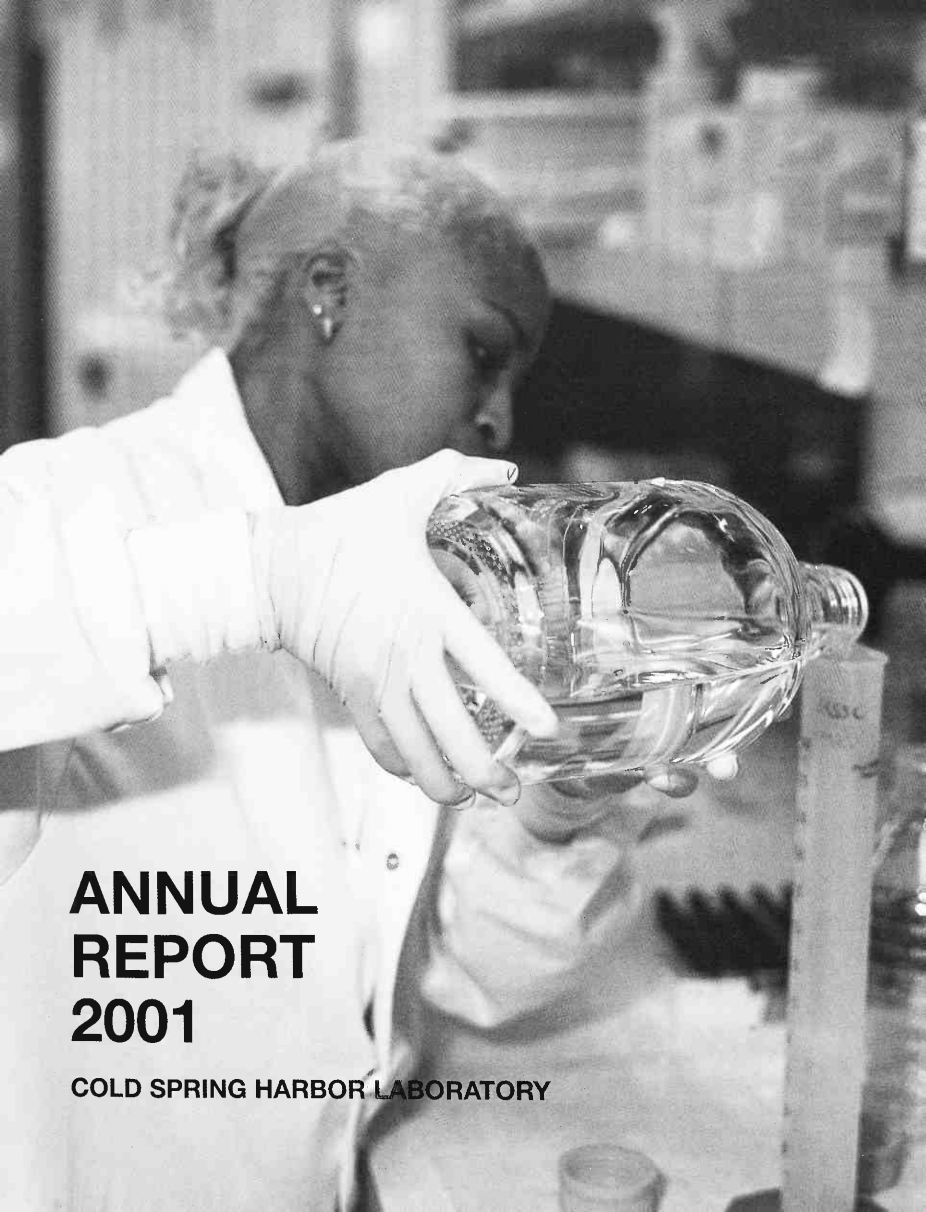
ANNUAL REPORT 2001

COLD SPRING HARBOR LABORATORY



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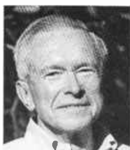

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DIRECTOR'S REPORT

The horrible events in September of 2001 in New York, Washington, and Pennsylvania changed our nation forever. No longer can we assume that those who are ignorant of decency and what America stands for will not interrupt our way of life. For those of us who work on research to improve the lives of people, it is difficult to understand how anyone could get to the point of needlessly killing so many. Immediately following September 11, our research goals seemed pale compared to the efforts of those who stood in the face of terrorism and who worked in the rescue efforts. But it did not take long to realize that our mission to fight cancer and other diseases is equally important. The paradox is that the nation which provides the most benefits to mankind through biomedical research is the one that is attacked because of what it stands for. But giving in to tyranny is not in the ethos of this nation. The best way to answer terrorism is to continue to do what we do best, and that is to help those who cannot help themselves. This is particularly true for those with cancer.

The modern era of our nation's effort to understand and treat cancer, which began with the signing of a new National Cancer Institute Act by President Nixon in December of 1971, is now 30 years old. Although the National Cancer Institute first came into existence in 1937 with the signing of an act by President Franklin D. Roosevelt, only a revolution in biological research in the latter half of the twentieth century enabled cancer to be studied in a rational way and with confidence that success might be possible. During the past 20 years, basic research into the causes that underlie cancer has opened the door to opportunities for diagnosis and treatment such that now, as we enter a new era in cancer research, meaningful progress is possible. To fully exploit these opportunities, however, academic research institutions and the private sector must approach the cancer problem in a fundamentally new way. Although investigator-initiated research should remain the backbone of publicly funded research, large projects that move basic research results into the clinic, commonly called "translational research," require close cooperation between academia and the private sector. Interdisciplinary approaches are the future for research that will make a real difference to patients, but achieving ambitious goals solely with public funds may not be possible. It is now time to re-think how translational cancer research should be assessed, supported, and performed.

The roots of the modern understanding of cancer came from a number of sources. Prominent among these was the study of viruses that caused tumors at the site of inoculation in experimental animals. These viruses carried genes that could change a normal cell into a cancer cell. Research using both RNA and DNA tumor viruses, with Cold Spring Harbor Laboratory preeminent among institutes studying DNA tumor viruses, showed that a small set of genes could transform otherwise healthy cells into cells that grew into a lethal tumor, eventually metastasizing and killing the animal. But relatively few virus genes proved to have any part in inducing human cancers. Notable exceptions were the transforming genes present in the DNA-containing papillomaviruses that, when carried into the epithelial or glandular cells of the cervix, initiate cervical cancer in women.

A few cancer-causing genes were found in RNA tumor viruses that had direct orthologs in human cells. These virus-related human genes, when altered by mutation or when overexpressed, contributed to human cancer. Michael Wigler codiscovered here at CSHL, at the same

time as Robert Weinberg at the Massachusetts Institute of Technology, one such gene in 1981, the so-called *ras* oncogene. Another notable example was the v-*myc* gene that was first defined in an avian retrovirus that caused myelocytic leukemia in chickens. Later, a related human gene called c-*myc* was found to be overexpressed in a variety of tumors, including lymphomas, leukemias, and lung, cervical, ovarian, breast, and gastric cancers. The c-*myc* gene was converted into an oncogene either as a result of chromosome translocation (the aberrant exchange between two unrelated chromosomes), by gene amplification, or by mutation directly in the gene itself.

Cancer progression can occur when another type of human cancer gene, called a tumor suppressor gene, is deleted in the cancer tissue, again contributing to the tumor cell's ability to proliferate uncontrollably. This type of cancer gene was first appreciated by studying the inherited predisposition of cancer in families that had a high incidence of rare cancers. Later, in the late 1980s and early 1990s, familial cancer genetics identified a series of important oncogenes and tumor suppressor genes, including the now well-studied *BRCA1* breast cancer susceptibility gene. Recent research, however, has shown that the number of people who have a higher probability of succumbing to a particular type of cancer by inheriting a defective gene from their parents is relatively small compared to those who have no obvious inherited predisposition, but nonetheless get the disease. It is therefore more likely that the main burden of cancer in our population occurs because of the accumulation of genetic changes that occur within a person's lifetime. Some of these changes may be promoted by environmental factors such as cigarette smoking, but others are a result of normal damage to the genome over time. Our longevity plays in favor of acquiring the necessary genetic changes that can result in cancer. In a quirk of fate, some cancer-promoting mutations actually cause further genome instability, thereby accelerating the process of acquiring more genetic changes and, ultimately, cancer.

During the past few years, a new view of cancer has emerged which suggests that the cancer cell itself, with all its genetic changes, is not the only culprit in the progression to metastatic tumor growth. This view of cancer has been best put forward in a review by Cold Spring Harbor Laboratory alumnus Douglas Hanahan and Robert Weinberg. They pointed out that for a tumor to develop to the stage where it is a clinical problem, the tumor must have a number of acquired characteristics. These include changes that allow the cancer cells themselves to produce and receive growth signals, to avoid being killed by a process called apoptosis, to proliferate with limitless potential, to attract a blood supply to sustain the increase in cell mass, and to invade and escape from the surrounding tissue. I would add another acquired characteristic—that of escaping from the body's immune surveillance that almost certainly helps in suppressing tumor growth, but of which little is known.

There are many ways of collecting the set of acquired tumor characteristics, and clearly accumulating genetic changes in the cancer cell is the primary driving force. But as Hanahan and Weinberg point out, cells that surround the cancer, such as invading immune system cells and the surrounding "normal" tissue, can provide many of the factors necessary to sustain and even change a cancer cell. Importantly, this new view of cancer progression offers a new way of thinking about cancer therapy. If the acquired characteristics are necessary to create a clinically dangerous tumor, then attacking one of them should provide new hope for cancer treatment. Attacking two different acquired characteristics might provide a benefit greater than the sum of the two alone, and so on. We are just entering an era where this thinking is being tested in the development of new cancer therapies.

The most common method of treating cancers now is to treat the tumor with agents that cause catastrophic damage to cells, such as chemotherapy with DNA intercalating drugs, with

drugs that damage the apparatus that ensures accurate segregation of chromosomes, or with radiation that both damages DNA and causes chromosome mis-segregation. These methods take advantage of the loss in cancer cells of the normal response to such external stresses, allowing the cancer cell to proliferate and eventually die due to the catastrophic accumulation of damage. But as we well know, such therapies are toxic to normal cells as well, and the window between killing cancer cells and normal proliferating cells is often all too small. Moreover, as clearly shown by Scott Lowe and his colleagues here at CSHL, cancer mutations such as those in the p53 tumor suppressor pathway can cause resistance to such treatments. This is why cancer therapies must be multidimensional, attacking the tumor from different angles. These new approaches include targeting the proteins in a cancer cell that initiate the tumor to proliferate in the first place, inhibiting the blood supply by anti-angiogenesis therapy, and inducing an immune reaction to the tumor cells.

A priori, it might seem that the protein products of the genetic changes that occur in a cancer cell might not be good targets for cancer therapy because they usually occur early in the life of the cancer cell. Since many genetic changes occur during the life of cancer cells, there is no guarantee that inhibiting one oncogene product will be sufficient to inhibit growth of the cancer cell and even shrink the tumor. But recent results from both basic and clinical research suggest that there is hope for this approach.

Recent research from Michael Bishop, one of the pioneers of cancer genetics, and his colleagues suggests that the primary changes in a cancer cell may well be an Achilles' heel. They created tumor cells that overexpressed the c-*myc* gene under conditions where it could be turned off at will. In experimental animals, c-*myc*-dependent tumors arose with predictable frequencies, but interestingly, and for many people unexpectedly, when the c-*myc* gene was turned off, not only did the tumors stop growing, but the cancer cells died and the tumor regressed. Thus, although a cancer might require multiple genetic changes, targeting a single oncogene product might be sufficient for therapy.

Concomitant with this basic research, a new, targeted therapy for chronic myeloid leukemia (CML) emerged with the advent of the Novartis drug Gleevec™, producing remarkable clinical effects. This small-molecule drug inhibits an enzyme produced by the *BCR-ABL* oncogene that is the principal cause of CML. Early in the clinical studies, patients responded dramatically, demonstrating that a therapy against a single oncogene product can be most effective. Later, however, some patients developed resistance to the drug and their tumors relapsed. When examined at the molecular level, the *BCR-ABL* oncogene either had further mutated or was now overexpressed at higher than initial levels. Although this was not good for the patients concerned, it proved a very important scientific point—that the drug was really making a difference by targeting the *BCR-ABL* oncogene that caused the tumor, rather than a combination of unknown targets that might be related to *BCR-ABL*. These clinical results validated the concept of single-target, specific anticancer cell therapy and rightly caused much excitement.

As I described in last year's Annual Report, Michael Wigler, Robert Lucito, and their colleagues here at CSHL, in collaboration with Scott Power's group at a nearby biotechnology company, have undertaken a large project to identify many of the genetic changes that occur in primary human tumors. Initially focusing on breast cancer in collaboration with clinical colleagues at Memorial Sloan-Kettering and Duke University School of Medicine, they developed techniques that are applicable to all cancers, given sufficient resources. The overrepresented or amplified oncogenes in cancer cells, like c-*myc*, are readily detected in cancer DNA derived from primary cancer biopsies. During the past year, this joint program between an academic research institution and the private sector has been enormously successful, with the identification of many

new human oncogenes that have the potential to become targets for anticancer therapy. Indeed, some of the gene products are already under preclinical investigation. Paradoxically, the success of the project points to a fundamental problem of how to fund such large cancer research projects.

In the not-too-distant past, basic research emerging from academic research laboratories would be published in the scientific literature. Only much later would private industry incorporate the published results into their disease programs. With the advent of the biotechnology industry, biotech companies more rapidly acquired the results from basic research laboratories and, with the help of the significant financial resources from venture capital, they could add commercial value and, in a very few cases, take products into the clinic. But very few basic research results are immediately applicable to clinical advances, and it often takes years of additional research to reach the stage where large pharmaceutical companies would invest the considerable sums needed to develop a drug. In the vast majority of cases, translational research is conducted, further developing the initial research so that it can be applicable in the clinic. The problem that arises, however, is that translational research is expensive and involves scale-up of the basic research capabilities that is beyond the resources of public funding mechanisms such as grants from the National Institutes of Health (NIH). A typical, investigator-initiated research grant from the NIH could not support such translational research, since it often involves the use of chemistry and other resources only available to large pharmaceutical companies. Bridging this large gap must be a high priority in disease-based research.

Mechanisms are needed that radically change the way such projects are viewed and supported. Clearly, public funds from granting agencies are not going to keep pace with the rising costs of research. Even though venture capital resources have grown significantly in the past decade, they will not carry the entire burden of the rapidly expanding biomedical research enterprise. The pharmaceutical industry is already inundated with drug targets, and they will only occasionally invest in the earliest stages of research outside of their internal programs. It seems that the most efficient method for translating interesting new research ideas into the preclinical stage is an intimate interaction between the biotechnology industry and academia.

Although interactions between academia and the biotech industry are going on all the time, and in many ways changing the research landscape, there are inadequate funding mechanisms to allow seamless cooperation. Individuals obtaining relatively small research grants for specific projects support the vast majority of traditional academic cancer research. This should continue, but the NIH should introduce new mechanisms that allow rapid scale-up of research when it is appropriate and at the same time allow seamless integration of funds from private sources, be they from private foundations or industry. Currently, it is very difficult to present a large translational research program to the panels that review smaller, investigator-initiated research grants. Large, multifaceted projects that need resources from the NIH and industry should be presented and reviewed as a single project, with the NIH grant funds supporting some of the research. The NIH already has the capacity to support clinical trials in collaboration with industry, but it is translational research, which links basic and clinical research and advances new ideas, that is not now planned and reviewed in the most efficient way. Many opportunities are being missed because of the lack of suitable funding mechanisms and flexibility.

There also needs to be a change in the way such projects are viewed by the NIH. Most cancer research in the United States occurs in Cancer Centers. These Centers receive core support based primarily on the grant funds that derive from the National Cancer Institute (NCI) and other peer-reviewed cancer research from public sources. But under current NCI guidelines, research supported by funds from private sources is not considered relevant for core funding when a

Cancer Center is periodically assessed for what it is doing in translating basic research into the clinic. As private-public cooperation in research funding increases, and it must in this new era, such impediments to translational research should not deter what Cancer Centers might do in the future.

Thirty years after initiating the modern era of cancer research, the NCI is in excellent shape and has the capability to rapidly respond to the opportunities made possible by the huge advances in basic research. I had the privilege of serving on an oversight board at the NCI for the past 6 years, the last 2 years as chairman, and seeing firsthand how large projects could work. Under the direction of Rick Klausner, the NCI was revitalized. Many opportunities were advanced with great success, particularly those that took advantage of the concomitant sequencing of the human genome. Now is the time to apply some of the same strategies to translational research, such as discovering new technologies for early diagnosis and, most importantly, for cancer treatment.

There has been a call for a complete rewriting of the National Cancer Institute Act to revitalize the nation's effort on cancer. The stated goals are to expand the number of researchers studying cancer, particularly those in translational research; to encourage the private sector to focus on cancers that as yet do not have standard therapies; to improve the number and efficiency of cancer trials; to increase research on cancer prevention; and to improve patient care. These are all laudable goals, but in achieving them, the mission of the NCI must be underpinned by strong basic research. As basic research provides opportunities for many new treatments, no amount of public funds will support the infrastructure to test all of the potentially beneficial approaches. A better mechanism for public-private cooperation is needed.

The new proposed legislation calls for an expansion of the Cancer Centers program to establish translational cancer centers to help move drugs and technologies into clinical trials. Again, this seems to be a valuable goal, but it is already the mission of existing Comprehensive Cancer Centers who are required to combine basic and clinical research. Effective translational research should be accomplished by the nation's best Comprehensive Cancer Centers. It is far from clear whether a new and potentially expensive physical infrastructure is needed. Furthermore, there is no guarantee that the most promising research applications will emerge from within the proposed new translational centers. Equally likely is that centers such as our own will contribute valuable new approaches that will have a large impact on translational cancer research.

More effective ways are needed to integrate vast private resources into partnerships with academic cancer research centers, and in a manner that will not penalize the core support for the Cancer Center. The existing peer-review mechanism for small, investigator-initiated research, which can take up to 1 year or more before a new proposal may be supported, is not adequate. Since academic institutions do not by themselves have the resources to establish such large translational programs in the hope that a proposal might be supported in 1 year's time, many opportunities are lost. One simple and effective way for such cooperative programs to be implemented is to institute a separate peer-review mechanism that can appreciate and assess translational research, particularly research that involves private and academic interactions. Precedents have been set at the National Institute of Human Genome Research, where very large, goal-oriented, peer-reviewed research involves industry and academic laboratories, with industry often assuming a large portion of the costs. Other institutes such as the NCI should rapidly move in this direction, establishing new mechanisms that could easily handle issues such as conflicts of interest and the expected large multicomponent budgets, and yet still move with a pace that is expected by a public calling out for meaningful action against cancer.

HIGHLIGHTS OF THE YEAR

The research and education programs at Cold Spring Harbor Laboratory continue their strong momentum. The Watson School of Biological Sciences recruited its third class of students this year, and the DNA Learning Center expanded both its physical plant and its educational objectives. The Meetings and Courses program and Banbury Center continue to be invaluable resources for scientific information, and the Cold Spring Harbor Laboratory Press added several new projects and properties to its long list of titles. Cold Spring Harbor Laboratory was once again a bustling center of scientific activity, discovery, and education.

Research

Bioinformatics

On February 15, 2001, some 20 genome research centers around the world, including W. Richard McCombie's group at Cold Spring Harbor Laboratory, jointly published the first draft of the complete human genome sequence and made it freely available to researchers throughout the world. The finished sequence is scheduled to be released in 2003, coincident with the 50th anniversary of the discovery of the structure of DNA. The complete human genome sequence can be used in a great many ways (see below) and has already fueled an explosion of biological and biomedical research—much of it directed toward improving human health.

Analysis of the raw DNA sequence through the use of various bioinformatics (computer-based) methods revealed several interesting features of the human genome, including the preliminary finding that our genome contains approximately 30,000–40,000 protein-coding genes. A similar analysis of restricted raw data by a private concern revealed most of the same features.

In a companion study that accompanied the publication of the human genome first draft, Lincoln Stein, his CSHL colleagues, and other members of the International SNP Map Working Group identified some 1.4 million *s*ingle *n*ucleotide *p*olymorphisms (or SNPs) distributed throughout the human genome. The high-density map of human DNA sequence variation will become useful for identifying disease-related genes, for tailoring therapies to those patients who are most likely to respond, and for several other applications. Importantly, this project was jointly funded by a consortium of pharmaceutical companies, private foundations, and the federal government, providing a model for future ways of funding large projects and making the data freely available to the broader scientific community.

Lincoln Stein

To study and manipulate genes for a wide variety of research, diagnostic, or therapeutic purposes, scientists need to determine the precise fine structure of genes against a backdrop of what is frequently a vast and complex genetic landscape. Conventional bioinformatics software fails when it comes to detecting two important features of genes—the very first segments of genes, and the nearby "on" switches of genes called promoters.

Michael Zhang and his colleagues have developed a computer program called First Exon Finder (or FirstEF) that is especially good at finding these first segments and "on" switches of genes. The program is tailored toward detecting these features in the human genome sequence, but it is also useful for annotating other mammalian genomes. Although the total number of genes in an organism's genome depends on subtle, nonuniversal definitions of what constitutes a gene, on the basis

of his analysis of the human genome using FirstEF, Michael believes that there are 50,000–60,000 fundamental protein-coding human genes and that the preliminary estimate of 30,000–40,000 human genes is too low.

We will test these computer predictions and have established a joint research initiative involving the laboratories of Michael Zhang, Greg Hannon, and Dick McCombie. These laboratories will experimentally verify the new predicted genes. Greg will incorporate the findings into a project whose goal is to determine the function of human genes that have heretofore not been assigned a function.

Michael Zhang

Cancer

Michael Wigler, Robert Lucito, and their colleagues are developing a reproducible, high-resolution technique for detecting changes in gene copy number that are associated with the initiation of breast, ovarian, and prostate cancer or with the progression of these cancers from a noninvasive to an invasive or metastatic state.

Michael Wigler

The technique, called *r*epresentational *o*ligonucleotide *m*icroarray *a*nalysis, or ROMA, combines DNA microarray technology with a method Michael previously invented to simplify the search for differences between two sets of DNA (representational difference analysis). Using ROMA, Robert and Michael can scan the entire genomes of both normal cells and cancer cells taken from the same patient and can detect the genetic differences between the two. To apply ROMA to the study of breast cancer, a needle biopsy of as few as 1,000–10,000 cells from breast tissue (followed by isolation of the normal and cancer cell DNAs) provides sufficient material.

Genes that are amplified (potential oncogenes) or deleted (potential tumor suppressor genes) in cancer cells—or in invasive/metastatic versus noninvasive tumors—are prime targets for improved methods to diagnose and treat cancer. A strong team has been established to develop this project: Robert and Michael are joined in several of their studies by clinical colleagues at Memorial Sloan-Kettering Cancer Center (Larry Norton), bioinformatics colleagues at CSHL (Bud Mishra, who holds a joint appointment at the Courant Institute of Mathematical Sciences at New York University), and Jonathan Melamed (also at the Courant Institute), and the local division of Tularik, Inc., a biotechnology company (Scott Powers). To date, these researchers have identified some 20 candidate genes associated with cancer by using ROMA and other genomics approaches.

Rob Lucito

Remarkably, the major limitation at this stage in the development of ROMA is neither theoretical nor technical, but economic. Robert and Michael estimate that the cost of synthesizing sufficient numbers of DNA oligonucleotides to reliably detect small deletions in the genomes of cancer cells is on the order of $2 million. However, once these oligonucleotides are available, they will be sufficient to fabricate 40,000 individual ROMA microarrays, enough to study many human cancers.

Epigenetics

Epigenetics is commonly defined as the study of heritable changes in chromosome structure or gene function (e.g., transcriptional activity) that occur without changes in DNA sequence. As work

at CSHL and elsewhere has shown, proper functioning of epigenetic mechanisms of inheritance, such as maintaining stable gene silencing over many cell divisions, is essential to many aspects of normal biology. Moreover, CSHL researchers have linked abnormal silencing of tumor suppressor genes to cancer.

In the past year, work in my own laboratory (in collaboration with Rui-Ming Xu), as well as studies by Greg Hannon, Shiv Grewal, David Spector, and Rob Martienssen, has revealed several molecular features of epigenetic phenomena.

Working with yeast, worms, flies, plants, and mammals, CSHL researchers are establishing comprehensive models for different aspects of epigenetic inheritance, including RNA interference, the modification of histone proteins and DNA (e.g., by methylation), and importantly, how these processes work together to establish and maintain silent versus active chromatin (DNA plus protein architecture). These models unify many genetic and biochemical observations and have a significant impact on how we view the biology of DNA.

RNA Interference: Double-stranded RNA triggers potent, specific gene silencing through a process called RNA interference (RNAi). A central component of RNAi is a nuclease, composed of both RNA and protein, which Greg Hannon and his colleagues first characterized and dubbed RISC (*R*NA-*i*nduced *s*ilencing *c*omplex). This nuclease seeks and destroys other RNA molecules (e.g., messenger RNA) homologous to the short guide RNAs or "siRNAs" (*s*mall *i*nterfering *RNA*s) it carries.

Greg Hannon

In 2001, members of Greg's lab reported two significant RISC-related observations. First, they showed that the siRNA molecules within RISC are derived from longer, double-stranded RNA through the action of an enzyme they aptly named Dicer. They noted that Dicer is evolutionarily conserved in yeast, worms, flies, plants, and mammals. Second, through biochemical purification of RISC, they showed that RISC itself contains a different RNA-cleaving enzyme, plus a protein called Argonaute2, which Greg believes is one of several such components that targets RISC-mediated inhibition of gene expression to various biochemical pathways. These findings are among several links being forged at CSHL between the genetics and biochemistry of RNAi, and between RNAi (post-transcriptional silencing) and other epigenetic phenomena such as transcriptional silencing and transposon activity.

As often happens, basic research on biology opens doors that are unexpected. It is now possible to synthesize RNAi molecules and to use them to individually and specifically shut off the expression of virtually every human gene. Greg Hannon and his colleagues have developed some of these technologies and have established a large project to use RNAi to investigate the function of human genes. The technology has spread like wildfire around the Cold Spring Harbor campus, and rightly so, because it has the potential to change how we study mammalian biology.

Transcriptional Silencing: By studying transcriptional silencing of mating-type genes in fission yeast, Shiv Grewal and his colleagues have shown that seemingly small differences between two varieties of histone H3 have profound effects on chromatin structure, gene expression, and recombination. They found that silent regions of chromatin—where genes are kept off and DNA resists genetic recombination—contain a particular variety of histone H3 (H3 Lys-9-methyl). In contrast, they observed that active regions of chromatin—where genes can be easily switched on and DNA can readily recombine—contain a different variety of histone H3 (H3 Lys-4-methyl). Through this and other work, Shiv has defined a highly conserved pathway wherein enzymes that modify histones (deacetylases and methyltransferases) act cooperatively to establish a "histone code" leading to epigenetic silencing.

In addition, Shiv showed that deleting "boundary elements" that mark the transition between silent and active chromatin allows the spreading of H3 Lys-9-methyl, and hence a silent chromosome architecture, into neighboring DNA normally occupied by H3 Lys-4-methyl. This finding has important implications for genetic disease caused by abnormal gene silencing. Indeed, in 2001, Scott Lowe, Yuri Lazebnik, and colleagues found that abnormal silencing of the tumor suppressor gene Apaf-1 leads to malignant melanoma.

My own laboratory has uncovered mechanisms of inheritance of epigenetically determined states of gene expression and has provided links to DNA replication proteins. Recently, in collaboration with Rui-Ming Xu, we have determined the three-dimensional structure of part of the origin recognition complex (ORC) that recruits gene silencing proteins to specific loci in the yeast genome. ORC was discovered here 10 years ago as a key regulator of genome duplication.

Shiv Grewal

X Inactivation: In species such as humans, where females have two X chromosomes and males have one, the proper proportion of X chromosome gene expression ("dosage compensation") is maintained in females through the global inactivation of one of the two X chromosomes. X inactivation stems from the initiation (at a locus called the *X-i*nactivation *c*enter or *Xic*) and spread of an inactive chromatin state. Establishment of the inactive chromatin state begins with the coating of the X chromosome by "Xist" RNA molecules, followed by large-scale remodeling of chromatin structure.

Until recently, little was known about either the molecular partners of Xist RNA that enable it to coat the X chromosome, or the precise nature of the chromatin remodeling induced by this coating. Visiting scientist Edith Heard suspected that the same histone H3 variety that Shiv Grewal found to establish silent chromatin in fission yeast (H3 Lys-9-methyl) might also mediate X inactivation. Using mouse embryonic stem cells, Edith and David Spector showed this to be true by measuring when and where (relative to other events) H3 Lys-9-methyl appears on X chromosomes marked for inactivation. They found that Lys-9 methylation of histone H3 occurs immediately *after* the coating of the X chromosome by Xist RNA and *before* the silencing of X-linked genes. Thus, Edith and David could conclude that Lys-9 methylation of histone H3 is a definitive early event in the X inactivation process.

DNA Methylation and Transposon Activity: Rob Martienssen explores the role of transposable elements (transposons) in epigenetic regulation and genome organization using both maize and the mustard relative, *Arabidopsis*. In plants and many other organisms, the DNA of inactive transposons is generally methylated, whereas the DNA of canonical genes and active transposons is generally unmethylated. One of Rob's interests is to determine how these methylated and unmethylated regions of chromatin are established and maintained, and to explore the biological consequences of normal and abnormal states of DNA methylation.

Several years ago, Rob isolated an *Arabidopsis* mutant called *ddm1* (*d*ecrease in *D*NA *m*ethylation) in which methylation of DNA was decreased. In 2001, Rob and his colleagues showed that transposons become demethylated in *ddm1* mutants and that such transposons become both transcriptionally active and activated for movement from place to place in the genome. Next, Rob and visiting scientist Vincent Colot showed that inactive transposons are preferentially associated with (you guessed it) the same variety of histone H3 that Shiv found ito be associated with silent regions of chromatin in fission yeast, and which Edith and David found to be involved in X inactivation (H3 Lys-9-methyl). Rob and Vincent also showed that transposons activated in *Arabidopsis ddm1* mutants become preferentially associated with the variety of histone H3 that Shiv found to be associated with active regions of chromatin in fission yeast (H3 Lys-4-methyl).

Finally, working together, Rob, Shiv, and their colleagues showed that fission yeast homologs of the RNAi components Dicer and Argonaute are involved in silencing ancient relics of transposons known as centromeric repeats. Moreover, *dicer* and *argonaute* mutations in fission yeast, like *ddm1* mutations in *Arabidopsis*, lead to the replacement of the silent chromatin-associated variety of histone H3 (H3 Lys-9-methyl) by the active chromatin-associated variety (H3 Lys-4-methyl).

These observations suggest a model in which components of the RNAi machinery target histone-modifying enzymes to regions of chromatin to be silenced or kept silent (centromeric repeats, transposons, and possibly other regions such as inactive X chromosomes in female mammals). Furthermore, Rob suspects that in those situations where DNA methylation is involved in epigenetic inheritance, the silent chromatin-associated variety of histone H3 (H3 Lys-9-methyl) may indirectly recruit enzymes that methylate DNA.

Molecular Biology

In the United States, approximately 70,000 deaths from cancer per year are associated with genetic alterations in the *myc* oncogene. This gene encodes a protein—the Myc transcription factor—that is a potent stimulator of cell proliferation. Because Myc is a potent growth stimulator, the level of Myc within cells is normally tightly regulated. This level is determined by how much Myc is synthesized in a given time frame and by how quickly it is destroyed.

Bill Tansey

William Tansey and his colleagues are studying how the destruction of the Myc protein is regulated and how defects in this process lead to abnormally high levels of Myc and to cancer. In so doing, they have uncovered an intriguing connection between two seemingly unrelated processes—protein degradation and transcriptional activation. Bill's findings support a "licensing" mechanism in which a particular protein modification (ubiquitylation) simultaneously activates transcription factors that switch on gene expression and primes them for destruction. The net result of this licensing mechanism is to limit how long genes remain switched on. Bill suspects that this mechanism is an efficient way for the cell to limit the effects of many of its most potent transcription factors.

Moreover, work in Bill's lab indicates that a link between transcription and protein degradation has been conserved since the evolutionary divergence of yeast and mammals approximately 1 billion years ago. Thus, Bill and his colleagues appear to have uncovered a phenomenon of fundamental biological importance.

Tim Tully

Neuroscience

Learning & Memory: Josh Dubnau and Tim Tully have used a genetic strategy in fruit flies to switch electrical activity on and off at will in the "learning center" of the insect's brain called the mushroom body. In so doing, they have made the surprising discovery that switching off electrical activity in this part of the fly brain blocks memory recall but not the initial formation of a particular kind of memory.

In some respects, fruit fly brains work very much as do the brains of other animals, including humans. Both flies and humans are capable of the kind of "associative learning" made famous by Pavlov's dogs. (After ringing a bell and presenting dogs with food several times over a few days, the Russian physiol-

ogist Ivan Pavlov [1849–1936] found that eventually his dogs would display dinnertime behavior [drooling, excitement] upon hearing the sound of the bell alone.)

Josh and Tim study olfactory associative learning by training flies to avoid a particular odor and later measuring the flies' odor avoidance behavior. They found that memories based on olfactory associative learning can be acquired and stored in the absence of electrical activity in mushroom bodies. However, those memories cannot be recalled in the absence of such electrical activity. These results suggest that memories are acquired and stored by chemical processes in mushroom bodies, and that electrical activity in this part of the brain is necessary only to recall those memories. In some instances, when it comes to learning and memory, the fly brain (and perhaps ours) must function electrically to recall what is stored chemically.

Brain Imaging: Karel Svoboda continues to pioneer the application of a high-resolution, real-time imaging technique called two-photon microscopy to brain research. Recently, Karel and his colleagues have used two-photon microscopy to examine abnormalities during brain development in a mouse model of Fragile X syndrome. In humans, Fragile X syndrome is the most commonly inherited cause of mental impairment. The syndrome is typically caused by a mutation in the *FMR1* gene leading to absence of the *F*ragile X *m*ental *r*etardation *p*rotein or FMRP.

By using two-photon microscopy to compare the earliest stages of postnatal brain development in normal and *FMR1* knock-out mice, Karel and Esther Nimchinsky have detected dramatic differences in the density and length of all-important neuronal structures called dendritic spines. Such spines form the familiar connections between neurons called synapses. When examined 1 week after birth, the "barrel" cortex in the brains of Fragile X mice displayed significantly increased density and length of spines. (Barrel cortex interprets neurological signals from the whiskers of newborn animals as they explore their world for the first time.) However, by 4 weeks after birth, differences in the density and length of spines between normal and Fragile X mice were largely undetectable. The transient nature of the spine abnormality in Fragile X mice suggests that FMRP functions soon after birth during a critical window of opportunity for brain development. In addition, Karel's findings indicate that FMRP probably has a role in linking experience (e.g., whisker signals) to the growth of dendritic spines and that such links are required to specify the elaboration of a fully functional neuronal architecture in the brain.

