F. Lacroute, CNRS, Gif-sur-Yvette, France: Genetic background on URE3 appearance frequency.

R.B. Wickner, National Institutes of Health, Bethesda, Maryland: URE3 and PS1 as prions of yeast: Genetic evidence.

D.C. Masison, National Institutes of Health, Bethesda, Maryland: Prion-inducing domain of yeast Ure2p and protease resistance of Ure2p in prion-containing cells.

S. Liebman, University of Illinois, Chicago: Variability of yeast prion-like elements affecting translational fidelity.

Y.O. Chernoff, Georgia Institute of Technology, Atlanta: Propagation of PS1 and chaperones.

S.L. Lindquist, HHMI, University of Chicago, Illinois: Hsp104 and maintenance of yeast prions.

M.F. Tuite, University of Kent, United Kingdom: The genetics of PS1: A non-Mendelian phenomenon explained?

SESSION 6: Cell Biology of Prion Protein Isoforms and Molecular Chaperones
Chairperson: P. Gambetti, Case Western Reserve University, Cleveland, Ohio

R.B. Petersen, Case Western Reserve University, Cleveland, Ohio: Effects of PrP mutations on PrP metabolism.

W.J. Welch, University of California, San Francisco: Molecular chaperones and prion propagation.

A. Taraboulos, The Hebrew University of Jerusalem, Hadassah Medical School, Israel: Cholesterol in the cellular metabolism of the PrP isoforms.

D.A. Harris, Washington University Medical Center, St. Louis, Missouri: Cell biology of prion diseases.

SESSION 7: Strains of Prions and Molecular Pathogenesis
Chairperson: P. Gambetti, Case Western Reserve University, Cleveland, Ohio

R.F. Marsh, University of Wisconsin, Madison: Strain-specific neuropathology of TME.
G.A. Carlson, McLaughlin Research Institute, Great Falls, Montana: Host genetics and prion diversity.
S.J. De Armond, University of California, San Francisco: Transgenic models of prion disease pathogenesis.
S.B. Prusiner, University of California, San Francisco, School of Medicine: Closing comments.

Vaccine Development and Delivery in the Era of Managed Care

November 12-November 15

FUNDED BY

The Albert B. Sabin Vaccine Foundation

ARRANGED BY

H. Bailit, Aetna Health Plans, Hartford, Connecticut
E.K. Marcuse, Children's Hospital and Medical Center, Seattle, Washington
M.T. Osterholm, Minnesota Department of Health, Minneapolis
P.K. Russell, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland
T. Vernon, Merck & Company, Inc., West Point, Pennsylvania

Opening remarks: P.K. Russell, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland and **H.R. Shepherd,** Albert B. Sabin Vaccine Foundation, New Canaan, Connecticut

SESSION 1: Vaccine Delivery in Pediatric and Family Practice
Chairperson: E.K. Marcuse, Children's Hospital and Medical Center, Seattle, Washington

B. Guyer, School of Hygiene & Public Health, The Johns Hopkins University, Baltimore, Maryland: Interaction of parental and provider factors in explaining vaccination coverage: Implications for policy.
B. Harvey, Palo Alto, California: Barriers to vaccine delivery in the private sector.
J.R. Almquist, Virginia Mason Pediatrics, Federal Way, Washington: Problems faced by practitioners.
S. Berman, University of Colorado, Children's Hospital, Denver: The doctors' dilemmas: Immunization barriers from a primary care provider's perspective.
S.L. Katz, Duke University Medical Center, Durham, North Carolina: Flexibility in new vaccine schedules.

SESSION 2: Vaccine Financing and Delivery by the Insurance and Managed Care Industry
Chairperson: H.L. Bailit, Aetna Health Plans, Hartford, Connecticut

C.M. Grant, Connaught Laboratories, Inc., Swiftwater, Pennsylvania: Partnerships in adult and pediatric immunization services, consumer expectations. CLI can emphasize "adult" vaccines if you have coverage for pediatrics.
A. McCollam, The Prudential Insurance Co. of America, Roseland, New Jersey: A managed care perspective on vaccine coverage and reimbursement.
R.A. Hansen, Aetna Health Plans, Hartford, Connecticut: How we factor the cost of immunization services into the price for managed care benefit plans at Aetna Health Plans.
D. Siegel, Health Alliance Plan of Michigan, Detroit: Managed care perspectives on enhancing appropriate immunization administration through managed care vehicles.

SESSION 3: Successes and Problems in National and State Vaccination Programs
Chairperson: W.A. Orenstein, Centers for Disease Control & Prevention, Atlanta, Georgia

R.H. Bernier, Centers for Disease Control, Atlanta, Georgia: Current status of the Childhood Immunization Initiative.
D.K. Alfano, Kansas Department of Health & Environment, Topeka: A states perspective.
M. Sheehan, Minnesota Department of Health, Minneapolis: Providing chilhood immunizations in a managed care environment: The Minnesota experience.

D. Siegel, D.A. Henderson

SESSION 4: Government/Industry Relationships
Chairperson: M.T. Osterholm, Minnesota Department of Health, Minneapolis

J.J. Totten, Mercer Management Consulting, Inc., Washington, D.C.: Government/industry relationships and the impact on U.S. vaccine industry economics.
L.B. Hackett, Mercer Management Consulting, Inc., Washington, D.C.: Children's immunization initiative.
S.K. Sharma, United States General Accounting Office, Washington, D.C.: Barriers and obstacles to vaccination.

SESSION 5: Introductions of New Vaccines
Chairperson: R.G. Douglas, Merck & Co., Inc., Whitehouse Station, New Jersey

L.K. Gordon, OraVax Inc., Cambridge, Massachusetts: A perspective from industry. Oral vaccines and mucosal immunity products in development for respiratory syncytial virus, *Helicobacter pylori,* and *Clostridium difficile.*

Colloquium Summary:
D.A. Henderson, Baltimore, Maryland
P. Freeman, University of Massachusetts, Boston

Human Molecular Genetics: A Hands-on Workshop

November 16-November 19

FUNDED BY

Ethical, Legal, and Social Issues Program and Department of Energy's Human Genome Project

ARRANGED BY

M. Bloom, DNA Learning Center, Cold Spring Harbor Laboratory
D. Micklos, DNA Learning Center, Cold Spring Harbor Laboratory
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1

D. Micklos, DNA Learning Center, Cold Spring Harbor Laboratory: Mendelian view of the gene: From peas to eugenics.
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory: Modern view of the gene.
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory: PCR, RFLPs, and (CA)n: What they are; what they do.

SESSION 2

B. Ward, Integrated Genetics, Inc., Waltham, Massachusetts: Cytogenetics in the age of DNA.
D. Micklos, DNA Learning Center, Cold Spring Harbor Laboratory: Laboratory I: Using restriction enzymes to construct chromosome maps.
D. Micklos, DNA Learning Center, Cold Spring Harbor Laboratory: Laboratory II: Construction of chromosome map.

SESSION 3

M. Bloom, DNA Learning Center, Cold Spring Harbor Laboratory: Cloning human disease genes.
P. Ward, Institute of Molecular Genetics, Baylor College of Medicine, Houston, Texas: DNA-based diagnosis for human genetic diseases.
J. Friedman, HHMI, The Rockefeller University, New York, New York: The genetics of obesity.
W.T. Brown, New York State Institute for Basic Research, Staten Island, New York: Molecular Genetics and biology of the fragile·X syndrome.

SESSION 4

M. Bloom and D. Micklos, DNA Learning Center, Cold Spring Harbor Laboratory: Laboratory: Fingerprinting your own DNA by polymerase chain reaction.

SESSION 5

M. Bloom and D. Micklos, DNA Learning Center, Cold Spring Harbor Laboratory: Laboratory results: Analyzing fingerprinting results.
T. Tully, Cold Spring Harbor Laboratory: Genetics and behavior.
M.G. McInnis, The Johns Hopkins University, Baltimore, Maryland: Genetics of psychiatric disorders.
K. Culver, OncorPharm, Inc., Gaithersburg, Maryland: Human gene therapy trials.
P. Reilly, Shriver Center for Mental Retardation, Waltham, Massachusetts: Future of genetic testing and screening.

Looking to the Next Generation of Genetic Analysis

November 28-December 1

FUNDED BY

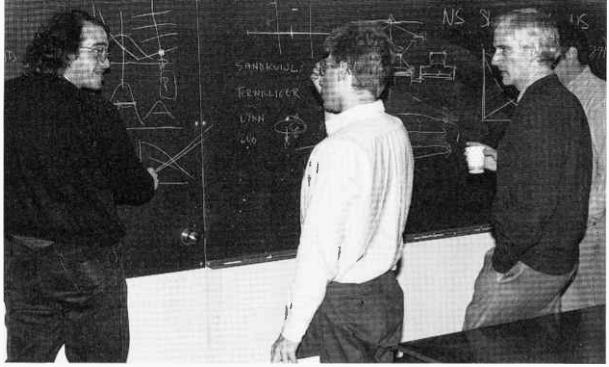
The Charles A. Dana Foundation

ARRANGED BY

A. Chakravarti, Case Western Reserve University, Cleveland, Ohio
E.S. Lander, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts

SESSION 1: 'Real World' Experience
Chairperson: K. Morgan, Montreal General Hospital, Canada

N.B. Freimer, University of California, San Francisco: IBD mapping of loci for human behavioral traits.
J.R. DePaulo and M.G. McInnis, The Johns Hopkins Hospital, Baltimore, Maryland: Studies of manic-depressive illness.
L. Peltonen, National Public Health Institute, Helsinki, Finland: Lessons from disease gene search in genetic isolates.
M.S. Georges, University of Liege, Belgium: The application of IBD mapping to locate disease genes in livestock: The example of syndactyly.



J.D. Terwilliger, L. Kruglyak, L.A. Sandkuijl

SESSION 2: Genomic Technologies
Chairperson: A. Chakravarti, Case Western Reserve University, Cleveland, Ohio

T.J. Hudson, Whitehead Institute, Cambridge, Massachusetts: Integrated genetic, physical, and transcript map development of 2-allele polymorphisms.

M.T. Boyce-Jacino, Molecular Tool Inc., Baltimore, Maryland: Identification and genotyping of diallelic polymorphisms.

D.G. Wang, Whitehead Institute, Cambridge, Massachusetts and Robert Lipshutz, Affymetrix, Santa Clara, California: Automated comparative sequencing of PCR products for large-scale screening of biallelic markers and genotyping them on DNA chips.

M. Zabeau, Keygene N.V., Wageningen, The Netherlands: AFLP: A robust high throughput diallelic marker system for the next generation of genetic analysis.

SESSION 3: IBD Mapping in Families
Chairperson: M.S. Georges, University of Liege, Belgium

L. Kruglyak, Whitehead Institute, Cambridge, Massachusetts: A new multipoint method for nonparametric linkage analysis of pedigree data based on identity by descent.

D.W. Fulker, Institute for Behavioral Genetics, University of Colorado, Boulder: Simple strategies for increasing power in sib-pair QTL studies.

J.M. Olson, Case Western Reserve University, Cleveland, Ohio: Two-locus linkage models for complex diseases.

N. Schork, Case Western Reserve University, Cleveland, Ohio: IBD sibship mapping with multiple phenotypes: Design and power issues.

A. Chakravarti, M. Zabeau

SESSION 4: Association Mapping
Chairperson: N. Schork, Case Western Reserve University, Cleveland, Ohio

L.A. Sandkuijl, Delft, The Netherlands: A search for shared DNA segments is most efficient in patients from recent founder populations.
J.D. Terwilliger, University of Oxford, United Kingdom: Behavior of true and false positive sib-pair statistics in genome screens.
A. Lynn, Case Western Reserve University, Cleveland, Ohio: Linkage disequilibrium mapping under heterogeneity.
S.-W. Guo, University of Michigan, School of Public Health, Ann Arbor: Linkage disequilibrium mapping: Problems and prospects.

SESSION 5: Population Genetics
Chairperson: E.S Lander, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts

E. Puffenberger, Case Western Reserve University, Cleveland, Ohio: Genetic variation in the Old Order Mennonites of Lancaster County, Pennsylvania.
K. Morgan, Montreal General Hospital, Canada: Genetic differentiation of the French Canadian population of Quebec.
R.R. Hudson, University of California at Irvine: Effects of background deleterious mutations on linked neutral variation.
K. Weiss, Pennsylvania State University, University Park: Population history and human variation.