Awards and Honors

Several members of the Laboratory community were honored in 2001 for their work and achievements.

Nick Tonks

CSHL Professor Nick Tonks was appointed a Fellow of the Royal Society of London, England's National Academy of Sciences. Since 1660, election to the Fellowship has been recognized as one of the highest honors in science. Dr. Tonks joins CSHL President Jim Watson and myself as CSHL's Fellows of the Royal Society.

Scott Lowe

Laboratory President James Watson received an honorary knighthood from the Queen of England in the New Year's Honours List. This is a rare honor for a United States citizen, and recognizes Dr. Watson's accomplishments from the double helix to the human genome, among others.

CSHL Professor Scott Lowe, the Deputy Director of the Cold Spring Harbor Laboratory Cancer Center, was honored this year with the Cornelius P. Rhoads Memorial Award of the American Association for Cancer Research (AACR). The

award recognizes groundbreaking contributions to cancer research and was established in 1979 to honor the memory of Dr. Cornelius P. Rhoads, the founder and first director of the Sloan-Kettering Institute for Cancer Research.

Song-Hai Shi

Song-Hai Shi, a 2000 graduate of the joint Genetics Program at Cold Spring Harbor Laboratory and Stony Brook University, was awarded the 2001 Amersham Biosciences and *Science* Grand Prize for work he carried out in the neuroscience lab of Robert Malinow at Cold Spring Harbor Laboratory. The award recognizes the most outstanding Ph.D. thesis among graduate students in molecular biology. He received the award in Stockholm at the time of the Nobel Prize ceremonies in December.

I was pleased this year to be elected as a foreign member of The European Molecular Biology Organization (EMBO), the organization that leads, guides, and promotes biological sciences in 24 European countries.

Symposium LXVI

From May 31 to June 5, an enthusiastic group gathered for the 66th annual CSHL Symposium, titled "The Ribosome." On Sunday, June 3, meeting attendee Dr. Venki Ramakrishnan, of the Laboratory of Molecular Biology, MRC, Cambridge, delivered the annual Dorcas Cummings Memorial Lecture to a scientific and public audience. This very successful annual event is hosted

"Sir" James Watson

Jim Watson and Venki Ramakrishnan

by the CSHL Association. Dr. Ramakrishnan's talk, "Protein Factories and Antibiotics," was timely and appealed to the broad range of interests represented in the audience. The Symposium celebrated recent, major breakthroughs in understanding how proteins are made in cells, a fundamental aspect of molecular biology.

William Maxwell Cowan

Watson School of Biological Sciences

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

Robert J. Glaser

On November 2, Cold Spring Harbor Laboratory held its 2001 Convocation in Grace Auditorium to celebrate service to science. Before an audience of nearly 300 people, the Watson School bestowed honorary degrees upon William Maxwell Cowan, Robert J. Glaser, and David L. Luke III, in recognition of their continuing service to science. All three honorees have made a vast difference to the world of scientific education and research, and the convocation provided an excellent opportunity to honor these individuals. Gail Mandel, a professor of Neurobiology and Behavior at Stony Brook University and an investigator for the Howard Hughes Medical Institute, gave the keynote address.

Lilian Gann, the Assistant Dean of the Watson School of Biological Sciences, was also appointed this year to Associate Dean, in recognition of her work in developing the programs and offerings of the Watson School.

James D. Watson, president of CSHL, spent a busy year promoting his newest book, *Genes, Girls and Gamow*. The book, a follow-up to the critically acclaimed *Double Helix*, profiles Jim's life after the landmark discovery.

David L. Luke III

Banbury Center

From time to time, Banbury Center holds a meeting on what might be called "science policy," i.e., discussions of topics that affect the way biomedical research is done. One of the most interesting, held in 2001, was on the ways in which the Internet is making, and provoking, changes in the dissemination of scientific information and data. *Electronic Access to the Scientific Literature* examined the initiatives intended to lower the barriers to electronic access to the scientific literature, for example, by providing free access to the full collection of scientific papers within a short period of publication. This is a highly controversial topic, and although no resolution was reached at the meeting, the discussions involving scientists, publishers of society journals, and commercial publishers were useful.

Cancer figured strongly in the Banbury Center program in 2001, with four meetings dealing with important issues in cancer treatment. Two discussed the current status of interferon therapy in cancer and were particularly interesting in that they reviewed the actions of interferon in virus infections as well as in multiple sclerosis. The third meeting on *New Concepts for Clinical Cancer Trials* discussed new findings indicating that the manner in which cancer treatments are given is important. It seems that different dosing schedules combined with lower doses could increase the efficacy and reduce the toxicity and side effects of traditional cytotoxic drugs, and perhaps also of radiotherapy and some investigational drugs. These topics were so interesting that we decided to make *Controlling Cancer* the subject of the 2001 Executives Conference. It was a great success, and we are very grateful to David Deming and JPMorgan H&Q for their continuing support of the meeting.

We turned to a neuroscience topic with strong psychological and philosophical underpinnings with *Can A Machine Be Conscious?* A wealth of new experimental information about the brain has been gathered by neuroscientists, and participants discussed how these data require a revision of classic thinking on consciousness. The participants were a most interesting mix, from philosophers and neuroscientists, to builders of robots, to robots themselves!

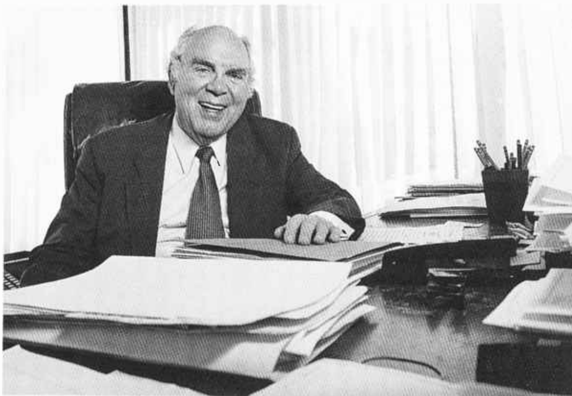
Robertson Research Fund

Since 1973, the Robertson Research Fund has been the primary in-house support for our scientists. The fund has grown from its original 1973 total of $8 million to more than $95 million. During 2001, Robertson funds supported cancer research in the labs of Shivinder Grewal, David Helfman, Winship Herr, Tatsuya Hirano, Yuri Lazebnik, Dick McCombie, Jacek Skowronski, William Tansey, and Rui-Ming Xu; neurobiology research in the labs of Grigori Enikolopov, Dimitri Chklovskii, Josh Huang, Roberto Malinow, Zachary Mainen, Anthony Zador, and Yi Zhong; and plant research in the labs of Robert Martienssen and David Jackson. Robertson funds also supported several new investigators, including Carlos Brody, Alea Mills, Bud Mishra, and Senthil Muthuswamy.

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

Cold Spring Harbor Laboratory Board of Trustees

Cold Spring Harbor Laboratory's Board of Trustees led the Lab through another challenging and eventful year, continuing to shape the direction and vision of this institution.



William E. Murray

This year, the Board welcomed the addition of three new trustees to its ranks: Eduardo G. Mestre, Vice Chairman of Salomon Smith Barney and Chairman of its Investment Banking Division; Douglas P. Morris, Chairman and CEO of Universal Music Group; and Thomas C. Quick, Vice Chairman of Quick & Reilly/Fleet Securities, Inc.

William E. Murray and Whitney D. Pidot both reached the end of their allotted terms as trustees and were honored at the board's November meeting for their outstanding service to the Laboratory. In early 2002, William E. Murray was elected as Honorary Trustee.

Whitney D. Pidot

CSHL Association

The CSHL Association (CSHLA) continues to support the wide range of research and education programs at Cold Spring Harbor Laboratory. The CSHLA is particularly important in funding the research of junior scientists in the formative years of their careers, when it is most difficult to obtain federal grants. Through its fund-raising efforts and community interaction, the CSHLA is vital to the Laboratory's success.

The CSHLA held its annual meeting on February 4, featuring a lecture by Dr. Shirley Tilghman on "Genomic Imprinting." Dr. Tilghman, at that time a professor at Princeton University, is a former trustee of Cold Spring Harbor Laboratory and has since been appointed as President of Princeton University. We extend congratulations to our former trustee and good friend.

I encourage you to closely review the report of the Cold Spring Harbor Laboratory Association, found later in this Annual Report, for a more comprehensive view of the many functions and successes of this special group. I extend my thanks to David H. Deming, President of the CSHLA, and Annette Gangitano, Executive Director of the CSHLA, for their hard work in making this another banner year for our Association.

Shirley Tilghman

DNA Learning Center

Charles and Helen Dolan with Dr. Peter Bruns (Right)

The DNA Learning Center (DNALC) had an exhilarating year. On June 8, the BioMedia Addition to the Center was dedicated, and the entire building was renamed the Dolan DNA Learning Center. The expanded administrative offices, additional teaching lab, computer lab, a lunchroom, and additional exhibition space were made possible primarily through the generosity of the Dolan Family Foundation, established by former and current CSHL Trustees Charles and Helen Dolan.

In addition to its expansion, the Dolan DNALC continued its heralded "Great Moments in Science" lecture series this year, and launched a new Web Site, "Your Genes, Your Health." The comprehensive Site profiles genetic diseases and explains their intricacies on an easy-to-understand level. The Site, which receives thousands of visitors each month, is quickly becoming a valuable resource for families and patients with genetic diseases. I encourage you to review the Dolan DNALC report in its entirety, later in this Annual Report.

CSHL Press

For Cold Spring Harbor Laboratory Press, 2001 was a year of new beginnings. The entirely rewritten, third edition of its classic *Molecular Cloning: A Laboratory Manual*, by Joe Sambrook and David Russell, recaptured its place as a best-selling, essential information tool in experimental biology. David Mount's *Bioinformatics* captured the suddenly burgeoning interest in this topic at the undergraduate level to become the textbook of choice at over 50 schools in the United States. A second textbook, the idea-driven *Genes & Signals*, by CSHL Trustee Mark Ptashne and staff member Alex Gann, was published to high praise in December. Among the other successes in a list of unusually interesting new titles was Elof Carlson's *The Unfit*, a history of the concept of eugenics, and Kathy Barker's *At The Helm*, a unique source of guidance for newly appointed leaders of research teams. Among the journals, *Genome Research* advanced strongly in circulation and manuscript submission, and the newly acquired *Protein Science*, being published for The Protein Society, was relaunched very successfully, with substantial increases in subscriptions and advertising sales. Amid all this activity, 40 members of the Press staff relocated to handsome new offices on the Woodbury campus, where for the first time in its history, all its key functions could be conducted under one roof, with ease and efficiency.

CSHL Library and Archives

In 2001, the CSHL Library continued its work toward creating a "virtual" library. To that end, it has maintained and substantially increased its digital library that includes all the necessary publications, software, and databases for the multidisciplinary research of the Laboratory's scientific staff. The Library continues its membership in consortia with two other major research institutions that began

in 2001: Rockefeller University and Memorial Sloan-Kettering Cancer Center. Membership in these consortia provides our scientists electronic access to many journals from these institutions. In addition, the *BioInformation Synthesis Collaborative Consortia* was formed with the libraries of the Woods Hole Marine Biological Laboratory, The Rockefeller University, and the American Museum of Natural History. The purpose of the consortia is to share human and information resources so that the collective strengths support and advance the research and learning of the members' constituents.

The Library enjoyed the second year of its automated cataloging system called *Sirsi*, which provides the Library's electronic catalog called *WebCat*, containing the library collection. Work on creating a new comprehensive database of all of the scientific laboratory's publications began this year. It will cover the period from its inception in 1890 to 1965 (pre *PubMed*). Two new digital projects were initiated this year: James D. Watson's Photo Archives and the CSHL Symposium photo collection. This collection contains a remarkable history of biology in the latter two thirds of the 20th century and into the new millennium.

New Major Gifts

2001 was a challenging year for fund-raising. Several major campaigns continue to be successful, despite the changed economy and the aftermath of the tragic events on September 11. The campaign to fund the Genome Research Center, the BioMedia Addition to the Dolan DNA Learning Center, and a continuing campaign for the Watson School of Biological Sciences were our major concerns, as was the ongoing effort to fund the cancer gene discovery program. We are very fortunate to have such generous and supportive friends at Cold Spring Harbor Laboratory, but for us to remain at the forefront of all areas of research and education, we need to increase support. Cold Spring Harbor Laboratory appreciates the major investment for these campaigns that provide the basis for many innovative initiatives. Through private funding, the Laboratory is changing the way we think about disease to provide a better future.

Institutional Advancement

The thoughtful commitment of $750,000 from Helen and Charles Dolan has enabled the planning and execution of initiatives to advance our education and research programs.

Watson School of Biological Sciences

The main focus of fund-raising for 2001 was the continuing campaign for the Watson School of Biological Sciences led by Laboratory Honorary Trustee David L. Luke III. New support was secured this year to fund student fellowships, lectureships, and courses. We are grateful for new gifts from Mr. and Mrs. Norris Darrell, Jr. for $200,000; The William Stamps Farish Fund for $200,000; the Annette Kade Charitable Trust for $100,000; Pfizer Global Research & Development for $300,000; Mr. and Mrs. Julian H. Robertson for $100,000; The Seraph Foundation for $75,000; and the Ziering Family Foundation for $300,000. A generous challenge gift of $350,000 was also made to the Watson School by an anonymous donor.

The Genome Research Center

This year heralded the completion of the 65,000-square foot Genome Research Center in Woodbury. The Center, just occupied in the middle of this year, is now running at close-to-full capacity, housing a Bioinformatics Center, a Cancer Research Center, the Genome Sequencing

Center, the Plant Genomics Center, the Cold Spring Harbor Laboratory Press, and the Laboratory's Purchasing Department. We are grateful for major new contributions to the facility in nearby Woodbury from the Goldfield Family Charitable Trust for $100,000 and the William and Maude Pritchard Charitable Trust for $280,000. The largest gift to date for this facility and its programs was $3,500,000 received from a generous donation of real estate by Kenyon Gillespie.

Dolan DNA Learning Center

Major support to fund the new BioMedia Addition continued this year with gifts from The Booth Ferris Foundation for $150,000 and $165,000 from the estate of Mrs. Dorothy H. Hirshon. Rotating exhibit space in *The Genes We Share* installation was made possible by a generous $150,000 pledge from Dr. Laurie J. Landeau. Once again, Brinkmann Instruments, Inc./Eppendorf AG has donated new lab equipment, this year valued at $75,000. We are also grateful to Arrow Electronics for their generous gift of $30,000; to Fisher Scientific International for $27,000 worth of equipment; and to Mr. and Mrs. Stephen A. Paolino/JPC Contracting for their gift of $10,000.

Private Research Support

Private funding in 2001 facilitated important research initiatives in cancer, neuroscience, and other areas, with major gifts in the $100,000 and above category, including the Oliver and Jennie Donaldson Trust for $250,000; the Louis Morin Charitable Trust for $125,000; the David and Lucille Packard Foundation for $970,000; the St. Giles Foundation for $183,000; Ann and Herb Siegel for $1,000,000; and the Whitehall Foundation for $225,000. The generosity of these and numerous other major private benefactors of Cold Spring Harbor Laboratory is crucial to our mission to perform the highest-quality basic research.

Breast Cancer Support

We greatly appreciate the support received in 2001 for breast cancer research from a number of breast cancer groups, including The Breast Cancer Research Foundation; the Long Beach Breast Cancer Coalition; Long Islanders Against Breast Cancer (L.I.A.B.C.); the Michael Scott Barish Human Cancer Grant sponsored by 1 in 9: The Long Island Breast Cancer Action Coalition; and the proceeds from Breast Cancer Awareness day in memory of Elizabeth McFarland. We remain grateful to and inspired by the dedicated individuals who donate their time and energy to advance cancer research through these organizations.

CSHL Planned Giving Advisory Board

The Cold Spring Harbor Laboratory Planned Giving Advisory Board was convened in 2001. Its members—drawn from the financial services, accounting and legal professions—total more than 20. The Board consists of bank officers, trust officers, investment advisors, investment bankers, accountants, tax advisors, and lawyers, representing 13 firms located on Long Island, in New York City, and in Connecticut. The purpose of the Board is to report to this influential group the Laboratory's activities and accomplishments and to engage Board members in the support of the Laboratory's goals. We welcome the board members to the Laboratory community.

President's Council

As in previous years, Jim Watson invited members of the President's Council to a 1-day meeting at the Laboratory, to meet the young scientists supported by the Council members and to hear about interesting advances in biomedical research. The topics for these meetings are carefully chosen for a combination of high-quality research and general interest, and for 2001 we selected a topic of importance to everyone—Nutrition: Facts and Myths.

The Council began with a talk by Nancy Etcoff (Massachusetts General Hospital) on "The Evolutionary Basis of Beauty," discussing why we find certain body forms attractive and showing that there are some fundamental forms that are cross-cultural. She was followed by Walter Willett (Harvard School of Public Health) who spoke on "Nutrition and Health" and gave us many good suggestions for improving our health through our diet—including some suggestions that were against our current wisdoms. Diet and cancer is a very controversial topic, but Bruce Ames (University of California, Berkeley) presented convincing data that micronutrients really do have a protective effect against cancer. With "Diets: Reality and Hype," Marc Jacobson (North Shore-Long Island Jewish Health System) reviewed diets and what does and does not work, and George Roth (National Institute on Aging) described how diet affects life span. This was a truly fascinating meeting and one that had a lasting impact on all participants.

Bruce Ames

Gavin Borden Visiting Fellow

Joan A. Steitz, Ph.D., Sterling Professor and Chair of Molecular Biophysics and Biochemistry at Yale University, was the Laboratory's 7th Gavin Borden Visiting Fellow. A former Trustee of CSHL, Dr. Steitz presented a lecture titled "Mechanisms of mRNA Export and Stability" on April 23 in Grace Auditorium. The Gavin Borden Lecture series was named in memory of Gavin Borden, a publisher whose *Molecular Biology of the Cell* and other books made a lasting impression on many scientists, both old and new.

Joan A. Steitz

Building Projects

Cold Spring Harbor Laboratory obtained some much-needed space this year, with the completion of two major projects.

On June 8, the Laboratory dedicated the Dolan DNA Learning Center, named for Helen and Charles Dolan. The Center is significantly expanded, with new administrative, laboratory, auditorium, and lunchroom space for school-aged visitors. In addition, the Learning Center nearly doubled its existing exhibit space and is in the process of designing new exhibits related to the history and future of biology.

Renovations were completed this year on the Genome Research Center in nearby Woodbury. The building, a 65,000-square-foot facility, now houses four research components: a

The Genome Research Center (*top*) and the Dolan DNA Learning Center (*bottom*).

Bioinformatics Center, a Cancer Research Center, a Genome Sequencing Center, and a Plant Genomics Center, as well as new administrative space for the CSHL Press and other administrative departments.

Community Outreach

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

- CSHL was well-represented at the annual Chase Corporate Challenge, held at Jones Beach on July 31. More than 32 Laboratory runners were on hand at the event. The CSHL Men's team placed 12th and the Women's team placed 59th in the 3.5-mile race. Top performers included Holly Cline, Linda Van Aelst, and Ivo Grosse.
- CSHL "road-runners" were also on hand at the Cigna 5K Walk/Run, to support 1 in 9: The Long Island Breast Cancer Action Coalition, held at Eisenhower Park in East Meadow on August 23. The CSHL group—grateful for more than 10 years of support from 1 in 9—performed well despite the rain. In all, the event raised more than $60,000.

Special Events

Harbor Lecture Series

Francis Collins

This year, the Laboratory hosted its first Harbor Lecture Series, three public lectures that attracted more than 900 visitors to Grace Auditorium. The first lecture was given by Dr. Francis Collins, M.D., Ph.D., Director of the National Human Genome Research Institute, on May 8. Dr. Collins's lecture, "Medical and Societal Consequences of the Human Genome Project," was well attended and much enjoyed. On May 21, John Coffin, Ph.D., the Director of the HIV Drug Resistance Program at the National Cancer Institute and the American Cancer Society Research Professor at Tufts University School of Medicine in Boston, delivered a lecture titled "HIV: Can This Disease Be Controlled or Cured?" The timely topic was fascinating for all participants. The final lecture, delivered on September 10, was by Jeffrey M. Friedman, M.D., Ph.D., a professor at The Rockefeller University in New York and an investigator for the Howard Hughes Medical Institute. Dr. Friedman addressed the audience about weight and body mass in an appealing lecture titled, "Molecular Mechanisms Regulating Body Weight."

Other Lectures

CSHL continued to host the Huntington Hospital Lecture Series, which ran from February to June. This year's topic was "Cardiac Health," part of Huntington Hospital's Heart Health Lecture program.

Cold Spring Harbor Laboratory expanded its popular West Side School Lectures Program, designed for students in local schools in grades four through six. Four CSHL Professors—Holly Cline, Eli Hatchwell, Yuri Lazebnik, and Robert Malinow—delivered entertaining and informative lectures to the budding scientists. The lectures, already a success, will again be expanded in 2002.

On March 23, Dr. Tom Kirkwood, a professor at the University of Newcastle in the United Kingdom, delivered a fascinating lecture on aging and genetics titled "Thread of Life: The Role of DNA in the Aging Process."

Holly Cline delivering a West Side School lecture.

Concerts and Exhibits

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

March 9	Cathie Ryan, Traditional Irish Female Vocalist
May 5	Stella Simakova, Pianist
May 19	Melvin Chen, Pianist
May 26	Jonathan Biss, Pianist
August 18	Anna Stoytcheva, Pianist
August 25	Alexis Pia Gerlach, Cellist
September 1	Antonio Pompa-Baldi, Pianist
September 8	Claremont Trio
September 22	Anthony Molinaro, Pianist
October 6	Eric Johnson, Jazz Guitar/Donald Axinn Poetry Reading

The Claremont Trio (*top*) and Cathie Ryan, Vocalist (*bottom*)

In addition to the concert series, the Laboratory hosted "FotoLab I," a collection of works from Laboratory staff and students. The event, inspired by Watson School student Rebecca Ewald, included Doug Fogelson, a Chicago-based photographer, as the "official" photographer in residence. Doug helped the staff and students select and capture their best work for the exhibit. Doug also gave special lectures and master classes during the exhibit, which ran in the Bush Auditorium from June 30 through August 5.

Laboratory Employees

Long-term Service

On June 22, employees celebrating milestone anniversaries with the Laboratory were honored at a special dinner at Robertson House, on the Banbury Center property. Congratulations to all! Honorees included:

30 Years	William Keen and John Maroney
25 Years	Guy Cozza, Joseph Ellis, Roberta Salant, Peter Stahl, Margaret Wallace, and Patricia Wendel
20 Years	Dorothy Brown, Rodney Chisum, David Helfman, Patricia Kurfess, Joseph Pirnak, and Philip Renna
15 Years	Russell Allen, Jennifer Blovsky, Mary Cozza, Nouria Hernandez, Christopher Hubert, Adrian Krainer, Susan Lauter, Carol Marcincuk, Vincent Meschan, Timothy Mulligan, Jacek Skowronski, and Diane Tighe

Back row (left to right): David Helfman, Russell Allen, Patricia Wendel, Adrian Krainer, Joseph Ellis, Peter Stahl, Jacek Skowronski, Bruce Stillman, Dill Ayres

Middle row (left to right): Susan Lauter, Nouria Hernandez, Roberta Salant, William Keen, Timothy Mulligan, John Maroney

Front row (left to right): Philip Renna, Carol Marcincuk, Margaret Wallace, Mary Cozza, Dorothy Brown, Guy Cozza, Jennifer Blovsky, Chris Hubert

Not pictured: Diane Tighe, Rodney Chisum, Patricia Kurfess, Joseph Pirnak, and Vincent Meschan

Carlos Brody

Alea Mills

Senthil Muthuswamy

New Faculty and Staff

Carlos Brody, Alea Mills, and Senthil Muthuswamy joined the Laboratory this year as assistant professors.

Promotions

Michael Myers and Marja Timmermans were both promoted to assistant professor this year. Shiv Grewal, David Jackson, and Andrew Neuwald were promoted to associate professors, and Dick McCombie was promoted to professor.

Departures

Departures this year included Ryuji Kobayashi, an associate professor; Edward Stern, a senior fellow; and Clifford Yen, a research investigator.

Visiting Scientists

Three visiting scientists joined us this year: Ann-Shyn Chiang joined the lab of Tim Tully; Xiaomin Wang joined the lab of Grigori Enikolopov; and Tilak Sharma joined the lab of W. Richard McCombie. Four scientists also wrapped up their stays at Cold Spring Harbor Laboratory: Antonius Holtmaat, Vincent Colot, Edith Heard, and Mary Sabatini.

Concluding Remarks

Science moves at a rapid pace at Cold Spring Harbor Laboratory—perhaps faster than at many places—for three principal reasons. First, our focus has always been on nurturing young scientists who are in the formative years of their scientific careers, giving them the reins and letting them go where they will. We are constantly surprised by what they achieve. Second, we teach in our courses program the latest research technologies to active scientists who visit from all over the globe. Third, administration and support staff are solely geared to the support of science. When combined, these factors make CSHL a center for truly innovative research. This is one of the reasons I decided to stay at CSHL after arriving as a postdoctoral fellow (not too long ago). Scientific research, by its very nature, is always moving into the unknown, trying to explain the ways of nature and how it can go wrong in diseases that afflict so many. I am confident that our approach will win the fight against some of the most important problems in biology and biomedicine.

With the slowing economy and the ever-increasing cost of high-technology-based science, our ability to support all that we need to do is a challenge that keeps me constantly worrying how we might "make ends meet" at the end of each year. The opportunities are significant, and I hope that as we move forward into a new year, we might feel more comfortable that at least the very best of what we do will receive the necessary funds. Ultimately, it is the fruits of this science that will benefit us, our children, and our children's children.

Bruce Stillman

ADMINISTRATION

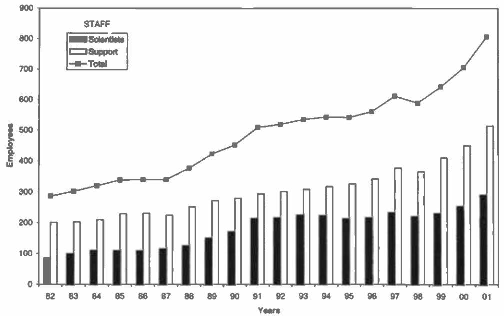
It is impossible to reflect on the year 2001 without one's thoughts going immediately to September 11. The "where were you when?" syndrome will be as strong for this moment as for any of the others in our history that are so indelibly inscribed in the national psyche. When my assistant, Judi Smith, came into my office at 8:45 that Tuesday morning to tell me that there was a fire burning at the Trade Center—apparently due to an airplane crash—a sense of foreboding began to set in. It was a brilliant fall morning in Cold Spring Harbor with bright blue sky and water—not conditions where I could imagine an aircraft accidentally crashing into the towers. I went immediately to the Richards conference room where there was already a group from the Facilities Department huddled around the television set. There, we watched in disbelief as the cascade of horrific images and events unfolded before our eyes on live television. Shaken and numb after the collapse of the second tower, I returned to my office. Immediate thoughts were of how many families of Laboratory personnel would be directly affected and how many friends who worked in the Trade Center were in their offices that morning. With 850 employees and their families living and working 35 miles from the site of such massive loss of life, it seemed highly unlikely that our community would be spared.

My concern about Laboratory personnel was sadly confirmed. Chief Facilities Officer Art Brings called to tell me that two of his veteran employees had sons missing. Joey Ellis' son Mark was a New York City Transit Authority police officer who had rushed to the scene to take part in the rescue effort. Lou Jagoda's son Jake worked for the ill-fated bond house, Cantor Fitzgerald. Such loss is tragic beyond description and our hearts go out to Joey, Lou, and their families. A long-time friend and supporter of the Laboratory also lost his life at the Trade Center. Lindsay Herkness, an executive at Morgan Stanley Dean Witter, was a familiar and popular figure at Symposium lectures and dinner parties. He will be sorely missed by the Laboratory community, his family, and his wide network of loving friends in New York City and Long Island.

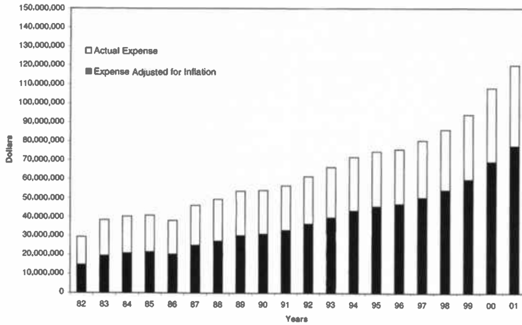
On a brighter note, the science at the Laboratory gets more exciting by the moment. Having the complete human genome sequenced allows our investigators to use novel techniques to compare diseased and normal tissue and to identify genes associated with cancer and other diseases. The good news for the nonscientists among us is that the progress of the research is getting us closer to the clinic. It is becoming easier to make the connection between the basic research being done in our laboratories and practical and promising therapeutics being developed for human disease. The heretofore challenging job of answering the familiar question—What is it that goes on at Cold Spring Harbor Laboratory?—has become easier to handle. Our Director of Public Affairs, Jeff Picarello, has done a commendable job communicating the Laboratory's work and mission. Articles on the Laboratory itself and on the field of molecular biology were more prevalent during the past year—witness cover stories in national publications such as *Time, Newsweek*, and *Forbes*.

Our graduate program, The Watson School of Biological Sciences, continues to thrive under the leadership of Dean Winship Herr. The third class of Ph.D. students arrived in the fall. The student body—now totaling 20 Watson School students, 50 SUNY Stony Brook students, and 140 postdoctoral fellows—provides an academically charged and stimulating atmosphere characteristic of an elite university. The campus is more alive than ever before, and the students add a welcome dimension of vitality to the daily life of the Laboratory.

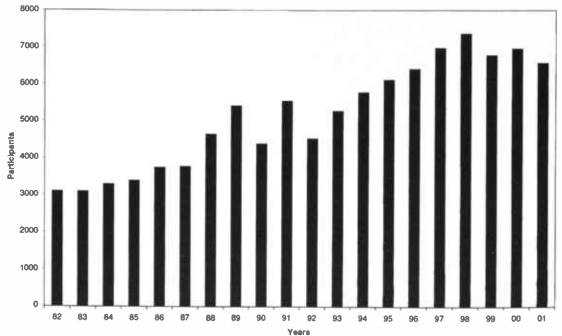
* Consists of Full-time and Part-time Technical Support, Core Services, Publications, Meetings, Library, Public Affairs, Building and Grounds, Administrative, Personnel, Banbury Center, and DNA Learning Center



OPERATING EXPENSE



MEETINGS & COURSE PARTICIPANTS



Two substantial projects were completed over the course of the year, the most challenging being the renovation and expansion of the Genome Research Center at Woodbury. The process of redesigning, gutting, and rebuilding this 65,000-square-foot building was complex to say the least. This is a very high-tech facility, housing not only the offices of the Cold Spring Harbor Laboratory Press and the Laboratory Purchasing Department, but also state-of-the-art laboratories with robotics and other sophisticated scientific equipment to facilitate genome sequencing, cancer gene discovery, and plant biology. The Center is already home to 120 employees, and our Food Services Department, led by Jim Hope, has opened an attractive new dining facility on site. This research facility is the Laboratory's first away from the main campus, and concerted effort has been made to make employees there still feel part of a cohesive and integrated community. Much credit for the success of this project goes to our own Facilities Department for their planning, execution, and oversight and to Centerbrook Architects and Fortunato Sons, Inc. for their work on the project.

The other successful project completed was the renovation and addition to the Dolan DNA Learning Center. Thanks to the generosity of the Dolan family and others, this innovative educational facility has undergone a complete facelift and has virtually doubled in size. The new Bio-Media addition includes a state-of-the-art computer laboratory for the students, new exhibit space, additional wet labs, a student lunchroom, and much-needed office space for staff. The innovative high school and middle school programs continue to attract thousands of eager students and teachers each year. Visits to the award-winning educational Web Sites developed by the Center's talented team are increasing rapidly. In June, the opening of the building was celebrated at a well-attended dedication featuring remarks by both Charles and Helen Dolan and followed by an operatic performance.

The library at the Laboratory is becoming an increasingly dynamic place. Mila Pollock, Director of Library Services, and her colleagues have undertaken an effort to create a state-of-the-art digital library to meet the growing requirements of our research scientists and students. Also in 2001, Jim Watson formally donated his book and reprint collection to the Laboratory Archives. With the help of a generous grant from Lewis Lehrmann, archivists are in the process of organizing the vast and valuable collection of Dr. Watson's manuscripts, letters, and other materials for the eventual benefit of scholars from around the world.

As always, we are indebted to our Cold Spring Harbor Laboratory Association Directors and other volunteers who do so much to draw positive attention to the Laboratory. The third *Jazz at the Lab* concert in April, organized by Joe Donohue, Susan Hollo, Cathy Soref, and Gil Ott, was a huge success from every perspective. Staffers Charlie Prizzi and Annette Gangitano both did superb jobs in working with the committee and managing the logistics of the event. Thanks are also due to the Corporate Advisory Board of the Dolan DNA Learning Center, led by Ed Chernoff, which organized another successful golf tournament at Piping Rock in June.

It has become evident that the field of biomedical research is at a turning point and that advances against human disease are likely to change our lives during the next few decades. This revolution, and Cold Spring Harbor Laboratory's central role in it, has been the catalyst for a new push in strategic planning. Over the course of the year, a great deal of effort was expended on the development of a long-term "master plan" for the Laboratory's future. This initiative was led by Drs. Stillman and Watson, who have both articulated a compelling vision. The process entailed mapping the future direction of the research and educational programs and then creating and developing an infrastructure plan to accommodate the growth. A team, including Trustees Helen Dolan and Mary Lindsay, was formed to work with Centerbrook

Architects. We are pleased with the resulting plan for a new research and academic cluster at the west end of our main campus. Great care was taken to preserve the lawns and open spaces at the eastern end of the property and to design buildings with the traditional "whaling village" character of the campus. The plan is well within the stringent zoning guidelines set out by the Village of Laurel Hollow in 1995 and has the enthusiastic support of our Board of Trustees. Needless to say, this project requires a large and comprehensive fund-raising campaign that must be well under way before any work begins. To that end, two new appointments have been made. As Vice President of Institutional Advancement, Rod Miller—who joined us from Brooklyn Polytechnic—will oversee the planning and execution of the campaign. Diane Fagiola, a Locust Valley native, has succeeded Rick Cosnotti as Director of Development. Again, we express our appreciation to the Dolan family for their generous grant enabling the groundwork for the master plan and for the campaign plan development.

Two other managerial changes of note took place at the end of the year. Cheryl Sinclair, Director of Human Resources, retired after 12 years of loyal service. During her tenure, the employee population grew from 463 to 850. Cheryl met the many challenges presented by this growth with grace and professionalism. We wish her well in her retirement. Katie Raftery was recruited from the Ericsson Corp. as our new Director of Human Resources. Katie is an experienced manager who has transitioned into the job with great energy and enthusiasm. Jerry Latter, our Director of Information Technology, was recruited to Rockefeller University in October. We were fortunate, in this case, to draw on existing talent and to promote Mark Kilarjian to the Director position.

As we face new challenges, the leadership and support of our Board of Trustees become increasingly important. In identifying potential Board members, we look for bright, prominent, and influential individuals who will take an active interest in the Laboratory. We welcome and look forward to working with newly elected Trustees Tom Quick and Eduardo Mestre. We also express our gratitude to two Board members whose terms expired at the end of the year. Both Bill Murray and Whitney Pidot served with great distinction, and their contributions will long be felt at Cold Spring Harbor Laboratory.

The Laboratory had a satisfactory year financially. There were concerns as we began the year that it would be a difficult one in which to achieve break-even results with our operating budget. I am pleased to report that results were better than forecasted and we were able to break even after $4.6 million in depreciation expense. Having said that, there was no operating surplus as in past years, and it was necessary to utilize $800,000 of an existing $2.3 million reserve account to achieve these results. Revenues grew 10% to $76,691,000, and $3.8 million in positive cash flow from operations was available for capital expenditures. A number of factors contributed to the better-than-expected performance. The Cold Spring Harbor Laboratory Press had an excellent year due in part to strong sales of the third edition of its popular laboratory manual *Molecular Cloning*. Royalty and licensing income also exceeded expectations, thanks to the hard work of John Maroney and the Technology Transfer Department. Good cost control by a number of administrative departments also contributed to the upside. These positives were enough to outweigh effects directly and indirectly related to the events of September 11. Two of our largest scientific meetings following 9/11 had to be postponed. Meetings and Courses Director David Stewart did an admirable job in rescheduling and conducting the meetings, but attendance was understandably down. We believe that our Annual Fund was also negatively impacted. The number of gifts was down substantially, and only as a result of a generous and concerted effort by our Board of Trustees were we able to avoid a commensurate decline in the

total dollars raised. Finally, we were most disappointed to cancel a gala fund-raising concert featuring Luciano Pavarotti. This event had been generously arranged and supported by Laboratory Trustee Doug Morris, CEO of Universal Music.

The Laboratory's endowment funds suffered this year, reflecting the poor performance of the public equity markets. At year end 2001, the endowment totaled $212 million, down from $227 million at the previous year end. The asset allocation of our managed funds is approximately 53%/47% equities to fixed income investments. Total return on these funds was a negative 4.8%, comparable to benchmark indices of similarly allocated funds. Most equity managers had a difficult year and ours were no exception. Our "growth" strategy equity managers, Vanguard Primecap and Essex Management, both underperformed the Standard & Poor's 500. "Value" manager U.S. Trust was able to outperform the Standard & Poor, but it was still down for the year. On the other hand, our fixed income manager, Miller Anderson, achieved excellent results and outpaced market indices. The Laboratory continues its relatively conservative policy of drawing down 4% per annum from the endowment based on the 3-year moving average of year-end market value. We rely on these funds to supplement government support of cutting edge research and to ensure a secure financial future for the Laboratory. Over time, the Laboratory has managed the endowment prudently and successfully and has achieved outstanding growth. The Finance and Investment Committee, chaired by Trustee Lola Grace, meets regularly to evaluate, review, and determine investment policy and guidelines. This responsibility is undertaken with diligence, especially in times of poor markets.

We face another year of financial challenges as we look ahead to 2002. The markets and the economy remain murky in the short term. At the Laboratory, the opening of the Genome Research Center, while exciting, carries with it substantial increases in operating costs and depreciation expense, not all of which is yet supported by new grant income. We are anticipating a difficult year and are taking measures to conserve cash. These measures include an administrative hiring freeze and a 2.5% cap on administrative salary increases, as well as a salary freeze for senior management. In addition, we are planning to spend $800,000 less than the budgeted depreciation expense on capital expenditures. The Laboratory is a healthy institution financially because it has been managed prudently and diligently. It is our intention to maintain this practice. I am indebted to Chief Financial Officer Morgan Browne and Comptroller Lari Russo for their counsel, wisdom, and leadership in this ongoing effort.

In the midst of this challenging environment, we have developed an ambitious plan that will require a capital-raising effort of unprecedented scope for the Laboratory. From a fund-raising perspective, the timing may not be perfect. From a scientific standpoint, the time is clearly now. Our research and education programs are of the very highest caliber because we have the best people and the best minds in the business. It is imperative that we provide them with the best facilities and the resources necessary to achieve their objectives. Cold Spring Harbor Laboratory did not gain the prominence it enjoys today without bold vision. In 1968, Jim Watson assumed the leadership of an institution with no endowment and an uncertain future. An opportunity was seized. Today, we face another turning point and yet another opportunity. Onward and upward!

W. Dillaway Ayres, Jr.
Chief Operating Officer

RESEARCH

CANCER: GENE EXPRESSION

The investigators in the Gene Expression Group are unified by a shared interest in understanding the mechanisms of control of gene expression. These shared interests result in a highly interactive unit. The members of this group study the following topics.

- Shiv Grewal studies the epigenetic control of gene expression in the yeast *Schizosaccharomyces pombe*.
- Nouria Hernandez studies the regulation of small nuclear RNA gene transcription in human cells and thus addresses the mechanisms of RNA polymerase selectivity.
- Winship Herr studies the regulation of gene transcription in human cells using herpes simplex virus as a model system.
- Leemor Joshua-Tor studies the structural basis of regulation of proteolysis and DNA functions.
- Adrian Krainer studies the control of pre-mRNA splicing, and thus an important mechanism for the generation of diversity in gene function.
- Mike Myers uses proteomic approaches to study how protein complexes regulate cell signaling and tumor progression.
- William Tansey studies the proteolytic destruction of the oncoprotein transcription factor Myc and the involvement of the ubiquitin-proteasome pathway in transcriptional control.
- Jacek Skowronski studies how the human and simian immunodeficiency viruses disrupt signal transduction and the expression of cell surface receptors in infected T cells.
- Rui-Ming Xu studies the structural basis of pre-mRNA splicing and transcriptional silencing.

See previous page for photos of the following scientific staff.
Row 1: Farida Emran; Eric Allemand; Tatsuhiko Kawaguchi; Laura Schramm; Linda Van Aelst; Chih-Chi Yuan
Row 2: Xavier Roma, Andriy Tovkach; Peter O'Brien; Jannic Boehn; Christine Von Klitzing, Masaaki Hamaguchi; Hirac Gurden; David Roberts
Row 3: Guy Karger; Lee Henry; Benjamin Boettner; Santanu Chakraborty, Sachin Ranade
Row 4: Ashish Saxena; Gowan Tervo; Tom Volpe; Koichi Iijima, Kanae Iijima; Julius Zhu
Row 5: Catherine Zhang; Ying-hua Zhu; Esteban Araya; Ingrid Ehrlich
Row 6: Ken-Ichi Noma; Christine Von Klitzing; Ed Ruthazer; Mike Wehr

EPIGENETIC CONTROL OF GENE EXPRESSION

S. Grewal I. Hall K. Noma
A. Malikzay G. Shankaranarayana
J. Nakayama G. Xiao

Research in our laboratory is focused on the epigenetic control of higher-order chromatin assembly. The organization of chromatin into higher-order structures governs diverse processes ranging from stable inheritance of gene expression patterns to other aspects of global chromosome structure essential for preserving genomic integrity. To understand the molecular mechanism of establishment and maintenance of higher-order chromatin structures, we are studying a gene-repression mechanism known as "silencing" at the mating-type region and centromeres of the fission yeast *Schizosaccharomyces pombe*. Our earlier studies have revealed that an epigenetic imprint marking the mating-type region contributes to maintenance of a higher-order chromatin structure, which controls silencing and recombinational suppression throughout the 20-kb silent mating-type interval. A chromodomain-containing protein Swi6, which shares structural and functional similarities to HP1 from higher eukaryotes, was found to remain stably associated with the silent mating-type region throughout the cell cycle, presumably providing a molecular bookmark to clonally propagate a specific chromatin configuration during cell division. We provided direct evidence showing that the Mendelian unit of inheritance, the gene, at the *mat* locus comprises DNA plus the associated Swi6-containing protein complex(es).

Differences in chromatin structure have been linked with distinct covalent modifications, such as acetylation, phosphorylation, ubiquitination, and methylation of histone amino-terminal tails that regulate transcription and influence chromosome condensation and segregation. Mutations in histone-modifying factors such as the methyltransferase Clr4 and deacetylases Clr3 and Clr6 affect silent chromatin assembly at centromeres and the mating-type region. How these histone modifications participate in the modulation of chromatin structure and the precise sequence of events leading to assembly of higher-order chromatin structures have remained elusive. One possible explanation is that they directly alter nucleosomal structure, thereby influencing histone-DNA and histone-histone interactions. Another attractive possibility is that distinct combinations of covalent histone modifications, also referred to as the "histone code," provide a mark on the histone tails to recruit other chromatin-modifying proteins. During the past year, we have made significant progress toward understanding the epigenetic control of higher-order chromatin assembly.

MOLECULAR MECHANISM OF HETEROCHROMATIN ASSEMBLY

Our work addressed the key molecular events leading to the assembly of higher-order chromatin structures. The covalent modifications of histone tails by deacetylase and methyltransferase activities are believed to act in concert to establish the histone code essential for heterochromatin assembly at centromeres and mating-type region of fission yeast. We provided the first in vivo evidence that methylation of histone H3 tails has a central role in heterochromatin-mediated silencing of large chromosomal domains. In collaboration with David Allis's laboratory (University of Virginia), we showed that lysine 9 of histone H3 (H3 Lys-9) is preferentially methylated at the silenced chromosomal regions. Clr4 protein, a homolog of mammalian SUV39H1, which contains the amino-terminal chromodomain and a carboxy-terminal SET domain, is the H3 Lys-9-specific histone methyltransferase (HMTase) in fission yeast. Although the highly conserved SET domain and surrounding cysteine-rich regions of Clr4 are sufficient for its HMTase activity, we found that both its chromo and SET domains are required in vivo. Methylation of H3 Lys-9 by the Clr4 enzyme is dependent on another factor, Rik1, which contains 11 WD40 repeat-like domains. It has been suggested that Rik1 might form a complex with Clr4 to recruit its HMTase activity to heterochromatic loci. Interestingly, the localization of Swi6 to the heterochromatic loci is tightly linked to H3 Lys-9 methylation. More importantly, we found that an H3 Lys-14-specific deacetylase Clr3 seems to be required for H3 Lys-9 methylation by Clr4 and Swi6 localization.

Although methylation of H3 Lys-9 is required for heterochromatin association of Swi6, mutations in Swi6 have no effect on H3 Lys-9 methylation, suggesting that Swi6 is dispensable for H3 Lys-9 methylation and most likely acts downstream from Clr4.

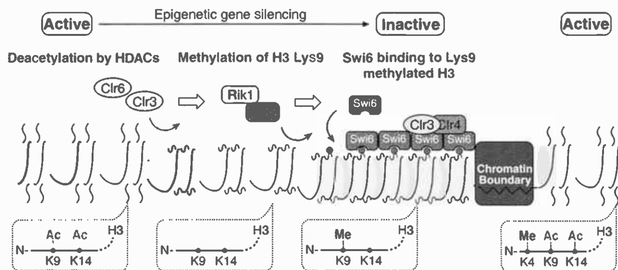


FIGURE 1 A stepwise model for epigenetic control of heterochromatin assembly in fission yeast. Histone deacetylase and methyltransferase enzymes cooperate with each other to establish a specialized histone code essential for heterochromatin assembly. Clr6/Clr3 deacetylases are believed to remove acetyl groups on lysines 9 and 14 of H3, whereas the Clr4/Rik1 complex methylates lysine 9 of H3. The chromodomain of Swi6 binds specifically to methylated H3 Lys-9 to continue heterochromatin assembly. The dimerization of Swi6 is believed to provide an interface for its interaction with other proteins, including histone-modifying enzymes, and might be a key to spreading as well as inheritance of heterochromatic structures.

Collectively, these studies helped us define a temporal sequence of events leading to heterochromatin assembly that may be conserved from fission yeast to humans (Fig. 1). The deacetylation of the histone H3 tail likely precedes the methylation of H3 Lys-9, which creates a binding site for recruitment of Swi6. The binding of Swi6 chromodomain to H3 Lys-9-methylated tails and the dimerization of Swi6 through its chromo shadow domain likely promote formation of heterochromatic structures.

BOUNDARIES OF HETEROCHROMATIN DOMAINS

Eukaryotic genomes are believed to be organized into discrete structural and functional chromatin domains that help separate independently regulated parts of the genome. Specialized DNA elements, known as insulators or boundary elements, have been suggested to act as barriers against the effects of enhancer and silencer elements from neighboring regions. However, the mechanism that prevents the spreading of heterochromatin into euchromatin in a natural chromosomal context is not well understood.

To address these fundamental questions related to genome organization, we created a 47-kb high-resolution map of the heterochromatin complexes at the mating-type locus of fission yeast. We found that a heterochromatic domain can be easily distinguished from a neighboring euchromatic domain on the basis of their distinct histone H3 methylation patterns. Our analyses revealed that histone H3 methylated at lysine 9 and its interacting Swi6 protein are strictly localized to a 20-kb heterochromatic domain (Fig. 2). In contrast, H3 methylated at lysine 4 (H3 Lys-4), only a few amino acids away, is specific to the surrounding euchromatic regions. Remarkably, we discovered that two inverted repeats (IR-L and IR-R) flanking the silent interval serve as boundary elements to mark the borders between heterochromatin and euchromatin. Deletions of these boundary elements lead to spreading of H3 Lys-9 methylation and Swi6 into neighboring sequences. We also showed that the H3 Lys-9-methyl modification and corresponding heterochromatin-associated complexes prevent H3 Lys-4 methylation in the silent domain. This study suggests that boundary elements might help separate specialized chromatin domains with distinct histone codes. Our work also supports the idea that position effect variegation in higher eukaryotes, such as *Drosophila*, results from the spreading of heterochromatin complexes into adjacent euchromatic regions. How boundary elements protect against encroachment of repressive heterochromatin complexes into euchromatic regions containing genes is the focus of our current research.

HETEROCHROMATIN AND CHROMOSOME DYNAMICS

Previous studies have shown that heterochromatin at centromeres has a pivotal role in proper kinetochore function and chromosome segregation. Mutations in heterochromatin proteins such as Swi6 result in defective centromeres leading to missegregation of chromo-

somes, although the precise mechanism is not clear. In collaboration with Yoshi Watanabe's lab (University of Tokyo), we showed that cohesin protein complexes, which are essential for sister chromatid cohesin and proper chromosome segregation, are preferentially enriched at heterochromatic regions in *S. pombe*. Moreover, cohesin enrichment at heterochromatic loci is dependent on Swi6, which directly interacts with cohesin subunit Psc3. Therefore, the role of heterochromatin in chromosome segregation might be coupled to its involvement in preferential recruitment of cohesin. We also found that cohesin is enriched at the silent mating-type region in a manner that depends on Swi6 and it is required to preserve the genomic integrity of this locus.

ROLE OF RNAi MACHINERY IN HETEROCHROMATIN ASSEMBLY

What defines a specific chromosomal domain as a preferred site of heterochromatin assembly? It has been suggested that heterochromatin formation might have originated in response to defense against transposable elements. According to this hypothesis, heterochromatin assembly might be linked to the presence of repeated DNA sequences, rather than any specific DNA sequence identifying these loci. Alternatively, aberrant RNA transcripts produced from repetitive DNA, such as present at *S. pombe* centromeres, might be recognized by RNA-mediated interference processes and serve as a trigger, targeting chromatin modifiers to the corresponding genomic locations. Interestingly, factors known to be involved in RNA interference (RNAi), such as RNA-dependent RNA polymerase (*rdp1*), Dicer (*dcr1*), and Argonaute (*ago1*), present in higher eukayotes with complex genomes, are present in *S. pombe*. Although it remains to be demonstrated whether RNAi exists in *S. pombe*, in collaboration with R. Martienssen's group here at CSHL, we have shown that deletion of *rdp1*, *dcr1*, or *ago1* affects silencing and heterochromatin assembly at centromeres. Specifically, RNAi machinery is essential for the targeting of histone-modifying activities and Swi6 to centromeric repeats. Consistent with a role for centromeric repeat sequences in heterochromatin formation, we also showed that these sequences possess the ability to recruit heterochromatin complexes and promote silencing at an ectopic site.

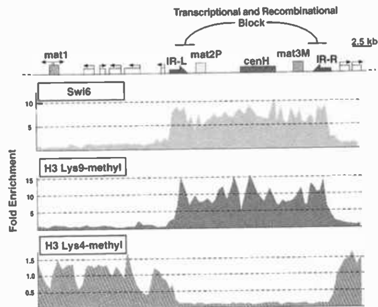


FIGURE 2 Distribution of Swi6 and distinct site-specific histone H3 methylation patterns marking the euchromatic and heterochromatic domains at the *S. pombe* mating-type region. Shown is a physical map of the mating-type region with *mat1*, *mat2*, and *mat3* loci. (*Thick arrows*) IR-L and IR-R inverted repeats flanking the *mat2-mat3* interval. *cenH* represents sequences sharing homology with the centromeric repeats. (*Open boxes*) Location of open reading frames; (*arrows*) direction of transcription. The graphs below represent results from high-resolution mapping of Swi6, H3 Lys-9 methylation, or H3 Lys-4 methylation levels determined by chromatin immunoprecipitation experiments. H3 Lys-9 methylation and Swi6 are specifically enriched throughout the 20-kb heterochromatic interval that displays transcriptional silencing and suppression of recombination. In contrast, H3 Lys-4 methylation is enriched in transcriptionally poised euchromatic regions containing genes. IR-L and IR-R inverted repeats act as boundaries of the heterochromatin domain and prevent spreading of repressive chromatin complexes into neighboring areas.

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MECHANISMS OF TRANSCRIPTION

N. Hernandez F. Emran S. Mayilvahanan A. Saxena C.-C. Yuan
P. Hu P.S. Pendergrast L.M. Schramm X. Zhao
B. Ma K. Samudre Y. Sun A. Zia

The specific transcription of genes is accomplished through a combinatorial mechanism in which different configurations of protein-DNA and protein-protein interactions are used to recruit the correct RNA polymerase at the correct place and the correct time. We use the human small nuclear RNA (snRNA) genes and the human immunodeficiency virus type-1 long terminal repeat (HIV-1 LTR) as model systems to study mechanisms of transcription. Most snRNA genes are transcribed by RNA polymerase II, but some are transcribed by RNA polymerase III. Yet, all snRNA promoters are quite similar to each other and recruit several common transcription factors. Thus, in this system, transcription initiation complexes that recruit different RNA polymerases are very similar and can be compared to identify determinants of RNA polymerase specificity.

The core RNA polymerase II and III snRNA promoters contain a proximal sequence element or PSE, which recruits a multisubunit complex we refer to as $SNAP_c$. The RNA polymerase III core snRNA promoters contain, in addition, a TATA box located downstream from the PSE that recruits the TATA-box-binding protein (TBP). $SNAP_c$ and TBP recruit at least two additional proteins referred to as B′′ and BRFU. BRFU is an interesting protein because it is related to both TFIIB, which in RNA polymerase II mRNA promoters bridges the promoter-binding proteins and RNA polymerase II, and BRF, which is involved in transcription from gene-internal RNA polymerase III promoters. The RNA polymerase II snRNA promoters require many of the factors involved in transcription from RNA polymerase II mRNA promoters including TBP, TFIIA, TFIIB, TFIIF, and TFIIE. However, it is not clear how these factors are recruited to the TATA-less RNA polymerase II snRNA promoters. We are characterizing the remaining factors required to reconstitute RNA polymerase II and RNA polymerase III transcription from snRNA promoters and studying how the RNA polymerase II and III snRNA initiation complexes assemble.

The HIV-1 promoter generates two types of transcripts: full-length transcripts and prematurely terminated transcripts. We have continued the characterization of FBI-1, a transcription factor that binds to the HIV-1 promoter element (the inducer of short transcripts or IST) that activates the formation of short HIV-1 transcripts.

Analysis of the Functional Domains of BRFU and TFIIB

L.M. Schramm, Y. Sun, N. Hernandez

The U6 snRNA promoter recruits $SNAP_c$, TBP, B′′, and BRFU. As mentioned above, BRFU is related in sequence to TFIIB. In TATA-box-containing RNA polymerase II mRNA promoters, TFIIB recognizes TBP or TFIID bound to the TATA box and recruits, through direct protein-protein contacts, RNA polymerase II complexed with TFIIF. Thus, it seems likely that BRFU has a similar role in bridging promoter-bound factors and, in this case, RNA polymerase III. Since the RNA polymerase II snRNA promoters, like mRNA promoter, require TFIIB for transcription, this in turn implies that the determination of RNA polymerase specificity is ultimately governed by the recruitment of either TFIIB or BRFU. We have constructed chimeric TFIIB-BRFU proteins and are determining which regions of these proteins provide the specificity of RNA polymerase recruitment.

Characterization of BRFU-containing Complexes

A. Zia, N. Hernandez

We isolated a cDNA encoding BRFU through database searches of proteins related to TFIIB and BRF. To determine whether BRFU exists in the cell as part of one or several complexes, we are purifying BRFU from HeLa cells.

Characterization of Endogenous B′′ and FBI-1

K. Samudre, N. Hernandez

B′′ is implicated in RNA polymerase III transcription. The gene from which it is derived appears to encode a number of other proteins as a result of alternative splicing patterns. We are interested in determining which of these proteins are actually present in the cell and what their functions are. We are also interested in determining whether FBI-1, which binds to the HIV-1 IST, is present as a complex in cells.

Cooperative Interactions between $SNAP_c$ and TBP on the U6 snRNA Promoter

B. Ma, N. Hernandez

$SNAP_c$ and TBP bind cooperatively to the human U6 promoter. By assembling subcomplexes of $SNAP_c$ that lack either entire subunits or subunit parts, we are mapping the regions in $SNAP_c$ required for cooperative binding with TBP and testing the role of these regions for transcription.

Mechanism of $SNAP_c$ Auto-regulation of DNA Binding

A. Saxena, N. Hernandez

$SNAP_c$ on its own binds poorly to DNA, but a subcomplex of $SNAP_c$ called mini-$SNAP_c$ and consisting of the amino-terminal third of SNAP190, SNAP43, and SNAP50 binds efficiently to the PSE. We are studying the mechanisms by which the full complex down-regulates its own binding to DNA.

Identification of $SNAP_c$-associated Factors

F. Emran, N. Hernandez

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

Reconstitution of Transcription from the Human U6 snRNA Promoter with Four Recombinant Factors and a Partially Purified RNA Polymerase III Complex

S. Sepehri Chong, P. Hu, N. Hernandez

We know that U6 transcription requires $SNAP_c$, TBP, BRFU, and B′′. In an effort to characterize the remaining factors required for basal U6 transcription in vitro, we developed antibodies directed against RNA polymerase III. We cloned a cDNA corresponding to the largest RNA polymerase III subunit, RPC155, and characterized a cDNA corresponding to one of the subunits unique to RNA polymerase III, RPC53 (BN51). We then used the predicted protein sequences to generate antipeptide antibodies directed against both subunits. With such reagents, we were able to show that an anti-RPC53 immunoprecipitate could direct U6 transcription when combined with a $SNAP_c$ fraction and TBP.

To determine which components in the RNA polymerase III fraction were required for U6 transcription, we used conventional chromatography to purify RNA polymerase III. The assay was reconstitution of transcription in the presence of the $SNAP_c$ fraction and TBP. A whole-cell extract was fractionated by successive ammonium sulfate precipitation, phosphocellulose, mono S and mono Q chromatography, and sucrose gradient centrifugation. In the last step, U6 transcription activity was recovered after the 669-kD size marker in a broad peak, which coincided with the elution profile of both the largest subunit and the RPC53 subunit of RNA polymerase III. These results suggested copurification of the U6 transcriptional activity with the bulk of RNA polymerase III. Furthermore, the recovery of U6 transcription activity in a single peak after sucrose gradient centrifugation suggested that the activity was contained within a complex, consistent with the observation that it could be immunoprecipitated with antibodies directed against an RNA polymerase III subunit.

To identify the components required in the $SNAP_c$ fraction, we combined the highly purified sucrose gradient RNA polymerase III fraction with recombinant

$SNAP_c$ and TBP. This combination was inactive. U6 transcription could be reconstituted, however, with a combination of sucrose gradient RNA polymerase III fraction, recombinant TBP, recombinant $SNAP_c$, recombinant B″, and recombinant BRFU. These results confirm the essential roles of B″ and BRFU in U6 transcription and open the way to reconstitution of U6 transcription from entirely defined components.

Characterization of a Minimal System for Basal U6 Transcription In Vitro

P. Hu, N. Hernandez

Basal levels of U6 transcription can be reconstituted in vitro with a combination of an RNA polymerase III complex and recombinant $SNAP_c$, TBP, BRFU, and B″. We are purifying the RNA polymerase III complex such that all of its components can be defined and functionally characterized.

Protein-Protein Interactions in the U6 Transcription Initiation Complex

C.-C. Yuan, N. Hernandez

We are close to having an entirely defined system for U6 transcription. This gives us the opportunity to understand how the different members of the U6 transcription initiation complex assemble, in particular how RNA polymerase III is recruited. We are studying protein-protein interactions between RNA polymerase III subunits and members of the U6 transcription initiation complex.

A Positioned Nucleosome on the Human U6 Promoter Allows Recruitment of $SNAP_c$ by the Oct-1 POU Domain

X. Zhao, P.S. Pendergrast, N. Hernandez

Oct-1 and $SNAP_c$ bind cooperatively to probes with a closely spaced octamer sequence and PSE. Cooperative binding is dependent on a direct protein-protein contact involving a glutamic acid at position 7 within the POU domain and a lysine at position 900 within SNAP190, the largest subunit of $SNAP_c$. In the natural snRNA promoters, however, the spacing between the octamer sequence and the PSE is typically about 150 base pairs, raising the question of how Oct-1 and $SNAP_c$ interact with each other in vivo on natural snRNA promoters.

To characterize the role of Oct-1 in natural snRNA promoters, we first performed some in vivo analyses. We used chromatin immunoprecipitations to show that both Oct-1 and $SNAP_c$ are found in close proximity to the endogenous RNA polymerase II U1 and RNA polymerase III U6 snRNA promoters, as expected if these factors are indeed used for snRNA gene transcription in vivo. Furthermore, the DNA between the octamer and the PSE in the U6 and U1 snRNA promoters was resistant to micrococcal nuclease digestion in exponentially growing cells, consistent with the idea that some kind of structure, perhaps a nucleosome, is located between the PSE and the octamer sequence in vivo.

We then performed in vitro chromatin assembly assays. Assembly of the U6 promoter into chromatin in an extract from *Drosophila* embryos resulted in a positioned nucleosome between the octamer and the PSE, regardless of whether the Oct-1 POU domain was included in the chromatin assembly reaction. This positioned nucleosome allowed cooperative binding of Oct-1 and $SNAP_c$ to the natural U6 promoter, and this cooperative binding required the same protein-protein contact as cooperative binding to closely spaced octamer and PSE sequences on naked DNA. We could also show that assembly of the chromatin template in the presence of the wild-type Oct-1 POU domain, but not an Oct-1 POU domain carrying a mutation at position 7, activated transcription. Together, these results suggest that the positioned nucleosome brings Oct-1 and $SNAP_c$ into close proximity, thus mediating a direct protein-protein contact that allows cooperative binding of the two factors. This is a striking example of a positioned nucleosome playing a positive architectural role that mediates efficient assembly of a transcription initiation complex.

Protein Transduction

S. Mayilvahanan, N. Hernandez

Proteins fused to a small peptide derived from the HIV-1 Tat protein can enter cells. We are using this

technique to test the ability of various fusion proteins to enter cells and elicit a biological response.

Function of FBI-1

P.S. Pendergrast, N. Hernandez

FBI-1 is a POZ domain protein that binds to the HIV-1 IST. In transfection experiments, overexpression of FBI-1 can modulate Tat-activated HIV-1 transcription, but both the mechanism by which this is accomplished and the cellular function of FBI-1 are unclear. In collaboration with Dr. S. Huang (Northwestern University Medical School, Chicago), we have examined the nuclear sublocalization of FBI-1. FBI-1 partially colocalizes with both Tat and the Tat cellular cofactor PTEFb within the splicing-factor-rich nuclear speckle domain. An insoluble subpopulation of FBI-1 displays a "peri-speckle" pattern of localization, which is dependent on the FBI-1 DNA-binding domain, on the presence of cellular DNA, and on active transcription. Together, these results suggest that FBI-1 is targeted to active chromatin and are consistent with the idea that FBI-1 modulates HIV-1 transcription elongation.

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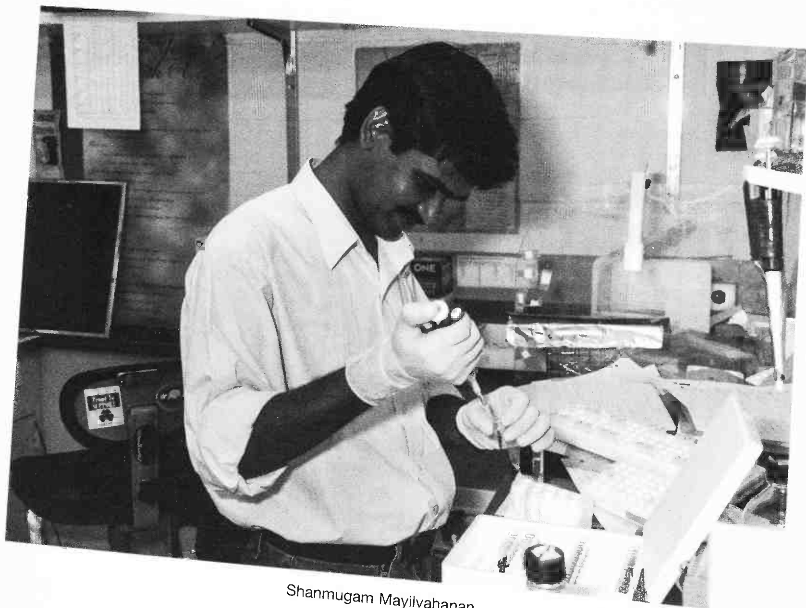

Shanmugam Mayilvahanan

TRANSCRIPTIONAL REGULATION

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

Soon, the complete sequence of the human genome will be determined and with it we will have the complete set of instructions—the blueprint—for human development. We are interested in how cells differentially access this complete set of instructions, which they nearly all possess, to generate the cellular complexity of the human body. We study this question by uncovering mechanisms of transcriptional regulation in human cells. We use the human herpes simplex virus (HSV) as a probe to uncover these mechanisms, by studying virus–host cell interactions. Viruses provide simple regulatory networks in which the cellular transcriptional machinery is manipulated to achieve the goals of virus infection. In a cell infected by HSV, the virus can grow lytically or remain latent for many years. In the lytic cycle, HSV-gene expression is initiated by a viral transcription factor called VP16, which is carried in the infecting virion. Before activating transcription, VP16 forms a multiprotein-DNA complex—the VP16-induced complex—on viral immediate-early promoters with two cellular proteins: HCF-1, an abundant chromatin-associated protein that regulates cell proliferation, and Oct-1, a POU-homeodomain transcription factor. Once the VP16-induced complex is assembled, VP16 initiates viral-gene transcription through a potent transcriptional activation domain.

Our research focuses on three principal issues: (1) How do transcriptional regulators activate the basal transcriptional machinery? (2) How do transcription factors, as in the VP16-induced complex, modify their transcriptional activity through selective protein-protein and protein-DNA interactions? (3) What are the natural cellular roles of HCF-1 and Oct-1, and how do they influence HSV infection? Our studies in each of these three areas continue to elucidate new mechanisms for transcriptional regulation in human cells. Below is a description of two important discoveries we had this year that involve the activity of the basal transcription factors TBP and TFIIB in the first instance and the structure and function of the family of HCF proteins in the second instance.

A REGULATED TWO-STEP MECHANISM OF TBP BINDING TO DNA: A SOLVENT-EXPOSED SURFACE OF TBP INHIBITS TATA-BOX RECOGNITION

A key to understanding transcriptional regulation is how transcription is initiated at the correct positions in the genome. One of the central players in the correct initiation of transcription is the TATA-box-binding factor TBP. TBP is involved in transcription by all three eukaryotic nuclear RNA polymerases—pol I, pol II, and pol III—whether or not the promoter contains a TATA-box core element. On TATA-box-containing promoters, however, the direct interaction between TBP and the TATA box is one of the key steps in the initiation of transcription.

Human TBP contains a bipartite structure. The TATA-box-recognition domain of TBP is a highly conserved 180-amino-acid carboxy-terminal domain, commonly referred to as the TBP core (TBP_{CORE}), and crystallographic studies have shown that when the TBP_{CORE} is bound to the TATA box, it unwinds and sharply bends the DNA to form a unique saddle-shaped structure over the bent DNA. In addition to the highly conserved TBP_{CORE}, human TBP contains a highly diverged species-specific amino-terminal region, which varies in both sequence and length. The structure of this region of TBP is not known and how it might influence TBP binding to the TATA box is not clear.

We have an on-going collaboration with Dr. N. Hernandez here at CSHL in which we have been examining the activities of full-length human TBP molecules with mutations located across the surface of the TBP_{CORE} in pol II and pol III transcription. During the course of these experiments, Xuemei Zhao discovered that some substitutions of solvent-exposed residues of the TATA-box-bound TBP_{CORE} induce the formation of an abundant TBP–TATA-box complex during electrophoretic mobility retardation analysis. By further mutagenesis, we discovered a solvent-exposed surface of the structured TBP_{CORE} domain that is important for inhibition of DNA binding and DNA

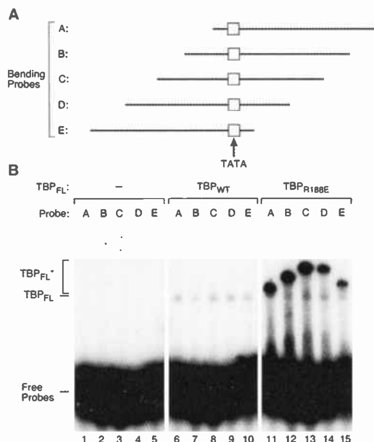

FIGURE 1 The DNA in the activated TBP_{R188E} complex, but not the wild-type TBP complex, is bent. (*A*) Schematic representation of the five probes used for the bending assay. The position of the TATA box is indicated. (*B*) Circular permutation analysis of wild-type (lanes *6–10*) and R188E mutant (lanes *11–15*) TBP molecules bound to the TATA box. Lanes *1–5* contained no TBP. The set of DNA-bending probes is as indicated in *A*.

bending by full-length wild-type TBP. Full-length wild-type TBP initially binds the TATA box to form an unstable complex containing unbent DNA and then slowly forms a stable complex containing bent DNA. The two complexes are shown in Figure 1, in which the binding activity of full-length wild-type human TBP is compared to a mutant R188E—in which the arginine at position 188 on the TBP_{CORE} surface has been replaced by a glutamic acid residue—on a set of "bending" probes.