Finding Genes: Experimental and Computational Methods

December 3-December 6

FUNDED BY

Cold Spring Harbor Laboratory Corporate Sponsor Program

ARRANGED BY

T.G. Marr, Cold Spring Harbor Laboratory, New York
D.L. Nelson, Baylor College of Medicine, Houston, Texas

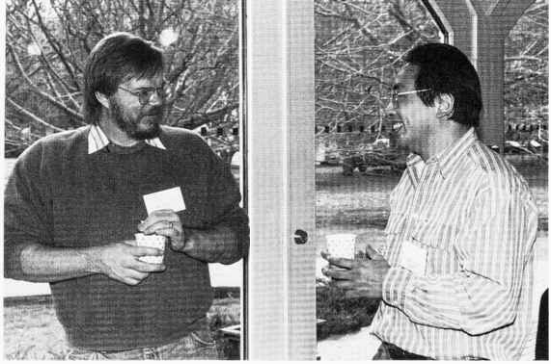
Introduction and perspective: D.L. Nelson, Baylor College of Medicine, Houston, Texas, and **T.G. Marr,** Cold Spring Harbor Laboratory

SESSION 1: Experimental Methods and Applications I: cDNA Selection
Chairperson: A.P. Monaco, Wellcome Trust Centre for Human Genetics, Oxford, United Kingdom

S. Weissman, Yale University School of Medicine, New Haven, Connecticut: cDNA detection and display.
J. Rommens, The Hospital for Sick Children, Toronto, Canada: Transcription maps.
R. Anand, Zeneca Pharmaceuticals, Cheshire, United Kingdom: Evaluation and optimization of preparative in situ hybridization: A tool for gene hunting in the 5–10-Mb region.

SESSION 2: Experimental Methods and Applications II: Trapping Plus
Chairperson: L.-C. Tsui, The Hospital for Sick Children, Toronto, Canada

A. Buckler, Massachusetts General Hospital, Charlestown: Exon amplification.
A.P. Monaco, Wellcome Trust Centre for Human Genetics, Oxford, United Kingdom: Hybridization of PAC inserts to cDNA libraries and exon trapping of PACs.
G. Lennon, Lawrence Livermore National Laboratory, California: Comparing exon trapping and hybrid selection of cDNAs with ESTs: Efficiency, comprehensiveness, or ease?
J.W. Foster, University of Cambridge, United Kingdom: Radiation hybrid mapping.
C.C. Lee, Baylor College of Medicine, Houston, Texas: Reciprocal probing for cDNAs and cosmids.



B. Koop, L.-C. Tsui

SESSION 3: Experimental Methods and Applications III: Use and Mutation
Chairperson: D.L. Nelson, Baylor College of Medicine, Houston, Texas

G. Borsani, Telethon Institute for Genetics and Medicine (TIGEM), Milan, Italy: Toward a transcription map of the distal short arm of the human X chromosome.
N.C. Dracopoli, Sequana Therapeutics, Inc., La Jolla, California: Finding genes for common disorders.
J. Liu, Merck Research Labs, West Point, Pennsylvania: High-throughput mutation detection.

SESSION 4: Computational Applications I: Sequence Production/Analysis
Chairperson: A. Buckler, Massachusetts General Hospital, Charlestown, Massachusetts

L.-C. Tsui, The Hospital for Sick Children, Toronto, Canada: Our experience of gene finding on chromosome 7.
D.L. Nelson, Baylor College of Medicine, Houston, Texas: Sequence-based gene finding.
B.F. Koop, University of Victoria, Canada: Lessons from comparative sequencing.
E.C. Uberbacher, Oak Ridge National Laboratory, Tennessee: Discovering and characterizing genes using sequence analysis.
R.F. Smith, Baylor College of Medicine, Houston, Texas: Sequence analysis for function.
M.Q. Zhang, Cold Spring Harbor Laboratory: What makes an exon an exon?

SESSION 5: Computational Applications II: Sequence Analysis
Chairperson: R.F. Smith, Baylor College of Medicine, Houston, Texas

W.R. McCombie, Cold Spring Harbor Laboratory: Strategies for sequence-based gene discovery.
J.-M. Claverie, CNRS, IBSM, Marseille, France: Progress in large-scale sequence analysis.
T.G. Marr, Cold Spring Harbor Laboratory: Summary and discussion of computational applications: How good are they? How can they be improved?

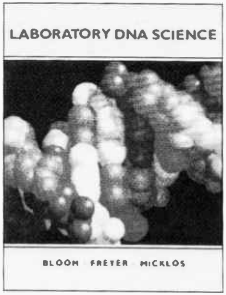

THE WALK OF LIFE
DNA LEARNING CENTER

DNA LEARNING CENTER

David A. Micklos, Director
Mark V. Bloom, Assistant Director
Susan M. Lauter, Creative Director
Judy Cumella Korabik, 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

Einstein's comment to the effect "God is subtle but not malicious," applies equally well to molecular genetics. On the face of it, this field must seem almost maliciously abstract to even bright and motivated students. Doing experiments is probably the only way to reduce this abstraction and give students a feeling for the subtle beauty of molecular mechanics. For better or worse, bands on gels, colonies on plates, and dots on filter paper are the major methods of inference in molecular genetics—the ways of knowing molecules. Only when these methods can be used to obtain predictable results, can students begin to believe that experiments, indeed, offer a window on the unseen molecular world. Teachers sometimes try to put the best spin on a failed experiment by saying that students can learn as much from failure as success. In fact, in students' eyes, a failed experiment can mean a failure of that mode of inference. A failed experiment throws students back into the abstractive quandary in which they began. We believe that experiments should work for students. Therefore, we have made it our *raison d'etre* to adapt and refine molecular genetics laboratories to increase the probability of success in student hands.

The publication in November of *Laboratory DNA Science: An Introduction to Recombinant DNA Techniques and Methods of Genome Analysis* marked another high point in our effort to bring reproducible and high-level molecular genetics laboratories into the biology classroom. The text incorporates the basic cloning sequence (10 laboratories) from our original DNA Science course, plus 13 new laboratories that cover gene library construction, hybridization, and polymerase chain reaction (PCR). The author team, Mark Bloom, Dave Micklos, and Greg Freyer (of Columbia University), believe these to be the most thoroughly tested labs available today for teaching molecular genetics at the college level. Each experiment incorporates insights from our own instruction of 2000 teaching faculty at training workshops across the country and from the 30,000 students taught in our *Bio2000* Biochemistry Laboratory. The entire lab sequence is supported by quality-assured reagents and kits available from the Carolina Biological Supply Company.



Nothing has been left to chance. Greg even engineered the teaching plasmids, pAMP, pKAN, and pBLU, specifically for the course. These plasmids transform well, give consistently high yields in plasmid preparations, simplify colorimetric screening for recombinant plasmids, and yield restriction products that are easily interpreted on agarose minigels. The laboratories also incorporate insights drawn from extensive contacts within the biological research community. Prescott Deininger and Mark Batzer, of the LSU Medical Center, introduced us to the *Alu* insertion polymorphisms—which proved to be ideal for educational demonstrations of human DNA typing. Collaborations with scientists at Cetus, Perkin-Elmer, and Roche Molecular Systems were key to the development of the

Dave Micklos, Mark Bloom, and Greg Freyer on the release of *Laboratory DNA Science*.

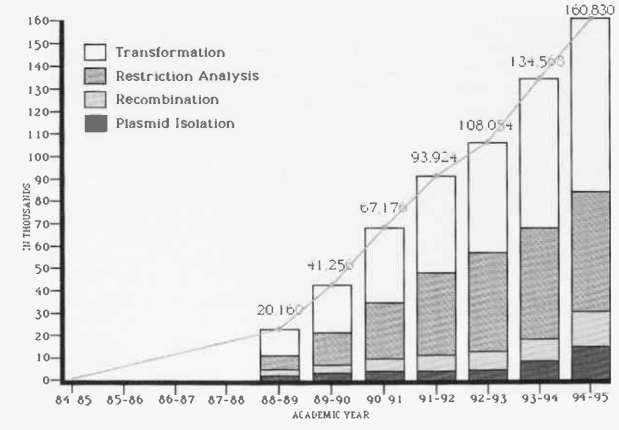
PCR experiments, while scientists at Boehringer Mannheim aided with colorimetric detection used in hybridization experiments.

We are all thankful to our editor, Cathy Pusateri, for keeping her promise to produce *Laboratory DNA Science* in only 10 months! The 434-page book features extensive prelab notes, flowchart diagrams, marginal notes, results and discussion, and for further research sections. Appendices include lists of needed equipment, supplies, and reagents; recipes for media, stock solutions, and reagents; restriction maps for plasmids; and complete sequence data for all DNAs used in the course. A 99-page *Instructor's Preparation Guide* includes hints on lab prep and answers to results and discussion questions. *Laboratory* DNA Science has gotten off to a good start, selling 1000 copies in its first month.

Tracing the Rise of Precollege Laboratory Instruction in Molecular Genetics

We hope that *Laboratory DNA Science* will encourage college instructors to increasingly bring molecular genetics labs into freshman biology and sophomore electives. This echoes the DNALC's original effort to bring student laboratories on molecular genetics into American high schools. In the summer of 1985, we were among the very first academic groups to initiate teacher training in recombinant DNA techniques. It is fair to say that prior to 1985, there was essentially no precollege lab instruction in molecular genetics. Thus, taking the 1984–1985 academic year as the zero point—and using sales data for needed reagents and teaching kits as an indicator of student exposures—one can trace the rise of an entirely new era in science education. The notion that recombinant DNA techniques are an important basis for student understanding was legitimized in 1989, when the Educational Testing Service recommended two DNA-manipulation labs for students who take its nationally administered Advanced Placement (AP) Biology curriculum. As shown in the figure, the AP "mandate" helped to catalyze the rapid adoption of several "core" techniques of recombinant DNA: bacterial trans-

Precollege Student Exposures to Four Molecular Genetics Laboratories
(extrapolated from sales of teaching kits and reagents by Carolina Biological Supply Company)



formation, DNA restriction analysis, DNA recombination, and plasmid isolation.

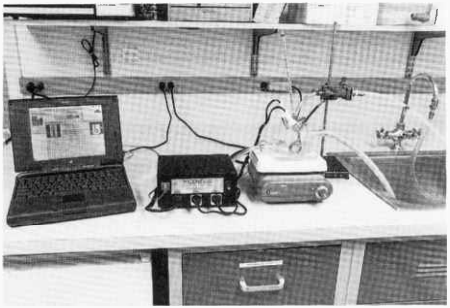
Our studies of teacher innovation suggest that the estimated 3500 teachers nationwide who have been trained using the *DNA Science* syllabus account for at least half of 160,000 student exposures to molecular genetics laboratories in the 1994–1995 academic year. In addition to experimentation in the context of AP courses, half- and full-year electives in molecular genetics/biotechnology are being instituted in growing numbers of American high schools. In some schools, it is filling the niche formerly occupied by anatomy and physiology or biology II; in other schools, it is filling the new "tech-prep" niche. For example, 14 of 23 participants in the 1995 *Leadership Institute* are currently teaching molecular genetics/biotechnology electives at their high schools.

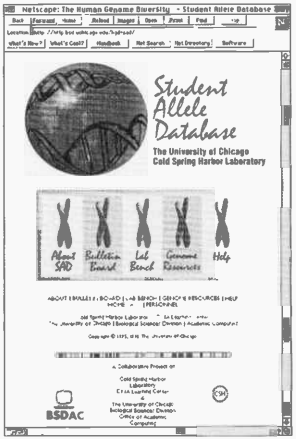
Further Progress in Making PCR Widely Available to Biology Students

With support from the Howard Hughes Medical Institute and the National Science Foundation (NSF), we continued our effort to make human DNA fingerprinting by PCR practical for widespread use in biology classes. In December, Mark Bloom and collaborator John Kruper taught the first of a series of NSF-supported workshops for college faculty, *Human Genome Diversity-Student Allele Database*. Held at the Air Force Academy in Colorado Springs, the workshop drew together 24 college educators for in-depth training to implement experiments on DNA fingerprinting in freshman and elective biology courses. The workshop revolves around experiments from *Laboratory DNA Science* that allow students to use PCR to analyze two types of human DNA polymorphisms—*Alu* insertions and variable number tandem repeats (VNTRs). *Alu* is an example of a "jumping gene" that has successfully reproduced and inserted about 500,000 copies of itself throughout the human chromosomes. Some *Alu*s inserted several million years ago, when humans were radiating out of Africa to populate the globe. These *Alu* insertions can be used as biological clocks to measure the genetic distance between populations and to study how humans have evolved. VNTRs are chromosome regions where a short DNA sequence is repeated numerous times. VNTRs are highly variable from person to person, making them especially useful in human identity. Several types of VNTR polymorphisms are used as evidence in rape, murder, and paternity cases.

Mark Bloom worked for several years to simplify the biochemical procedures for screening for *Alu* and VNTR polymorphisms. In collaboration with John Kruper's bioscience computing group, at the University of Chicago, we also have made significant progress in developing computer programs and instrumentation to make the technology more accessible to students. During the year, the Chicago group developed a prototype *Student Allele Database* to run on their World Wide Web server (http//:http.bsd.uchicago.edu/hgd-sad) and also reachable through a link on the DNALC server (http//:darwin.cshl.org). The program allows students to tabulate *Alu* insertion data, test Hardy-Weinberg equilibrium, and compare two populations by contingency Chi-square and genetic distance. The database is seeded with *Alu* data from a number of isolated populations from around the world, which have been collected by researchers Prescott Deininger and Mark Batzer, of LSU Medical School. Eventually, we hope the database will also contain data submitted by thousands of students from around the United States and Europe. In that way, students can participate in an evolving research project on human diversity.