When DNA is bent, its mobility during polyacrylamide gel electrophoresis is retarded, and the retardation becomes more significant the closer the bend is to the center of the DNA molecule. Therefore, if a protein bends DNA when bound to it, it will cause the mobility of the protein-DNA complex to decrease the closer the protein-binding site is to the center of the DNA molecule. Thus, the bending activity of a DNA-binding protein can be assessed by comparing the mobility of the protein-DNA complexes on a series of DNA probes in which the protein-binding site is progressively moved from one end of the DNA molecule to the other. Figure 1A shows such a probe set in which the position of the TATA box has been progressively moved, and Figure 1B shows the results of using this set of radiolabeled probes with wild-type and the R188E mutant TBP in an electrophoretic mobility retardation assay. The wild-type TBP formed a single complex, modest in quantity, with an identical mobility on all five probes, indicating that under these conditions, TBP is not bending the DNA. In contrast, the mutant TBP binds very effectively under these conditions and bends the DNA as indicated by the characteristic bell-shaped mobility exhibited by the mutant TBP-DNA complexes. Thus, the R188E mutation—as well as many others at neighboring positions—activates both DNA binding and DNA bending by TBP. We refer to the wild-type surface of the TBP_{CORE}, which inhibits TATA-box binding and bending, as the inhibitory DNA-binding (IDB) surface.

The basal factor TFIIB associates with TBP on DNA, and we have found that it greatly accelerates TBP binding to bent DNA in the form of a TFIIB–TBP–TATA box complex. The IDB surface of TBP, by inhibiting DNA binding by TBP in the absence of TFIIB, contributes to the cooperativity of binding with TFIIB. Using TBP and TFIIB, we showed that TBP can bind the TATA box to form first the unbent TATA-box complex and then transition into a bent complex without leaving the DNA. The transition from an unbent complex to a bent complex promoted by TFIIB may be an important step for TBP to direct the correct formation of an active initiation complex. We suggest that through this two-step unbent-to-bent TBP-binding mechanism, TBP may constantly probe the genome for TATA-box-like sequences without committing itself to forming an active bent complex. Once multiple signals are received (e.g., interaction with TFIIB), then an appropriate bent TATA-box complex can form. We are intrigued by the possibility that the ability of TBP to recognize unbent DNA may permit it to recognize a TATA box in DNA that cannot undergo the gross deformation involved in TBP bending of DNA, as, for example, might be expected of a TATA box within a nucleosome in a cell. Then through the action of other proteins recruited by TBP, the nucleosome could be destabilized and permit bent TBP–TATA box complex formation and subsequent transcriptional initiation.

STABILIZATION BUT NOT THE TRANSCRIPTIONAL ACTIVITY OF HSV VP16-INDUCED COMPLEXES IS EVOLUTIONARILY CONSERVED AMONG HCF FAMILY MEMBERS

In the second discovery, Soyoung Lee found that the ability of HCF proteins to stabilize the VP16-induced complex has been conserved among HCF family members but not their ability to support transcriptional activation by VP16. The VP16-associated factor HCF-1 is an unusual protein. It is translated as a large polypeptide of 2035 amino acids which undergoes proteolysis at a series of centrally located 26-amino-acid repeats; the resulting amino- and carboxy-terminal polypeptides are stable and remain noncovalently associated. At its amino terminus, HCF-1 contains a predicted β-propeller structure which is responsible for binding VP16 and sufficient to stabilize the VP16-induced complex.

Consistent with the role of HCF-1 in cell proliferation, elements of HCF-1 are highly conserved in metazoans. For example, extracts from worms and insects can replace HCF-1 in VP16-induced complex formation, implying the presence of functional HCF-1 homologs in these organisms. Indeed, the worm *Caenorhabditis elegans* contains a functional HCF-1 homolog called CeHCF that can support VP16-induced complex formation. Human HCF-1, however, differs in some respects from CeHCF: Although the amino- and carboxy-terminal regions of HCF-1 are highly conserved in CeHCF, the middle of the protein with its processing repeats is missing in CeHCF. Thus, CeHCF is much smaller than HCF-1 (782 vs. 2035 amino acids) and does not undergo proteolytic processing.

A second human HCF protein called HCF-2 has been described by A. Wilson and colleagues (New York University) that, like CeHCF, is smaller than HCF-1, and in which the amino- and carboxy-terminal regions, but not the middle of the protein, are conserved. Unlike HCF-1 and CeHCF, however, the interaction of HCF-2 with VP16 was reportedly weak, which led to the conclusion that HCF-2 is unlikely to have a role in transcriptional activation by VP16 and HSV infection.

To understand better the relationship of HCF-1 and HCF-2 to each other and to CeHCF, we compared all three proteins directly in both in vitro and in vivo assays. We found, unexpectedly, that all three full-length HCF proteins can associate with VP16 and stabilize the VP16-induced complex effectively, demonstrating that this is a conserved function of these molecules. Unlike HCF-1 and CeHCF, however, HCF-2 failed to support VP16-induced transcriptional activation effectively in vivo. Thus, in this instance, an HCF protein can promote VP16-induced complex formation, but the resulting complex is inactive. These results suggest that HCF-2 might interfere with VP16 activation of HSV gene expression and therefore HCF-2 could have a negative effect on HSV infection.

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STRUCTURAL BIOLOGY OF REGULATION OF DNA REGULATORY MOLECULES AND PROTEOLYSIS

L. Joshua-Tor A. Auster, E. Enemark, T. Messick, P.R. Kumar, P. O'Farrell, N. Tolia, E. Zarkowski, H. Zhou, S. Bechis (URP), O. Martin (PFF)

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

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

DNA Binding and Assembly of the Papillomavirus Initiator Protein E1

E. Enemark, A. Auster, S. Bechis [in collaboration with A. Stenlund, Cold Spring Harbor Laboratory]

Papillomaviruses are a large family of closely related viruses that give rise to warts in their hosts. Infection of the genital tract by the human papillomaviruses (HPV) from this group represents one of the few firmly established links between viral infection and the development of cervical cancer, as HPV DNA is found in practically all cervical carcinomas. The E1 protein belongs to a family of multifunctional viral proteins whose main function is related to viral DNA replication. These proteins bind to the origin of DNA replication, melt the DNA duplex, possess DNA helicase activity, and recruit other cellular replication proteins such as DNA polymerase α and replication protein A (RPA). Most likely, different oligomeric forms of the two proteins are responsible for the different activities, and the sequential assembly of T antigen and E1 complexes ensures an ordered transition between these different activities. Ultimately, E1 forms a hexameric ring helicase on each strand that serves as replicative DNA helicases that unwind the DNA in front of the replication fork.

In collaboration with Arne Stenlund's group here at CSHL, we embarked on structural studies to provide high-resolution structural information about E1, its DNA-binding activity, and its assembly on DNA. These would provide general insight into the biochemical events that are involved in viral DNA replication. They can also provide a basis for the development of clinical intervention strategies. Second, the viral DNA replication machinery itself represents an obvious target for antiviral therapy, and detailed information such as high-resolution structures of viral proteins required for replication will greatly facilitate the development and testing of antiviral agents.

The initial binding of an E1 dimer together with E2 serves to recognize the double-stranded origin of replication (*ori*) with high specificity. The subsequent binding of additional E1 molecules leads to *ori* melting, most likely through the positioning of E1 molecules in the proper arrangement necessary for melting of double-stranded DNA. Ultimately, two hexameric rings are formed on the *ori* that could unwind the DNA double helix. Thus, the transition between these complexes represents a transition from a tethering function for E1 to a function that modifies the DNA structure. We have captured structural snapshots of two sequential steps in the assembly process (Fig. 1). Loading of an initial E1 dimer on the origin occurs at adjacent major grooves on one face of the helix. Subsequently, a second dimer is loaded on another face of the helix. Binding occurs through two DNA-binding modules, a DNA-binding loop (DBL), and a DNA-binding helix (DBH), that bind to the two separate strands of DNA. The majority of contacts are made by the DBL, a long loop that is preorganized for binding. Binding of two individual strands by separate regions of the protein can provide a basis for a transition from double-stranded to single-stranded DNA binding, leading to strand separation, with protein maintaining binding to one strand while dissociating from the other. Additional subunit-subunit contacts

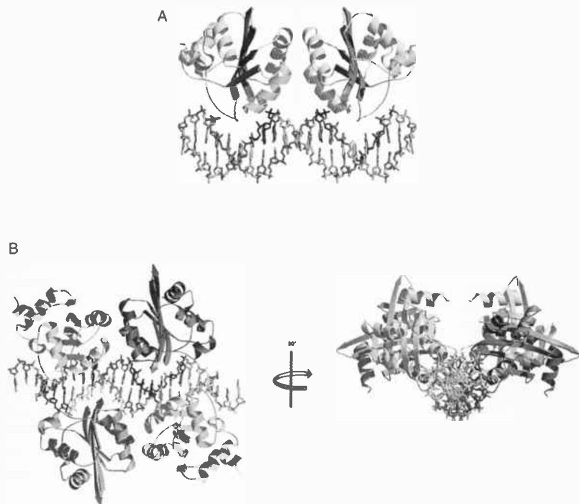

FIGURE 1 Crystal structures of two intermediates in the assembly of E1-DBD subunits on the origin of replication. (*A*) Structure of the E1-DBD dimer bound to sites 2 and 4 determined from the [(E1-$DBD_{159\text{-}307})_2(DNA_d)$] crystal structure. (*B*) Structure of the E1-DBD tetramer bound to E1-binding sites 1–4 determined from the [(E1-$DBD_{159\text{-}303})_4(DNA_l)$] crystal structure viewed perpendicular and parallel to the DNA helical axis.

formed in the assembly of the hexameric ring would then compensate for lost protein-DNA interactions. A more common recognition motif, such as "helix in the groove," in which the same structural element contacts both strands on either side of the groove, would not allow such a facile transition. Since interactions between DNA and the DBH are less extensive than interactions with the DBL, the DBL is a more likely candidate for the binding module that retains binding. Moreover, in the tetramer, the two DBLs of the upstream monomers of each dimer bind to the top strand, whereas the DBLs of the downstream monomers of each dimer bind to the bottom strand. We therefore suggest that one hexameric helicase assembles around the top strand on one side of the *ori* from the proteins at sites 3 and 4, and the other hexameric helicase assembles around the bottom strand on the other side of the *ori* from the proteins at sites 1 and 2. As the hexameric helicases travel in opposite directions around their respective strands, the correct 3′→5′ polarity would occur (Fig. 2).

Bleomycin Hydrolases

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

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

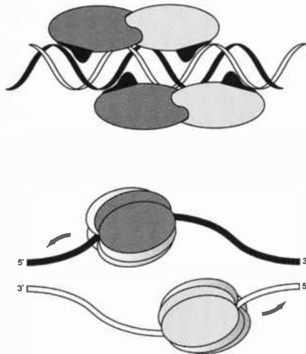

FIGURE 2 Schematic diagram of the proposed role of the DBL in strand selection. A cartoon depicting a model for the assembly of two hexameric helicases around single strands at the *ori*. The two DBLs (*black*) of the upstream monomers of each dimer bind to the top (*black*) strand, whereas the DBLs of the downstream monomers of each dimer bind to the bottom strand (*white*). One hexameric helicase assembles from the upstream monomers at sites 3 and 4 (*dark gray*) around the top strand, and the other hexameric helicase assembles from the downstream monomers at sites 1 and 2 (*light gray*). This arrangement would result in the correct 3′→5′ polarity for the helicases.

genetic linkage of an allelic variant to the nonfamilial form of Alzheimer's disease. BH was also found to bind amyloid precursor protein (APP).

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

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

In collaboration with Dan Rich's laboratory at the University of Wisconsin, Madison, we embarked on an iterative study to design specific tight-binding active site inhibitors of BH activity to increase the efficacy of bleomycin, and perhaps reduce dosage levels in cancer treatment. The emerging link between hBH and Alzheimer's disease is another incentive, since an inhibitor to hBH may be useful in understanding this connection and may also be useful in therapy. We are using our crystal structure to create novel inhibitors by use of computerized structure-generating programs. Using this approach, we can create both peptide-like and nonpeptide inhibitors by a combinatorial process. We identified four compounds in our initial screens that are good inhibitors and have solved two cocrystal structures of hBH with these compounds. These structures are the basis of further drug design.

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PROTEIN CHEMISTRY

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

Our laboratory has focused on the analysis of the primary structure of novel proteins and posttranslational modification. The combination of mass spectrometry and genome sequence database has allowed us high-throughput identification of proteins. Our electrospray ionization ion-trap mass spectrometer showed its power through sensitivity and high-throughput identification. With this tandem mass spectrometry analysis, one can identify many proteins in a large complex that interact with a specific protein. We hope that identification and characterization of the proteins will contribute to our fundamental understanding of biology.

We continued to collaborate with scientists at Cold Spring Harbor Laboratory. We identified protein complexes of the p88 ORC-binding protein of *Saccharomyces cerevisiae* in collaboration with Bruce Stillman; protein complexes of human RNA polymerase III in collaboration with Nouria Hernandez; and human caspase 9 complexes and XIAP complexes in collaboration with Yuri Lazebnik. We also identified the first RNAi components of *Drosophila* in collaboration with Greg Hannon.

This is my last annual report at Cold Spring Harbor Laboratory. I closed my lab in August after working here for ten and a half years. I accepted a position at the University of Texas M.D. Anderson Cancer Center in April. Aysha Ahmed went back to Montreal in May and found a job at McGill University. Lenka Hurton went on to graduate school at the Virginia Polytechnic Institute and State University in June to study marine biology. Denise Wurtz and I left Cold Spring Harbor Laboratory at the end of August. Stacy Lopez decided to stay at the Protein Chemistry Facility with Mike Myers. My experiences at Cold Spring Harbor Laboratory were extremely valuable and my stay here was a stimulating period in my life. I had a very fruitful time here thanks to my colleagues and friends at Cold Spring Harbor Laboratory.

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RNA SPLICING

A.R. Krainer E. Allemand L. Cartegni L. Friend M. Hastings L. Manche H. Okunola X. Roca S. Shaw P. Smith Q.-S. Zhang J. Zhu

MECHANISMS OF CONSTITUTIVE AND ALTERNATIVE PRE-mRNA SPLICING

RNA splicing is an essential step for the expression of most eukaryotic protein-coding genes. The selection and pairing of authentic splice sites within the spliceosome occur with extremely high fidelity, requiring precise interpretation of limited and dispersed sequence information present throughout introns and exons. In humans, ~60% of genes are expressed via alternative splicing, giving rise to multiple protein isoforms. The choice of alternative splice sites is commonly regulated to alter gene expression, either tissue-specifically or in response to a developmental program or to extracellular signals. The fact that multiple protein isoforms can be expressed from individual genes demonstrates that the classic "one gene–one enzyme" paradigm is no longer valid, and provides an explanation for the unexpectedly small number of genes uncovered by genome-sequencing projects.

Both constitutive and alternative splicing mechanisms involve multiple protein components, as well as RNA components that are part of small nuclear ribonucleoprotein (snRNP) particles. The work in our lab focuses on the identification and molecular characterization of protein factors and sequence elements that are necessary for the catalysis and fidelity of splicing and/or for the regulation of alternative splice-site selection. Our recent progress in understanding how exons are identified by the spliceosome is summarized below.

MECHANISMS OF SPLICING SILENCING

We investigated the mechanisms of splicing silencing mediated by elements present in the exons of a human immunodeficiency virus (HIV) *tat* pre-mRNA. Splicing enhancer elements are present near the 5′ end of exon 3, and a splicing silencing element is located near the 3′ end of the same exon. Interestingly, splicing of exons 2 and 3 is efficient in the presence of the SR protein SF2/ASF, but it does not occur in the presence of a different SR protein, SC35; however, when the silencer element was mutated, efficient splicing could take place in the presence of either SR protein. This type of selective silencing is probably important for modulating alternative splicing in different cell types, according to the levels of each SR protein present, and also for allowing inefficient splicing, which is necessary for retroviral replication and gene expression. We found that heterogeneous nuclear RNP (hnRNP) A1 and closely related proteins recognize the silencer and repress recognition of the exon, depending on which SR protein is present. Selective depletion of hnRNP A1-type proteins from splicing extracts derepressed splicing, as did deletion of the silencer element (Fig. 1). Adding back recombinant hnRNP A1 restored silencing in the presence of a wild-type silencer. Moreover, hnRNP A1 bound specifically to an RNA fragment encompassing a wild-type, but not a mutant, silencer (not shown).

Silencing required the carboxy-terminal domain of hnRNP A1, which is necessary for cooperative binding to RNA. This and other observations led us to propose that initial binding of hnRNP A1 to a high-affinity binding site within the silencer is followed by cooperative binding of additional hnRNP A1 molecules, which propagate toward the 5′ end of the exon (Fig. 2). To test this model, UV cross-linking experiments were carried out with RNA probes assembled by ligation of labeled and unlabeled fragments. The results demonstrated that hnRNP A1 binding to the upstream region of the exon depends on the presence of a wild-type silencer in the downstream region. Moreover, cooperative binding of hnRNP A1 was blocked in the presence of wild-type SF2/ASF but not SC35 (Fig. 2), which correlates with the activities of these proteins in splicing of the *tat* pre-mRNA. The RS domain of SF2/ASF is not required for blocking hnRNP A1 binding upstream or for splicing of this substrate. We presume that as hnRNP A1 polymerizes, SC35 binding to a splicing enhancer motif is prevent-

FIGURE 1 hnRNP A1 specifically silences *tat* pre-mRNA splicing. (*a*) Depletion of hnRNP A1 measured by immunoblotting. HeLa cell nuclear extract (NE) was depleted of hnRNP A/B proteins by selective binding to immobilized single-stranded telomeric DNA oligonucleotide; mock depletion was carried out with a control oligonucleotide. hnRNP A1, or as a control, SF2/ASF, was measured in the indicated extracts by immunoblotting with the appropriate monoclonal antibody. (*b*) In vitro splicing of radiolabeled transcripts from an HIV *tat* minigene (Tat23) and from a mutant in which the exonic splicing silencer in exon 3 was deleted (ΔS3). The reactions were carried out in untreated extract (NE; lanes *1* and *5*), mock-depleted extract (lanes *2* and *6*), hnRNP A1-depleted extract (ΔA1) alone (lanes *3* and *7*), or in the presence of recombinant hnRNP A1 (lanes *4* and *8*). The positions of the pre-mRNA, mRNA, and exon 1 intermediate are indicated; the asterisk indicates a band that results from aberrant RNA cleavage unrelated to splicing.

FIGURE 2 Mechanism of splicing silencing. The model shows a molecule of hnRNP A1 (*bold circle*) bound to a high-affinity site within the silencer near the 3′ end of *tat* exon 3. Initial binding of hnRNP A1 is followed by cooperative nonspecific binding of additional hnRNP A1 molecules along the exon. To test that binding of hnRNP A1 upstream depends on initial binding at the silencer, two RNA probes were designed for cross-linking experiments. Each probe was constructed by ligating two separate RNA fragments: the 78-nucleotide 5′ fragment is identical for both probes and was uniformly labeled with ^{32}P; the 23-nucleotide 3′ fragment is unlabeled and has either a wild-type silencer (E3 probe) or a mutant silencer with seven point mutations (E3m probe). Each probe was incubated in HeLa S100 extract with or without SR proteins under splicing conditions, followed by irradiation with UV light. The RNA was digested with RNase and the protein-oligonucleotide covalent adducts were immunoprecipitated with hnRNP A1 monoclonal antibody and analyzed by SDS-PAGE and autoradiography. Labeled hnRNP A1 was recovered from the S100 extract with the E3 probe (lane *1*), but much less efficiently with the E3m probe (lane *5*). SF2/ASF with or without (ΔRS) its RS domain blocked cooperative binding of hnRNP A1 to the upstream region of the exon (lanes *2* and *3*), whereas SC35 had little effect (lane *4*).

ed, whereas tighter binding of SF2/ASF to its cognate enhancer motif blocks the propagation of hnRNP A1. Indeed, strengthening the interaction of SC35 with the exon by improving the binding site allows SC35 to promote splicing in the presence of the silencer element and hnRNP A1 (not shown).

EXON-SKIPPING MUTATIONS AND MECHANISMS

We completed a mechanistic study of a nonsense mutation in exon 18 of the *BRCA1* breast cancer susceptibility gene which causes skipping of that exon. The phenomenon of nonsense-associated altered splicing (NAS) has been observed for many alleles in human disease genes, and various models have been proposed to explain it. We were able to reproduce NAS in vitro and demonstrated that the *BRCA1* nonsense mutation inactivates a splicing enhancer heptamer motif that is recognized by the SR protein SF2/ASF. We further showed that not all nonsense mutations

cause skipping and, conversely, that some missense mutations do cause skipping; it is the inactivation of the degenerate SR protein motif that determines whether the exon can no longer be recognized as such.

We also applied the analysis of SR protein recognition motifs to the *SMN1* and *SMN2* genes, which are involved in spinal muscular atrophy (SMA), a common pediatric neurodegenerative disease. SMA patients lack a functional *SMN1* gene but possess at least one copy of the nearly identical *SMN2* gene. It was known that exon 7 in *SMN1* is constitutively included, whereas exon 7 in *SMN2* is skipped 80% of the time, giving rise to a defective protein. Moreover, this difference between the two homologous exons is due to a single, translationally silent point mutation. The low level of functional SMN protein arising from the 20% of *SMN2* pre-mRNA that is spliced correctly is essential for viability but is not sufficient to prevent the disease. We demonstrated that the single-base change between *SMN1* and *SMN2* exon 7 inactivates a splicing enhancer motif recognized by SF2/ASF. We tested our ability to predict the presence of functional enhancer motifs by introducing a compensatory mutation five nucleotides downstream of the variant site to restore a high-score SF2/ASF motif in *SMN2*; this second-site suppressor mutation effectively restored inclusion of exon 7.

These studies can explain how single-base changes in exons allow genes to evolve, e.g., transforming a constitutive exon into an alternative one, or vice versa. A further implication is that many mutations traditionally classified as nonsense, missense, or silent, as well as some polymorphisms, can give rise to exon skipping, such that the mutant or variant site is no longer present in some or all of the mature mRNA. Thus, the effect of the mutation or polymorphism on the protein product is very different from, and usually more drastic than, what would be predicted from analysis of the genomic sequence. Analysis of mRNA splicing can therefore be critical for correct disease diagnosis and assessment of phenotypic risk in individuals with familial predispositions. Finally, the mechanistic understanding of exon definition may lead to new therapies for SMA and for certain alleles in other genetic diseases.

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PROTEOMIC APPROACHES TO STUDYING SIGNAL TRANSDUCTION

M. Myers S. Lopez
L. Schmidt

The completion of the human genome has ushered in a new era of discovery. Essentially, this accomplishment has identified all of the primary players governing human biology. Now the important work of assigning functions to this myriad of proteins has become the principal task of modern biology. Although the functions of some of these proteins will be assigned by genetic or computational means, the lion's share of the functional characterization of these proteins will be determined by proteomics—a new word that describes the fusion of biochemistry and analytical chemistry.

I left Nicholas K. Tonks' lab this year to start working on proteomics approaches to signal transduction and tumorigenesis. This transition was part of the natural progression from the work that I was performing with Nick on the role of protein tyrosine phosphatases (PTPs) in signal transduction, especially related to their function during tumorigenesis. As part of this transition, I also took over the Protein Chemistry facility from Ryuji Kobayashi who left this year. Although much of Ryuji's staff also left this year, I was pleased when Stacy Lopez decided to stay and when Lise Lotte Schmidt decided to join the lab.

Since my appointment in August of this year, my work has focused on finishing up studies on PTP substrate specificity that were initiated while I was a member of the Tonks lab and in improving the mass spectrometry facilities at Cold Spring Harbor Laboratory.

Optimization of Protein Characterization

S. Lopez, L. Schmidt

Most of our efforts this year have been in optimizing protein analysis by mass spectrometry (MS). When I took over the mass spectrometry facility, 100–200 fmoles of protein was considered to be the lower limit for protein identification; in other words, 200 fmoles might give you an answer... if you were lucky. To maximize the sensitivity and the speed (throughput) of the analysis, it was necessary to rework every step, from the way the samples were processed to the way the data are analyzed. In fact, these improvements required that several pieces of equipment be completely replaced or retooled in order to maximize the sensitivity of our analyses. Currently, we are able to successfully analyze most samples at the 100-fmole level, and we estimate that the lower limit for successful analysis is in the 5–10-fmole range. Examples of some typical chromatograms before and after optimization are shown in Figure 1.

Proteomic Approaches for the Analysis of PTP Function

M. Myers [in collaboration with N. Tonks, Cold Spring Harbor Laboratory]

The phosphorylation of tyrosine residues in proteins is often the key event in controlling signaling cascades. Importantly, these modifications are removed through the action of the PTP family of enzymes. Fortunately, all the members of this family have an easily recognizable catalytic core, which is also called the PTP signature motif. Studies of the human genome, utilizing this signature, have revealed approximately 90 members of the PTP family. In an on-going collaboration with Nick Tonks, we are attempting to enrich this genomic information by developing a proteomics-based strategy for defining the complement of PTPs from various cellular or subcellular sources. Importantly, this analysis is expected to lead to insights into PTP function, as well as allowing us to examine the roles of PTPs in various pathological conditions, such as diabetes and cancer, or as targets for therapeutic intervention. The strategy takes advantage of the chemical nature of the PTPs and is based on a

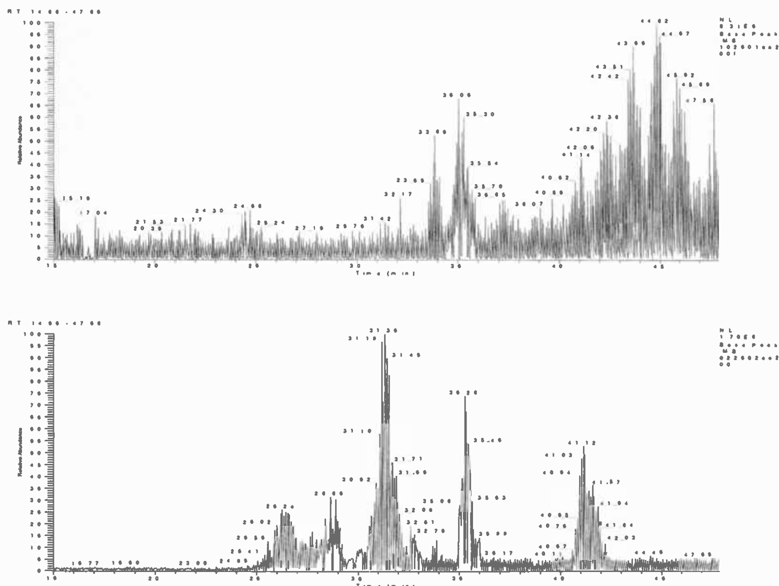

FIGURE 1 Optimization results in a significant improvement in sensitivity. Both chromatograms represent a high-performance liquid chromatography (HPLC)-reverse phase separation of 200 fmoles of a tryptic digest of carbonic anhydrase using the LCQ-ion trap mass spectrometer as a detector. The upper chromatogram was produced prior to the optimization procedure, whereas the lower chromatogram was produced postoptimization. Please note that the *y* axis is normalized to the tallest peak in the chromatogram and the value used for this normalization level (N.L. in the upper left corner of each chromatogram) is indicative of the strength of the signal. The N.L. value for the postoptimization chromatogram is 20-fold greater than for the preoptimization chromatogram, indicating a 20-fold increase in sensitivity.

battery of PTP inhibitors, which bind to a catalytically required cysteine found in the signature motif. Importantly, these inhibitors can be used as affinity reagents, and we have successfully adapted them for the visualization of PTP family members. In the past year, we have been optimizing this strategy and have begun to identify the proteins that are enriched by this scheme and to further optimize this strategy.

Substrate Specificity of PTP1B

M. Myers [in collaboration with N. Tonks and J. Andersen, Cold Spring Harbor Laboratory]

The substrate specificity of protein kinases has long been established; however, the substrate specificity of the PTPs is a much more controversial issue. The controversy is largely fueled by in vitro studies in which members of the PTP family traditionally exhibit weak intrinsic substrate specificity. This year, we extended findings from the previous year, which suggested that PTP1B preferred substrates with a tandem phosphorylation motif. From this study, we were able to develop a consensus dephosphorylation motif for PTP1B (E/D-pY-pY-R/K). A search of the human database with this motif uncovered more than 700 proteins that contain this motif. Somewhat surprisingly, this search identified a large proportion of the tyrosine. Importantly, this motif was found in the activation loop of these kinases where phosphorylation of the tandem tyrosine residues is required for kinase activation, suggesting that PTP1B has a major role in antagonizing these tyrosine kinases. We focused our

analysis on the JAK/TYK2 subfamily, in which JAK2 (E-pY-pY-R) and TYK2 (E-pY-pY-K) represent perfect matches to the motif, whereas JAK1 (E-pY-pY-T) does not meet the consensus. In response to the cytokine interferon-α (IFN-α), JAK1 and TYK2 are activated by cross-phosphorylation. Similarly, in response to IFN-γ, JAK1 and JAK2 become activated. Since these cytokines are simultaneously activating kinases, which do (JAK2 or TYK2) or do not (JAK1) fit the predicted PTP1B consensus dephosphorylation site, stimulation with IFN-α or IFN-γ provides the perfect opportunity to study the in vivo substrate specificity of PTP1B. In fact, substrate trapping experiments with PTP1B revealed that TYK2 and JAK2 are in vivo substrates of PTP1B, whereas JAK1 does not become associated with PTP1B trapping mutants in response to either IFN-α or INF-γ. Importantly, PTP1B-deficient mouse embryo fibroblasts exhibit enhanced sensitivity to INF-α or INF-γ stimulation, indicating that PTP1B is required to antagonize these responses. This study demonstrated that it is possible to use consensus dephosphorylation motifs to predict PTP substrates, as well as demonstrating that PTP1B acts as a negative regulator of INF-α, INF-γ, and other cytokine signals.

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Mike Myers

CELL SIGNALING IN HIV PATHOGENESIS

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Our interest is in defining the molecular mechanisms underlying the pathogenesis of AIDS, and in particular, understanding the functional consequences of interactions between viral proteins and the cellular regulatory machinery. The focus of our research is to delineate the functions of Nef, a regulatory protein of human and simian immunodeficiency viruses (HIV and SIV), which is an important determinant of virulence. Viruses with defects in Nef replicate poorly in the host, and this correlates with attenuated development of AIDS.

NEF FUNCTIONS

Natural Nef protein isolates have several conserved classes of effects on signal transduction and protein-sorting machineries that probably enhance virulence in vivo. In T cells, Nef disrupts antigen-specific signal transduction machinery. This is accomplished both by down-regulating cell surface CD4, an essential component of the T-cell antigen receptor (TCR) on class II major histocompatibility complex (MHC)-restricted T lymphocytes, and by blocking aspects of signal transduction via the CD3 signaling component of the TCR. At the same time, Nef activates downstream signaling cascades, thereby likely bypassing the events that normally control the functional activation of T lymphocytes. In macrophages, Nef promotes the production of chemokines that attract T lymphocytes to infected cells, thus facilitating the spread of the virus (the infectious process). Additionally, Nef disrupts the antiviral response of the host by decreasing the expression of class I MHC on the surface of HIV-1/SIV infected cells. Class I MHC molecules constitute the critical element of the immune system detection machinery that allows elimination of virally infected cells. This effect of Nef may enable infected cells to evade the immune response in the infected host. Finally, Nef also stimulates SIV/HIV particle infectivity and replication in primary T cells, which probably reflects Nef effects on signal transduction and protein-sorting machineries.

In previous years, we probed and described the molecular mechanisms that mediate the effects of HIV-1 Nef on CD4 and class I MHC expression and initiated structure-function analysis of the Nef protein encoded by a pathogenic strain of SIV. More recently, our research focused on three major areas:

- We discovered that Nef down-regulates cell surface expression of the CD28 molecule, a major co-stimulatory receptor that is necessary for effective T cell activation, and described the underlying mechanism.
- We initiated biochemical purification of protein complexes that associate with Nef in T cells and antigen-presenting cells. We anticipate that this approach will lead us to identification of novel Nef effectors in these cell types.
- We developed novel transgenic mouse models to study and genetically dissect the effects of Nef on the function of T cells and antigen-presenting cells. These two latter sets of experiments will continue to be the major focus of our research in the future.

NEF DOWN-REGULATES THE CELL SURFACE EXPRESSION OF CD28: THE MAJOR COSTIMULATORY RECEPTOR OF CD4-POSITIVE T CELLS

Nef proteins were found to disrupt two separate aspects of normal antigen recognition machinery by interfering with the expression of the CD4 and by interfering with expression and/or signaling of the CD3/TCR complex. Productive antigen-initiated response, in addition to CD4 and TCR/CD3, also requires the CD28 receptor. CD28 is the major co-stimulatory receptor of CD4-positive T cells, and interference with the CD28 signaling pathway could result in suppression of the immune response and

anergy. Given the conserved effects of Nef proteins on other aspects of antigen-specific signaling machinery, we suspected that Nef might also interfere with the expression or signaling of CD28, and we set up experiments to address this possibility. These experiments revealed that both HIV-1 and SIV Nef proteins down-regulate the cell surface expression of the CD28 molecule.

The ability of Nef to down-regulate CD28 cell surface expression is genetically separable from other known functions of Nef such as CD4, CD3, and class I MHC down-regulation, and from its ability to associate with serine/threonine kinases. Hence, CD28 down-regulation is selected independently from those other Nef functions.

MECHANISM FOR DOWN-REGULATION OF CD28 BY NEF

We studied the mechanisms underlying the down-regulation of CD28 expression on the cell surface by HIV-1 and SIV Nef proteins. Genetic and functional evidence from our studies indicates that Nef induces CD28 endocytosis via an AP-2 clathrin-adaptor-dependent pathway and that this is mediated by direct interactions between Nef, AP-2, and CD28. To further address the interactions between Nef, CD28, and the endocytic machinery, we studied amino acids in the cytoplasmic domain of CD28 that are required for the effects of the HIV-1 and SIV Nef proteins. Interestingly, we found that the induction of CD28 endocytosis by SIV and HIV-1 Nef proteins requires partially overlapping but distinct sets of amino acid residues in the cytoplasmic domain of CD28. The use of different target sites in the CD28 cytoplasmic domain by HIV-1 and SIV Nef proteins implies that they contact CD28 directly and argues against the possibility that they bind CD28 via a common bridging protein.

Interestingly, the Nef target sites in CD28 are located in a close proximity to the native endocytic signals in the cytoplasmic domain yet do not overlap these signals. That Nef target sites are located close to the likely normal site of interaction of CD28 with the endocytic machinery suggests that CD28 contacts the endocytic machinery in a similar manner in the absence or presence of the viral protein, and Nef probably strengthens these normal interactions. This model is reminiscent of previous data which indicate that Nef-induced endocytosis of CD4 and class I MHC requires intact native endocytic signals in these target molecules. Thus, it appears that in all cases, Nef facilitates the normal interactions of these molecules with AP-2, or with other components of the endocytic machinery that are normally required for constitutive endocytosis, rather than replacing these interactions with new ones. Our observations have begun to provide new general insights into the mechanisms used by Nef to recruit target molecules to the endocytic machinery.

BIOCHEMICAL PURIFICATION OF NEF EFFECTORS

Identification of cellular proteins that mediate Nef functions is critical for our understanding of the molecular mechanisms that mediate various Nef functions and for our ability to develop drugs that disrupt Nef's function(s). Previous approaches to identify Nef effectors in T cells and macrophages that relied on binding of Nef to candidate proteins in heterologous systems such as yeast or in vitro in biochemical assays using GST-fusion proteins failed to identify downstream effectors for several Nef functions. To identify additional critical effectors of Nef, we developed a protocol to biochemically purify protein complexes that associate with HIV-1 and SIV Nef in T cells.

For these studies, we used the SIV mac239 and HIV-1 NA7 natural Nef proteins, which have been studied extensively in our laboratory during the last several years. Both Nef proteins were tagged at the carboxyl terminus with heterologous peptide-epitopes to which high-affinity monoclonal antibodies are available in quantity. We confirmed that the addition of carboxy-terminal epitopes does not negatively affect conserved functions of Nef such as the ability to down-regulate cell surface expression of class I MHC, CD4, CD28 (and CD3 by SIV Nef), or Nef's ability to associate with PAK2 kinase.

Genes encoding epitope-tagged Nef proteins were stably expressed in a Jurkat-derived T-lymphoblastic cell line. Cell proteins associated with Nef were purified from cytoplasmic detergent extracts by immunoaffinity chromatography using monoclonal antibodies specific for the epitope tags. Using this technique, we consistently detect a set of approximately eight polypeptides having molecular masses between 20 kD and 300 kD that copurify with both the HIV-1 and SIV Nef proteins. We are presently focusing on purification of Nef-associated proteins in quantities required for their identification using mass spectrometry. We are

also extending this approach to study Nef-associated proteins in macrophage cell lines.

INDUCIBLE EXPRESSION OF NEF IN T CELLS AND ANTIGEN-PRESENTING CELLS IN TRANSGENIC MICE

The benefit of reducing levels of CD28 on the surface of Nef-expressing T cells for SIV/HIV replication in the host has not yet been investigated, but can be speculated based on known functions of CD28 and on known modulating effects of Nef on signal transduction in T cells. During antigen presentation, T cells form tight conjugates with antigen-presenting cells (APCs). CD4, CD28, and T-cell antigen receptor are critical components of the immunologic synapse, a macromolecular structure that mediates the interactions of T cell with APCs. These interactions and antigen-initiated signaling regulate several important aspects of T-cell biology, such as entry into cell cycle, clonal expansion, effector responses, and apoptosis of the expanded T-cell clones, that underlie the immune response. The concerted down-modulation of CD28 and CD4 by both HIV-1 and SIV Nef, and of CD3 by SIV Nef, probably limits the adhesion of a Nef-expressing T cell to the APC following a productive antigen-presentation event. Furthermore, it could promote the disengagement of the activated T cell from the APC and its subsequent movement to other APCs, thus facilitating the spread of the virus. Another possible consequence of the coordinated down-modulation of CD3, CD4, and CD28 is compromised duration and strength of antigen-specific signaling in the infected T cell.

To address the impact of Nef and the contribution of its individual functions on T-cell biology, we directed inducible expression of HIV-1 Nef to T cells and APCs in transgenic mice. We anticipate that these animals will provide important novel insights into the consequences of Nef expression in these cells, consequences that are likely critical for the progression of HIV-1 infection in humans.

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TRANSCRIPTION AND PROTEOLYSIS

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E. Ejkova M. Muratani K. Tworkowski
A. Herbst

Normal cellular growth and development depend on processes that regulate the abundance, distribution, and activity of proteins within the cell. Transcription and proteolysis are two processes that have key roles in regulating protein abundance. Transcription is the first step in the life of a protein and is fundamental in determining when a protein will be synthesized and at what level. Proteolysis is the last step in the life of a protein, and it is ultimately responsible for establishing correct steady-state protein levels. As "bookends" in the life of any protein, these two processes have apparently very little in common, yet it is the connection between transcription and proteolysis that is the focal point of our research. We study how the proteins that control transcription—transcriptional activators—are regulated at the level of their own stability, and how these proteins use components of the proteolytic system to regulate gene expression.

MYC

As a paradigm for transcription factor destruction, we study Myc. Myc is a basic helix-loop-helix transcription factor that features prominently in the regulation of cell proliferation and in cancer. Myc is also a highly unstable protein that is destroyed within minutes of its synthesis. The rapid destruction of Myc plays an important part in keeping intracellular levels of the Myc protein low and responsive to environmental stimuli. We have previously shown that Myc is an unstable protein because it is destroyed by ubiquitin-mediated proteolysis, a process in which covalent attachment of Myc to ubiquitin (Ub) signals Myc destruction by the proteasome.

Protein ubiquitylation is a highly specific process that begins when an element within the target protein—termed a "degron"—is recognized by a Ub-ligase. The Ub-ligase binds to the degron and catalyzes the transfer of Ub to a lysine residue within the target protein. This process is then repeated many times to produce a highly ubiquitylated substrate that is rapidly destroyed by the proteasome. For Myc, the degron lies within the amino terminus of the protein, overlapping the transcriptional activation domain (TAD) and two highly conserved elements called Myc boxes I and II. Curiously, although the degron has a crucial role in Myc proteolysis, it is not the only element that contributes to Myc turnover. Within the interior of Myc—overlapping highly conserved Myc box III—there is an element (the D-element) that collaborates with the degron to facilitate efficient Myc destruction. The interactions between the Myc degron and the D-element demonstrate that Myc destruction is regulated at multiple levels and reinforce the notion that the stability of Myc is governed by the actions of the most highly conserved regions of the protein.

A CONSERVED PROTEOLYTIC MACHINERY SIGNALS MYC DESTRUCTION

A number of years ago, we discovered that the Myc degron can signal Ub-mediated proteolysis in the yeast *Saccharomyces cerevisiae*. This was a surprising discovery because yeast have no direct homolog of the Myc protein, and it suggests that Myc proteolysis proceeds through a highly conserved mechanism shared by yeast and humans. In the last year, we have further probed the evolutionary conservation of Myc destruction. We have discovered that cancer-associated and transforming mutations within the degron that stabilize Myc in human cells also stabilize Myc in yeast, and we have found that the recently identified D-element can also function to direct Myc proteolysis in this species. Moreover, we have characterized the cellular machinery that targets Myc for Ub-mediated destruction in yeast and found that this, too, is conserved. Using a directed genetic approach, we have identified a number of genes that are required for Myc proteolysis in yeast. These genes encode members of a conserved Ub-ligase complex called SCF^{Grr1}. At the heart of this complex is the Grr1 protein, which is directly responsible for mediating substrate recognition. Yeast with mutations in Grr1 (or indeed any other component of the SCF^{Grr1} complex) are defective for Myc proteolysis. We have used this defect to search for human proteins that can direct Myc turnover in yeast and have identified a human homolog of Grr1 that complements loss of Grr1 and directs a rapid rate of Myc destruction in yeast cells. The interchangeability of human and yeast proteins in

this way further reinforces the conservation of the mechanism that directs Myc destruction and provides valuable insight into the mechanistic basis of Myc proteolysis.

IDENTIFICATION OF A METABOLICALLY STABLE POOL OF MYC PROTEIN

One of the reasons we study Myc destruction is because Myc is an exceptionally unstable protein; the typical half-life of Myc in a human cell is approximately 20 minutes. The importance of rapid Myc turnover is evidenced not only by the evolutionary conservation of Myc destruction processes, but also by the link between increased Myc stability and oncogenic transformation by Myc: Mutations within Myc that increase its stability increase its transforming ability. Given the extreme instability of Myc, therefore, we were surprised to find a pool of Myc protein, present in many cells, that escapes rapid proteolysis.

The identification of this metabolically stable pool of Myc protein was made during our attempts to understand the complicated kinetics of Myc destruction. We have known for some time that the rate of Myc decay in many cells does not obey simple first-order kinetics. Rather, Myc destruction appears to be biphasic; there is an initial phase in which the rate of Myc destruction is very rapid, followed by a latter phase in which the rate of Myc decay is quite slow (Fig. 1A). Using a detergent-extraction process, we have learned that this biphasic decay is due to the existence of two distinct forms of Myc (Fig. 1B): An extractable pool that decays rapidly and with first-order kinetics (the "S1" pool), and an extraction-resistant pool that decays more slowly ("S2"). The partitioning of Myc into the S2 pool occurs rapidly following Myc synthesis, although the relative amount of Myc that enters S2 is dependent on the cell type. In some cell types, greater than 10% of newly synthesized Myc enters S2; in other cell types, Myc is restricted exclusively to the S1 pool. We have identified a carboxy-terminal element within Myc that is responsible for this partitioning and have shown that proteins which interact with Myc in this region differentially affect S1/S2 partitioning. Curiously, we have also found that Myc in the S2 pool is efficiently ubiquitylated, despite its stability. Together, these observations suggest that the proteasomal destruction of Myc can be regulated by environmental partitioning, and raise the interesting issue of why some cells would maintain a portion of the Myc protein in a ubiquitylated, but metabolically stable, state.

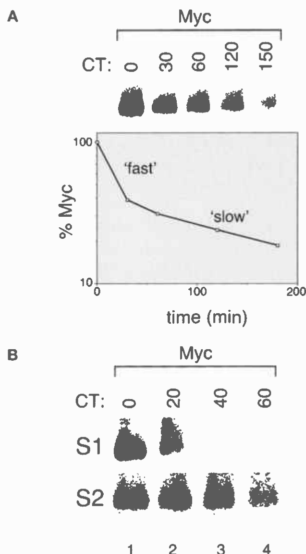

FIGURE 1 Myc protein exists in two forms with different metabolic stabilities. (*A*) Myc decay is biphasic. (*Top panel*) Pulse-chase experiment showing the decay of total radioactively labeled, epitope-tagged, human Myc in HeLa cells. CT refers to "chase-time," in minutes. (*Bottom panel*) Graphical representation of the above pulse-chase experiment. The percentage of radioactively labeled Myc protein present at each time point is shown on a logarithmic scale. In this representation, if Myc decayed with first-order kinetics, we would expect to see a straight line. Instead, we observe that there is an initial rapid phase of decay (fast), followed by a later, slower, phase of decay (slow). (*B*) Two forms of Myc exist with different metabolic stabilities. Pulse-chase experiment showing the decay of radioactively labeled, epitope-tagged, human Myc in HeLa cells. For this experiment, labeled cell extracts were separated into detergent-extractable (S1) and detergent-resistant (S2) pools prior to recovery of Myc proteins by immunoprecipitation. Note that Myc is partitioned into both pools, but that the rate of decay of Myc in each pool is very different.

TRANSCRIPTION FACTOR LICENSING BY UBIQUITIN

In addition to dissecting the molecular basis of how Myc is destroyed, one of the research objectives in our laboratory is to understand *why* Myc is destroyed. Many transcription factors, particularly those involved in the

control of cell growth (e.g., p53 and E2F), are unstable proteins that are destroyed by Ub-mediated proteolysis. In almost all of these proteins (including Myc), the element that signals their destruction overlaps with the transcriptional activation domain. Indeed, the overlap between TADs and degrons is intimate, and we have not been able to separate these two activities in a number of transcription factors that we have examined. The curious relationship between TADs and degrons suggests some involvement of the Ub-proteasome pathway in transcriptional activation, but until recently, we have not understood the nature of this involvement.

To determine why transcriptional activation domains signal Ub-mediated proteolysis, we have examined the role that components of the Ub-proteasome pathway have in transcriptional activation. For these studies, we fused the prototypical acidic activation domain from VP16 to the bacterial LexA DNA-binding protein and expressed the chimeric protein in yeast where it potently activates transcription and is rapidly destroyed by Ub-mediated proteolysis. Using a directed approach, we found that the Ub-ligase required for LexA-VP16 destruction is an SCF complex, SCF^{Met30}, the substrate-recognition component of which is Met30. We found that Met30 associates specifically with the VP16 TAD—and not other activation domains examined—and that deletion of Met30 not only stabilizes LexA-VP16 in vivo, but also blocks its ability to activate transcription. Remarkably, the requirement for Met30 in transcription can be circumvented by direct fusion of Ub to the LexA-VP16 protein (Fig. 2). This result suggests that in order to activate transcription, LexA-VP16 must be ubiquitylated (either by SCF^{Met30} or by some other process). The requirement for activator ubiquitylation provides a simple explanation for why it is that transcription factors are destroyed so readily—because they cannot activate transcription unless they have first signaled their own ubiquitylation.

But why would a transcription factor need to be primed for destruction in order to stimulate transcription? We suggest that the requirement for ubiquitylation serves to tightly couple transcription factor activity and destruction. By using the same signal—ubiquitin—both to activate and to destroy a transcription factor, the cell is in effect granting a "license" to that transcription factor for a limited period of activity before it gets destroyed. We suspect that this is a mechanism which prevents unlimited activation by any one promoter-bound transcriptional activator and links continual gene activation to the ongoing synthesis and activation of transcription factors.

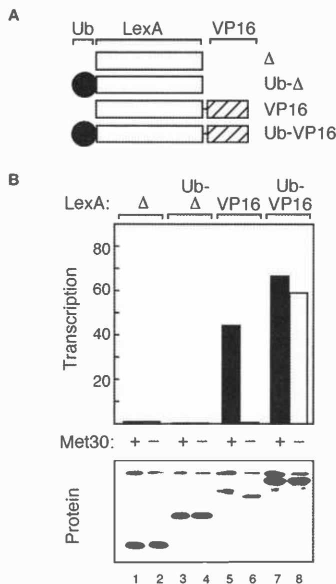

FIGURE 2 Regulation of VP16 activation domain function by ubiquitin. (*A*) Structure of chimeric activators. To probe the relationship between transcription and proteolysis, we generated synthetic activators consisting of the bacterial LexA DNA-binding protein fused to the prototypical acidic activation domain from VP16. Where indicated, we also fused ubiquitin (Ub) to these proteins. (*B*) Transcriptional activity of LexA-VP16 depends on ubiquitylation. The LexA fusion proteins shown above were expressed in yeast strains either expressing (+) or not expressing (–) Met30, the Ub-ligase for the VP16 activation domain. (*Top panel*) Transcriptional activity, in arbitrary units. (*Bottom panel*) Steady-state protein levels, as determined by immunoblotting. Note that the LexA-VP16 fusion protein is transcriptionally inactive in the absence of Met30 (lane *6*) but that its activity can be rescued by direct fusion to ubiquitin (lane *8*).

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STRUCTURAL BIOLOGY

R.-M. Xu J. Min J. Vitali
O. Merkel Y. Zhang
H. Shi

During 2001, Olaf Merkel returned to Austria and Jacqueline Vitale completed her 1-year appointment as a visiting scientist. After spending 4 years as a research technician in my laboratory, Ying Zhang joined the Novartis Pharmaceuticals Corporation in New Jersey.

We continued to focus our attention on structural studies of proteins involved in pre-mRNA splicing and proteins that affect chromatin structure and dynamics. We completed a high-resolution structural analysis of the RNA-binding domain of the heterogeneous nuclear ribonucleoprotein (hnRNP) A1 (UP1) in collaboration with Adrian Krainer here at CSHL, and in collaboration with Zhiguo Zhang in Bruce Stillman's lab, we carried out structural and functional studies of the amino-terminal bromo-adjacent homology (BAH)-containing domain of ORC1 in transcriptional silencing in *Saccharomyces cerevisiae*. These studies are highlighted below.

HIGH-RESOLUTION ANALYSIS OF THE RNA-RECOGNITION MOTIF OF hnRNP A1

Human hnRNP A1 is an abundant nuclear protein that has been implicated in many cellular processes, including packaging of nascent RNA polymerase II transcripts in the nucleus, regulating alternative 5′ splice-site selection in pre-mRNA splicing, mediating the splicing-inhibitory effects of certain exonic splicing silencers, nuclear-cytoplasm transport, RNA annealing, and telomere-length regulation. The protein consists of 320 amino acids and has two RNA recognition motif (RRM) consensus sequences at the amino terminus, followed by a glycine-rich carboxy-terminal region. Both the amino-terminal domain, known as UP1 and the carboxy-terminal domain are required for the function of hnRNP A1 in alternative splicing.

We have previously solved the crystal structures of UP1 and its complex with single-stranded human telomeric DNA. In the present study, we extended the resolution to 1.1 Å, the highest-resolution structure for any RRM-containing proteins. The 1.1-Å revealed that several critical residues involved in RNA binding have alternative side-chain conformations (Fig. 1). These residues, Phe-17, Phe-59, and Val-44, are all located in the RRM1. Furthermore, the alternative conformations of these residues are spatially correlated, namely, alternative conformation of one residue also forces the others to adopt alternative conformations. The newly observed side-chain conformations are incompatible with the observed mode of RRM-RNA interactions studied to date. It is not clear at this point whether this new conformation of these residues inhibits RNA

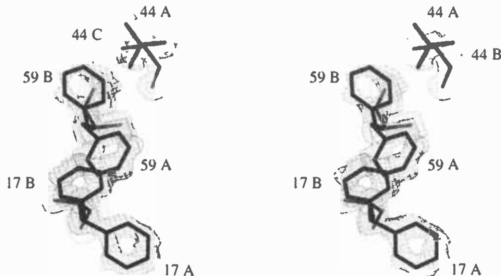

FIGURE 1 Stereoview of the electron density map showing correlated alternative side-chain conformations of Phe-17, Phe-59, and Val-44. The amino acid side chains are numbered and shown in a stick model. Different side-chain conformations are denoted with the suffixes A, B, and C. The electron density map is a σ_A map contoured at 1.2 σ level.

binding or utilizes a new mode of RRM-RNA interaction. Interestingly, amino acids located in the closely related RRM2 of hnRNP A1 do not have alternative conformations. Previous studies in Adrian Krainer's lab showed that the two RRMs have distinct functions in pre-mRNA splicing. Different conformational states in RRM1 and RRM2 may contribute to the functional differences between the two RRMs. Given that RRM is the most common RNA-binding motif, it is likely that the observed alternative side-chain conformation of the conserved aromatic residues also occurs in other RRM-containing proteins. In future studies, it will be of interest to determine the effects of alternative side-chain conformation on RNA binding directly.

STRUCTURAL STUDY OF THE BAH-CONTAINING DOMAIN OF ORC1P IN EPIGENETIC SILENCING

Genes located in certain regions of eukaryotic chromosomes are permanently repressed. This heritable, transcriptionally silent state is caused by an altered chromatin structure that can be propagated from one generation to the next. In *S. cerevisiae*, silencing is observed for at least three genomic loci: the silent mating-type loci, *HML* and *HMR*, telomeres, and the ribosomal DNA region. Several protein complexes are known to be critical for the establishment and maintenance of transcriptional silencing in yeast, including the silent information regulator (SIR) proteins, Sir1p, Sir2p, Sir3p, and Sir4p, and several sequence-specific DNA-binding proteins, the origin recognition complex (ORC), Rap1p, and Abf1p.

The amino-terminal domain of Orc1p, the largest subunit of ORC, functions specifically in transcriptional silencing, but it is dispensable for DNA replication. This silencing domain of Orc1p shares about 50% identity with the amino-terminal region of Sir3p. This Orc1p domain interacts with Sir1p, and in *Drosophila*, the corresponding region of dORC1 interacts with HP1. In all species, this region of Orc1p contains a BAH domain that is also present in Sir3p, and other chromatin-associated proteins such as mammalian DNA-(cytosine-5)-methyltransferases, components of the yeast RISC chromatin-remodeling complex, and histone deacetylase complexes.

We have solved the crystal structure of the amino-terminal domain of Orc1p (amino acids 1–235) to 2.2-Å resolution using the multi-wavelength anomalous diffraction (MAD) method. The structure has an elongated shape consisting of two domains: a large domain composed of mainly β strands and a small helical domain (Fig. 2). The BAH domain is in the large domain and it forms the core of the structure. The small, nonconserved helical domain, referred to as the H-domain, is inserted between two β strands, β6 and β7, of the BAH domain. In collaboration with Zhiguo Zhang in Bruce Stillman's lab, we showed that the H-domain interacts with Sir1p and is necessary for targeting the SIR protein complex to the silent mating-type loci. In the future, we plan to carry out structural studies of this Orc1p domain in complex with Sir1p.

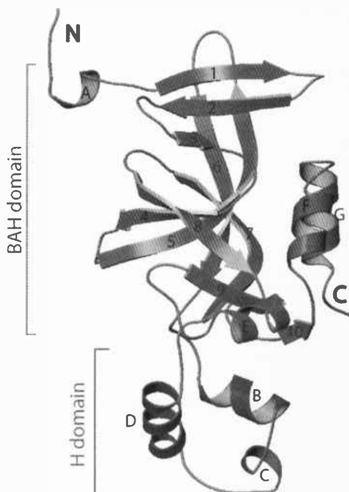

FIGURE 2 Crystal structure of the amino-terminal domain of Orc1p is shown in a ribbon representation.

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CANCER: GENETICS

The highlights of efforts in this group are described below.

- Dr. Masaaki Hamaguchi's group has discovered a new tumor suppressor candidate, *DBC2*, a member of RAS-BTB family, that is likely to be involved in breast cancer development and was discovered by application of RDA.
- Dr. Greg Hannon's group has made significant strides toward understanding the mechanisms of double-stranded RNA-induced gene silencing (RNAi) with the cloning of the initiating enzyme, Dicer. Genetic studies of Dicer mutants in worms have led to the realization that RNAi may represent a common mode of cellular gene regulation in addition to its role in the response to exogenous double-stranded RNAs and endogenous mobile genetic elements. These advances in understanding the mechanisms of RNAi have led to the development of RNAi as a powerful tool for manipulating gene expression in mammalian cells.
- Dr. Eli Hatchwell and collaborators have recently discovered a small microdeletion on chromosome 8q24.3 in a patient with Kabuki syndrome, a condition originally described more than 20 years ago, for which no genetic clue had previously been uncovered.
- Dr. Yuri Lazebnik's lab has continued to develop approaches to dissect oncogene-dependent apoptosis.
- Dr. Scott Lowe's group has continued to refine and deepen their models for the role of *p53* in senscence, apoptosis, and drug resistance.
- Dr. Rob Lucito and collaborators have made strides in developing arrays of oligonucleotide-based microarrays useful for measuring gene copy number.
- Dr. Alea Mills, who has recently joined CSHL, has continued collaborations to create mouse models of human disease using chromosome engineering. She has also developed a model system that will be used to study the role of p63 in stem cell maintenance, differentiation, and cancer.
- Dr. Vivek Mittal, in collaboration with Robert Benezra and Marianna Russinova (Memorial Sloan-Kettering Cancer Center, New York) have been exploring the helix-loop-helix protein Id which is required to support angiogenesis in tumors. They have discovered novel candidates, such as Pleiotrophin, a 6b4 integrin, and their receptor Laminin 5, which may mediate its action.
- Dr. Michael Wigler's group has continued the development of methods for the representational analysis of complex genomes. In collaboration with Rob Lucito, they have shown that low-complexity representations and oligonucleotide microarrays can be used to measure gene copy numbers in cancers and mutant humans. They have developed methods for annotating a genome with the frequency of its constituent mers and are using this method to develop new insights into genome evolution and to design of hybridization probes. They have also demonstrated the use of transcription profiling to observe cell-cell interactions.

TUMOR SUPPRESSOR GENES INVOLVED IN BREAST CANCER DEVELOPMENT

M. Hamaguchi M. Kanis M. Reimels
J. Meth D. Roberts
T. Odawara R. Von Roeschlaub

Our lab is interested in the discovery and characterization of cancer-related genes, especially tumor suppressor genes that are ablated in cancer cells. The recent history of tumor suppressor gene investigation has been revolutionary. The identification of these genes and subsequent studies into their biological and pathological functions have provided vast new insights into the development of cancer. A great deal is now known about the relationship between cancer development and cellular functions.

In the case of breast cancer, the isolation of tumor suppressor genes *BRCA1* and *BRCA2* has had an impact on several aspects of cancer treatments as well as cancer research. *BRCA1* and *BRCA2* genes can be utilized for identification of individuals at high risk of developing breast cancers. This has been crucial in the development of risk assessment. There are also differences in clinical outcomes between breast cancers with *BRCA1* mutations and those with *BRCA2* mutations. These findings suggest that specific types of breast cancers require specific screening methods and therapeutic approaches. However, tumor suppressor genes responsible for sporadic breast cancer remain to be discovered. We have investigated genetic alterations, especially deletions in breast cancer, to discover new tumor suppressor genes. Among the candidate tumor suppressors we isolated, *DBC2* (*d*eleted in *b*reast *c*ancer 2) seems promising. It is homozygously deleted in 3.5% (7/200) of breast tumor samples and silenced in 55% (12/22) of breast cancer cell lines. Activation of *DBC2* in a breast cancer cell line resulted in growth arrest of the cells. Our mutation and expression analyses of two neighboring genes implied that they were unlikely to be tumor suppressor genes. Studies of the other genes from this region have been well characterized and no relation to breast cancer has been inferred. These data led to the conclusion that *DBC2* is the best candidate for a tumor suppressor in this region. One of our research goals is to validate *DBC2* as a tumor suppressor gene and to elucidate its role in breast cancer development.

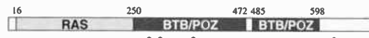

FIGURE 1 Structure of *DBC2*. Predicted functional domains of *DBC2*. The numbers refer to the deduced amino acid sequence of *DBC2*. The asterisks denote missense mutations we have discovered.

DBC2 is a member of a new gene family RAS-BTB (Fig. 1). The RAS domain shows highest homology with RRAS family members (Fig. 2). The BTB/POZ (broad complex tramtrack bric-a-brac/poxvirus and zinc finger) is a putative protein-protein interacting domain. *DBC2* also has a DNA-binding domain (MYND) at its carboxyl terminus. We hypothesize that it is a G-protein-coupled transcription factor. Another goal of our research is to understand the physiological function of DBC2 and other RAS-BTB proteins.

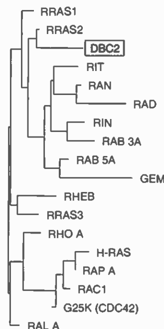

FIGURE 2 The phylogenic tree demonstrates overall homology between DBC2 and RAS family members. A table of the pairwise distances between the genes was prepared by the GCG Wisconsin Package, and a phylogram was then created from the table using the neighbor-joining method. The length of a horizontal line is proportional to estimated divergence along each branch.

Mutation Analysis of *DBC2*

M. Hamaguchi, J. Meth

Tumor suppressor genes are frequently inactivated by mutations. The first step of validating *DBC2* as a tumor suppressor was mutation analysis. The coding region of *DBC2* was screened for mutations by DHPLC (denatured high-performance liquid chromatography) and SSCP (single-strand conformation polymorphisms). The detected mutations were confirmed by sequencing. The mutation analysis of cell lines revealed two missense mutations in the BTB/POZ domain, found in lung and breast cancer cell lines. Then, 95 primary tumors and matched normals were screened by either DHPLC or SSCP for mutations in either of these two genes. Samples showing variants in retention time (DHPLC) or mobility shifts (SSCP) were sequenced, and tumors were compared to matched normals. Two missense mutations were again discovered within tumors (but not in matched normals), one in the BTB/POZ domain. In total, four missense mutations in the coding region of *DBC2* were found from cell lines and tumors (Fig. 1).

Expression of *DBC2*

M. Hamaguchi, M. Reimels

Tumor suppressor genes are inactivated by transcriptional suppression in some cases. In fact, *DBC2* expression is extinguished in more than half of breast tumors while its expression is detected in other types of cancer. On the contrary, other genes in the same region were expressed in at least 80% of all tumors. These results indicated that *DBC2* was inactivated at a transcriptional level specifically in breast cancer.

Hypermethylation of the promoter region has been demonstrated to cause transcriptional inactivation. The promoter region of *DBC2* was screened for methylation status. First, we determined the genomic sequence of *DBC2* including the 5′ end (GenBank accession number AF315385). We then located CpG islands and found one approximately 500 bp upstream of exon 1. We examined two cell lines, T-47D, which does not express *DBC2*, and HeLa, which does, for methylation status. We analyzed methylation status by a bisulfite modification that converts only unmethylated cytosines to uracil but not methylated cytosines. As a result, sequences of the modified fragments were different depending on the methylation status. The differences were detected by polymerase chain reaction (PCR) and then confirmed by sequencing. CpG islands of T-47D cells were hypermethylated but those of HeLa cells were not. Our findings suggest that hypermethylation may have an important role in inactivation of *DBC2* in at least one of the breast cancer cell lines. Other breast cancer cell lines with *DBC2* silencing have not yet been examined.

Transcriptional inactivation by hypermethylation can be reversed in many cases when methylation is chemically blocked. A commonly used chemical for this purpose is 5-aza-2′-deoxycytidine (5-Aza-C). We cultured both HeLa and T-47D cells with 5-Aza-C. Neither *DBC2* expression nor growth of HeLa cells was affected by addition of 5-Aza-C. T-47D cells treated with 5-Aza-C demonstrated only weak expression of *DBC2*. As we describe below, induction of *DBC2* hindered the growth of T-47D, and the negative selection might have contributed to the observation of the faint expression of *DBC2*.

Characterization of *DBC2*

M. Hamaguchi, T. Odawara, D. Roberts [in collaboration with M. Wigler, Cold Spring Harbor Laboratory]

We utilized an inducible gene expression system developed in Dr. Wigler's lab here at CSHL to study *DBC2* function in breast cancer cells. With this system, *DBC2* expression can be regulated by administration of an inducer, Muristerone A. We first analyzed HeLa cells, which expressed endogenous *DBC2*. Induction of foreign *DBC2* did not cause noticeable phenotypic changes of the HeLa cells. When breast cancer cell line T-47D was examined, induction of *DBC2* hindered the growth of T-47D.

To determine the subcellular localization of DBC2, we fused it with green fluorescent protein (GFP). Both GFP and DBC2-GFP fusion protein were expressed in a mammalian cell line. Signals of the fusion protein were observed primarily in the nucleus, whereas those of GFP were observed in both the cytosol and nucleus. We concluded that *DBC2* was localized in the nucleus at least under certain conditions. However, it remains to be investigated whether *DBC2* shuttles between the nucleus and the cytoplasm like some G-proteins including human RAN and *Dictyostelium* Rac B.

Mouse *DBC2*

M. Hamaguchi, M. Kanis, R. Von Roeschlaub [in collaboration with M. Zhang, Cold Spring Harbor Laboratory]

Isolation of mouse counterparts has two purposes: to take advantage of well-studied mouse genetics and to prepare materials to establish mouse models. Evolutionary conservation of *DBC2* between the human and mouse was first confirmed by zoo blot analysis with human and mouse DNAs. The mouse counterpart of *DBC2* was then cloned by cDNA library screening and database searches. There are three RAS-BTB proteins in both human and mouse. The genes were compared using "distance analysis" of the GCG Wisconsin package. A mouse gene, AF420001, demonstrated by far the highest homology with human *DBC2*, and therefore, it is considered the mouse counterpart of *DBC2*. The genomic sequence of mouse *DBC2* was also determined by sequencing of BAC (bacterial artificial chromosome) clones (GenBank accession number AF420002).

Jennifer Meth

RNAi: MECHANISMS AND APPLICATIONS

G. Hannon E. Bernstein A. Caudy J. Du Y. Seger
S. Boettcher D. Conklin S. Hammond J. Silva
M. Carmell A. Denli P. Paddison I. Sujka

My laboratory continues to pursue two parallel interests. First, we strive to understand the process of neoplastic transformation using cultured normal and cancer cells as models. Second, we investigate the mechanistic basis of double-stranded RNA-induced gene silencing (RNAi) in experimental systems ranging from fungi to mammals.

This year, we have made considerable progress in deciphering the mechanism of RNA interference (RNAi) with the cloning of several proteins that are key to this gene silencing process (see Fig. 1). Among these was Dicer, the enzyme that initiates RNAi by processing double-stranded RNAs into discrete ~22-nucleotide small interfering RNAs (siRNAs). Genetic studies of Dicer in *Caenorhabditis elegans*, in collaboration with Ronald Plasterk of the Hubrecht Laboratories in Holland, revealed that Dicer had a role in regulating endogenous genes which control developmental timing (see report by Bernstein below). This prompted a paradigm shift in the field which now recognizes that the RNAi machinery may have a broad role in regulating cellular gene expression. An understanding of the enzymology of Dicer also led to two key discoveries, which may ultimately enable a fusion of our two core interests. Other laboratories (Elbashir and colleagues at the Max-Planck Institute in Germany) demonstrated that chemically synthesized Dicer products are effective inducers of gene silencing in mammalian cells. The use of siRNAs is rapidly becoming a standard methodology for probing gene function in mammals. We have extended their seminal discovery through the development of cost-effective, high-throughput methodologies for constructing siRNAs and are now beginning a project to assess loss-of-function phenotypes on a whole-genome scale. We have recently found (see report by P. Paddison, D. Conklin below) that the endogenous triggers of the RNAi pathway, which were linked to RNAi via genetic analyses of Dicer, can be retargeted to suppress a gene of interest. These small hairpin RNAs (shRNAs) can be stably expressed in vivo to create continuous cell lines, and perhaps even animals, in which RNAi is used to create a gene knock-out (or more precisely, knock-down).

One goal of these studies is to develop tools that will allow us to dissect the mechanisms of neoplastic transformation through the use of genetics in cell culture. A prerequisite to such an approach is the devel-

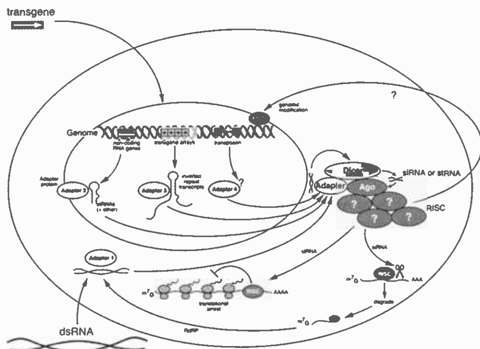

FIGURE 1 A mechanistic model for RNAi.

opment of genetically defined human cancer models. During the past 2 years, we have identified a combination of viral and cellular oncogenes that can transform normal human cells into cancer cells in vitro (see report by Y. Seger below). We can now combine these defined models with RNAi-based loss-of-function approaches to search for genes that are essential to the survival and proliferation of cancer cells. Through such approaches, we hope to define new potential targets for selective anticancer therapies.