By year's end, we also had a stable, working prototype of a programmable





The working model of the BioGENEartor, above, and the SAD web page at the University of Chicago, right.

thermal cycler to control the PCR. Commercial thermal cyclers cost several thousand dollars and have put automated cycling out of reach of most educators. Our *Biogenerator*, which will be available for about $700 in 1996, should remove a major obstacle to the use of PCR in education. Water temperature in a Plexiglas reaction vessel is raised by activating heater coils while temperature is lowered by activating a valve that controls cold water flow into the vessel. Heating and cooling are controlled by an analog-digital (A-D) interface that receives commands from a personal computer and temperature information from a thermocouple. In addition to its low cost, *Biogenerator* has several advantages over research-grade thermal cyclers that make it more suitable for the teaching laboratory:

- The simplicity of the apparatus helps students grasp the physical principles of thermal cycling. The open design allows students to observe the mechanisms of heating and cooling, as well as feedback between the computer and the thermocouple.
- Animated screen icons provide quick references to the cycling state (activation of heater coils or cold water valve) and PCR state (DNA denaturing, primer annealing, and primer extension).
- A computer "strip chart" plots temperature change in real time, allowing students to follow the course of the reaction, review the reaction profile, and calculate temperature ramping.

Programs for Long Island Students Continue to Expand

The DNALC had its roots in the Cold Spring Harbor Curriculum Study, a teacher-training program initiated in 1985 in cooperation with eight local school districts.

Visitors and Program Participants, 1988-1995

Visitors and Participants	1988	1989	1990	1991	1992	1993	1994	1995
Student Labs (on-site)	2,031	3,753	3,758	4,248	4,624	3,422	3,961	4,682
Student Labs (off-site)				291	435	1,305	1,434	2,328
Teacher Labs	58	278	270	234	270	254	302	379
Student Workshops	32	13	24	176	234	351	361	503
Teacher Workshops	496	285	314	333	441	249	177	101
Lab Subtotal	**2,617**	**4,329**	**4,366**	**5,282**	**6,004**	**5,581**	**6,235**	**7,993**
Student Lecture Series	553	449	660	600	1,000	734	575	520
Exhibit Visitors	3,231	2,547	2,964	1,480	848	6,416	9,943	13,064
Total Visitors/Participants	**6,401**	**7,325**	**7,990**	**7,362**	**7,852**	**12,731**	**16,753**	**21,577**

The *DNA Science* curriculum that arose from this collaboration achieved national prominence and was the basis for our nationwide teacher-training program. It also provided the template for the development of *Laboratory DNA Science* and its nationwide training program. Although our commitment to curriculum development and dissemination continues, we devote increasing energy to developing the DNALC facility as a resource for local students. In this sense, we are returning to our roots in collaboration with Long Island and New York City schools.

The Curriculum Study continues as our major mechanism to work intensively with local school systems. Over the years, it has grown from the original eight members to 28 districts and private schools in 1995. As shown in the table above, our visitation has nearly tripled since our opening in 1988. Cablevision's *Long Island Discovery* drew 10,366 visitors in 1995, and the *Story of a Gene* Exhibit drew 2698 visitors. Of special importance, lab participation has contributed equally to our growth as exhibit visitation. Although it is hard for a science center or museum to gauge the exposure received by an casual exhibit visitor, we know that each lab participant has had a 1–3-hour, hands-on experience conducted by a DNALC staff member, while workshop participants have had at least 20 hours of intensive instruction.

Over the past several years, we have devoted much effort to developing programs for middle-school students, including summer camps, academic-year lab field trips, and in-school instruction. Under the direction of our trio of middle school specialists, Jane Conigliaro, Diane Jedlicka, and Malissa Hewitt, these programs became the major source of growth in 1995. Eight school districts were members of the *Genetics as a Model for Whole Learning Program*—Half Hollow Hills, Lawrence, Great Neck, Jericho, Locust Valley, Roslyn, South Huntington, and Syosset—nearly doubling student participants to 2328. Although each program is customized according to district needs, the typical program includes in-school instruction and teacher mentoring by DNALC staff, as well as a field trip to the DNALC for intensive use of the *Story of a Gene* Exhibit and experimentation in the *Bio2000* Laboratory. Science supervisors in these districts believe that they are already beginning to see increased interest in science courses, as graduates of our middle school programs move on to high school. Fortunately, these high schools offer research and elective courses that will allow these gifted students to follow up on their interest in biology.

Dianne Jedlicka (standing) instructs students in the *BioMedia* Lab during a *Fun with DNA* Camp.

Student participation in lab field trips to the *Bio2000* Laboratory increased 18% to 4682 students, thanks to excellent instruction offered by part-time instructors Flavio Della Seta, Diane Esposito, Diane Jedlicka, and Malissa Hewitt. High school students (2894) performed labs on bacterial transformation, DNA restriction analysis, and human DNA fingerprinting, and middle school students (1788) performed experiments on Mendelian genetics, cell study, and DNA extraction. The *Great Moments in DNA Science* Honors Student Seminar Series, now in its 11th year, continued as a popular element of our annual calendar of events, drawing the attendance of 520 local students and teachers. Speakers and topics were:

Syd Mandelbaum, Imagen Instrumentation. Historical DNA Cases: The Solving of the Anastasia Mystery.

Dr. Michael Hengartner, CSHL. Apoptosis: Why and How Cells Commit Suicide.

David Micklos, DNALC. DNA and OJ.

Student participation in summer workshops increased dramatically from 361 in 1994 to 558 in 1995, due to collaborations that enabled us to hold additional workshops at four other sites in the New York metropolitan area. We continued our collaboration with Portledge School (Nassau County) to serve the great demand for middle school workshops on the North Shore of Long Island. Central Islip School District (Suffolk County), Roslyn Middle School (Nassau County), and the American Museum of Natural History (AMNH, New York City) were sites for middle school and high school workshops targeting primarily minority students. We were especially pleased with a new collaboration that was made possible through the support of AMNH President Ellen Futter and Chairman Anne Sidamon-Eristoff, and a 2-year grant from the Barker Welfare Foundation. Eleven sessions of *Fun With DNA* (grades 5–6) served a total of 315 participants, including 202 minority students. Three sessions of the workshop, *World of Enzymes* (grades 7–8), attracted 77 participants, including 26 minority students. Four sessions of *DNA Science* (grades 10–12) drew 131 participants, including 83 minority students. New for 1995 were two sessions of *Advanced DNA Science* (grade 12) held at Central Islip High School and the Beckman Neuroscience Center that served 35 participants, including 25 minority students.

Mark Bloom (center, top) discusses gel electrophoresis results with *Advanced DNA Science* Workshop participants.

DNA Boot Camp participants after a discussion led by Dr. Watson.

Training Workshops Reach Faculty and Opinion Leaders Nationwide

This year marked the ninth summer of NSF funding for our *DNA Science* Workshop. In keeping with our commitment to bring high-level training to teachers from rural and nonurban areas, workshops were held in Lawrence, Kansas, and Lewiston, Maine, drawing the participation of 55 high school faculty. An additional 23 faculty, representing 17 states and Puerto Rico, attended the month-long *Leadership Institute in Human and Molecular Genetics*, held at the DNALC and Cold Spring Harbor Laboratory. Also known as "DNA Boot Camp," this intensive experience includes practical laboratory and computer work, as well as an array of seminars presented by CSHL scientists and visiting faculty:

Karen Buchavich, CSHL: Telomeres, Cancer, and Aging.
Xiaodong Cheng, CSHL: X-ray Structures Solved at CSHL.
Rob DeSalle, American Museum of Natural History: Ancient DNA and Comparative Biology.
Mike DeStio, Half Hollow Hills School District: Sequenced Genetics Instruction—A District Perspective.
Anil Dhundale, Oncogene Science: Expression Screening for Drug Discovery.
Bruce Futcher, CSHL: Cell Cycle Control.
Fred Gillam, Sachem North High School: Molecular Genetics Electives at the High School Level.
Marvin Grubman, Plum Island Animal Disease Center: Molecular Approaches to a Foot and Mouth Vaccine.
Mike Hengartner, CSHL: Apoptosis and Cell Suicide.
Gerry Latter, CSHL: 2-Dimensional Electrophoresis.
Rob Martienssen, CSHL: Transposon Tagging in *Arabidopsis*.
Dick McCombie, CSHL: Sequencing Genomes.
Bruce Stillman, CSHL: Research Highlights at CSHL.
Tim Tully, CSHL: *CREB*, Memory and Intelligence.
James Watson, CSHL: Perspectives on the Gene Revolution.
Jan Witkowski, CSHL: Human Genetics.

With funding from the Department of Energy, we continued our collaboration with Banbury Center to increase knowledge of advances in human genetics among influential "opinion leaders" in various fields. Continuing a shift in emphasis begun last year, the 1995 workshop drew 17 medical education directors representing hospitals in 10 states, with the expectation that they can help hospi-

tals better appreciate how the molecular genetics perspective is changing modern medicine. In addition to hands-on experiments on gene mapping and DNA diagnosis, participants heard high-level seminars about topics on the interface of molecular genetics, medicine, and society:

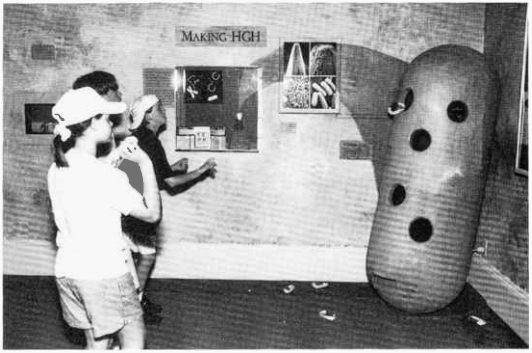
Ted Brown, NY State Institute for Basic Research: Molecular Genetics and Biology of Fragile-X Syndrome.
Jeffrey Friedman, The Rockefeller University: Genetics of Obesity.
Ken Culver, OncorPharm: Human Gene Therapy Trials.
Melvin McInnis, Johns Hopkins University: Genetics of Psychiatric Disorders.
Philip Reilly, Shriver Center for Mental Retardation: Future of Genetic Testing and Screening.
Tim Tully, CSHL: Genetics and Behavior.
Brian Ward, Integrated Genetics: Cytogenetics in the Age of DNA.
Patricia Ward, Baylor College of Medicine: DNA-based Diagnosis for Human Genetic Diseases.

We Open Our First In-house Exhibit on Human Growth Hormone

In 1987, when the decision was made to begin development of our facility, the Cold Spring Harbor Laboratory Trustees were adamant that we emphasize the active process of acquiring knowledge. Then and now, our primary mission is to develop and disseminate novel methods for students to actively experience the world of molecular genetics. Thus, museum exhibits have always been considered ancillary to the hands-on experience students receive in our *Bio2000* Biochemistry Laboratory and *BioMedia* Computer Laboratory.

For our opening in 1988, we were lucky enough to obtain loan of the Smithsonian Institution exhibit, *The Search For Life*, which allowed us effectively to dodge exhibit development during our first 4 years. Prior to dismantling the Smithsonian exhibit, in preparation for building renovations in 1993, we had made ambitious plans to develop our first in-house exhibit on the technology of gene hunting used in the Human Genome Project. However, by the time we were ready to begin exhibit development in 1994, we were forced to reconsider our

Dave Micklos and Malissa Hewitt place authentic Fore artifacts on sculpture of Fore tribesman. Educators preview *Story of a Gene* at the second of three exhibit openings in May.



Top right: middle-school students transform the giant *E. coli* in the *Plasmid Toss*; Top left: visitors take the *Walk of Life*, and Nicole Costa (bottom left), who receives HGH therapy, stands by her likeness.

plans. Two new programs in 1993—Cablevision's *Long Island Discovery* and *Genetics as a Model for Whole Learning*—had significantly changed our predominantly high school clientele to include large numbers of elementary students. It had become clear that our original exhibit concept was far too technical for younger students; we had to scrap our previous plan and find a friendlier approach to a difficult subject matter.

The solution we came upon was, simply, to tell the *Story of a Gene*, i.e., to organize the entire exhibit around a single gene. This case study approach allowed us to cut a cross-section through modern biological research, touching upon the hard science topics of molecular genetics, cell biology, and physiology, as well as medical, personal, and social issues. This approach compelled us to tell the story of human growth hormone (HGH), which is produced by the pituitary gland within the brain. This is the best single example of a gene affecting a visible human trait; HGH is largely responsible for a person's height. Problems with this gene most commonly cause short stature, which is not life-threatening and is treatable. The HGH gene was one of the first human genes isolated using modern gene-hunting technology, and HGH was among the first recombinant DNA therapeutics approved for use in humans. At the same time, there is continuing controversy over the use of recombinant HGH for nonclinical purposes and to increase milk production in dairy cows. The exhibit blends art and science and juxtaposes both micro- and macroscopic perspectives. A hand-painted mural covering the walls and ceiling of the main gallery creates the environment

within a single pituitary cell. The *Cellarium* mural is the backdrop for sculptured molecular models, video displays, full-size human sculptures, and interactive games.