Characterization of RISC, the RNAi Effector Complex

S. Hammond

This project is centered on the purification of the RISC (RNA-induced silencing complex) effector nuclease of RNAi. During the past year, I identified a putative protein component of this complex, Argonaute2, and have further characterized the role of Argonaute2 in the RISC complex. I generated antibodies to Argonaute2, confirming that it cofractionated with RISC nuclease activity. Interestingly, the protein shifts from a 300-kD complex to a 500-kD active complex after exposure to double-stranded RNA. I cloned the full-length coding sequence of Argonaute2 and expressed it transiently in *Drosophila* S2 cells. Affinity purification of this protein yielded active material, confirming its presence in the RISC complex. Argonaute2 coimmunoprecipitates with the enzyme Dicer, another component of the RNAi machinery. This suggests cross-talk between the two mechanistic steps of RNAi, i.e., the double-stranded RNA processing step and the effector (RISC) step. My efforts at this time are directed at identification of remaining components of the RISC complex, and how they accomplish the mRNA degradation aspects of RNAi.

Dicer: Does It Control Everything?

E. Bernstein

The mechanism of RNAi is not fully understood, but it has recently begun to be elucidated. The field of RNAi has been advanced by various methods, including biochemical purification, genetics, and candidate gene approaches. These systems have yielded the identities of key players in the silencing process.

In particular, we have identified the enzyme Dicer from *Drosophila* embryo extracts, which is responsible for cleaving the double-stranded RNA into small fragments of approximately 22 nucleotides (siRNAs). These species, which were first observed in plants undergoing silencing, presumably confer target specificity to the interference process through their incorporation into the effector nuclease complex, RISC.

The Dicer protein has a unique domain structure and is highly conserved in evolution. Homologs exist in organisms such as *Arabidopsis, C. elegans, Neurospora, Schizosaccharomyces pombe,* mouse, and humans. To probe the biological function of this gene and confirm its role in RNAi, we sought out the *C. elegans* Dicer mutant.

dcr-1 mutants are indeed RNAi-deficient in certain tissues and show a range of developmental abnormalities. These phenotypes are similar to those caused by loss of function of *let-7*, a gene encoding a small temporal RNA (stRNA) necessary for the proper timing of *C. elegans* development. stRNAs are thought to interact with their target genes at the 3′ UTR and to negatively regulate gene expression at the translational level. The *let-7* precursor RNA forms a stem-loop structure of approximately 70 bp, which is processed into a mature form that is 21 nucleotides in length. Dicer can process the *let-7* precursor into its mature form in vitro, and *dcr-1* mutant animals have increased levels of precursor *let-7* accompanied by reduced levels of the mature form in vivo. This suggests a role for Dicer in processing stRNAs as well as double-stranded RNAs, two regulatory molecules involved in mechanisms with distinct outcomes.

Several laboratories have recently demonstrated that *let-7* is a prototype for a large family of endogenously encoded small RNAs. To date, more than 300 have been characterized in *Drosophila, C. elegans*, and mammals. This has led to the notion that regulation of gene expression by small RNAs may be a common motif in the control of gene function, which was not even suspected to exist only 1 year ago.

RNAi: Multiple Silencing Triggers and Distinct Effector Complexes?

A. Caudy

For several years, we have anticipated the existence of cellular genes that would be regulated by RNAi. This

was confirmed by findings from several laboratories, including our own (see report by E. Bernstein above). On the basis of this hypothesis, I devised a method for cloning small RNAs from cells with the hope of identifying endogenous targets of RNAi. I identified sequences corresponding to viruses and transposons, which had been predicted targets of RNA interference based on genetic evidence from plants and worms. I also identified some microRNAs, which are small RNAs that may regulate translation through a mechanism related to RNAi.

These data led to an interest in how different RNAi complexes might regulate expression through different effector pathways, translation and RNA degradation. I have therefore been purifying complexes that contain microRNAs from *Drosophila* and human cells. I have found that microRNAs associate with a specific member of the *Argonaute* gene family, *Ago-1*. Other workers in the lab have shown that a different *Argonaute* family member, *Ago-2*, is part of the complex that specifically degrades messenger RNA. From genetic work in *C. elegans*, we predict that the microRNAs in the Ago-1 complex may regulate message translation rather than initiating message degradation. Efforts are under way to identify the proteins associated with this complex and the manner in which it may regulate gene expression.

Human Cell Transformation: Shortened Telomeres and Extended Graduate Careers

Y. Seger [in collaboration with R. Maestro, CRO-Aviano, Italy]

One long-standing interest in our lab is the elucidation of the minimum genetic requirements for the transformation of human cells. Utilizing the technique of retroviral gene transduction, we have previously shown that normal human fibroblasts could be transformed with the oncogene combination of adenovirus E1A, MDM2, and Ha-RasV12. This transformation model is significantly different from those previously published in that there is a distinct absence of telomerase expression, either through direct introduction of the catalytic subunit, hTERT, or by an oncogene capable of activating hTERT. Analysis of telomerase status in these engineered cells and the resultant primary tumors indicates that telomerase is not activated, indicating that telomerase activity is not essential in the initial transformation event.

Upon explantation into culture, however, cells derived from these primary tumors undergo widespread apoptosis and senescence, phenomena indicative of telomere crisis, although a small percentage of explanted cells are able to survive this crisis event. Interestingly enough, the cells that survive after explantation are telomerase-positive. When these cells are injected into a second nude mouse, the kinetics of tumor formation are similar to those of the primary tumors, indicating that no additional genetic alterations were acquired in vivo or in vitro other than telomerase activation. It is interesting to note that telomerase activation does not appear to enhance tumorigenicity, but serves as an enabling event to prevent telomere shortening and widespread genomic instability characteristic of cells undergoing mitotic crisis.

Through our studies utilizing this E1A-based system, we have begun to elucidate the minimum genetic alterations that must occur to transform a normal human cell into one that is cancerous. Currently, we are focusing on taking this tractable transformation system and adapting it for use in an epithelial cell system to more accurately model events that lead to tumorigenesis. In addition, we are continuing to make progress on the development of a human cell transformation model composed entirely of cellular oncogenes.

Short-hairpin-activated Gene Silencing (SHAGging): A New Approach to Functional Genomics and Nucleic Acid-based Therapies

P. Paddison, D. Conklin

RNAi was first recognized in *C. elegans* as a biological response to exogenous double-stranded RNA, which induces sequence-specific gene silencing. RNAi represents a conserved regulatory motif, which is present in a wide range of eukaryotic organisms. Recently, we (Ketting et al. 2001) and other investigators have demonstrated that endogenously encoded triggers of gene silencing act through elements of the RNAi machinery to regulate the expression of protein-coding genes. These stRNAs are transcribed as small hairpin precursors (~70 nucleotides), processed into

active, 21-nucleotide RNAs by Dicer, and recognize target mRNAs via base-pairing interactions. We have found that small hairpin RNAs (shRNAs) can be engineered to suppress the expression of desired genes in cultured *Drosophila* and mammalian cells. shRNAs can be synthesized exogenously or can be transcribed from RNA polymerase III promoters in vivo, thus permitting the construction of continuous cell lines or transgenic animals in which RNAi enforces stable and heritable gene silencing. The ability to encode a constitutive silencing signal may also permit the marriage of shRNA-induced silencing with in vivo and ex vivo gene delivery methods for therapeutic approaches based on stable RNAi in humans.

Centrosome Duplication

J. Du

In the past year, we have continued to probe centrosome function by studying the centrosomal kinase STK15, a serine/threonine kinase, which upon ectopic expression in mammalian cell culture causes centrosome amplification, aneuploidy, and cellular transformation. Through a two-hybrid screen using STK15 full-length coding sequence as the bait, we isolated several gene products with which STK15 potentially interacts. Of these, we chose two for further study. One of them is Nm23, a putative tumor suppressor gene. We found STK15 and Nm23 not only interact in the two-hybrid system, they also coimmunoprecipitate with each other and cofractionate on a gel-filtration column (Superose 6). Both proteins localize to the centrosome. The colocalization appears most significantly during the mitotic phase of the cell cycle, when both proteins significantly increase at the centrosome.

The other protein that shows interaction with STK15 is DEEPEST. This is a mitotic spindle-associated protein that associates with the centrosome and its surrounding mitotic microtubule structure at the M phase but not at the interphase. DEEPEST is an STK15 kinase substrate in vitro. Overexpression of DEEPEST in human IMR90 cells induces premature senescence and causes centrosome amplification in about 40% of the cells. We are currently testing pathways through which DEEPEST induces these effects.

We also continue our effort to purify the STK15 complex (STK15-associated factor, SAF) in normal human cells. We isolated and characterized nine proteins that coimmunoprecipitate specifically with STK15 and identified them through mass spectrometry analysis (SAF-160: p160 ROCK1; SAF-68: DDX3; SAF-55: CDC20*; SAF-50: p54 RCK; SAF-44: STK15; SAF-38/36: pyrroline-5carboxylate reductase 1/reductase; SAF-30: HSP27; SAF-20: HSP20). p160 ROCK1 can be phosphorylated by STK15 in vivo.

Dicer Proteins from Fungi to Plants

A. Denli

RNAi is a highly conserved gene silencing mechanism. Genetic studies have mainly been done in plants and *C. elegans*, whereas a good biochemical system has been set up only in *Drosophila*, by our lab and others. My main interest has been setting up a system that will have the advantages of bringing genetic and biochemical approaches together.

Many gene-silencing mutants have been isolated in plants, making plants a good system in which to pursue biochemical studies. I started working on tobacco suspension cells, which have the advantage of offering enough material for biochemistry. An additional advantage of this model is that tobacco is a host for many viruses that encode suppressors of gene silencing, which may help us to map the details of the silencing pathways. To that end, we prepared cell extracts which have dicer activity that can cut the long double-stranded RNAs into short RNAs. Presence of small (~21–25 nucleotides) RNAs is a hallmark of RNAi. In plants, two kinds of small RNAs are present. Other investigators have suggested that these two populations seem to be involved in different aspects of silencing. In our hands, the activities producing these different populations seem to be biochemically separable.

Another organism with good genetics is *Neurospora*, in which different gene-silencing mechanisms have been described. We were able to prepare dicer extracts from *Neurospora* as well. These extracts may help us map some genetic components upstream of dicer in the silencing pathways. Recently, we have started working on *S. pombe* in collaboration with the Martienssen group here at CSHL. We are interested in setting up a biochemical system in *S. pombe*, which

offers many advantages, especially the lack of redundancy of genes involved in RNAi. Overall, I am hoping to contribute to the characterization of RNAi mechanism.

Functional Analysis of the *Argonaute* Family in Mice

M. Carmell

Argonaute family members have been shown to be essential for RNAi/PTGS in several organisms, including *Neurospora* (QDE-2), *Arabidopsis* (AGO1), and *C. elegans* (*rde-1*). In *Drosophila*, Scott Hammond has shown that Ago2 is a component of the RISC complex and interacts with Dicer. Interestingly, argonaute proteins have also been implicated in control of development in several organisms. *Arabidopsis* AGO1 is necessary for meristem cell maintenance, and mutants have pleiotropic developmental defects. In addition, PIWI, one of four argonaute proteins in *Drosophila*, is required for maintenance of germ-line stem cells. It is unknown what roles of the RISC complex are mediated by argonaute family members and whether these developmental phenotypes result from loss of discrete regulatory functions or from a more general misregulation of silencing mechanisms.

I have been working on characterizing the argonaute family in humans and mice with the ultimate goal of creating knockout mice for each member. Each argonaute protein, of which there are seven in human and eight in mice, has a PAZ domain (thought to mediate interaction with Dicer), and a carboxy-terminal 300-amino-acid PIWI domain of unknown function. Along with Zhenyu Xuan in Michael Zhang's lab here at CSHL, we have determined the chromosomal locations, gene structures, predicted cDNAs and protein sequences, as well as the ortholog and paralog relationships among the genes. In addition, I am currently investigating the expression pattern of each gene using multiple-tissue northern blots.

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SPORADIC HUMAN GENETIC DISEASE

E. Hatchwell S. Kantarci
T. Lee

Much of the success in dissecting human genetic disease at the molecular level has been restricted to Mendelian (familial) disorders. This is in large part because a robust algorithm exists that allows disease-associated loci to be mapped to specific chromosome regions, namely, linkage analysis. The availability of genome-wide polymorphic DNA markers has made linkage studies almost routine.

No general algorithm, however, exists for the analysis of sporadic disorders in humans. Since by definition, such disorders are not familial, linkage analysis is of no value. Any approach that is likely to succeed in defining causative genetic lesions in such disorders must allow for an objective assessment of the whole genome of an individual. Furthermore, it is likely that alternative technologies will be developed for each category of mutation (from single-base changes to large chromosomal rearrangements). Historically, for those sporadic conditions whose etiology is known, that information has invariably relied on serendipity. For example, Williams syndrome, a condition that results in short stature, moderate mental retardation, and highly specific behavioral abnormalities, is now known to result in more than 95% of cases from a submicroscopic deletion (encompassing ~1.5 Mb) at chromosome 7q11.23. The deletion is almost always de novo, having arisen in a parental germ cell. The chance discovery, in 1993, of a balanced translocation involving chromosome 7q11.23 segregating with affected individuals from a unique family with a forme fruste of Williams syndrome led to the suspicion that chromosome 7q11.23 might be implicated in this condition. Had that family not been ascertained, it is almost certain that Williams syndrome would still be idiopathic today. The emphasis of this laboratory is twofold:

- To develop methodologies that will allow whole genomes to be screened for subtle copy number variations of the type known to be important in conditions such as Williams syndrome. Such methodologies must permit genome scanning at high resolution and be able to differentiate dosage ratios of 2:1 and eventually, 3:2 (for detection of duplications). The most robust technology currently appears to be large clone genomic microarrays, made with DNA isolated from bacterial artificial chromosomes (BACs). We are currently designing and testing "homemade" BAC arrays, in collaboration with colleagues at the University of California at San Francisco and the Sanger Centre, United Kingdom.
- To screen selected patient groups and analyze in detail any abnormalities that are discovered.

We recently detected an interstitial deletion at chromosome 8q24.3 in a patient with Kabuki syndrome. This condition results in moderate learning difficulties, short stature, typical facies, and very high rates of congenital heart disease (50 times that of the general population). The deletion was initially detected on a genome-wide BAC array, and subsequently confirmed by FISH (fluorescence in situ hybridization) analysis of metaphase chromosomes from the

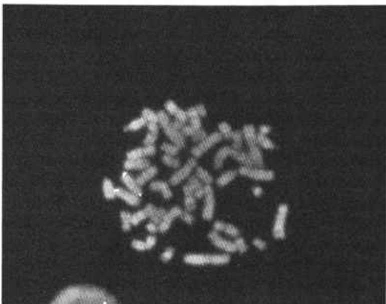

FIGURE 1 BAC array output for chromosome 8 in a child with Kabuki syndrome. One BAC located near the end of the long arm of chromosome 8 appears to be deleted. This was subsequently confirmed on FISH analysis (see Fig. 2).

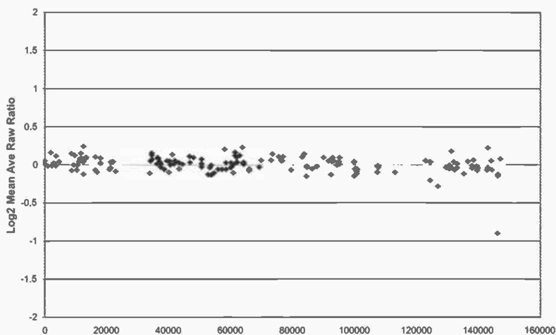

FIGURE 2 FISH analysis of metaphase chromosomes from a patient with Kabuki syndrome. Two clones were used: One maps to chromosome 8ptel (a signal is seen on both homologs) and the other to chromosome 8q24.3, the region suspected to be deleted on the BAC array. This probe only yields one signal, consistent with the presence of a heterozygous deletion in this patient.

patient. We have begun a detailed molecular analysis of this microdeletion and estimate its size to be no greater than 500 kb, well below the level of cytogenetic resolution. We plan to analyze as many Kabuki patients as possible in order to determine the frequency of this deletion in that population, as well as the background frequency in the general population. To this end, we are designing an array that will contain a very dense set of DNA markers in the affected region and that will allow analysis at a resolution of 10 kb.

REGULATION OF APOPTOSIS IN CANCER CELLS

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

Apoptosis is a type of cell death that is critical for maintaining tissue homeostasis. An intense effort to understand apoptosis has been fueled in large part by the hope of using the apoptotic machinery for curing cancer. The accumulated knowledge is sufficient to design and implement tools that kill cells quickly and efficiently by inducing apoptosis. However, because the apoptotic machinery is present in most if not all mammalian cells, the major problem is how to induce apoptosis in cancer cells selectively. One approach to solving this problem is to learn how apoptosis is induced by oncogenic transformation. This approach is based on a paradoxical observation that some oncogenes, including *myc* and adenovirus *E1A*, either induce apoptosis or sensitize cells to cytotoxic agents, including those used for chemotherapy. One implication of this observation is that some oncoproteins are pro-apoptotic activities that are specific for transformed cells. We think that understanding how the apoptotic machinery is activated in response to the expression of oncogenes, and how apoptosis is prevented in transformed cells, will help to develop ways to kill cancer cells selectively.

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

To investigate how caspases are activated by oncogene expression, we previously developed a cell-free system that mimics apoptosis dependent on expression of *E1A*, an adenoviral oncogene. Using this system, we found that *E1A*-dependent apoptosis is mediated by caspase-9 and that the expression of *E1A* sensitizes cells to apoptosis by facilitating activation of this caspase. This is achieved by at least two ways: (1) facilitating the release from mitochondria of cytochrome *c*, a cofactor required for caspase-9 activation, and (2) regulation of an unidentified subsequent step in caspase-9 processing.

Our subsequent experiments suggested that *E1A* regulates cytochrome *c* release both by promoting activation of proteins that can permeabilize mitochondria and by inactivating an activity, which we named IODA (inhibitor of oncogene-dependent apoptosis) that prevents cytochrome *c* release. One of the goals of our laboratory during the last year was to develop approaches to identify IODA.

MONOCLONAL ANTIBODIES AS A TOOL TO IDENTIFY MOLECULES THAT REGULATE MITOCHONDRIAL PERMEABILITY

The current view is that cytochrome *c* release from mitochondria is regulated by interactions between cytosolic proteins, such as Bax and Bad, and as yet unidentified receptors on the mitochondrial surface. To us, this is reminiscent of the interaction between cytokines and their cellular receptors in the immune system. We then reasoned that a significant number of these receptors were identified by producing monoclonal antibodies to complex cell populations and then testing the antibodies for interaction with a particular subset of cells. Therefore, we thought that by using a similar approach, we may be able to identify mitochondria-associated proteins that are involved in regulation of apoptosis and that mediate the response to *E1A*. This approach seemed to be particularly attractive because of our substantial experience in making monoclonal and polyclonal antibodies by producing and

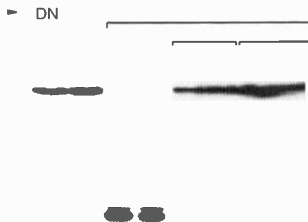

FIGURE 1 Expression of ode and primus (pr) depends on expression of *E1A* as detected by immunoblotting.

characterizing more than 150 antibodies to caspases, APAF-1, and other proteins involved in apoptosis.

As a result of our first attempt, we identified multiple antigens that change their localization, mobility on SDS-PAGE, or expression, depending on *E1A* expression or drug treatment. For example, an antigen called primus is a protein that is localized at mitochondria and is repressed in cells expressing *E1A*, whereas the antigen ode is induced by *E1A* (Fig. 1). Although the majority of antigens are associated with mitochondria, we also found a number of antigens affected by *E1A* that are located in other cellular compartments. We have been identifying the antigens and are beginning to investigate their role in apoptosis. This task will be substantially facilitated by the advent of RNA interference as a technology that will allow us to easily obtain cells that lack a protein of interest.

BCL-2 PROTEINS AS TRANSMEMBRANE TRANSLOCATORS

Because Bcl-2 proteins, which are inhibitors of apoptosis, regulate mitochondria permeability, there is a possibility that IODA is a known or yet to be identified Bcl-2-like protein. How Bcl-2 and its relatives function is not known. The observation that the Bcl-2 family regulates the release of pro-apoptotic proteins from mitochondria led to the concept that the family controls cell survival by regulating permeability of these organelles.

Several models have been proposed to explain how this regulation is achieved. A popular model comes from the intriguing observation that the tertiary structure of the Bcl-2 proteins is reminiscent of that of colicins and diphtheria toxin (DT), which are bacterial toxins. Colicins kill other bacterial strains, whereas diphtheria toxin is toxic to humans. Because these toxins can make pores in lipid membranes, it was suggested that the Bcl-2 proteins function by making pores in mitochondrial membranes.

Although some Bcl-2 proteins were indeed found to form pores in synthetic lipid membranes, the model has been facing substantial challenges. For example, the structures of Bax and Bcl-x (Bcl-2 relative) are remarkably similar, suggesting that if one of the proteins makes the pores, another would likely make them as well. Yet, Bax promotes the release of pro-apoptotic molecules from mitochondria, whereas Bcl-x prevents it. This puzzle has not been made easier by the difficulty of explaining how Bcl-x or Bcl-2 would prevent release of proteins from mitochondria by making pores.

These and other unexplained observations argue that either the similarity between the Bcl-2 family and the toxins is a coincidence or they share a common function that is unrelated to the formation of pores. We proposed that the structural similarity between the Bcl-2 proteins and the diphtheria toxin translocation domain indicates that the common function of these proteins is to translocate other molecules or themselves through membranes, rather than to form pores. This hypothesis is yet to be tested.

MITOCHONDRIAL ACTIVATORS OF APOPTOSIS

This work was done in collaboration with Emad Alnemri, Kimmel Cancer Center. Our interest in IODA led us to investigate whether mitochondria contain unidentified molecules that regulate apoptosis. In collaboration with Dr. Emad Alnemri, we found that a mitochondrial protease called Omi is released along with cytochrome *c* and promotes apoptosis, apparently by inactivating inhibitors of caspases. We also identified another protein whose function we are currently investigating.

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REGULATION OF APOPTOSIS AND SENESCENCE BY CANCER GENES

S. Lowe E. de Stanchina, G. Ferbeyre, J. Fridman, A. Lin, M. McCurrach, Z. Nahle, M. Narita, M. Narita, S. Nunez, E. Querido, C.S. Ray, C. Rosenthal, A. Samuelson, C. Schmitt, M. Soengas, J. Zilfou

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

Oncogene-induced Apoptosis

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

Normal cells possess natural fail-safe mechanisms that limit the consequences of aberrant proliferation. One of these mechanisms involves their ability to couple excessive proliferation to cell death. For example, deregulation of either the c-Myc or Rb pathway in normal cells can force aberrant S-phase entry and predispose cells to apoptotic cell death. This increased sensitivity to apoptosis appears to limit tumor development and, as a consequence, mutations that disable oncogene-induced apoptosis can contribute to carcinogenesis. Consistent with this view, we have previously shown that oncogenes can engage the ARF-p53 pathway to promote apoptosis and that disruption of this pathway cooperates with oncogenes to promote oncogenic transformation in vitro and tumor development in vivo.

We are currently using a primary fibroblast system as well as innovative gene expression technologies to identify additional components of the apoptotic and senescent processes and how they function in a "tumor suppressor network." Our current questions include: What are the cellular targets of E1A in promoting apoptosis? How does $p19^{ARF}$ couple oncogenes to p53? How do oncogenes promote apoptosis independently of p53? To what extent does disruption of oncogene-induced apoptosis contribute to tumor development in vivo?

One example of our progress in this area involves demonstrating how different signaling pathways cooperate to promote apoptotic cell death. The adenovirus E1A oncogene sensitizes cells to apoptosis, in part by deregulating normal cell cycle control to activate the ARF-p53 tumor suppressor pathway. Previously, we showed that at least two separate functions of E1A are required to promote apoptosis, one involving deregulation of the pathway and the other thought to be required for E1A to physically associate with the p300/CBP transcriptional coactivators. However, we now have shown that E1A promotes apoptosis through its interaction with the p400-TRRAP chromatin remodeling complex and not its interaction with p300/CBP. Significantly, the p400-TRRAP complex is also an essential cofactor for the c-Myc oncoprotein and, interestingly, c-Myc rescues the apoptotic defect of E1A mutants unable to interact with p400-TRRAP.

The results described above suggest that E1A promotes apoptosis by deregulating two endogenous pathways intimately involved in cell cycle control, the Rb and c-Myc pathways. Consistent with this notion,

c-Myc and cyclin E (which also deregulates the Rb pathway) cooperate to induce ARF and p53 and to sensitize primary cells to apoptosis. Thus, by studying E1A, we show that Myc activation and disruption of the Rb pathway act synergistically to promote cell death. This suggests that the c-Myc and Rb pathways do not act in a simple linear manner, but rather target distinct steps in the apoptotic program. The molecular nature of these targets is currently being investigated.

p53 Effector Functions

J. Fridman, C. Schmitt, M. Soengas, J. Zilfou
[in collaboration with Y. Lazebnik and D. McCombie, Cold Spring Harbor Laboratory; J. Herman, Johns Hopkins University; W. Gerald and C. Cordon-Cardo, Memorial Sloan-Kettering Cancer Center; A. Giaccia, Stanford University]

The p53 tumor suppressor participates in several cell-cycle checkpoints, DNA repair, senescence, apoptosis, angiogenesis, and the surveillance of genomic integrity. In principle, the simultaneous inactivation of all p53-associated activities could provide a multilateral advantage to a developing tumor cell that mutates *p53*; conversely, inactivation of only some p53 functions may provide an essential new capability, whereas others may simply be by-products of p53 loss. A major effort of our laboratory is to understand how p53 executes different biological programs, and which of its many activities is relevant to tumor suppression in vivo. Our current questions include: How does p53 execute its apoptotic and senescence programs? What factors determine which process will be engaged upon p53 activation? What p53 activities are essential for p53 tumor suppressor function in vivo?

Because mutations in cancers necessarily produce a selective advantage to emerging tumor cells, the identification of mutated components and their frequency of mutation highlight critical regulatory points in survival and proliferative processes. Therefore, one way to determine the contribution of specific p53 effector functions in tumor suppression is to see whether molecules that act specifically in one process are mutated or altered in human tumors. In this regard, we have shown that *apaf-1*—a downstream effector of p53 in apoptosis—is silenced in a substantial percentage of human melanomas (Soengas et al. 2001). Interestingly, *p53* mutations are rare in melanoma, and those melanoma cell lines are resistant to p53-dependent apoptosis in response to chemotherapy and following overexpression of p53 itself. This suggests that some melanomas disable the p53 pathway by disrupting apoptosis downstream from p53 and provide an example where disruption of the apoptotic function of p53 may be essential for tumorigenesis. This result is supported by a separate series of experiments in which we have examined the contribution of apoptosis to p53-mediated tumor suppression in Eμ-myc transgenic mice (C.A. Schmitt et al., in press).

We are also interested in how p53 executes its biological functions. Previously, with L. Attardi and T. Jacks, we showed that the ability of p53 to *trans*-activate target genes was essential for its ability to promote apoptosis. This year, with A. Giaccia, we see that transcriptional repression can be an important component of apoptotic function of p53, particularly in cells responding to hypoxia (Koumenis et al. 2001). Future work will continue to evaluate the contribution of specific p53 effector molecules to apoptotic cell death, and to explore how the senescence program is executed.

Control of Cellular Senescence

E. de Stanchina, G. Ferbeyre, A. Lin, M. McCurrach, M. Narita, M. Narita, E. Querido [in collaboration with G. Hannon, Cold Spring Harbor Laboratory]

Cellular senescence was originally described as the process that accompanies replicative exhaustion in cultured human fibroblasts and is characterized by a series of poorly understood markers. Senescent cells remain metabolically active, but are unable to proliferate, and they display changes in gene expression that could alter tissue physiology. As such, they are genetically "dead" and cannot contribute to tumor development. Although "replicative" senescence is triggered by telomere attrition and can be prevented by telomerase, an identical endpoint can be produced acutely in response to activated oncogenes, DNA damage, oxidative stress, and suboptimal cell culture conditions.

We have proposed that senescence acts in parallel with apoptosis as a cellular response to stress. Like apoptosis, senescence can be induced by diverse stimuli that appear to engage a common effector program.

For this reason, our studies on apoptosis have guided those on senescence. We are currently addressing several questions: How does Ras signaling activate a permanent cell cycle arrest? How do tumor suppressors such as p53, p16, and p19^{ARF} initiate and maintain this arrest? How do epithelial cells respond to Ras? How do cells maintain the senescent state?

This year, we have shown that as in fibroblasts, oncogenic *ras* induces keratinocyte cell cycle arrest in a manner involving the p19^{ARF}, p16^{INK4a}, and p53 tumor suppressors (Lin et al. 2001). This arrest clearly limits tumor development, since *ARF*-null keratinocytes expressing oncogenic *ras* rapidly formed carcinomas in immunocompromised mice, whereas their wild-type counterparts were not tumorigenic. These results establish the importance of the ARF-p53 pathway in epithelial cells and imply that senescence-like processes suppress epithelial cell transformation.

Using a conditional p53 expression system, we have begun to evaluate the contribution of p53 to the initiation and maintenance of *ras*-induced senescence (G. Ferbeyre et al., in press). Interestingly, p53 activation efficiently induced a reversible cell cycle arrest but was unable to induce features of senescence. In contrast, coexpression of oncogenic *ras* or an activated *mek1* with *p53* produced an irreversible cell cycle arrest that displayed features of cellular senescence. These results indicate that oncogenic activation of the MAP kinase pathway in murine fibroblasts converts p53 into a senescence inducer. Future work will continue to investigate how p53 initiates senescence and the factors that contribute to the maintenance process.

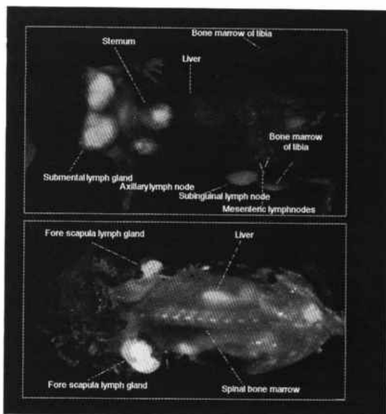

FIGURE 1 Imaging lymphomas in live mice. Primary lymphomas arising in *Eμ-myc* transgenic mice are "tagged" with a GFP-expressing retrovirus ex vivo, and then injected into an immunocompetent recipient. After several weeks, a lymphoma forms that is histopathologically similar to the original transgenic animal, but now can be monitored using whole-body fluorescence imaging. The images were produced in collaboration with R. Hoffman (AntiCancer, Inc., San Diego, California).

Molecular Genetics of Drug Sensitivity and Resistance

J. Fridman, Z. Nahle, C.S. Ray, C. Rosenthal, C. Schmitt
[in collaboration with R. Hoffman, AntiCancer, Inc.,
San Diego, California]

A major goal of our research is to understand the biological and molecular basis of drug sensitivity and resistance in tumors. Conventional approaches to identify factors that dictate treatment sensitivity rely on human tumor cell lines treated in vitro or as ectopic xenografts. As an alternative approach, we are using transgenic mouse models to study drug action in spontaneous tumors. Our system exploits the *Eμ-myc* transgenic mouse, which develops B-cell lymphomas at short latency with high penetrance. Using this system, we have identified biologic (apoptosis) and genetic (p53, bcl2, INK4a/ARF) determinants of treatment sensitivity in vivo. We continue to identify new processes and genes that modulate treatment sensitivity, and we are gearing up to use our mice as a physiologic system to test novel compounds that might circumvent conventional resistance mechanisms.

Most of our progress in the last year has been with improving the technology for manipulating the genetics of primary lymphomas and for monitoring treatment responses. This year, we have (1) adapted retroviral gene transfer methods to introduce genes into hematopoietic stem cells from transgenic and knockout mice, allowing rapid generation of tumors with compound genetic lesions (C.A. Schmitt et al., in press); (2) made practical advances in studying treat-

ment responses and producing drug-resistant tumors (Schmitt and Lowe 2001); and (3) shown that tumor behavior can be monitored by whole-body fluorescence imaging (Fig. 1). The latter approach will allow both spatial and temporal monitoring of lymphoma behavior in live mice, for example, during tumor progression or following cancer therapy.

With support from the Ann L. and Herbert J. Siegel Philanthropic Fund, we are scaling up our efforts to study drug action in mice. Although the extent to which these models will be predictive of responses in human cancer remains to be determined, we believe that the intractable nature of the resistance problem demands new thinking and new experimental approaches. With this in mind, our goal is to use these mouse models to provide a rational framework for predicting response of human tumors to therapy and developing strategies to overcome or circumvent resistance.

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GENOMIC MICROARRAY ANALYSIS

R. Lucito S. Rostan A. Buglino
A. Brady J. Healy
J. Alexander R. McCombie

Progression of cancer is a multistep process. Mutations occur in the genome of precancerous cells and accumulate until the growth of these cells proceeds unchecked. The large majority of these mutations occur sporadically, i.e., they are not inherited. The genes responsible for sporadic cancer must be identified if we are to understand why a cell becomes cancerous. To identify and characterize other genes affected by sporadic mutation, we can look to the genome of the cancer cell, identifying regions that have undergone increased or decreased gene copy number, namely, amplifications or deletions, since these changes can be used as markers for the locations of oncogenes or tumor suppressor genes, respectively. Once these regions are identified and tumor suppressors and oncogenes are found, their function can be investigated to understand the role that these genes have in the progression to tumorigenesis. The regions themselves can be used for studies in prognosis and diagnosis.

To facilitate the identification of prognostic and diagnostic indicators and new genes involved in tumor progression, it is critical to be able to survey gene copy number changes over the whole genome of the tumor. We have developed a genomic microarray technique, borrowing the methodology of complexity-reducing representations developed for representational difference analysis (RDA) to increase hybridization efficiency and increase signal-to-noise ratio. A representation is a reproducible sampling of the genome, produced by first cleaving the genome with a restriction enzyme such as *Bgl*II, ligating the adapters, and amplifying by polymerase chain reaction (PCR). Amplification in the presence of many fragments results in preferential amplification of the smaller fragments, so that the size range of the representation is 200–1200 bp. Many of the restricted fragments are lost during this preferential size selection caused by the PCR amplification, resulting in a reduction in complexity of approximately 3% of the genome.

By taking full advantage of the sequence of the human genome, the array can be designed so that it is composed of oligos derived from *Bgl*II fragments. *Bgl*II representations of tumor and normal differentially labeled with two different fluorescent tags are compared on such an array. The intensity of the fluorescent tag in each channel is measured, and the ratio one-to-the-other is indicative of copy number measurement of the gene. The resolution of this type of array can be increased simply by adding more *Bgl*II fragment-derived oligos, up to an average of one probe every 20 kb at theoretical maximum. Furthermore, if this is not enough coverage or if there is a region poor in *Bgl*II fragments, a second array can be constructed based on a second enzyme. The oligos are designed in collaboration with M. Wigler here at CSHL who, with John Healy, has designed a program that identifies the repeat nature of a *Bgl*II fragment and also designs the best 70-nucleotide oligo. This allows us to design non-repetitive oligos from fragments that contain highly repetitive elements.

We have constructed an oligo array with five loci represented at a resolution of one probe every 75 kb to test the ability of the format to detect gene copy number fluctuations. These loci are *erbB2*, c-*myc*, a region on 8p22 found deleted in breast cancer, a region on 8q24 found amplified independently of c-*myc* in breast cancer, and a region on chromosome 22q11 that is lost hemizygously in patients with velo cardio facial syndrome. These arrays have been hybridized to samples that have been characterized by us or other investigators as being mutated for these regions, to determine how accurately this method can identify gene copy number fluctuations.

Analysis of a tumor cell line published to have genomic amplification of *erbB2* and c-*myc* at 8q24, and 17q21 or c-*myc* and *erbB2*, respectively, demonstrated that our method is robust and can detect amplifications as well as the more standard techniques can. The genomic profiles easily identified the two loci as being amplified, with regions of single copy on either side of the amplicon. Analysis was performed on a primary breast tumor. The tumor was separated by fluorescence-activated cell sorting (FACS) analysis into aneuploid and diploid, which were used as tumor and normal, respectively. This tumor was identified as having an 8q24 region amplified by other methods. When

analyzed on the array, it became apparent that there were several amplicons in this region and a possible deletion. Two of the regions have been identified previously in other tumor samples and are being followed further. The method is also sensitive enough to detect deletions of one out of two alleles, or hemizygous deletions. By comparing a son with velo cardio facial syndrome to his unaffected mother, we have shown that one copy of the 3 Mb that is commonly deleted is missing in the affected son, and the regions flanking this 3-Mb region are retained.

We are planning to expand the array to a genome-wide scale; 70-mer oligonucleotides are designed from representational *Bgl*II fragments such that they have the least repetitive content. Eventually, we will reach 60,000–90,000 oligos distributed throughout the genome, giving us a resolution of one probe every 30–50 kb. The oligonucleotides must also be characterized to determine which will yield the most reliable data. We are utilizing a combination of genomic clone pooling, restriction-killed representations, and genomic fractionation strategies.

Although this microarray method will be used to categorize the mutations that occur in many tumor types, we will use this method initially to analyze primarily two tumor types. Breast cancer will be analyzed addressing two questions: The first question is the amplification of *erbB2* and the treatment with Herceptin, a monoclonal antibody to the *erbB* gene product. Herceptin is prescribed as a treatment for patients who test positive for *erbB* gene amplification, but only in a minority of cases do patients respond completely to Herceptin and chemotherapy. It is our intention to take a set of patients who are *erbB2*-positive and have had varied response to Herceptin, to identify other genomic mutations and to make correlations between the mutations and response. The second question will address the conflicting evidence of whether ductal carcinoma in situ (DCIS) is the precursor lesion to invasive ductal carcinoma (IDC). It seems likely from the literature that DCIS sometimes progresses to IDC. If this were the case, it would be useful to identify which cases of DCIS will progress to invasiveness. We hope to identify regions of the genome that can serve as markers for the clinician to identify patients with DCIS that will progress to IDC so that they can be treated more aggressively.

The second cancer we will focus on is prostate cancer. When a patient presents with a high prostate-specific antigen (PSA) level, a radical prostatectomy is performed. Within this group, there is a subgroup in which the PSA levels again rise after ~1 year , often as a result of metastasis. We will go back to the original tumor samples, classified aggressive, and compare these to a set that does not metastasize. By comparing the genomes of these two classes of primary tumors, those that have recurrent PSA expression with those that do not, we hope to identify useful markers that can distinguish between the two groups. If a correlation can be made between the profiles obtained and the clinical information, the clinician can use this information to judge how to treat the patient.

The regions identified from these studies will serve as valuable diagnostic and prognostic indicators. Of equal importance, these regions will serve to identify new oncogenes and tumor suppressor candidate genes. The advantage to using genome-wide analysis on a large set of tumors is that the time-consuming step of positional cloning will be done, although it will be necessary to compile the data. Once compiled, the epicenter, or common region of mutation, will be located and candidate genes identified informatically in collaboration with R. McCombie here at CSHL.

Colon Cancer

A. Brady, S. Gass [in collaboration with M. Wigler and R. McCombie, Cold Spring Harbor Laboratory]

We are currently investigating a possible tumor suppressor gene candidate located on chromosome 20p12. This region of the genome is rarely found homozygously deleted in cancer unless the tumor is of gastrointestinal origin. In the GI tumor cell lines that were tested, the deletion frequency approaches 4%, quite high for homozygous deletions. The deletion patterns of the cell lines were mapped and compared to identify the epicenter, or the minimal region of deletion. Once identified, the region was used for high-quality sequencing in collaboration with R. McCombie. The sequence generated was used for gene finding using several programs, but no gene candidates were identified. After the draft of the human genome sequence was completed, the region was used for the informatic identification of gene candidates. No known genes were identified in the region, and only one spliced expressed sequence tag (EST) was identified. It was identified as a spliced EST because the sequence of the EST splits into eight pieces and each maps to the genome. This EST, a 5′ read, is ~700 bp, but spreads

across a region of ~500 kb. The coding region does not have any clear homology. The exons were tested for their presence or absence in eight of the cell lines which have homozygous deletions. Six of the eight tumor cell lines have a deletion of exons 1 and 2. We are presently amplifying the EST sequence to use it to probe northern blots to identify the size of the full-length product. It may be necessary to clone the full-length gene by RACE or phage library screening. Once identified, the sequence will be used to initiate screening of primary colon tumor samples to identify point mutations. We already have approximately 100 tumor samples that can be used for this purpose.

RAC SIGNALING

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

MAIZE MUTAGENESIS LIBRARY SCREENING

This work, being done in collaboration with Robert Martienssen, Pablo Rabinowicz, and Erik Vollbrecht here at CSHL, is the development of a high-throughput screen of a maize insertion mutagenesis library. The probes on the array are picked from a gene-enriched methyl-filtering library. This library relies on the fact that much of the maize genome is methylated repeat sequences, but the gene-rich regions are not methylated. This method enriches the genes represented by subcloning unmethylated genomic regions. The mutant plant library was produced by random insertion of a transposable element. The samples are produced by first pooling the individual plant DNAs in a plate-wise fashion accumulating 50 individual plants per pool. Representations are prepared from these samples by PCR amplification on this pool using one oligo to the transposable element inserted and a second random oligo. The representations are then used for hybridization to the arrays to identify which individual plants have a gene insertion. This information will be cross-referenced to phenotypic data to identify the gene regions with transposon insertions.

In Press

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MAMMALIAN FUNCTIONAL GENOMICS

A. Mills J. Kui
E. Garcia
A. Bagchi

Our goal is to understand the genetic basis of human disease. Although genome sequencing efforts have generated an impressive amount of information about the content of the mammalian genome, we know relatively little about how specific genes function. Thus, functional genomics—dissecting the genome by determining the role of specific genes—is essential for understanding normal development and the disease process.

We are using the mouse as a model system to probe the function of human genes. The mouse provides an excellent opportunity to explore gene function because of its reproductive, anatomical, physiological, and genomic similarity to humans. Our ability to genetically modify the mouse genome makes it possible to create mouse models that allow us to address a multitude of specific biological questions.

In our laboratory, we use gene targeting in embryonic stem cells to generate mouse strains that have precise modifications of the genome; these strains serve as experimental models that allow us to define gene function. We are using two different approaches to achieve this goal: (1) analyzing the role of *groups of genes* within specific regions of the genome and (2) analyzing the role of *individual genes* known to be associated with the disease process. The first approach uses chromosome engineering to generate mouse strains that have precise chromosome rearrangements which mimic those found in human diseases. These mouse strains are useful for providing models of human disease and for determining the function of genes within specific regions of the genome. The second research emphasis in the laboratory is to perform an in-depth exploration of a single gene known to be causal in several human disease syndromes. This gene, *p63*, is related to the *p53* tumor suppressor gene—a gene that is mutated in approximately one half of all human cancers. Understanding the molecular and genetic mechanisms of *p63* action will allow us to gain insight into the processes of development, differentiation, stem cell maintenance, and cancer.

Generation of Megabase Chromosome Rearrangements: *human chromosome 1p*

A. Bagchi

A substantial number of human diseases are associated with chromosomal rearrangements such as deletions, duplications, and inversions. These abnormalities are complex because they affect large numbers of genes within specific chromosomal regions. To understand how these rearrangements cause disease, we can make mouse strains that have the same rearrangement as those found in human patients.

Defined chromosome rearrangements are made using chromosome engineering—an approach that combines the power of gene targeting with Cre/*loxP* technology. Similarities between the human and mouse genome make it possible to generate relevant models of human disease. We are currently using this approach to generate models for hereditary cancer syndromes. We are focusing our efforts on *human chromosome 1p*—a region containing several as yet unidentified tumor suppressor genes. A diverse array of human tumors have deletions at *1p*, suggesting that genes underlying the tumorigenic process are located in this region of the genome. We are creating models of hereditary cancer by creating mouse strains that have the same deletions as those found in human tumors. We first make specific deletions in embryonic stem cells, and then use these cells to generate mice that transmit the modified allele in the germ line. To make this approach feasible on a genome-wide scale, we have created a system that greatly reduces the substantial amount of effort required for generating gene-targeting constructs. This system is composed of two genomic libraries of essentially pre-made gene-targeting vectors that contain all of the features required for generating Cre-induced chromosome rearrangements. In addition, constructs isolated from these libraries

contain genes that alter the color of mice that harbor them; this feature has the advantage that mice containing a specific rearrangement in their genomes are visibly distinguishable from normal mice. This greatly reduces the cost and labor that are normally required for maintaining mouse colonies and also allows these models to be implemented in genetic screens.

Analyzing the Role of Specific Disease Genes: The *p63* Locus

J. Kui, E. Garcia

The *p53* tumor suppressor gene has an important role in cancer progression. Approximately one half of all human cancers either have lost *p53* altogether or have inactivated it by mutation. Although mice lacking *p53* are viable, they develop tumors at a very early age. Thus, *p53*-deficient models are extremely valuable for investigating the molecular and genetic events associated with tumor formation.

We discovered *p63,* a gene that has striking similarity to the *p53* tumor suppressor gene. We used gene targeting to create mice that lack *p63;* these mice have severe developmental defects that affect craniofacial, limb, and skin development. Mice lacking *p63* are devoid of all structures that are normally derived from the ectoderm during development; they lack hair, teeth, mammary and sebaceous glands, nails, and prostate. These observations provided an important clue which led to the discovery that mutations in *p63* cause the human developmental syndrome ectrodactyly ectodermal dysplasia clefting (EEC syndrome).

We are currently investigating the role of *p63* in morphogenesis of the ectoderm and its related structures. We have generated a conditional *p63* model that allows us to first generate viable mice, and then to ablate *p63* function within specific tissues at particular stages of development. Using this approach, we will examine the role of *p63* within the adult to assess how it functions in stem cell maintenance, differentiation, and cancer.

We are also using several mutagenesis approaches to determine how specific alterations of *p63* affect its function. The first approach is to use site-specific mutagenesis to generate mouse models which have the same *p63* mutations that are present in EEC patients. We used gene targeting to replace the normal copy of *p63* with this mutated version. This model will allow us to determine how mutations in *p63* lead to the abnormalities found in individuals afflicted with EEC syndrome.

We are also investigating the role of *p63* using a phenotype-driven mutagenesis strategy—one that makes random mutations throughout the entire genome. The mutagen that we use is ENU; it is a potent mouse mutagen that typically generates single-nucleotide mutations. If an ENU-induced mutation happens to occur within the *p63* gene, it will be detectable in the screen because a visible phenotype can be observed. This approach is extremely valuable because it is random; it is unbiased because it is based entirely on the resulting phenotype and not on our understanding of gene structure. This approach will provide a series of mouse models that harbor different *p63* mutations. This resource will be invaluable for assessing the role of *p63*.

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DNA MICROARRAY TECHNOLOGY AND CELLULAR PATHWAYS

V. Mittal R. Kumar O. Morris
R. Schoer D. Chertoff
K. LaVine R. Lindsay
A. Pirro

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

My laboratory is interested in using the DNA microarray technology in understanding cellular pathways in mammalian cells. Below is a brief description of individual projects being pursued in my laboratory.

DNA Array Technology: Fabrication and Methods Development

K. LaVine, A. Pirro, O. Morris

We have set up a reliable system for the fabrication of high-density human and mouse expression arrays and developed informatics tools for quantitative analyses of the array data. We have assembled a collection of 15,000 mouse cDNAs and expressed sequence tags (ESTs) from the National Institute of Aging and 40,000 human cDNAs and ESTs from the Image Consortium. These clones have been processed, and microarrays of greater than 20,000 cDNA probes have been successfully printed, hybridized, and analyzed. Last year, we focused mainly on developing standards for all steps in the process including microarray design, printing, sample preparation, labeling, hybridization, and data analysis.

Delineating Angiogeneic Pathways in Tumors

R. Schoer [in collaboration with Marianna Russinova and Robert Benezra, Memorial Sloan-Kettering Cancer Center, New York]

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

Two in vivo mouse model systems were used: wild-type (PTEN +/–, Id +/+) that forms lymphoma and a mutant (PTEN +/–, Id –/–) that forms lymphoma but lacks the ability to form blood vessels. Tumors were excised from these animals and RNA was isolated. RNAs from these tumors were differentially labeled with fluorescent dyes and hybridized to murine cDNA microarrays containing 15,000 mouse cDNAs and ESTs. Informatics analysis was used to identify differentially expressed genes. These were subjected to hierarchical clustering methods to decipher inherent patterns of coexpressions.

The candidate genes are undergoing validation by an independent method such as northern blotting. Their localization is being confirmed by in situ hybridization of tumor sections. We will next explore the various connections between these genes and order the genes into the specific, complex pathways of angiogenesis. The resulting gene set will advance understanding of the molecular mechanisms of angiogenesis and will pinpoint ideal targets for specific therapeutic initiatives.

Pathways in Stem Cell Differentiation

R. Kumar [in collaboration with Sang Yang Kim,
Cold Spring Harbor Laboratory]

In multicellular organisms, cell-cell communication has an important role in the regulation of fundamental biological processes such as proliferation, differentiation, and development. This communication involves a variety of intercellular and intracellular signaling events that operate through a complex system of biochemical pathways whose functioning is poorly understood. Although available molecular and genetic tools have revealed many of the players involved in these processes, an overall integrated and unified view of the complex responses and their dynamic interactions is clearly lacking. We are developing methods for deciphering complex systems of interacting signal transduction pathways by using cell coculture experiments, high-density DNA microarrays, and bioinformatics to reconstruct precise gene interaction networks from large-scale gene expression data. We are applying this approach to understand the mechanism of stem cell fate determination in response to microenvironment cues. We have begun generating double-transgenic mice in which a progenitor cell-specific promoter drives expression of green fluorescent protein (GFP) and a differentiated cell-specific promoter drives expression of a red fluorescent protein (RFP). Stem cells from these double transgenics will be isolated and enriched by fluorescence-activated cell sorting (FACS). Clonally derived stem cells (as judged from their ability to self-renew) will be grown alone or cocultured with equal proportions of differentiated adult cells. Transplantation of differentiated stem cells has considerable therapeutic potential for debilitating diseases such as muscular dystrophy, Parkinson's, and other degenerative diseases.

Functional Analysis of Cancer Genes

V. Mittal [in collaboration with M. Wigler,
Cold Spring Harbor Laboratory]

We are interested in understanding the mechanistic role of tumor suppressors and oncogenes in cancer by determining their specific cellular and physiological functions. In collaboration with the Wigler laboratory here at CSHL, we are examining the mechanistic role of the human tumor suppressor, PTEN (protein tyrosine phosphatase family member). A robust ecdysone-inducible mammalian expression system was developed for these studies. The effects of wild-type PTEN induction on transcription shared common signatures with the effects of inhibition of PI3-kinase, consistent with the model positioning PTEN as an inhibitor of PI3-kinase signaling. A majority of PTEN-regulated genes were involved in cell proliferation, cytoskeleton restructuring, and signal transduction pathways. Strikingly, at least five genes in the cluster were involved in the sterol biosynthesis pathway. The role of this pathway in the pathophysiology of the PTEN loss is under investigation. These results illustrate the power of a robust inducible expression system in conjunction with cDNA microarrays in understanding gene expression patterns induced by a tumor suppressor gene. Such an approach is directly applicable to the functional analysis of other cancer genes.

PUBLICATIONS

Stolarov J., Chang K., Reiner A., Rodgers L., Hannon G.J., Wigler M.H., and Mittal V. 2001. Design of a retroviral-mediated ecdysone-inducible system and its application to the expression profiling of the PTEN tumor suppressor. *Proc. Natl. Acad. Sci.* **98:** 13043–13048.

MAMMALIAN CELL GENETICS

M. Wigler J. Alexander, A. Brady, J. Brodsky, A. Buglino, K. Chang, M. Chi, J. Douglas, D. Esposito, S. Gass, J. Healy, R. Lucito, A. Reiner, M. Riggs, L. Rodgers, S. Rostan, E. Thomas, J. Troge, R. von Roeschlaub, J. West

THE CENTRAL PROBLEM OF CANCER

Cancer is a disease caused by genetic mutations. The particular combinations of genes mutated in the different tissues of origin probably determine the characteristics of individual cancers: their degree of malignancy and their particular therapeutic vulnerabilities. Science knows some of the genes that become mutated in cancer, much about the cellular pathways on which they act, and a little about the way that cancer cells interact with the host. Molecular oncology has finally begun to make very promising contributions to the treatment and diagnosis of cancers. So it is beguiling to think that the "Cancer Problem" has been solved, or will be solved by the routine cranking of the wheel, and thus be tempted to turn one's attention elsewhere, to other biological or biomedical problems that appear to hold greater mystery. This would be a grave mistake. The adequate treatment, diagnosis, and prevention of cancer in patients will require a detailed understanding of the specific molecular mechanisms that go awry in specific cancers, both within the cancerous cell and between the cancer and the host. Without this knowledge, we cannot improve diagnosis, prognosis, and treatment.

Science is far from having a complete list of either oncogenes or tumor suppressor genes that are mutated in even the most common types of cancer. Our work during the past years attests to this, as do the efforts of our direct collaborators and other scientists using similar approaches. Major new oncogenes and tumor suppressor genes abound, and, probably, relevant new cellular pathways. Science does not yet have a significant handle on the ways in which cancer cells interact with their host, or the cooperative ways in which cancer cells within a tumor may help one another. Moreover, medicine does not yet have a practical method for assessing the mutations of individual cancers. During the past years, we have developed new approaches to these problems that we expect will translate into improved diagnosis, prognosis, and treatment. We expect that the same approaches we are developing will lead to solutions for other complex human genetic diseases, problems in genomic mapping, and genome evolution.

REPRESENTATIONAL GENOMIC ANALYSIS

In the early 1990s, we developed a powerful approach to genomic analysis called RDA (representational difference analysis). RDA was designed to solve this problem: Find A when one genome is $X + A$ and the other genome is X (Lisitsyn et al., *Science 258:* 946 [1993]). Many biological searches can be reformulated as this problem, including the search for oncogenes, tumor suppressors, pathogens, or even genetic differences between individuals. Essentially, RDA was a combination of a new method for difference cloning, based on differential DNA hybridization, and a method for reducing the complexity of genomes. Complexity reduction was achieved using a process that we call representation (see Fig. 1), in which a reproducible subset of the genome is derived from the entirety. The value of representations lies in the

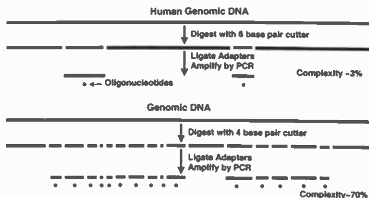


FIGURE 1 Schematic of the preparation of a low-complexity (*upper panel*) and high-complexity representation (*lower panel*). In designing the array, we chose probes from the amplified representational fragments.

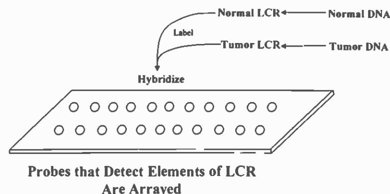


FIGURE 2 A typical format for our gene copy number arrays.

enabling of many protocols dependent on hybridization of genomic DNA. The entire human genome is just too complex in sequence to hybridize efficiently and specifically.

RDA led to the discovery of several new oncogenes and tumor suppressor genes by our collaborators and us. One of these, *DBC-2*, is reported jointly with Hamaguchi and another, *DIGIT*, by Lucito, both in this Annual Report. PTEN, which was discovered jointly with Ramon Parson's group at Columbia (Li et al., *Science 275:* 1943 [1997]), is described here. Additionally, many candidate oncogenes were found with our collaborators at Tularik. Several of these oncogenes belong to novel classes of gene families and have gone into drug discovery screens at Tularik. They will not be discussed here.

Although RDA has been a useful method for finding genetic lesions in cancer, and more effective than any technique before it, it is not sufficiently powerful to satisfy the needs of the cancer problem. We therefore began to fashion a microarray-based version of RDA wherein representations of two samples are compared by array hybridization (see Fig. 2). Our array method builds upon the success of microarrays to analyze gene expression (Schena et al., *Science 270: 467* [1995]; Chee et al., *Science 274:* 510 [1996]), the use of long oligonucleotide probes (Van't Veer et al., *Nature 415:* 530 [2002]), and comparative genomic hybridization, as originally developed by Joe Gray and his group for chromosomal analysis (Kallioniemi et al., *Sem. Cancer Biol. 1:* 41 [1993]). Unlike those groups, we use representations to create a system of unparalleled resolution that can examine the imbalances in the genome from minute samples of diseased (cancerous) tissues. The same methodology can also be used to study other complex human genetic diseases, assemble physical maps of probes from any genome, and enable us to compare the genomes of related species. Such comparative genomic studies will provide maps of rearrangements, segmental duplications, and deletions between related organisms.

OTHER RELATED PROJECTS

Our representational analysis using microarrays has led us, by necessity, to develop a novel genome-informatics algorithm for examining exact sequence matches within a genome. The application of this tool to genome analysis has led to a series of unexplained and interesting observations about genome and gene structure. We will describe our method and observations below, together with some theories. Finally, we describe a new effort to use expression microarray profiling to study the important and largely neglected subject of cancer cell communication: the study of the messages that cancer cells send and receive that may influence their persistence, growth, and spread in the host, and their cooperativity during tumor formation.

PTEN

The *PTEN* tumor suppressor gene was identified as a collaboration between Ramon Parson's laboratory at Columbia University and our own (Li et al., *Science 275:* 1943 [1997]). A deletion locus was first detected using RDA on breast cancer biopsies, which was then recognized as coincident with a locus implicated in a rare genetic disease, Cowden's syndrome, that predisposes affecteds to breast cancers. The *PTEN* gene was found to be mutated in a number of other cancer types, particularly brain cancers (glioblastomas) (Wang et al., *Cancer Res. 19:* 4183 [1997]). The gene has homology with protein phosphatases, and subsequently, Nick Tonks and Mike Myers here at CSHL showed that *PTEN* encodes a protein and phosphatidyl inositol (PIP3) phosphatase (Myers et al., *Proc. Natl. Acad. Sci. 94:* 9052 [1997]; Myers et al., *Proc. Natl. Acad. Sci. 95:* 13513 [1998]). In a collaboration with our group, the PIP3 phosphatase activity of PTEN, which opposes the action of PI3 kinase, a known oncogene and a candidate effector for the *Ras* oncogenes, was determined to be the most likely function that suppresses tumorigenicity.

Last year, in a collaboration with Vivek Mittal here at CSHL, we published a gene expression array analysis of PTEN (Stolarov et al. 2001). Using an efficient ecdysone-inducible promoter derived from the work

of Evans and co-workers (No et al., *Proc. Natl. Acad. Sci. 93:* 3346 [1996]) and a retrovirus-mediated delivery system, we showed that the genes which were under the control of PTEN, both positively and negatively, are also the genes under the control of a small molecule inhibitor of PI3 kinase. These experiments perhaps most clearly demonstrate that PTEN acts by controlling the PI3 kinase pathway. Among the genes that we observed to be negatively controlled by PTEN were those encoding transforming growth factor-β (TGF-β) and genes of the cholesterol biosynthesis pathway, including HMG-coA synthase and reductase, and squalene synthetase. Since PTEN may have a role in opposing the insulin response (Butler et al., *Diabetes 4:* 1028 [2002]), it is possible that these observations may indicate a link between the insulin signaling pathway and elevated cholesterol biosynthesis.

We have pursued these studies by investigating the role of TGF-β in the response of cells to PTEN. TGF-β is a growth factor that has a role in development and differentiation and has been suggested to have a role in tumor development and angiogenesis. When PTEN is induced in a glioblastoma cell line, the cells flatten out and form adherent sheets. In fact, these morphological effects are reversed by the administration of TGF-β. However, expression analysis has so far failed to provide a convincing link between TGF-β and PTEN. The genes controlled by both are a partially overlapping set, but the transcriptional effects of PTEN induction are not reversed by administering TGF-β. In fact, many of the PTEN responses are enhanced. The interaction between TGF-β and PTEN is thus likely to be complex.

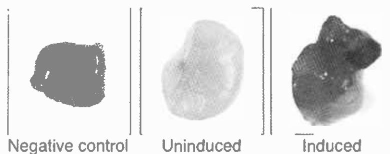
Last year, we reported the development of an effective ecdysone-inducible viral vector system, the system that was used in the above studies. Initially, we were unable to determine if the system would function in vivo. That difficulty was illusory and has been overcome (Fig. 3). Tumors that carry an ecdysone-inducible β-*GAL* gene can be induced to express that gene upon injection of an ecdysone homolog into the peritoneum of tumor-bearing mice (Fig. 3, top). This has enabled us to test the expression of PTEN in the glioblastoma cell line U87MG that has deleted its endogenous *PTEN* gene. The growth curves shown in Figure 3 (middle) suggest that expression of PTEN can arrest tumor growth. Considering that U87MG has been maintained in cell culture for many years after its removal from a patient, this is quite remarkable. Our observations support one of the fundamental hypotheses of cancer genetics: that pharmacological correction of the critical genetic defects of a cancer can lead to a cure. Figure 3 (bottom) shows the reverse result, as a control: When an oncogene (H-*ras*) is induced in U87MG, the cells form tumors more rapidly. These studies illustrate the utility of the ecdysone-inducible system for assaying both tumor suppressor genes and oncogenes in vivo.

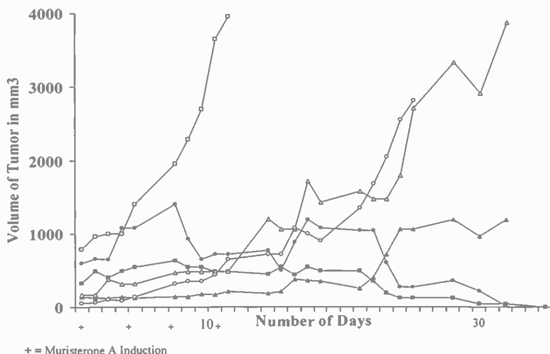
REPRESENTATIONAL MICROARRAY ANALYSIS OF THE GENETIC LESIONS IN CANCERS

The majority of our research effort is, in fact, directed to the use of microarray analysis to determine the genetic defects of cancer cells. As mentioned in the introduction, this effort has evolved from our early RDA studies, which clearly indicated that there were many oncogenes and tumor suppressor genes that had not yet been discovered. The essential core of our approach is the representation, which simplifies a complex genome so that its analysis by hybridization becomes more feasible. As illustrated in Figure 1, and described in past Annual Reports, we make representations by cleaving genomic DNA with a restriction endonuclease and then select only small DNA fragments by polymerase chain reaction (PCR) amplification. This enables us to reduce complexity in a computationally predictable manner and also analyze genomic DNA from minute amounts of specimen. A long history of the application of representations supports its reproducibility and reliability (Lisitsyn et al., *Proc. Natl. Acad. Sci. 92:* 151 [1995]; Lucito et al., *Proc. Natl. Acad. Sci. 95:* 4487 [1998]). If a sequence is present in a representation made with a particular restriction endonuclease, it derives from a small restriction fragment, and if a sequence is in a small restriction fragment, it is in the representation made with that enzyme.

The application of representations to microarray analysis is illustrated in Figure 2. Samples are represented and hybridized to arrays of probes that are present in representations. We call this RMA, for representational microarray analysis.

In our earlier studies, we made libraries from representations and arrayed fragments from the library as probes (Lucito et al., *Genome Res. 10:* 1726 [2000]). Our first experiments, published in 2000, demonstrated the feasibility of our approach. There were several problems with this approach, however. First, it was necessary to derive map positions for our probes. We accomplished this by sequencing (a collaboration with Richard McCombie here at CSHL). Second, these





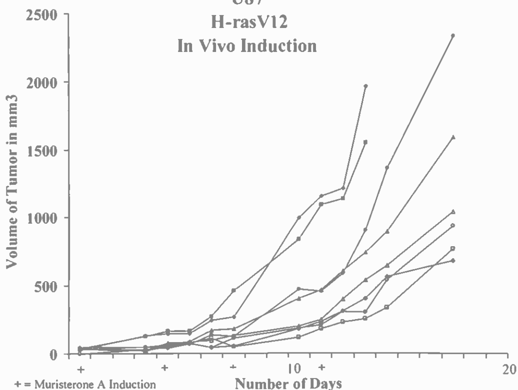


FIGURE 3 (*Top*) Induction of β-galactosidase expression under the control of the ecdysone promoter in a tumor. (*Middle*) Inhibition of tumor growth resulting from induction of PTEN expression under the control of the ecdysone promoter. (*Bottom*) Increased tumor growth in tumors due to the induction of oncogenic *ras* under the control of the ecdysone reporter. (*Open symbols*) Uninduced; (*closed symbols*) induced.

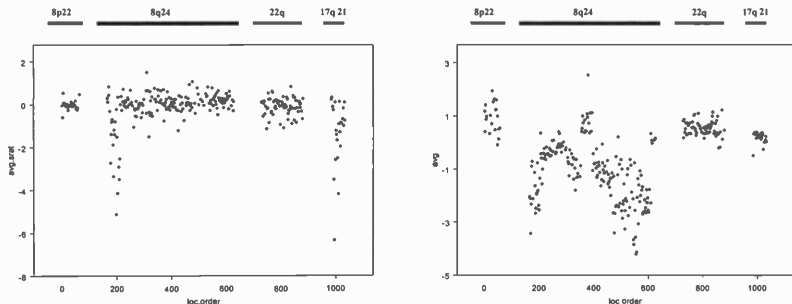


FIGURE 4 Two breast cancer samples are examined by probes distributed among four loci, on 8p11, 8q24, 22q, and 17q21. Each point in the graphs is the average of four measurements at each probe. Probes have been arranged according to their chromosomal position. Gene amplification is seen on the vertical axis as values in the negative range.

studies indicated that repeats were more widely distributed in the genome than we had expected, and probes with repeats were not useful for measuring gene copy number in a sequence-specific way. As a consequence, fewer probes were usable than we had expected. A third disadvantage of our first approach was that our probe set was randomly distributed in the genome, and hence we could not focus probes in regions of interest. Fourth, the infrastructure of maintaining large physical collections of probes was daunting. Finally, the arrays made from fragments are generally of a variable quality.

We were encouraged by the success of arrays of long oligonucleotide probes, which had been used by other investigators for expression analysis. Our first test of oligo arrays was successful (see Fig. 4). Importantly, we can design oligonucleotide probes directly from the published human genome assembly inferred to be in representations (see Fig. 1). In this way, we can avoid repeats and have many more probes that are usable. We can therefore make arrays with higher resolving power. In addition, we can place oligonucleotide probes at will near genes of interest, and thus increase our resolution in important regions of the genome. We call this procedure ROMA, for representational oligonucleotide microarray analysis.

Figure 4 illustrates results of the microarray analysis of two breast cancer specimens. The microarrays were designed with probes distributed over a region containing the c-*Myc* oncogene on chromosome 8 and the *ErbB-2* gene on chromosome 17, as well as concentrations of probes from elsewhere on the genome. As can be readily seen from the figure, amplification at these loci, and at another locus distant from c-*Myc*, can be readily detected. These arrays contain about 2000 probes, arranged in chromosomal order of the horizontal axis. The vertical axis displays the concentration in the cancer sample relative to their concentration in a normal sample for each probe, measured as a ratio of intensity of cancer to normal. From the peak of Figure 4 (left), we can see this patient has an amplification at *ErbB-2* and one at the c-*Myc* locus. Figure 4 (right) shows the analysis of a tumor from a patient with amplification around c-*Myc* and another locus at the other end of that segment, but no amplification about *ErbB-2*. These experiments demonstrate the ability of our microarray system to characterize the cancers of patients, and at the same time, point to new loci that are likely to be relevant to our understanding and treatment of breast cancer.

Although amplification is a hallmark of oncogenes, deletion is a hallmark of tumor suppressor genes. In Figure 5 (top), we demonstrate the ability to detect (the equivalent of) homozygous deletions. We prepared two representations, a *Bgl*II rep and a *Bgl*II rep "killed" with *Eco*R1. The latter is a *Bgl*II representation from which fragments containing *Eco*R1 sites are mainly destroyed. On the left of the figure are all of the probes derived from *Bgl*II fragments containing *Eco*RI sites, and on the right are those derived from *Bgl*II fragments that do not contain *Eco*R1 sites. The vertical axis is the ratio of hybridization signal, one representation versus

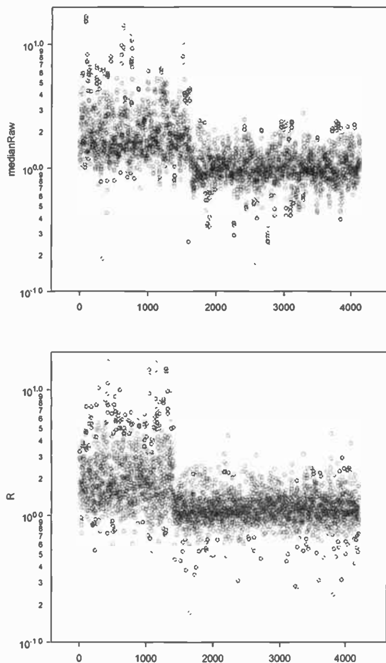


FIGURE 5 *(Top)* Actual data from a control experiment. In this control experiment, probes are not in chromosomal order. Rather, probes that are expected to show increased ratios, because they derive from *Bgl*II fragments with *Eco*RI cleavage sites, are on the left. *(Bottom)* Simulation of the top panel, based on sampled probe parameters.

the other. It is clear that the probes set on the right "report" differently from those on the left.

SIMULATION OF ARRAY RESULTS: PREDICTIONS OF PERFORMANCE

It is clear from Figure 5 (top) that not all probes report equally well. Some probes fail to perform. There are a variety of possible reasons for this: Some oligonucleotides are not properly synthesized, some *Bgl*II fragments do not amplify well, the genome sequence from which we derive our probes is inaccurate, some probes recognize repeat sequences, and some probes hybridize poorly due to subtleties of their secondary structure. However, microarray hybridizations such as that shown in Figure 5 (top) enable us to characterize our probes, and future results can be corrected for the performance character of each probe.