At the microscale, visitors can follow the flow of genetic information from DNA to RNA to protein through which HGH is manufactured within a pituitary cell. In "The Walk of Life," visitors play the role of a cellular structure, the ribosome, with each step along a walkway activating computer-controlled lights to illustrate the translation of the genetic code into the HGH protein. On the macroscopic level, visitors are asked to consider their own stature by comparing themselves to life-size portraits of famous people, by placing themselves on a life-size growth chart, and by visualizing themselves taller/shorter using a video morph. The secretion of HGH by the pituitary and its stimulation of bone growth are traced by a digital circuit of light-emitting diodes within a see-through human model. Our long-time friends, Greg and Edna McLaughlin, of CBS News, produced an engaging, "MTV-like" video following a day in the life of Nicole Costa, a local elementary student being treated with HGH. A "ring-toss" game allows visitors to toss the HGH gene into a giant bacterium, illustrating the modern method for making HGH.

Leaving the main gallery, and its macro/micro worlds of the present, the visitor enters a small gallery with four scenes representing past and future issues of HGH. "Gruesome Treatment" is a horror-story parody about extracting HGH from cadaver brains, the medical source of HGH prior to recombinant methods. "The Kuru Connection" shows how a deadly brain disease, first discovered among cannibals in New Guinea, could contaminate HGH extract prepared from human brain tissue. "Who Should Take Growth Hormone" takes place in a family room, where a television shows news videos that explore the growing use of HGH to increase athletic ability and counteract the aging process. "Milk and More Milk" allows one to eavesdrop on four cows as they discuss the pros and cons of using HGH in the dairy industry.

The development timeline for *Story of a Gene* was condensed to only 8 months—from storyline brainstorming in September, 1994, to opening in May, 1995. At the same time, we are very proud that essentially all exhibit elements, including three-dimensional models, digital control circuitry, video morphs, and casework, were developed in-house by DNALC staff and the CSHL carpentry shop. Creative Director Sue Lauter worked tirelessly designing graphical and computer components, developing technical specifications, interfacing with subcontractors, and supervising staff and art interns. Her effort is all the more extraordinary in light of the fact that it occurred during her first year of motherhood. Our team of elementary educators, Jane Conigliaro, Diane Jedlicka, and Malissa Hewitt, carefully streamlined the narrative to improve readability for younger students. They also developed curriculum guides, including vocabulary, questions/answers, and suggested readings, to help students get the most out of their visit. The exhibit articulates seamlessly with our extensive program of hands-on laboratories and computer experiences for 5th to 12th grade students. Development of *Story of a Gene* was supported by major grants from the Weezie Foundation and Genentech, Inc.

We Renovate the Last Available Space in Our 1925 School Building

In the fall, we completed renovation of 500 square feet of space in the east basement to create a computer/design office, intern work area, and storage closets.

The computer/design office provides a focus for our growing effort to develop and distribute multimedia programming in biology. The office is occupied by two senior staff members (currently Sue Lauter and Dave Micklos) and has four workstations for Macintosh, IBM/PC, and Unix machines. The adjacent intern area has three additional computer workstations. In combination with the *BioMedia* Computer Laboratory and the *Multitorium*, the computer/design office gives us almost unmatched facilities for testing innovative, interactive methods for introducing students to modern biology. Our aim is to add additional staff in the areas of computation and video production that will increase our capability to integrate many types of audio and visual information into a modern learning system.

Through renovation, we also gained office and laboratory storage rooms. The lab storage space was critical, considering that we maintain the equivalent of four classrooms worth of laboratory equipment, as well as supplies for providing in-school instruction for approximately 5000 elementary students. The east basement renovation also provided the impetus to renovate the west office, which had grown very cramped and had suffered water damage on several occasions. New carpeting and a more open plan gave an entirely new look. By reducing the number of workstations, we also gained room for an attractive library/meeting area.

Before and after in the new east basement office.

Staff and Interns

The DNALC continues to operate very cost-effectively. According to survey statistics published by the Association of Science-Technology Centers, the current DNALC staff of 8.5 full-time equivalents (FTEs) is well below an average staff of 12–14 FTEs for science centers with similar budgets, square-footages, and visitation.

In July, Susan Lauter was promoted to Creative Director in recognition of her long-term service to CSHL, her leadership in all phases of project design, and her managerial role in the daily operation of the DNALC. While still a student at the Cooper Union, in 1985, Sue began work at CSHL as an intern in the Development and Public Affairs Department. She became designer at the DNALC in 1989. Over a 10-year period, Sue has proven herself a brilliant and adaptable "generalist" capable of doing whatever is required to get the job done. Beyond her obvious artistic talent, Sue is also a capable manager who knows virtually every aspect of day-to-day operation of the DNALC. Sue was assisted by Denise Sauer, an art student who graduated from SUNY Farmingdale in May with a Bachelor of Technology degree in Visual Arts. Denise is continuing her studies at the C.W. Post campus of Long Island University, pursuing a graduate degree in Art History.

Chris Como, a senior at Cold Spring Harbor High School and a veteran intern of the DNALC, continued to assist us as a computer intern. Michael Bellino, a student at the Rhode Island School of Design, interned at the DNALC during the summer in a computational position. Newcomers Trevor Carlson of Central Islip High School and Michael Romanelli of Cold Spring Harbor High School joined the DNALC staff late in 1995, filling the void left when Chris Como began his college studies at Cornell University and Michael Bellino resumed his degree program at the Rhode Island School of Design.

The laboratory instructional staff was ably assisted by high school interns Ken Bassett (Massapequa), Jermel Watkins (Central Islip), Salley Ann Gibney (Cold Spring Harbor), and Stacey Trotter (Walt Whitman). In the fall, we bid farewell to

Ken, who began his freshman year at Drew College, while Jermel began a pre-med course of study at the New York Institute of Technology, continuing to work at the DNALC on a part-time basis. Newcomers SooJin Kim (Syosset), Chun-Der Li (Half Hollow Hills), Rachael Neumann (Syosset), and Trevor Sammis (Huntington) joined the staff late in 1995. Assisting at *Fun With DNA* summer camps were lab aides Aaron Bronfman (Long Island School for the Gifted), Brian Herz (Cornell University), Todd Rebori (Walt Whitman High School), Matt Robbins (Jericho High School), Paul Tanck (Massapequa High School), Stacey Trotter, Kristy Wagner (Huntington High School), and Jermel Watkins.

Staff associates Twana Adams of Bronx Alternative School and Jerry Watkins of Central Islip High School assumed lead teaching responsibilities at seven minority workshops hosted at the American Museum of Natural History in Manhattan. A resident and community organizer in Harlem, Twana taught five *Fun With DNA* Camps, with support from Kofi Brown of Farmingdale College, Shukrani Tyehemba of Tuba City High School, and Sterling Brinson of Aviation High School. In addition to instructing the workshop, Twana and her assistants handled the complicated arrangements to chaperone and transport the young students by public transport from Harlem. Jerry Watkins, father of DNALC intern Jermel, is a graduate of our the *DNA Science* Workshop and the NSF *Leadership Institute*. In addition to instructing two *DNA Science* Workshops at the Museum of Natural History, Jerry also taught two student workshops in his home district, where he is currently offering an elective course in molecular genetics.

Corporate Advisory Board

Although the DNALC's national curriculum efforts and programs for minority students are funded by federal and private grants, activities for Long Island children receive no foundation support. Tuition pays for the direct cost of lab field trips and summer camps, but it is not enough to pay the true costs of maintaining a vital, innovative institution with an annual operating budget of approximately $1 million. The Corporate Advisory Board was formed in 1992 to help close this funding gap and provide a sustainable level of unrestricted annual giving. The Annual Fund is essentially "think money," that pays for staff time needed to do the advance work on new curricula and programs. This flexibility allows us to move aggressively into new areas—in advance of specific funding. Thus, we were overjoyed to learn at year's end that the Annual Fund had met its $125,000 goal for 1995! This is incredible, considering that as few as 5 years ago we received virtually no annual giving from Long Island companies.

The great cheer of meeting the 1995 goal was tempered by news that Corporate Advisory Board Chairman Rick Clark left New York at year's end to become the chief financial officer of Insession, a computer software company based in Boulder, Colorado. The loss was a double blow to Cold Spring Harbor Laboratory, because we also lost the extremely capable services of Rick's wife Jill, associate development director at the main campus. A partner at Price Waterhouse, Rick was brought onto the Advisory Board by former Chairman Doug Fox. As a new member to the Advisory Board in 1994, he organized the first annual golf tournament at Piping Rock Club. This single event, which grew dramatically in 1995, has been successful beyond our wildest expectations, making up about half the Annual Fund. We will miss Rick and Jill tremendously.

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; Arthur 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. A complete listing of Corporate Advisory Board members and details on the Annual Fund are located in the "Financial Support" Section.

Publications

Bloom, M., G. Freyer, and D. Micklos. 1995. *Laboratory DNA Science: An Introduction to Recombinant DNA Technology and Methods of Genome Analysis.* Benjamin/Cummings, Redwood, California.

1995 Workshops, Meetings, and Collaborations

January 10	Advisory Committee Meeting, National Marfan Association, Port Washington, New York
January 13–14	National Science Foundation Follow-up Workshop, *DNA Science*, University of Idaho, Moscow
January 18	Corporate Advisory Board Meeting, DNALC
January 19–20	Laboratories for *Women in Science and Engineering* Program, SUNY Stony Brook, DNALC
January 20–21	National Science Foundation Follow-up Workshop, *DNA Science*, Colorado College, Colorado Springs
January 26	Intensive Enrichment Planning Meeting, DNALC
January 26–28	National Science Foundation Grant Review, Washington, D.C.
January 28	Laboratory for *1 in 9 Breast Cancer* Group, DNALC
February 2–4	National Science Foundation Grant Review, Washington, D.C.
February 13	Corporate Advisory Board Meeting, DNALC
February 22	Presentation to New Orleans Public Schools, John McDonogh High School, New Orleans, Louisiana
March 11–12	National Science Foundation Follow-up Workshop, *Advanced DNA Science*, Trinity University, San Antonio, Texas
March 17–18	National Science Foundation Grant Review, Washington D.C.
March 23–25	National Science Teachers Association Meeting, Philadelphia, Pennsylvania
March 25–26	National Science Foundation Follow-up Workshop, *Advanced DNA Science*, Boston University, Massachusetts
March 30	Presentation to SEED Program, SUNY Westbury, DNALC
April 8	Laboratory for Corporate Advisory Board, DNALC
April 12	*Great Moments In DNA Science* Honors Student Seminar, DNALC
April 19	Corporate Advisory Board Meeting, DNALC
April 20	*Introduction to Biotechnology* Workshop, for Business Professionals, DNALC
April 25	*Great Moments in DNA Science* Honors Students Seminar, DNALC
April 26	Site visit by Captain Craig Maki, United States Air Force Academy, Colorado Springs, Colorado
April 28	Student Laboratory at A. Philip Randolph High School, Harlem, New York
May 2	*Great Moments in DNA Science* Honors Students Seminar, DNALC
May 6	Seminar for Harvard-Radcliffe Club, DNALC
May 10	Site visit by Claire Pillsbury and Adam Aaronsen, Tech Museum of Innovation, California
May 11	*Long Island Business Association* Meeting, DNALC
May 17, 19, 21	*Story of a Gene* Exhibit Openings, DNALC

May 22	Television Program, *New York State Courts in the Community*, Bellport, New York
May 30	Site visit by Dr. Isadore Edelman, Columbia University, New York
May 31	Advisory Committee Meeting, American Association for the Advancement of Science, Washington, D.C.
June 8	Seminar and Tour, Institute of Social Sciences, DNALC
June 12–16	*DNA Science* Workshop, Fiskbackskill and Jonkoping, Sweden
June 14	Award Presentation at BioPharm Conference, Boston, Massachusetts
June 26–30	Access Excellence Summit, San Francisco, California
	DNA Science Workshop, DNALC
	Computational Biology Workshop, DNALC
	Howard Hughes/Barker Welfare Minority Workshop, *DNA Science*, American Museum of Natural History, New York
July 3–28	National Science Foundation *Leadership Institute*, DNALC
July 6	Site visit by Dr. Susanna Benner, Museum of Technology and Work, Mannheim, Germany
July 10–14	*Fun With DNA* Workshop, Portledge School, Locust Valley, New York
July 17–21	*Fun With DNA* Workshop, Portledge School, Locust Valley, New York
	Howard Hughes/Barker Welfare Minority Workshop, *Fun With DNA*, American Museum of Natural History, New York
July 24–28	*Fun With DNA* Workshop, Portledge School, Locust Valley, New York
	Howard Hughes/Barker Welfare Minority Workshop, *Fun With DNA*, American Museum of Natural History, New York
	DNA Science Minority Workshop, Central Islip, New York
July 31–Aug 4	National Science Foundation Workshop, *DNA Science*, University of Kansas, Lawrence
	World of Enzymes Workshop, DNALC
	Howard Hughes/Barker Welfare Minority Workshop, *Fun With DNA*, American Museum of Natural History, New York
August 7–11	National Science Foundation Workshop, *DNA Science*, Bates College, Lewiston, Maine
	Fun With DNA Workshop, DNALC
	Fun With DNA Minority Workshop, Central Islip, New York
	Howard Hughes/Barker Welfare Minority Workshop, *Fun With DNA*, American Museum of Natural History, New York
August 14–18	*DNA Science* Workshop, Frederick Cancer Research and Development Center, Maryland
	Fun With DNA Minority Workshop, Central Islip, New York
	Howard Hughes/Barker Welfare Minority Workshop, *Fun With DNA*, American Museum of Natural History, New York
	Computational Biology Workshop, DNALC
August 17–25	*Advanced DNA Science* Workshop, Beckman Neuroscience Center, CSHL
August 21–25	*World of Enzymes* Workshop, DNALC
	Howard Hughes/Barker Welfare Minority Workshop, *DNA Science*, American Museum of Natural History
August 28–Sept 1	*DNA Science* Workshop, DNALC
	Fun With DNA Workshop, DNALC
September 6	Corporate Advisory Board Meeting, DNALC
September 11–13	Howard Hughes Precollege Director's Meeting, Chevy Chase, Maryland
September 26	Site visit by Bonnie Kaiser, Rockefeller University, New York, New York
September 27–29	Brinkmann Business Meeting, Denver Colorado
October 6	Filming at DNALC by Alligator Films, Bergen, Norway
October 10	Seminar for Kiwanis Club, Huntington
October 20	Queens Gateway Project Meeting, DNALC
October 25–28	National Association of Biology Teachers Convention, Phoenix, Arizona
October 28	*Infectious Diseases: Ancient Plagues, New Epidemics* Laboratory for Business Executives, DNALC
November 4	Benjamin/Cummings Strategies Workshop, Sacramento, California
November 7	Queens Gateway Project Meeting, DNALC
November 11	Benjamin/Cummings Strategies Workshop, DNALC
November 16–18	Department of Energy *Human Genetics and Genome Analysis* Workshop, DNALC and Banbury Center
November 30	Cold Spring Harbor Laboratory Lecture, CSHL
December 2	Benjamin/Cummings Strategies Workshop, University of Cincinnati, Ohio
December 2–4	National Science Foundation Workshop *Human Genome Diversity-Student Allele Database*, United States Air Force Academy, Colorado Springs, Colorado

Sites of Major 3-10 Day Faculty Workshops 1985-1995

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
	United States Air Force Academy, Colorado Springs	**1995**
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	University of Idaho, Moscow	1994
IOWA	Drake University, Des Moines	1987
KANSAS	University of Kansas, Lawrence	1995
KENTUCKY	Murray State University	1988
	University of Kentucky, Lexington	1992
	Western Kentucky University	1992
LOUISIANA	Jefferson Parish Public Schools, Harvey	1990
	John McDonogh High School, New Orleans	1993
MAINE	Bates College, Lewiston	1995
MANITOBA	Red River Community College, Winnipeg	1989
MARYLAND	Annapolis Senior High School	1989
	Frederick Cancer Research Center, Frederick	1995
	McDonogh School, Baltimore	1988
	Montgomery County Public Schools	1990–1992
	St. John's College, Annapolis	1991
MASSACHUSETTS	Beverly High School	1986
	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 and Science, Columbus	1990–1991
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	1988–1995
	DNA Learning Center	**1990, 1992, 1995**
	DNA Learning Center	***1990–1992***
	Fostertown School, Newburgh	1991
	Huntington High School	1986

	Irvington High School	1986
	Junior High School 263, Brooklyn	***1991***
	Lindenhurst Junior High School	***1991***
	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
PANAMA	**University of Panama, Panama City**	**1994**
PENNSYLVANIA	Duquesne University, Pittsburgh-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


COLD SPRING HARBOR
LABORATORY PRESS

1995 PUBLICATIONS

General Books

Molecular Genetics of Cancer
Symposia on Quantitative Biology 59

Vaccines 95: Molecular Approaches to the Control of Infectious Diseases
R.M. Chanock, F. Brown, H.S. Ginsberg, and E. Norrby (eds.)

Archaea: A Laboratory Manual
F.T. Robb, A.R. Place, K.R. Sowers, H.J. Schreier, S. DasSarma, and E.M. Fleischmann (eds.)

Archaea: Halophiles
S. DasSarma and E.M. Fleischmann (eds.)

Archaea: Methanogens
K.R. Sowers and H.J. Schreier (eds.)

Archaea: Thermophiles
F.T. Robb and A.R. Place (eds.)

Genetic Analysis of Pathogenic Bacteria: A Laboratory Manual
S.R. Maloy, V.J. Stewart, and R.K. Taylor

Methods in Plant Molecular Biology: A Laboratory Course Manual
P. Maliga, D. Klessig, A. Cashmore, W. Gruissem, and J. Varner

Methods in Yeast Genetics: A Laboratory Course Manual, 1994 Edition
C. Kaiser, S. Michaelis, and A. Mitchell

PCR Primer: A Laboratory Manual
C.W. Dieffenbach and G.S. Dveksler (eds.)

Strategies for Protein Purification and Characterization: A Laboratory Course Manual
D.R. Marshak, J.T. Kadonaga, R.R. Burgess, M.W. Knuth, W.A. Brennan, Jr., and S.-H. Lin

The Cells of the Body: A History of Somatic Cell Genetics
H. Harris

The Statue Within
F. Jacob

Discovering Molecular Genetics: A Case Study Course with Problems & Scenarios
J.H. Miller

CSHL Monograph Series

Translational Control
J.W.B. Hershey, M.B. Mathews, and N. Sonenberg (eds.)

Telomeres
E.H. Blackburn and C.W. Greider (eds.)

Cancer Surveys Series

Vol. 22: *Molecular Mechanisms of the Immune Response*
W.F. Bodmer and M.J. Owen (eds.)

Vol. 23: *Preventing Prostate Cancer: Screening versus Chemoprevention*
R.T.D. Oliver, A. Belldegrun, and P.F.M. Wrigley (eds.)

Vol. 24: *Cell Adhesion and Cancer*
N. Hart and I. Hogg (eds.)

Vol. 25: *Genetics and Cancer: A Second Look*
B.A.J. Ponder, W.K. Cavenee, and E. Solomon (eds.)

Journals

Genes & Development (Volume 9, 24 issues)
T. Grodzicker and N. Hastie (eds.)

PCR Methods and Applications (Volume 4, 3 issues)
D. Bentley, E. Green, R. Gibbs, and R. Myers (eds.)

Genome Research (Volume 5, 5 issues)
A. Chakravarti, R. Gibbs, E. Green, R. Myers, and M. Boguski (eds.)

Learning & Memory (Volume 2, 5 issues)
R. Davis, E. Kandel, R. Morris, C. Shatz, L. Squire, and C. Stevens (eds.)

Videotapes

A Decade of PCR

Audiotape/CD

More Songs for a Cynical Scientist
R. Laskey

Other

The Lab Manual Source Book

A Crack in the Shield: Our Unvaccinated Children
Report prepared for the Sabin Foundation

CSHL Annual Report 1994

Banbury Center Annual Report 1994

Administration and Financial Annual Report 1994

Abstract/program books for 14 CSHL meetings

COLD SPRING HARBOR LABORATORY PRESS

The growth and expansion of our publishing program continued in 1995. Twenty books were published, bringing the total in print to over 220. A new videotape series and a new audiotape/CD were produced. The journals continued to expand in scope and frequency, as the *PCR Methods and Applications* journal was relaunched as a monthly research journal in genome science. The first issue of an annual directory of laboratory products and their sources of supply was published, and a start was made on the development of a searchable database of products and suppliers that could be accessed via the World Wide Web. Program income rose by 14% to $5.12 million.

Book Publishing

For the past several years, the commissioning of new titles has focused primarily on laboratory manuals, scholarly monographs, and books appropriate for graduate student teaching. Among the 1995 titles were eight manuals, two monographs, and an advanced textbook on molecular genetics. The manuals on bacterial genetics, plant molecular biology, yeast genetics, and protein purification each had roots in practical courses taught recently at the Laboratory, but there the resemblance ended. Each book was individually crafted by its authors to reflect the needs of its particular audience and required correspondingly painstaking in-house development. The most immediately successful of the new crop of manuals was *PCR Primer*, edited by Carl Dieffenbach and Gabriella Dveksler. This volume originated in a series of articles commissioned for our journal *PCR Methods and Applications*, reviewing the use of the polymerase chain reaction from basics to advanced applications. The eventual reworking of this material into step-by-step protocols was explicit in the way the series was created. The result is an extremely useful book with a long life in subsequent editions as variants on amplification technology continue to appear.

The book's appearance coincided with the tenth anniversary of the invention of PCR by Kary Mullis, an event marked by a two-day meeting in September that was captured on videotape and subsequently published. Mullis first described his discovery at the Laboratory's annual Symposium in 1985. The extraordinary importance of PCR as a genetic technique is indicated by the fact that in this institution alone, two books, two meetings, a journal, and a videotape series have been solely devoted to it.

Two titles were added to the distinguished list in the CSHL Monograph series, thanks in large measure to the dedication of senior Laboratory scientists. Michael Mathews and colleagues John Hershey and Nahum Sonenberg edited a volume on the translation of RNA into protein that will stand as this mature field's central reference for some years. The study of telomeres, on the other hand, has recently entered a new and rapidly changing phase, and the decision to create a book on the topic came from the recognition that the older information in the field was fragmented and unfocused. Carol Greider and her mentor Elizabeth Blackburn assembled a volume that concisely summarized what is known about these interesting chromosomal structures and the important future directions that beckon from the fields of cancer biology and aging research.

Not every new title can be described in detail here (see opposite for a complete list), but three deserve particular mention because of the very personal nature of their creation. Jeffrey Miller's *Discovering Molecular Genetics* is an innovative textbook based on his much-praised graduate course at the University of California, Los Angeles. The course is centered on Miller's unique selection and analysis of historically important papers. The book reprints the papers and captures the imaginative exercises in thinking that Miller uses to illuminate the lessons they convey.

The author's personality also emerges strongly from *The Cells of the Body*, a history of somatic cell genetics by one of its most distinguished scholars, Sir Henry Harris. Written for a broad sci-

entific audience, the book begins with the discovery of cells and their origins and guides the reader elegantly through the development of the concepts and techniques which made possible the present era of molecular analysis.

One of the founding visionaries of that era, François Jacob, graciously agreed to the publication of a special Cold Spring Harbor edition of his out-of-print autobiography *The Statue Within*. This was accomplished in time for the meeting celebrating the fiftieth anniversary of the phage course in August, and many of the participants were thrilled to be able to take home a copy of this unusually personal and moving memoir signed by its legendary author.

Journal Publishing

As in each of the previous seven years, we entered 1995 bracing for further significant changes in the journal program. The countdown began in June with the transformation of the successful, established journal *PCR Methods and Applications* into the novel *Genome Research*. Planning for this event had begun in mid 1994 with the conviction that basic PCR technology was becoming a laboratory mainstay, no longer the engine of the many innovative variations and applications seen four years earlier when the journal began. Our response was to reposition the journal as a place for new and exciting original research in genome studies. *Genome Research* appeared first in August, guided by an enthusiastic and influential editorial board led by the editors Aravinda Chakravarti, Richard Gibbs, Eric Green, and Richard Myers (joined by Mark Boguski in December) and reviews editor, Alison Stewart. After some anxieties concerning the printer's ability to maintain the new monthly production schedule and an initially sluggish paper flow, the year finished strongly with issues of high quality, fair size, and wide scope. Moreover, the journal's financial objectives were met. This was no small achievement given the scale of the transformation undertaken—a simultaneous change of title, scope, audience, and renewal cycle. A further source of quiet satisfaction was the use made of electronic supplements to the print issues. Each issue contained an article for which additional information—video clips, large data sets, or software programs—were made available through the CSHL World Wide Web site. The article itself was available online, enhanced by the provision of references hyperlinked to the corresponding abstracts in Medline. *Genome Research* was the first major science journal to employ web technology in this way and its use taught us much that was helpful.

A good deal of concern was also directed toward the development of our other fledgling journal *Learning & Memory*. Launched in mid 1994, this journal serves a scientific community that is producing some of the most fascinating results in current neuroscience, so competition from established journals for good papers is fierce. Our journal is so far still a newcomer with promise, but progress was made on the foundation created in 1994, and the journal's editors, Ron Davis, Eric Kandel, Richard Morris, Larry Squire, Carla Schatz, and Chuck Stevens, resolutely maintained a high standard of published papers. All concerned realized that starting this journal while the molecular analysis of learning and memory is still in its infancy was a major challenge. Nevertheless, our commitment to success was renewed in 1995, and we were delighted when Jack Byrne accepted the role of Editor from 1996 onward, building on the achievement of the founding editors.

The administrative and financial challenges presented by changes such as these can be taken on only by a healthy journal publishing program. The essential foundation for that program is our flagship journal *Genes & Development*, which had another year of widening scope, increased circulation, and strong financial results. The editors Terri Grodzicker and Nick Hastie, leading a distinguished editorial board, continued to provide readers with papers describing first-class, reliable science in some of the most innovative areas of molecular biology. More review articles were published than ever before. The annual citation analysis conducted by the Institute for Scientific Information on papers published in 1994 once again placed *Genes & Development* in the topmost tier of biology journals.

The Lab Manual Source Book

Our strong position in the publishing of laboratory manuals prompted us to embark in 1994 on a new project, the creation of a comprehensive "Yellow Pages" of the products and services needed by the users of laboratory manuals. Beginning early in 1995, more than 30,000 copies of *The Lab Manual Source Book* were distributed free of charge to scientists worldwide. The goal was to create a publication that would command the attention of both scientists and the companies that supply them with products. The enthusiastic response from both camps indicated that a good start had been made. Work began immediately on the preparation of the second annual edition, for publication in February 1996. Improvements were made in the product classification scheme, new product categories were added, and the supplier list was expanded. Advertising sales efforts were redoubled in order to fund greater circulation of the 1996 issue.

Alongside these activities, we initiated the creation of a dedicated World Wide Web site that contained searchable versions of the product and supplier databases. Its title, BioSupplyNet, was chosen to convey the concept of an extended network of information about laboratory materials and their sources of supply that extended far beyond the information published in the *Source Book*. Vendors were encouraged to make available through BioSupplyNet technical specifications, performance data, and special pricing information—any kind of information valuable to end users that would otherwise have to be conveyed statically and expensively in print. BioSupplyNet opened for access in August and considerable efforts were made to promote awareness and use of it. It was quickly recognized as the most advanced of several competing efforts to create a focus of product information on the Web, and traffic to the site was heavy. We also became aware that the maintenance of the online database, the enhancement of the site as Web technology evolved, and the full exploitation of the site's capabilities for advanced marketing and online purchasing would require investment on a larger scale than the Press could undertake unaided. As the year ended, discussions were advancing with potential partners about the many exciting ways in which BioSupplyNet could be developed to best advantage.

Marketing and Distribution

The year's direct mail marketing program centered on a widely dispatched Genetics and Cell Biology catalog, a Plant Biology catalog, two issues of the Press newsletter announcing new titles, and a variety of smaller brochures and flyers advertising specific titles.

The Press World Wide Web server also took its place as a valuable marketing tool this year, offering a complete online catalog, announcements of new books on publication, and the opportunity to place orders electronically. This initiative was extremely valuable not just for the income generated, which was surprisingly high, but because it offered the opportunity for direct interaction with users of our publications, particularly overseas. Many valuable lessons were learned.

Another important marketing channel is the series of major scientific meetings attended each year. Our exhibit stand is modestly sized, but still miraculously effective in displaying the ever-increasing number and diversity of our publications. The conference landscape is changing as the fortunes of the large scientific societies wax and wane. Two previously valuable meetings were disappointing this year and will not be revisited, but new candidates were sampled and at least one was found to fill the gap. However mixed the huge society meetings may be scientifically (and several this year were excellent), the face-to-face encounter with tens of thousands of our customers presents many valuable opportunities, not all quantifiable in dollars, and must not be missed.

A smaller-scale but equally valuable interaction takes place each year with the 5000 attendees at Laboratory meetings and courses, via the campus bookstore. A remarkable degree of commerce occurs in this small space, involving our publications, general science books, and items visitors find useful. The bookstore had a successful year financially and after lobbying for relocation

for some years, we were delighted with the November decision to move the bookstore into a three-times larger space in the basement of Grace Auditorium. At the turn of the year, several options were being considered on the best way to manage the much larger operation this new venue would accommodate.

The compilation and analysis of sales data from the various marketing channels take place in the computer system at our Plainview facility. First occupied by us five years ago, this building has proved adaptable and adequate for our present and short-term future needs. A new lease was negotiated in February. Regrettably, the computer system itself has aged less well. Left behind by seismic changes in computing environments, the system is based on software that runs only on outmoded components for which maintenance contracts may soon be impossible. The search for an alternative began early in 1995. Our objective is to find a cost-effective, flexible, low-maintenance, publishing-specific system as compatible as possible with other databases operated in the Laboratory that will allow effective management of records from over 50,000 accounts worldwide and the simultaneous administration of both book purchases and journal subscriptions.

Despite its limitations, the computer system provided data for an increasing array of sales reports and analyses necessary for informed decision making. This facility was particularly helpful in a series of extended conversations in mid year on the nature and direction of our international marketing program. Thirty percent of book sales already go overseas, but led by a consultant, we assessed the extent of sales in each foreign country and targeted six in which special attention was likely to increase sales cost-effectively. Contact with potential distributors in those countries was made at the Frankfurt Book Fair and negotiations have ensued. We plan to develop significant business for the first time in certain Pacific Rim and South American countries and increase our market penetration in Europe.

In the meantime, we continued to supply books and journals direct to overseas customers. The costs of international dispatch were continually reviewed, and in this ever-changing marketplace, carriers were switched as necessary to ensure that the service provided to purchasers was both economical and efficient.

Publishing is a team activity: Neither the most able author nor the most remarkable publication can succeed without the ministrations of editors and typesetters, proofreaders and production staff, marketers and order processors, and people who pick, pack, and post in the warehouse. The Press is fortunate to have capable staff throughout the house who bring intelligence and care to what they do. Their names are listed at the back of this volume: Here there is room only to acknowledge their vital contribution to the program and to thank them for it. However, special mention must go to Nancy Ford and Judy Cuddihy, Ingrid Benirschke, Nancy Hodson, Guy Keyes, and Joan Boyce. These department heads provided the management skills and direction that got things done and ensured the program's high quality of output and service.

New Products

During the year, agreements were reached with authors and editors of more than 20 new books. At the same time, many other conversations were initiated about possible new publishing projects in a variety of media, both print and electronic, in stand-alone and subscription formats. Such conversations, particularly with younger scientists, fuel the new publications necessary to sustain our growth in the immediate future. We are also looking to the back list of distinguished books upon which the current program is based, creating new editions as appropriate and employing new publishing technologies to adapt and reuse classic material from the past. Both the book and the journal programs are rising to more complex editorial and production challenges, and investment in new, skilled staff is required. The book program, for example, urgently needs another developmental editor capable of managing a project all the way from conceptual discussions with potential authors to the delivery of a bound book from the printer.

Meanwhile, the wired world beckons insistently, promising radically to transform the means of communication in science. Electronic mail is now ubiquitous, substituting for letters, faxes, and phone calls. The communication of important science remains print-based . . . for now. We will continue to experiment with additional and alternative media, examining the potential of the latest technologies but conscious of the uncertainties that underlie the current business models for their application. The promised transformation may be neither swift nor sudden, but we will be prepared.

John R. Inglis

FINANCE

FINANCIAL STATEMENTS

STATEMENT OF FINANCIAL POSITION
December 31, 1995

Assets:	
Cash and cash equivalents	$ 13,459,548
Marketable securities	94,979,330
Accounts receivable	959,727
Grants receivable	3,028,747
Contributions receivable	500,000
Publications inventory	1,394,720
Prepaid expenses and other assets	2,078,234
Investment in employee residences	1,825,641
Land, buildings, and equipment:	
Land and improvements	7,588,532
Buildings	59,706,386
Furniture, fixtures, and equipment	4,239,740
Laboratory equipment	10,554,994
Library books and periodicals	365,630
	82,455,282
Less accumulated depreciation and amortization	24,634,265
Land, buildings, and equipment, net	57,821,017
Construction in progress	190,382
Total assets	$ 176,237,346
Liabilities and Net Assets:	
Accounts payable and accrued expenses	$ 949,079
Notes payable	667,520
Bonds payable	30,000,000
Deferred revenue	5,351,669
Total liabilities	36,968,268
Net Assets:	
Unrestricted	
General operating	5,693,880
Designated:	
For research program	1,150,000
Capital expenditures	34,010,961
Board designated endowment	49,544,118
Total unrestricted	90,398,959
Temporarily restricted	470,000
Permanently restricted	48,400,119
Total net assets	139,269,078
Total liabilities and net assets	$ 176,237,346

STATEMENT OF ACTIVITIES
Year Ended December 31, 1995

	Unrestricted	*Temporarily Restricted*	*Permanently Restricted*	*Total*
Support and revenue:				
Public support	$ 10,436,932	–	754,427	11,191,359
Government grant awards	12,105,851	–	–	12,105,851
Indirect cost allowances	9,007,870	–	–	9,007,870
Other revenue:				
Program fees	1,773,800	–	–	1,773,800
Rental income	435,555	–	–	435,555
Publications	5,119,484	–	–	5,119,484
Dining services	1,983,219	–	–	1,983,219
Rooms and apartments	1,664,609	–	–	1,664,609
Gain on sale of marketable securities	1,179,323	–	3,231,282	4,410,605
Investment income	6,903,504	–	–	6,903,504
Royalty & licensing fees	1,222,390	–	–	1,222,390
Recovery of valuation allowance	522,774	–	–	522,774
Miscellaneous	134,334	–	–	134,334
Total other revenue	20,938,992	–	3,231,282	24,170,274
Net assets released from restrictions:				
Expiration of time restrictions	1,090,000	(1,090,000)	–	–
Total support and revenue	53,579,645	(1,090,000)	3,985,709	56,475,354
Expenses:				
Program services:				
Research	15,837,311	–	–	15,837,311
Summer programs	5,201,484	–	–	5,201,484
Publications	5,078,559	–	–	5,078,559
Banbury Center conferences	920,781	–	–	920,781
DNA Education Center programs	486,367	–	–	486,367
Total program services	27,524,502	–	–	27,524,502
Supporting services:				
Direct research support	1,243,713	–	–	1,243,713
Library	591,520	–	–	591,520
Operation and maintenance of plant	5,514,513	–	–	5,514,513
General and administrative	4,655,785	–	–	4,655,785
Dining services	1,814,174	–	–	1,814,174
Interest	1,526,387	–	–	1,526,387
Depreciation and amortization	2,820,555	–	–	2,820,555
Total supporting services	18,166,647	–	–	18,166,647
Total expenses	$ 45,691,149	–	–	45,691,149
Change in net assets before cumulative effect of a change in accounting principle	$ 7,888,496	(1,090,000)	3,985,709	10,784,205
Cumulative effect in method of accounting for contributions	–	1,560,000	–	1,560,000
Net assets at beginning of year	82,510,463	–	44,414,410	126,924,873
Net assets at end of year	$ 90,398,959	470,000	48,400,119	139,269,078

COMPARATIVE OPERATING HISTORY 1991-1995
(Dollars in Thousands)

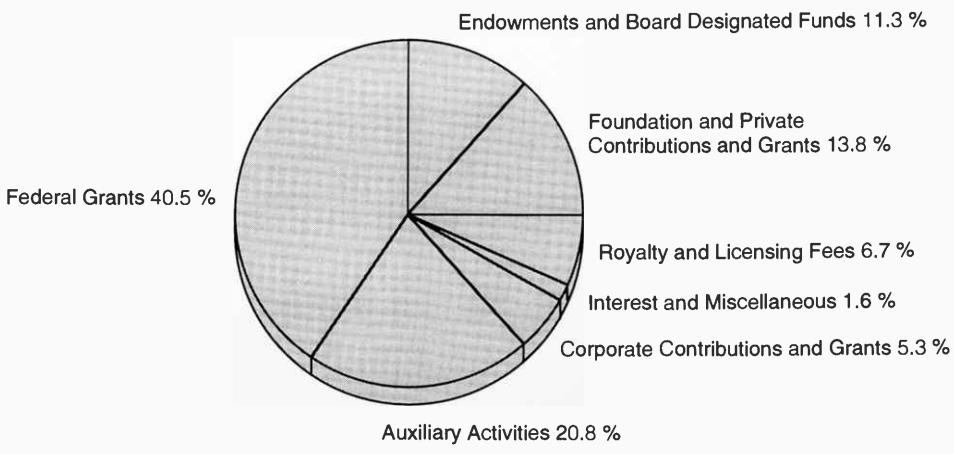
	1991	*1992*	*1993*	*1994*	*1995*
Income:					
Main Lab:					
Grants & contracts	$ 15,172	16,800	18,136	19,293	19,653
Indirect cost reimbursement	7,170	8,388	8,383	8,460	8,881
Other	5,056	5,520	6,049	6,808	7,461
CSH Press	3,079	3,709	4,319	4,390	5,119
Banbury Center	1,090	1,104	1,281	1,569	1,732
DNA Learning Center	744	822	796	824	954
Total income	32,311	36,343	38,964	41,344	43,800
Expenses:					
Main Lab:					
Grants & contracts	15,172	16,800	18,136	19,293	19,653
Operation & maintenance of plant	3,904	4,241	4,777	5,141	5,266
General & administrative	2,468	2,634	2,785	2,909	3,329
Other	3,375	4,141	4,385	4,847	4,959
CSH Press	3,488	3,548	4,134	4,309	5,079
Banbury Center	1,063	1,070	1,226	1,498	1,643
DNA Learning Center	752	843	768	798	958
Total expenses	30,222	33,277	36,211	38,795	40,887
Excess before depreciation and (designation) release of funds	2,089	3,066	2,753	2,549	2,913
Depreciation	(1,898)	(2,358)	(2,522)	(2,668)	(2,821)
(Designation) release of funds (1)	(100)	(600)	0	200	0
Net operating excess	$ 91	108	231	81	92

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.

COLD SPRING HARBOR LABORATORY

SOURCES OF REVENUE

YEAR ENDED DECEMBER 31, 1995



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 1995.

GRANTS January 1, 1995–December 31, 1995

COLD SPRING HARBOR LABORATORY

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*1995 Funding*+
FEDERAL GRANTS			
NATIONAL INSTITUTES OF HEALTH			
Program Projects	Cancer Research Center, Dr. Herr	1/92–12/96	$3,496,771
	Cancer Center Support, Dr. Stillman	8/95–7/00	2,669,134*
Research Support	Dr. Arndt	1/95–12/98	278,871*
	Dr. Beach	5/94–4/96	292,366
	Dr. Beach	4/93–3/97	189,211
	Dr. Beach	8/95–5/00	250,005*
	Dr. Cheng	4/93–3/97	199,974
	Dr. Cheng	9/95–8/97	50,000*
	Dr. Cline	12/95–11/98	265,296*
	Dr. Enikolopov	9/94–8/98	204,056
	Dr. Futcher	4/93–3/97	261,180
	Dr. Futcher	1/91–12/99	190,088
	Dr. Greider	12/94–11/98	271,168
	Dr. Greider	8/91–7/96	269,308
	Dr. Helfman	4/94–3/98	286,913
	Dr. Helfman	8/93–5/98	228,960
	Dr. Hengartner	5/95–4/00	213,983*
	Dr. Hernandez	7/92–6/96	238,834
	Dr. Hernandez	9/91–8/96	239,433
	Dr. Herr	3/92–2/96	219,732
	Dr. Krainer	7/94–6/98	323,426
	Dr. Lisitsyn	7/94–6/99	203,495
	Dr. Malinow	5/94–4/97	118,534
	Dr. Malinow	4/95–2/98	247,205*
	Dr. Marr	6/95–5/98	797,610*
	Dr. Mathews	2/92–1/97	291,477
	Dr. Mathews	9/93–8/98	317,582
	Dr. Mathews	9/94–9/97	33,845
	Dr. McCombie	4/94–3/97	432,959
	Dr. Skowronski	12/93–11/97	308,538
	Dr. Spector	4/95–3/99	338,536*
	Dr. Silva	7/95–6/00	241,373*
	Dr. Stillman	7/91–6/95	197,428
	Dr. Tonks	8/91–5/96	345,659
	Dr. Tully	4/94–3/97	327,494
	Dr. Wigler	7/95–4/99	1,809,369
Fellowships	Dr. Dai	8/94–10/97	29,900
	Dr. DeZazzo	1/95–12/95	31,200*
	Dr. Edwards	9/95–8/98	22,608*
	Dr. Mainen	10/95–9/97	22,608*
	Dr. O'Gara	12/95–12/96	23,700*
	Dr. Zhang	9/92–8/97	102,12
Training Support	Training in Cancer Cell Biology and Tumor Virology	7/94–2/99	179,001

* New Grants Awarded in 1995
\+ Includes direct and indirect cost

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*1995 Funding*+
Course Support	Advanced Bacterial Genetics	5/93–4/98	56,260
	Cancer Research Center Workshops	4/92–3/97	243,444
	Neurobiology Short-term Training	5/82–4/96	141,885
	CSHL Analysis Large DNA Molecules	1991–1996	104,625
	Essential Computational Genomics for Molecular Biologists	1991–1996	27,337
	Advanced In Situ Hybridization and Immunocytochemistry	1992–1997	50,759
	Molecular Biology and Development of *Xenopus Laevis*	4/93–3/96	12,154
	Automated Genome Sequencing	4/95–3/98	71,250 *
Meeting Support	Genome Mapping and Sequencing	4/91–3/96	32,394
	Mechanisms of Eukaryotic Transcription	7/95–6/96	3,000 *
	Programmed Cell Death	7/95–6/96	8,000 *
	Molecular Genetics and Phage	8/95–7/96	2,000 *
	Eukaryotic DNA Replication	9/95–8/96	6,000 *
	Neurobiology of *Drosophila*	9/95–9/96	12,000 *
	Tyrosine Phosphorylation and Cell Signaling	4/95–3/96	7,000 *
	Cell and Molecular Biology of *Aplysia*	4/95–3/96	15,000 *
NATIONAL SCIENCE FOUNDATION			
Research Support	Dr. Cline	9/94–8/96	79,373
	Dr. Grotewold	11/94–10/98	115,556
	Dr. Ma	5/94–4/97	134,500
	Dr. Ma	8/94–7/98	110,000
	Dr. Martienssen	8/94–7/98	150,000
	Dr. Peunova	9/95–8/98	90,000 *
Fellowship Support	Dr. Springer	12/93–11/96	32,400
Training Support	Undergraduate Research Program	6/91–5/97	50,000
Course Support	*Arabidopsis* Molecular Genetics	6/95–5/96	60,000 *
	Molecular Biology and Development of *Xenopus laevis*	4/93–3/96	7,550
	Macromolecular Crystallography	8/94–7/97	15,000
Meeting Support	Programmed Cell Death	8/95–7/96	3,000 *
	Tyrosine Phosphorylation and Cell Signaling	4/95–3/96	6,000 *
	Eukaryotic DNA Replication	6/95–5/96	15,000 *
	Signaling in Plant Development	7/95–6/96	4,000 *
	Molecular Genetics of Phage	8/95–7/96	5,000 *
	Neurobiology of *Drosophila*	9/95–8/96	10,000 *
	Cell and Molecular Biology of *Aplysia*	4/95–3/96	2,500 *
	The Cytoskeleton and Cell Function	4/95–3/96	4,000 *
DEPARTMENT OF ENERGY			
Research Support	Dr. Marr	7/94–2/97	450,000
Meeting Support	CSHL 60th Symposium: Protein Kinesis	1995	10,000 *
	Molecular Genetics of Bacteria and Phage	1995	11,800 *
	Programmed Cell Death	1995	7,500 *
	Signaling in Plant Development	1995	3,500 *

* New Grants Awarded in 1995
+ Includes direct and indirect cost

Grantor	Program/Principal Investigator	Duration of Grant	1995 Funding+
UNITED STATES DEPARTMENT OF AGRICULTURE			
Research Support	Dr. Ma	9/94 – 9/96	69,322
	Dr. Ma	9/95 – 9/97	50,000 *
	Dr. Martienssen	9/95 – 9/97	63,444 *
	Dr. Martienssen	3/95 – 2/98	92,000 *
	Dr. Sundaresan	8/94 – 9/97	70,000
	Dr. Sundaresan	9/94 – 9/97	74,000
Meeting Support	Signaling in Plant Development	8/95 – 7/96	6,000 *
	Molecular Markers for Plant Breeding and Plant Genetics	11/95 – 1/96	10,000 *
UNITED STATES DEPARTMENT OF ARMY			
Research Support	Dr. Futcher/Dr. Marshak	6/94 – 6/99	203,926
	Dr. Wigler/Dr. Lisitsyn	7/94 – 8/98	200,000
UNITED STATES DEPARTMENT OF NAVY			
Meeting Support	The Cytoskeleton and Cell Function	4/95 – 3/96	5,000 *
NONFEDERAL GRANTS			
Research Support			
American Cancer Society	Dr. Enikolopov	7/95 – 6/96	100,000 *
	Dr. Ma	7/94 – 6/97	28,500
	Dr. Ma	1/95 – 12/96	90,000 *
	Dr. Marshak	7/93 – 6/95	103,000
	Dr. Wigler, Professorship	1986 – 2012	50,000
	Dr. Wigler, Supply Allowance	1995	10,000
American Heart Association	Dr. Helfman	7/91 – 6/96	35,000
Amplicon Corporation	Dr. Wigler	6/94 – 5/97	596,000
Arnold & Mabel Beckman Foundation	Dr. Silva	7/94 – 6/96	100,000
Sara Chait Foundation	Dr. Marshak	12/91 – 11/96	25,000
Council for Tobacco Research	Dr. Arndt	8/95 – 7/98	75,000 *
	Dr. Helfman	7/91 – 6/96	80,000
	Dr. Tonks	1/95 – 12/97	85,000 *
Geron Corporation	Dr. Greider	2/94 – 1/97	217,175
Irving Hansen Memorial Fund	Dr. Tonks	8/95 – 7/97	10,000 *
Johns Hopkins University/NIH	Dr. Wigler	7/95 – 9/95	40,000 *
Esther A. & Joseph Klingenstein Fund, Inc.	Dr. Cline	7/94 – 6/96	33,333
	Dr. Enkolopov	7/95 – 6/98	40,000 *
	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 – 6/96	50,000 *
Mathers Charitable Foundation	Dr. Malinow	8/91 – 7/94	233,000
McKnight Endowment Fund	Dr. Silva	7/95 – 6/96	50,000 *
Memorial-Sloan Kettering/NIH	Dr. Kobayashi	9/94 – 7/97	175,000
	Dr. Tonks	9/94 – 7/97	185,944
	Dr. Wigler	9/95 – 8/98	220,261 *
John Merck Fund	Dr. Silva	5/95 – 4/96	60,000 *
NYU Consortium/NIH	Dr. Kobayashi	5/92 – 4/97	74,643
Nanoprobes, Inc./NIH	Dr. Spector	6/94 – 5/96	29,087
National Down Syndrome Society	Dr. Cline	7/95 – 6/97	25,000 *
Lauri Strauss Leukemia Foundation Felix Schnyder Memorial Fund	Dr. Tonks	5/95 – 4/96	15,000 *
St. Giles Foundation	Dr. Beach/Dr. Wigler	3/93 – 2/96	50,000
Westvaco, Inc.	Dr. Martienssen/Dr. McCombie	6/95 – 5/96	290,000 *
Whitehall Foundation	Dr. Silva	9/93 – 8/96	45,000
	Dr. Zhong	1/94 – 12/96	40,000

* New Grants Awarded in 1995

\+ Includes direct and indirect cost

Grantor	Program/Principal Investigator	Duration of Grant	1995 Funding+
Equipment Support			
Dextra Baldwin McGonagle Foundation	Equipment	10/95–9/96	10,000*
Lorraine Grace	Equipment	4/95–3/96	15,000*
E.S. Moore Foundation	Equipment	9/95–12/95	10,000*
Fellowships			
Rita Allen Foundation	Dr. Hengartner	9/94–8/99	30,000
American Cancer Society	Dr. Ellison	7/95–6/98	24,000*
American Heart Association	Dr. Temm-Grove	7/95–6/97	21,650*
Austrian Science Foundation	Dr. Gimona	4/94–4/96	27,800
Swedish Cancer Society	Dr. Berg	9/93–9/95	25,000
Cancer Research Fund of the Damon Runyon-Walter Winchell Foundation	Dr. Conklin	11/93–10/96	32,500
	Dr. Kim	1/95–12/97	27,000*
	Dr. Liang	9/94–8/97	31,000
	Dr. Weinreich	1/94–12/96	31,000
CSHL Association	Fellowship Support	4/95–9/96	162,000*
Jane Coffin Childs	Dr. Mirzayan	7/93–6/96	28,500
	Dr. Verreault	10/94–9/97	27,500
The Charles A. Dana Foundation	Dr. Witkowski	1993–1995	360,000
Demerec-Kaufmann Hollaender Fellowship in Developmental Genetics	Dr. Grotewold	6/95–5/96	5,000*
Deutsche Forschungsgemeinscaft	Dr. Giese	1994–1996	34,000
Eton Student Internship	Dr. Hoffman	8/95–7/96	3,000*
Fonds de la Recherche en Sante du Quebec	Dr. Autexier	5/92–4/95	6,000
Foundation for Promotion of Cancer Research, Japan	Dr. Iizuka	1995	42,660*
Glaxo Inc.	Fellowship Support	1/95–12/95	100,000*
Goldring International Group, Inc.	Fellowship Support	7/94–6/97	30,000
Human Frontier Science Program	Dr. Donovan	6/95–5/96	35,400*
	Dr. Grossniklaus	11/94–10/96	49,700
	Dr. Hamaguchi	8/94–7/96	43,700
	Dr. Misteli	10/95–9/96	35,400*
	Dr. Steiner	4/94–3/96	30,400
	Dr. Vignais	8/93–7/95	40,500
Leukemia Society	Dr. Autexier	7/95–6/98	31,320*
	Dr. Li	7/94–6/97	33,480
	Dr. Serrano	7/95–6/99	31,320*
C.J. Martin Fellowship (NHMRC, Australia)	Dr. Tiganis	1995	24,000*
Ministerio De Education Y Ciencia, Madrid	Dr. Blasco	6/94–6/95	16,000
	Dr. Carnero	8/95–7/96	19,000*
Medical Research Council of Canada	Dr. Demetrick	11/91–11/95	43,260
Pew Scholars Program	Dr. Krainer	7/92–6/96	50,000
	Dr. Lazebnik	7/95–6/99	50,000*
	Dr. Zhong	7/94–6/98	50,000
Andrew Seligson Memorial Fellowship	Fellowship Support	9/90–5/96	75,000
Wellcome Trust	Dr. Frenguelli	1995	12,403*
Wendt Fellowships	Neurobiology Fellowship Support	1/94–12/96	50,000
Training Support			
Burroughs Wellcome Foundation	Summer Undergraduate Program	1995	40,260*
Jephson Educational Trust	Summer Undergraduate Program	1995	7,500*
Howard Hughes Medical Institute	Graduate Student Support	1995	26,000*
Phillips Petroleum Co.	Summer Undergraduate Program	1995	1,000*

* New Grants Awarded in 1995
+ Includes direct and indirect cost

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*1995 Funding+*
Course Support			
The Grass Foundation	Scholarships	5/94 –4/96	18,240
Howard Hughes Medical Institute	Advanced Neurobiology	1991 –1999	285,063
Esther A. & Joseph Klingenstein Fund, Inc.	Advanced Neurobiology	5/94 –4/97	60,000
Monsanto Company	Plant Breeding	3/95 –3/96	1,000 *
Pioneer Hi-Bred	Plant Breeding	9/95 –8/96	9,000 *
Meeting Support			
American Cyanamid	Molecular Approach to the Control of Infectious Diseases	10/95 –9/96	15,000 *
Anheuser-Busch Companies	Yeast Cell Biology	6/95 –5/96	500 *
CIBA-GEIGY Corporation	Signaling in Plant Development	9/95 –8/96	500 *
DuPont Corporation	Signaling in Plant Development	9/95 –8/96	500 *
Genetics Computer Group, Inc.	Molecular Genetics and Phage	8/95 –7/96	1,000 *
Pharmacia Biotech	Molecular Approaches to the Control of Infectious Diseases	10/95 –9/96	12,000 *
Pioneer Hi-Bred	Signaling in Plant Development	9/95 –8/96	1,000 *
Promega Corporation	Molecular Genetics and Sequencing	8/95 –7/96	1,000 *
QuantumSoft General Atomics	Genome Mapping and Sequencing	5/95 –4/96	500 *
The National Neurofibromatosis Foundation, Inc.	Neurofibromatosis	10/95 –9/96	20,000
University of Wisconsin/Madison (NSF Funds)	RNA Process	5/95 –4/96	2,500 *

BANBURY CENTER

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*1995 Funding+*
FEDERAL SUPPORT			
NATIONAL INSTITUTES OF HEALTH			
	HIV and the Pathogenesis of AIDS	1995	25,000 *
	Lyme Disease Conference	1995	10,000 *
DEPARTMENT OF ENERGY			
	Human Genetics for Nonscientists: Practical Workshops for Policy Makers and Opinion Leaders	1994 –1995	66,579
NONFEDERAL SUPPORT			
Meeting Support			
Connaught Laboratories	Lyme Disease Conference	1995	10,000 *
The Charles A. Dana Foundation	Genetic Basis of Manic-Depressive Illness	1993 –1995	361,000
Finisterre Fund	Imaging Meeting	1995	23,670 *
Fort Dodge Laboratories	Lyme Disease Conference	1995	5,000 *
MedImmune, Inc.	Lyme Disease Conference	1995	2,000 *
Mothers' Voices, Inc.	HIV and the Pathogenesis of AIDS	1995	5,000 *
OncorMed	DNA Repair Meeting	1995	31,298 *
Pediatric AIDS Foundation	HIV and the Pathogenesis of AIDS	1995	4,914 *
Private Contributions	Neurofibromatosis Meeting	1995	15,000 *
Albert B. Sabin Vaccine Foundation, Inc.	HIV and the Pathogenesis of AIDS Vaccine Development	1995	2,000 *
SmithKline Beecham	Lyme Disease Conference	1995	35,149
Viaticus, Inc.	HIV and the Pathogenesis of AIDS	1995	5,000 *
The William Stamps Farish Fund	Molecular Genetics of Diabetes	1993 –1996	2,000 *
The Wilson Foundation	Neurofibromatosis Meeting	1995	50,000
			5,000 *

* New Grants Awarded in 1995

+ Includes direct and indirect cost

DNA LEARNING CENTER

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*1995 Funding+*
FEDERAL GRANTS			
NATIONAL SCIENCE FOUNDATION	A Two-Part Program to Develop and Support Nationwide Corps of Human and Molecular Genetics Resource Teachers at the Secondary Level, David Micklos	4/93–3/96	242,193
	Laboratory-based Instruction in Molecular and Human Genetics for Teaching Faculty, Mark Bloom	5/93–4/95	35,835
	A Novel Mechanism for Introducing Human Genome Research in Freshman Biology, Mark Bloom	4/95–4/98	33,395 *
NONFEDERAL GRANTS			
Barker-Welfare Foundation	MS Biology Camp/AMNH	6/95–6/97	5,369 *
Genentech, Inc.	*Story of a Gene* Exhibit	4/95–4/96	32,747 *
Howard Hughes Medical Institute	Precollege Science Education Initiative for Biomedical Research Institutions	7/94–8/99	85,516
Stone Foundation	Biomedia Teaching Laboratory	7/92–6/96	61,360
Weezie Foundation	*Story of a Gene* Exhibit	12/91–12/95	15,940

The following schools each awarded a grant of $5,000 in 1995 for the *Genetics as a Model for Whole Learning* Program:
Locust Valley Central School District
Great Neck Public Schools
Jericho Union Free School District

The following schools awarded a grant for *Curriculum Study* in 1995
of $950:
Commack Union Free School District
East Meadow 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
Roslyn Public School
Sachem Central School District
South Huntington Union Free School District
Syosset Central School District

of $1,500:
Ramaz School

of $2,000:
West Hempstead

* New Grants Awarded in 1995
+ 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, 1995–December 31, 1995

Contributions of $5,000 and above, exclusive of Annual Fund

In 1995, the Laboratory received significant support in the form of capital and program contributions from individuals, foundations, and corporations.

Anonymous
Arrow Electronics, Inc.
Banbury Fund
Barker Welfare Foundation
Burroughs Wellcome Fund
John P. Cleary
D'Egville Foundation (Dr. Mark Ptashne)
Dextra Baldwin McGonagle Foundation
Dolan Family Foundation
William Stamps Farish Foundation
The Garfield Foundation
Garland Publishing (Elizabeth Borden)
Genentech
Dr. H. Bentley Glass
Glaxo Research Institute
Goldring Family Foundation
Mrs. Oliver R. Grace
Irving A. Hansen Memorial Fund
Estate of Maxine Harrison
Jephson Educational Trust
David L. Luke III
Edwin S. Marks
William and Marjorie Matheson
McKnight Endowment for Neuroscience
John Merck Fund
Edward S. Moore Foundation
1 in 9: Long Island Breast Cancer Coalition
William and Maude Pritchard Charitable Trust
Estate of Joan Read
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The Corporate Sponsor Program continues to be a major source of support for the meetings held in both Grace Auditorium on the main Laboratory campus and at Banbury Center. The Program covers the cost of six meetings at Banbury Center and helps underwrite the costs of the meetings in Grace Auditorium. The 1995 membership remained at the same level as in 1994, with 35 members. It is clear that companies are rethinking their contributions to outside programs, but we have been able to maintain the membership fee at the same level for four years and that fee, $20,500 in 1995, is a relatively small sum in the research budgets of the companies we approach. In addition, substantial benefits return to member companies:

- All on-site fees are waived for eight representatives of each company attending 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 the Cold Spring Harbor Laboratory Press publications including the journals *Genes & Development*, *Learning & Memory*, and *Genome Analysis*.
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- Corporate Sponsors are acknowledged in all relevant publications, including the books of abstracts given to every participant at meetings; the names of the sponsoring companies are listed on the poster describing the meetings which is mailed to approximately 7,000 scientists throughout the world.

On behalf of all the scientists that attend meetings at Cold Spring Harbor Laboratory, we thank the Corporate Sponsors listed below and encourage other companies to help us promote research through this Program.

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President's Report

1995 was a year of consolidation and growth for the Cold Spring Harbor Laboratory Association. Our mission in making new friends and maintaining old friends, in fund-raising for the support of postdoctoral scientists, in providing support services for our scientific personnel, and in attempting to maintain and improve community relations was conducted with enthusiasm and resolve. Membership in the Association now stands at 746. Moreover, efforts were made to diversify the constituency of Laboratory support through mailings, personal contact, discussions with visitors, communications with different school districts, and other community organizations.

Our year started out with our annual meeting held on February 5th. The experience was enriching in that our principal speaker was Dr. Rich Roberts, a 1993 Nobel Laureate who did the scientific work resulting in his prize at Cold Spring Harbor Laboratory. His talk entitled "From Split Genes to Stockholm" covered his research work leading up to the award and also provided us with vignettes of the ceremony in Stockholm.

On March 25th, a fabulous evening was held at the Laboratory; we were entertained by Flicka von Stade and three accompanists, Bruce Brubeck, Bill Crofut, and Joel Brown. The music was truly enchanting, with a medley of different musical work expressed through voice, piano, guitar, and banjo. The proceeds from the evening went to the Undergraduate Research Program. With the help and support from the Association, the performance was a unique and interesting musical experience.

In April, at the instigation of Jordan Saunders, a luncheon was held in New York with our Director, Bruce Stillman, as the principal speaker. The meeting was a success, and kudos go as well to Holly Brooks, Maureen Augusciak, and Mary Lindsay.

The Spring mailing for the Association Annual Fund began in May, and we thank each of our solicitors for their ardent efforts in the fund-raising effort and also for the energy expended to acquire new friends for the Laboratory.

On June 4th, during the annual Cold Spring Harbor Symposium, the Dorcas Cummings Memorial Lecture was held, an annual event that is organized and sponsored by the Association. Dr. Günter Blobel, a member of the Board of Trustees and a research scientist at the Rockefeller Institute, spoke on "How Proteins Find Their Addresses in the Cell." As is tradition, the lecture was followed by special dinner parties hosted by members of the Association and friends of the

Yuri Lazebnik (son of CSHL scientist Yuri Lazebnik) and Nobel prize winner Rich Roberts at the CSHL Association Director's meeting.

Laboratory. Here, scientists who attended this important meeting met and mixed with our wonderful friends. Special thanks go to each of our host and hostesses:

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With the fall came the introduction of a new lecture series called Next Generation Initiative, which was held on four separate Thursday nights in October and November. The idea for this new series was initiated by members of our community who felt that an approach should be developed to introduce new and younger members of the community to the Laboratory. Each meeting was preceded by a sandwich and salad buffet, which was followed by a lecture by members of the scientific staff at the Laboratory. The talks were enriching. It is our hope that we can now improve upon this good start. In addition, during the fall, through the efforts of Skip Hargraves, the Association sponsored an estate-planning seminar conducted by Jennifer Jordan, a Trusts and Estates partner at Cadwalader, Wickersham & Taft. Jennie was supported in this presentation by Townie Knight, and for those who attended, it was certainly an informative experience. A significant new event benefiting the Association and raising nearly $8,000, namely, the Tree Symposium, brought a new constituency to the Laboratory. Carol Large, our Vice President and a Trustee of Old Westbury Gardens, organized the event, which was a collaborative effort with Old Westbury Gardens and the Cornell Cooperative Extension of Nassau County. It drew many new faces not only from Long Island, but also from other parts of the northeast. More than 300 people attended this symposium.

In the fall, the efforts of our solicitors, and also an improved computer capability, allowed us to solicit approximately three times as many friends as we have in the past. These new prospects came to us from a variety of sources, including visits to the Laboratory, public inquiries, and suggestions from members of people expressing interest in Laboratory activities. Prospecting is a worthwhile exercise. We hope to continue to enlarge our membership base in the forthcoming year. The final report reflects gifts by 162 major donors of $1,000 or more. The total number of donors increased to 767 with total receipts in excess of $662,000, which exceeded last year's receipts by

more than 9% and our goal of $600,000. All in all, it was a tremendous effort conducted by many people including our directors.

On Sunday evening, October 22nd, John and Lola Grace gave an elegant buffet at their home in Cove Neck to thank the Association's major donors. Guests included Laboratory scientists and staff, and those in attendance were treated to a night of informal conversation and discussion. The pleasure of the evening was enhanced by active participation of John and Lola's daughters, Lorraine and Victoria, and the introduction of John, Jr.

It is my privilege to acknowledge the significant effort given to the Laboratory and the Association during the past six years by Jane Greenberg and Doug Rogers. In their different ways they fostered considerable support and expended effort in furthering the interest of the Association and the Laboratory for which we are very grateful. In addition, a special thank you to Dr. Donald Kent for his service as a Director in 1995. A special thanks to Jean Schwind who completes her first year as our staff director and to the other members of the Laboratory development organization who have given of their time and effort to support the work of the Association.

February, 1996

John P. Cleary

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Benefits	8,308
Other	785
Total	**$ 653,785**

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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 regional high school students, is to produce future research scientists and to encourage awareness among young people in general.

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A genetic education seminar was held in April 1995 for professionals who interface with genetic technology. Participants included employees of Oncogene Science and other New York metropolitan area companies. The seminar raised $2,005 for the Annual Fund.

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