Experiments such as Figure 5 (top) also allow us to simulate overall array performance, using statistical sampling protocols. We assume that the basic equation for probe hybridization is given by

$$I = \alpha(Ax + B) + \beta$$

where, for each channel, I is hybridization intensity, α is a multiplicative system noise, A is the specific hybridization parameter of the probe, x is the concentration of complementary sequence to the probe in the sample, B is the nonspecific hybridization parameter, and β is the additive system noise. We can then simulate array performance for any number of probes, assuming that the distribution of probe parameters A and B of the probes we have sampled will be representative. Figure 5 (bottom) illustrates a simulation of the data shown in the top panel.

We can judge from our simulations how many probes with what probe parameters enable us to detect which kinds of lesions. For example, a 60K probe set with the characteristics of our current probe set will be able to detect reliable fivefold amplification of a 400-kb region, similar-sized homozygous deletions, and 3-Mb hemizygous deletions. However, if we use 120K probes with twice the performance characteristics of our current set, we will be able to detect even 150-kb hemizygous deletions. If probes are arranged six to a gene, we believe we can detect hemizygous deletion of a single gene. These predictions have important applications to the analysis of the genome in individuals with inherited genetic disease.

APPLICATIONS OF ROMA TO GENETIC DISEASE

The applications of ROMA for the analysis of cancer have been illustrated. The method provides a rapid way to define the regions of the chromosome of cancer cells where amplifications and deletions take place. This analysis can proceed in a highly parallel manner, at high resolution, from small amounts of cancer biopsies. The benefits from this study would be a catalog of lesions that contain oncogenes and tumor suppressors and could quickly narrow the search for those genes involved in human cancer. My estimate is

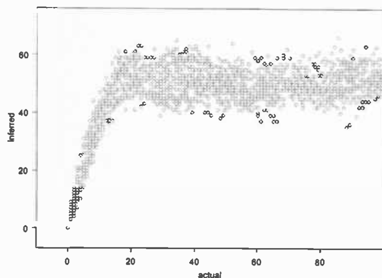


FIGURE 6 Hamming distance. Horizontal is Euclidean distance between probes. Vertical is Hamming distance. The Hamming distance provides a local metric.

that we know fewer than a quarter of the genes that are frequently mutated in cancers. When these data are correlated with clinical data, then we also derive knowledge about which genes are most commonly mutated in which kinds of cancer, and which mutations or combinations of mutations have the greatest value in predicting malignancy and outcome to particular therapies. Knowledge of the new oncogenes and tumor suppressor genes is likely to lead to new therapeutic approaches to cancer and new forms of diagnosis and detection.

The applications of ROMA, however, are likely to extend beyond cancer to gene identification in other complex human genetic diseases. Many of the mutant genes that cause genetic disease are mutated by deletion. I estimate that 5–10% of loci associated with disease are lost through deletion rather than point mutation. With our method, we can detect hemizygous deletion. We compared a patient with velo-facial cardio-syndrome with a normal. This patient had a 3-Mb deletion of chromosome 22, which was readily seen by array hybridization. Our simulations predict that with a dense enough microarray, we will be able to detect hemizygosity at individual genes. Thus, we could be in a position to scan the entire genome of affected and unaffected humans for such deletion events. This may be an efficient way to analyze humans with spontaneous deletions in the germ line, or with complex genetic diseases, as it is postulated that the aggregation of nonlethal mutations (e.g., hemizygous loss) of genes with a common function can lead to disease susceptibility. For example, a cohort of unrelated schizophrenics might show a statistically high level of hemizygous deletion of certain genes critical in brain function.

PHYSICAL MAPPING OF GENOMES AND COMPARATIVE GENOMICS

There are major applications of ROMA to the physical mapping of the genome. It is widely recognized that the publicly available sequence assembly of the human genome is quite flawed. Many regions of the genome are misassembled, there are large gaps, and in particular, the duplicated regions are poorly assembled. The extent of the defects of assembly is largely unknown but could adversely affect the techniques we have developed, in particular since our oligo-based representational methods are strongly dependent on the colocalization of probes by map position.

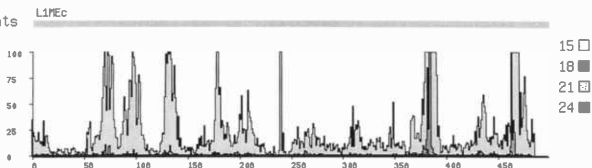
Last year, in a collaboration with Bhubaneswar Mishra and Will Casey of the Courant Institute of Applied Mathematics at New York University, we described algorithms whereby the order of arrayed probes could be inferred from a series of hybridizations of arrays with random pools of BACs (bacterial artificial chromosomes). The central concept is that probe proximity will be inferred by a similar pattern of hybridization signals from nearby probes throughout the series of hybridizations. Probes less than a BAC's length away will tend to show related patterns, and the closer the probes, the closer their patterns will match. The Hamming distance gives a quantitative measure of pattern matching, and for nearby probes, the Hamming distance is proportional to Euclidean distance (see Fig. 6) over the short range. The Hamming distance provides a local metric from which large stretches of ordered linear map information may be inferred (Casey et al. 2001).

We estimate that on the order of 100 hybridizations are sufficient to create correctly ordered contigs of about 75 probes, assuming an average arrayed probe density of five probes per BAC. The boundaries of regions of segmental duplications should also be evident from the analysis. The relative order of the contigs can be established by a variety of other methods. We can also map regions of the genome that have not yet been entered into the public databases, as we have a set of sequences from representations that are not yet in the published databases (a collaboration with McCombie, CSHL).

Sequences between humans and primates are sufficiently close that the same arrays we make for

>chr7:157303476-157304001.L1MEc.- from 8 to 508

Exons



>chr1:18067744-18068244

Exons

Repeats

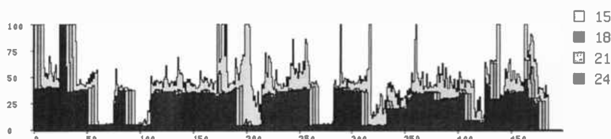


FIGURE 7 (*Top*) Region annotated as repeat, but with low mer frequencies. (*Bottom*) Reverse of the top panel, a sequence of chromosome 1 that is not annotated as a repeat, but that has high mer frequencies. The sequence in the bottom panel is found repeated about 40 times on chromosome 1 only. Frequencies of mers of lengths 15, 18, 21, and 24 are the vertical axis (see Key at right of figure). Each horizontal pixel is a single base pair.

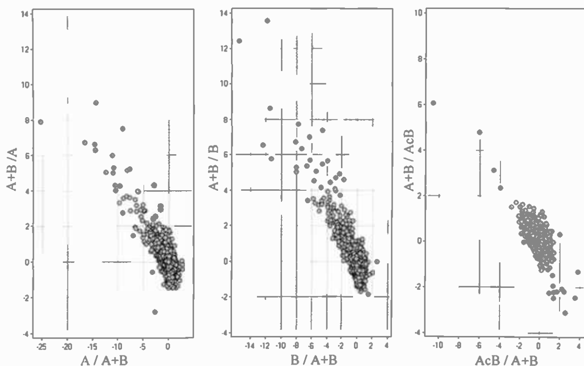


FIGURE 8 Probes that are complementary to genes that are expressed relatively abundantly in the carcinoma are in the upper left of the left panel; those complementary to genes that are abundant in the sarcoma are in the upper left of the middle panel; those complementary to genes that are induced upon coculture of the two cell lines are in the lower right of the right panel; and those complementary to genes that are repressed upon coculture are in the upper left of the same panel.

humans can be used to map primates. All that is needed are BAC libraries of the primate genomes. From such studies, the points of rearrangements between the genomes can be inferred. Perhaps of greater importance, and more accessible, the comparison of patterns resulting from array hybridization of primate and human genomes can indicate which regions of the genomes have been duplicated or deleted in the respective species recently in evolution.

GENOME ANNOTATION BY EXACT MATCHING

The oligonucleotide probes we use in ROMA are 70 mers. To maximize probe performance (i.e., specific to nonspecific hybridization), we decided to minimize the coincident exact matches between smaller "mers" of the 70 mers and other regions of the genome. We accomplished this by creating algorithms that can compute the number of exact matches for mers of any length in a given genome. We then select the best 70 mers for each member of a representation that minimize the number of exact 15-mer matches elsewhere. We demonstrated that 70 mers with frequent 15-mer matches do indeed have poor specific to nonspecific hybridization characteristics.

When we compared our method to repeat masker—a standard method for determining homology of sequences to known repeats—we observed that some mers with matches of high frequency are not annotated as repeats and, conversely, that some sequences which are annotated as repeats have low frequencies of extraneous matches in the genome (Fig. 7). We are now exploring the use of our algorithm for automated annotation of the genome and are finding new families of repeats, many of which are chromosome-specific. Many other features of gene structure and genome evolution become apparent when the genome is annotated this way, and we are continuing genomic analysis with this tool.

CELL-CELL COMMUNICATION

One of the most neglected areas of cancer biology is the study of host–cancer cell and cancer cell–cancer cell interactions. Although angiogenesis has received a lot of attention, many other host-cancer interactions will be important for tumor growth and spread. Furthermore, although most cancers are monoclonal, they are not clonal monoliths. They are genetically heterogeneous, and perhaps functionally heterogeneous and interdependent. We therefore have sought to devise a method to examine cell-cell communication, starting initially with a model system based on carcinoma-sarcoma interactions.

Through the use of expression microarrays, we may detect whether cocultivation of cells alters their joint transcriptional state. To test this idea, we profiled the transcriptional state of two colon carcinomas and one fibrosarcoma cell line, each grown independently. We then cocultivated them in all pairs and examined the joint transcriptional state. The results (see, e.g., Fig. 8) indicate that the colon carcinoma cell lines do not alter each other's transcriptional state, whereas cocultivation of the sarcoma with either carcinoma results in new transcriptional states. We observe both gene repression and induction and are in the process of determining which changes occur in which cells. The effects we observe may be mediated by soluble factors, or interaction between molecules on the surfaces of the cells, or even be the consequence of differential modulation of the culture medium. These experiments suggest that we can at least observe retention of the capacity for cancer cell communication, and they encourage us to believe that we can use this technique to catalog and correlate interactions.

PUBLICATIONS

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CANCER: CELL BIOLOGY

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

- David Helfman's group is studying how specific actin assemblies are organized and regulated and how alterations in actin filament assembly contribute to abnormal cell growth control in cancer. They have found that two functionally different oncogenes, namely, the small GTPase Ras and the tyrosine kinase v-Src, use similar biochemical pathways to achieve transformation-associated changes in the actin cytoskeleton, i.e., inactivation of Rho-mediated stress fiber assembly through activation of MEK.
- Tatsuya Hirano's laboratory studies the molecular mechanisms of chromosome cohesion and condensation in eukaryotic and prokaryotic systems. During the last year, they have successfully visualized the molecular architecture of condensin and cohesin and have obtained important insights into the regulatory network that coordinates their dynamic interaction with chromosomes during the cell cycle.
- Senthil Muthuswamy joined Cold Spring Harbor Laboratory in 2001. His research interests address the mechanisms by which oncogenes, particularly the ErbB family of receptor protein tyrosine kinases, initiate transformation of mammary epithelial cells in the development of breast cancer. He has developed novel methods to examine the dimerization and activation of these receptors and cell culture procedures to allow epithelial cells to form three-dimensional acini-like structures that are reminiscent of the physiological situation. He is using these approaches to characterize the signal transduction pathways that are important for transformation of mammary epithelial cells.
- David Spector's laboratory studies the structural-functional organization of the mammalian cell nucleus. During the last year, they demonstrated that a subset of PML nuclear bodies move within the nucleoplasm via a metabolic energy-dependent mechanism and that methylation of histone H3 at Lys-9 is an early mark on the X chromosome during X-inactivation.
- Arne Stenlund's group analyzes the assembly of viral replication initiator complexes. The highlights for the past year include the definition of the recognition sequence for the E1 initiator and determination of how the multiple E1-binding sites are arranged in the viral origin of DNA replication. The arrangement of E1-binding sites provides a blueprint for the assembly of E1 complexes involved in unwinding and helicase activity and determines both the order of assembly and the final architecture of the biologically active E1 complexes.
- Bruce Stillman's laboratory continues to study the replication of chromosomal DNA in eukaryotic cells. During the past year, he has expanded its focus to include the interaction between DNA replication and other cellular events, including gene silencing, nutrition, and the production of new ribosomes.
- Nick Tonks' laboratory studies the structure, regulation, and function of the protein tyrosine phosphatase (PTP) family of enzymes. They defined a consensus substrate recognition motif for PTP1B and identified the JAK protein tyrosine kinases as physiological substrates of the enzyme. These observations suggest that PTP1B may be a regulator of leptin signaling and thus a target for treatment of obesity. They also developed approaches for the study of regulation of PTP function by reversible oxidation. These approaches are expected to provide insights into the function of PTPs as regulators of a variety of signal transduction pathways.
- Research in the laboratory of Linda Van Aelst is focused on defining the role and mechanisms by which Ras and Rho GTPases exert their effects on specific aspects of tumorigenesis and neuronal development. During the last year, they have observed that the Ras-GAP-associated protein, $p62^{dok}$, functions as a negative regulator of growth-factor-induced proliferation and BCR-ABL-induced oncogenic transformation, at least in part by negatively influencing the Ras/MAPK pathway. They further obtained evidence for a role of the Ras-related GTPase, Rap1, in epithelial morphogenesis and obtained important insights into the underlying mechanism.

THE CYTOSKELETON AND ONCOGENIC TRANSFORMATION

D.M. Helfman E. Araya E. Kim G. Pawlak
L. Connell S.-W. Lee X. Wang

We are interested in the relationship between the cytoskeleton, oncogenic transformation, and tumor suppression. It is well known that transformation of cells in tissue culture results in a variety of cellular changes, including alterations in cell growth, adhesiveness, motility, morphology, and organization of the cytoskeleton. Morphological and cytoskeletal changes are perhaps the most readily apparent features of transformed cells. Studies by us and other investigators have demonstrated that changes in the organization of actin filaments are causally related to both abnormal growth and tumorigenicity, indicating critical functions for actin filaments in cell growth control. Changes in the expression patterns of various cytoskeletal proteins and their regulators, e.g., kinases and small GTPases, have been observed in a number of transformed cells. In some cases, the loss of specific proteins was found to be causally related to the transformed phenotype, because restoring their expression was accompanied by reversion of the transformed phenotype. Likewise, antisense experiments have demonstrated that inhibiting the expression of specific cytoskeletal proteins also resulted in properties associated with transformation. Thus, the cytoskeleton has important roles in the regulation of various cellular processes linked to transformation, including proliferation, contact inhibition, anchorage-independent cell growth, and programmed cell death (apoptosis). We are directing our efforts to determine how alterations in actin filament assembly contribute mechanistically to aberrant cell growth control and how specific actin structures are deregulated following oncogenic transformation. Below is a description of our studies during the past year to better understand the role of the cytoskeleton in normal and transformed cells.

MEK Mediates Ras- and v-Src-induced Morphological Transformation and Inactivation of Rho-dependent Stress Fiber Formation

G. Pawlak

Alterations in actin filament assembly are a common characteristic of transformed cells and are believed to contribute to aberrant cell growth. We investigated Ras-dependent signaling pathways involved in cytoskeleton disruption and demonstrated the involvement of the Ras/MEK (mitogen extracellular-regulated protein kinase) pathway in this process. Oncogenic activation of this pathway in normal NRK fibroblasts is sufficient to disrupt stress fibers and focal contacts, whereas inhibition of this pathway in Ras-transformed NRK cells restores stress fibers and focal contacts. While investigating the mechanism by which activation of MEK can lead to cytoskeleton disruption, we found that chronic activation of MEK in NRK/*ras* cells induces down-regulation of ROCKI and ROCKII expression, two downstream effectors of Rho required for stress fiber assembly. Upon MEK inhibition, ROCK expression and cofilin phosphorylation were increased, demonstrating that the ROCK/LIMK/cofilin pathway was functionally restored. Finally, using dominant-negative or constitutively active mutants, we demonstrated that expression of ROCK was both necessary and sufficient to promote cytoskeleton reorganization in NRK/*ras* cells. These findings establish the Ras/MEK pathway as the critical pathway involved in cytoskeleton disruption during Ras transformation and suggest a new mechanism, involving alteration in ROCK expression, by which oncogenic Ras can uncouple Rho from stress fiber formation and therefore achieve morphological transformation of the cells.

We extended this study to v-Src-transformed fibroblasts and reached the same conclusion that MEK is an effector of v-Src-induced morphological transformation, participating in v-Src-mediated inactivation of the Rho-ROCK-LIMK pathway. This work, together with other studies, demonstrates that two functionally different oncogenes, namely, the small GTPase Ras and the tyrosine kinase v-Src, use the same signaling pathway to achieve morphological transformation of fibroblasts, i.e., inactivation of Rho-mediated stress fiber assembly through activation of MEK. Further characterization of the pathway linking MEK to Rho-dependent signaling is therefore a crucial step toward understanding the morphological effects of these oncogenes, and experiments are in progress to elucidate this link, both in Ras-transformed and in v-Src-transformed cells.

Regulation of Caldesmon Expression and Function in Normal and Ras-transformed Fibroblasts

S.-W. Lee

Caldesmon is a cytoskeleton protein containing actin-, myosin-, and tropomyosin-binding domains. It is known that nonmuscle caldesmon contributes to the stabilization of actin stress fiber structures in normal cells. Consistently, Ha-RasV12-transformed NIH-3T3 cells in which stress fibers are disorganized have significantly decreased expression of caldesmon. RasV12 is known to activate three distinct effectors, namely, Raf, phosphatidylinositol 3-kinase (PI3K), and RalGDS. We have studied the contribution of these different signaling pathways in regard to disruption of the actin cytoskeleton and down-regulation of caldesmon. Interestingly, treatment of Ha-RasV12-transformed cells with a MEK inhibitor, U0126 (25 μM), caused reversion to normal phenotype (e.g., restoration of actin stress fibers) as well as an increased level of caldesmon. This indicates that sustained MEK/ERK activation in Ras-transformed cells contributes to the suppression of caldesmon, thereby possibly leading to disruption of actin stress fibers. It is well established that signaling pathways mediated by ROCKI/II, which lead to stress fiber formation, are compromised during Ras transformation. Consistently, inhibition of the MEK/ERK pathway failed to restore actin stress fiber when dominant-negative ROCKI was expressed or the ROCKI/II inhibitor (Y27632) was applied. This suggests that restoration of the ROCKI/II pathway is necessary for the phenotypic reversion caused by MEK inhibition. Furthermore, we found that the inhibition of the MEK/ERK pathway caused up-regulation of caldesmon at the mRNA level. Work is in progress to determine if the restored RhoA/ROCKI/II pathway is directly involved in the transcriptional regulation of caldesmon. We also have been applying an RNAi approach for knocking out caldesmon in mammalian cells to determine if caldesmon is necessary for the maintenance of the actin cytoskeleton.

Oncogenic-mediated Alterations in the Cytoskeleton and Cell Adhesion

X. Wang

Transformation of cells in tissue culture results in a variety of cellular changes, including alterations in cell growth, adhesiveness, motility, morphology, and organization of the cytoskeleton. In transformed cells, microfilament bundles exist in a more dispersed state and are reduced in both size and number. These changes in microfilament structure are highly related to both anchorage-independent growth and cellular tumorigenicity. In addition, adhesion of cells to the extracellular matrix (ECM) and neighboring cells has a critical role in various cellular processes, including differentiation, growth, motility, and programmed cell death (apoptosis). We are studying two aspects of oncogene-mediated transformation. First, we are studying signaling pathways that contribute to alterations in the actin cytoskeleton. Second, we are investigating the regulation and role of β-catenin signaling in *src*-transformed cells. β-catenin, together with cadherins and α-catenin, has a critical role in the structural organization of cell-cell adhesions. In addition, β-catenin is involved in transcriptional control of various target genes including *c-myc* and *cyclin D1* following its translocation to the nucleus. In particular, we are studying which signals contribute to translocation of β-catenin to the nucleus and thus activate critical regulators of cell proliferation.

The Cytoskeleton and Programmed Cell Death

L. Connell

The ability of a tumor cell to grow outside its local environment (metastasize) is a major problem in the development and therapeutic treatment of cancer. Attachment of cells to the ECM is important for the generation of signals that regulate normal cell proliferation and programmed cell death (apoptosis). The loss of the requirement for cell-matrix interactions has a critical role in the development of cancer. Normal epithelial and endothelial cells require attachment to the ECM not only for proliferation, but also for survival, as disruption of cell-ECM interactions can result in irreversible cell growth arrest and induction of apoptosis, a phenomenon termed "anoikis." Elucidating the mechanisms by which the interactions of cells with the ECM generate and regulate downstream signals that promote cell growth and survival is critical to understanding cancer. Previous work by us and other investigators has demonstrated that activation of myosin II (actomyosin contractility) is an essential step during adhesion-dependent signaling. On the

basis of this observation, we hypothesize that changes observed in transformed cells in cellular proteins involved in the regulation of myosin function will lead to constitutive activation of actomyosin contractility and the corresponding downstream signaling pathways that contribute to aberrant growth control and the insensitivity of cells to undergo apoptosis following detachment from the ECM. Thus, when normal cells detach from the extracellular matrix, they undergo apoptosis. Transformed cells do not undergo apoptosis, and loss of anchorage dependence in cells correlates with tumor growth. By utilizing pharmacological agents and dominant-negative proteins that can inhibit myosin, we are studying the role of myosin and the actin cytoskeleton in the generation of survival signals in normal and transformed epithelial cells.

When normal MDCK cells are grown in suspension, the cells undergo anoikis. By contrast, MDCK cells transformed with Ras are resistant to anoikis. One downstream target of Ras is myosin II. Therefore, we hypothesized that the resistance of MDCK Ras cells to undergo anoikis may be due in part to the activation of downstream signals that protect cells from apoptosis via activation of myosin II. To determine if myosin and actin contribute to survival signals, we have studied whether the inhibition of myosin or actin leads to apoptosis in normal MDCK or MDCK Ras cells using myosin and actin inhibitors. Using agents that disrupt actin filaments (latrunculin A and cytochalasin D), or inhibit myosin (BDM and ML-7), we have found that both normal and transformed epithelial cells undergo apoptosis following disruption of actin filaments or inhibition of myosin. These results suggest that myosin and actin filaments have an important role in generation of survival signals. We are currently investigating whether Ras acts directly upstream of myosin and the role of integrins in this process.

Characterization of the Metastasis-associated Protein, S100A4

E. Kim

Distinct changes occur to propel a tumor from benign to malignant. Increased motility, invasiveness, and survival in foreign environments are some of the changes that confer a metastatic phenotype. One potentially key protein involved in generating these changes is S100A4, a member of the S100 family of small calcium-binding proteins. Although the proteins in this family share relatively high homology, their expression distribution, protein partners, and putative roles are quite diverse. Elevation of A4 expression has been correlated with increased invasiveness and with a worse prognosis in the case of breast cancer. In addition, increased levels of S100A4 have been reported in metastatic colon, pancreatic, and bladder cancers, suggesting a critical role for this protein in a number of different human cancers. In vitro biochemical assays have suggested calcium-dependent protein-protein interactions between A4 and three cytoskeletal proteins: actin, tropomyosin, and myosin. We have confirmed a calcium-dependent interaction between A4 and the heavy chain of nonmuscle myosin in vivo. Specifically, we have demonstrated that A4 interacts with the myosin heavy-chain II-A isoform preferentially over the II-B isoform in vivo. A mutant A4 that is incapable of binding to calcium is unable to interact with myosin heavy-chain II-A.

To further characterize the effect of A4 in breast cancer cells, we have established clonal MCF7 sublines that stably express the A4 protein. These sublines are being characterized in both in vitro and in vivo metastasis-related assays. In comparison to both the parental cell line and sublines stably expressing the mutant A4 incapable of binding calcium, the wild-type A4-expressing cells appear to be more motile in vitro. Analysis of the in vivo metastatic capacity of these different sublines when transplanted orthotopically into nude mice is currently under way.

Molecular Organization and Regulation of Actin Filaments

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

In nonmuscle cells, the formation of actin filaments and their assembly into various structures, e.g., stress fibers, contractile ring, filopodia, and lamellipodia, are dynamic processes. How these different structures are formed and regulated within a single cell is not known. In addition to actin and its associated motor molecules (myosin I and II), other actin-filament-associated proteins, such as tropomyosin (TMs), have essential roles in the assembly, function, and regulation of these structures. We have been studying the dynamic localization of tropomyosin isoforms using

GFP-tagged constructs. Tropomyosins are a family of actin-binding proteins that bind to both grooves of filamentous actin. Although they are expressed in all eukaryotic cells, different forms are characteristic of specific cell types. In fibroblasts, TMs exist as high-molecular-weight isoforms (HMW) containing 284 amino acids (TM-1, TM-2, and TM-3) or low-molecular-weight isoforms (LMW), containing 248 amino acids (TM-4, TM-5[NM-1], TM-5a, and TM-5b). The multiple TMs found in fibroblasts are an integral part of the actin filaments, although how the different isoforms contribute to actin filament assembly and function is poorly understood. In addition, in fibroblasts, HMW TMs exist as homodimers, whereas LMW TMs can exist as heterodimers. How these coiled-coiled interactions are regulated and how they affect the interaction of TMs with actin filaments and their subsequent cellular function are under study. We have found that specific TM isoforms are involved in different subsets of actin filaments. For example, we have found that during cytokinesis, only a subset of tropomyosin isoforms are found in the contractile ring. The mechanism(s) involved in isoform-specific sorting is currently under study. We anticipate that elucidating the molecular basis for the differential localization of the specific TM isoforms during various cellular events involving reorganization of the actin cytoskeleton will provide important new insights into the regulation of distinct actin structures.

We have begun to use RNAi to inhibit the expression of specific isoforms of tropomyosin as well as other cytoskeletal proteins. By using RNAi technology, we plan to carry out a systematic screen targeted to structural and regulatory components of the cytoskeleton. These studies will determine the effects of disrupting the expression of specific proteins. For example, we will determine if loss in the expression of specific proteins leads to changes in the structure of the actin cytoskeleton, focal contacts and cell-cell contacts, as well as the growth properties of cells. It is anticipated that these studies will identify key components of the cytoskeleton involved in growth control.

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HIGHER-ORDER CHROMOSOME DYNAMICS

T. Hirano O. Cuvier A. Losada
P. Gillespie D. MacCallum
M. Hirano

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

Molecular Architecture of Condensin and Cohesin

A. Losada, T. Hirano [in collaboration with H.P. Erickson, Duke University]

Previous electron microscopy revealed that a bacterial SMC homodimer has a V-shaped structure in which two long coiled-coil arms are connected by a flexible hinge. During the past year, we have collaborated with H. Erickson's group to determine the molecular architecture of condensin and cohesin. Both complexes are purified from HeLa cell nuclear extracts or *Xenopus* egg extracts, rotary-shadowed, and visualized by electron microscopy. We find that condensin and cohesin display the two-armed structure characteristic of SMC proteins, but their conformations are remarkably different. The hinge of condensin is closed, and the coiled-coil arms are placed close together. In contrast, the hinge of cohesin is wide open, and the coiled-coils are spread apart from each other. The non-SMC subunits of both condensin and cohesin form a globular complex bound to the catalytic domains of the SMC heterodimers. We propose that condensin and cohesin are structurally differentiated to support their specialized biochemical and cellular functions. In the case of condensin, the two catalytic domains of the closed SMC dimer would bind to contiguous DNA segments, thereby allowing the complex to act as an intramolecular DNA cross-linker. In the case of cohesin, an open hinge would facilitate the interaction of the two catalytic ends of the SMC dimer with noncontiguous DNA segments, allowing the complex to function as an intermolecular DNA cross-linker.

Role of Aurora B in Chromosome Condensation and Chromatin Remodeling

D. MacCallum, A. Losada, T. Hirano

Histone H3 is phosphorylated at Ser-10 during mitosis in many, if not all, eukaryotic organisms. Spatial and temporal distribution of this modification is tightly correlated with chromosome condensation. Emerging lines of evidence suggest that a protein kinase, known as aurora B, is responsible for the mitosis-specific phosphorylation of histone H3. To test whether H3 phosphorylation by aurora B is directly involved in condensation, we have immunodepleted this kinase from *Xenopus* egg extracts. In the absence of aurora B and its binding partner INCENP, mitosis-specific phosphorylation of histone H3 is drastically reduced. We find, however, that neither chromosome condensation nor chromosomal targeting of condensin is compromised under this condition. We find instead that depletion of aurora B and INCENP suppresses dissociation of ISWI chromatin-remodeling complexes from chromosomes during mitosis. Our results suggest that H3 phosphorylation by aurora B does not

have a direct role in recruiting condensin to chromosomes in the cell-free extracts. Thus, the role of H3 phosphorylation in chromosome dynamics appears to be more complex than previously anticipated.

Regulation of Vertebrate Cohesin by Mitotic Kinases

A. Losada, T. Hirano

Cohesin is a highly conserved protein complex essential for the establishment and maintenance of sister chromatid cohesion. In *Saccharomyces cerevisiae*, a cysteine-protease, known as separase, cleaves one of the cohesin subunits at the onset of anaphase, thereby promoting a single-step separation of sister chromatids. In vertebrate cells, however, cohesion is dissolved by a two-step mechanism. The first step takes place during prophase when ~95% of cohesin is dissociated from chromosomes in a separase-independent manner. The second step occurs at the metaphase-anaphase transition when the residual population of cohesin is cleaved by a separase-mediated mechanism similar to that observed in yeast. Our effort is now focused on understanding how the first step of cohesin dissociation is regulated during prophase in vertebrate cells. Our previous results suggested that this process correlates with phosphorylation of the XSA1 subunit of cohesin. Purified cdc2-cyclin B could phosphorylate XSA1 in vitro, but it was not sufficient to induce dissociation of cohesin from chromosomes. It is therefore likely that an additional chromosome-associated kinase(s) has a critical role in regulating this process. A prominent candidate is aurora B that distributes along chromosome arms in prophase and is concentrated on centromeres by metaphase. A second candidate is Plx1, a member of the family of polo-like kinases that have a variety of roles in mitotic progression. We have initiated the biochemical and functional characterization of the two mitotic kinases in the egg extracts. In vitro phosphorylation assays with purified components as well as immunodepletion experiments are under way to determine whether the activities of these kinases might affect the behavior of cohesin. We hope to be able to depict a comprehensive molecular picture of the regulatory network that regulates coordinated structural changes of chromosomes during mitosis.

Establishment of Sister Chromatid Cohesion

P. Gillespie, T. Hirano

The establishment of sister chromatid cohesion is a complex process that involves at least two separable steps. The first is the binding of cohesin to chromatin, which occurs in late G_1 independently of DNA replication. The second step is the construction of a cohesin-mediated linkage between the sister chromatids during S phase. Although several gene products required for each step have been identified in yeast and fungi, it remains totally elusive, at a mechanistic level, how they functionally interact with each other in establishing sister chromatid cohesion. To address this question, we focus on three conserved factors: Scc2, Eco1, and DNA polymerase σ (Pol σ).

- Scc2: Genetic studies in yeast suggest that Scc2 plays a part in directing the association of cohesin with chromatin. Sequence analysis shows that Scc2 is composed of multiple HEAT repeats, tandem repeats of an α-helical structural unit. Since HEAT repeats are also found in two non-SMC subunits of the condensin complex, we speculate that HEAT proteins may regulate the SMC subunits of cohesin and condensin by a common mechanism.
- Pol σ: Pol σ is a Pol-β-like DNA polymerase whose mutations cause a defect in sister chromatid cohesion in *S. cerevisiae*. Characterization of this protein in *Xenopus* egg extracts should provide new insights into how the establishment of cohesion is functionally coupled with DNA replication.
- Eco1: This protein is unique in that it is required solely for the establishment of cohesion during S phase, but not for its maintenance in G_2 phase. Our sequence analysis reveals that *Xenopus* and humans have two different Eco1 isotypes (designated Eco1A and Eco1B). Each of the vertebrate Eco1 paralogs has a carboxy-terminal domain that shares a similarity to yeast Eco1, but also contains a unique amino-terminal domain that is missing in the yeast counterpart.

We have cloned *Xenopus* cDNAs for these putative cohesion factors. Preparation of specific antibodies

against synthetic peptides and recombinant fragments is in progress. Once these immunological tools are available, we will characterize these proteins biochemically and determine their specific roles in sister chromatid cohesion by using powerful *Xenopus* egg-cell-free extracts.

Structural and Functional Dissection of Mitotic Chromosomes

O. Cuvier, T. Hirano

Our previous studies suggested that condensin and topoisomerase II (topo II) make distinct contributions to the establishment and maintenance of mitotic chromosome condensation in *Xenopus* egg extracts. The precise actions of the two condensation factors in these processes, however, remain to be determined. During the past year, we have extended our efforts to gain new insights into the structure of mitotic chromosomes. Depletion of either condensin or topo II from the extracts prevents the formation of mitotic chromosomes in a morphologically distinctive manner. The loss of higher-order structure is correlated with an increased accessibility of chromosomal DNA to nucleases, although nucleosome assembly is apparently normal even in the absence of condensin or topo II. When mitotic chromosomes are exposed to dextran sulfate (or high salt) after their assembly is completed, a large halo of DNA is formed around an "axial core" structure of chromosomes. Under this condition, histones and most of topo II dissociate from the structure, whereas condensin remains bound to the core. We interpret this core-like structure to be artificially induced as a result of protein aggregation (possibly condensin itself) accompanying a loss of topo II and histones. Interestingly, the formation of the core structure can be prevented when chromosomes are pretreated with AMP-PNP, a nonhydrolyzable ATP analog, before being exposed to dextran sulfate. Under this condition, bulk histones are removed, but no DNA halo is observed. topo II remains bound to chromosomes and colocalizes with condensin on a helical domain within chromosomes, a structure that is often observed in unextracted, intact chromosomes. Pretreatment of chromosomes with ICRF, a specific inhibitor of topo II, has a similar, yet weaker effect. Furthermore, we find that condensin, but not topo II, is preferentially removed from chromosomes when they are exposed to ethidium bromide, a DNA intercalating agent. This treatment induces a drastic disruption of the overall shape of chromosomes. These results provide strong evidence that condensin has a central role in maintaining higher-order chromosome structure in this cell-free system. They also suggest that stabilization of topo II prevents chromosomes from undergoing artificial structural changes under certain extraction conditions.

Structural Dissection of Bacterial and Eukaryotic SMC Dimers

M. Hirano, T. Hirano

On the basis of electron microscopy of a bacterial SMC homodimer from *Bacillus subtilis* (BsSMC), it was proposed that SMC proteins dimerize by coiled-coil interactions between the two different subunits (Fig. 1, Model I). We have unexpectedly found, however, that introduction of point mutations into the hinge domain of BsSMC results in a conversion from dimers to monomers. This observation leads us to propose an alternative model of SMC dimerization: The two subunits may be self-folded to form two separate coiled-coil rods, which in turn dimerize by a hinge-mediated interaction (Fig. 1, Model II). To distinguish between the two models, we have taken two complementary approaches. In the first approach, we employ protein-protein cross-linking of BsSMC using a cross-linker specific for free sulfhydryl groups. We have

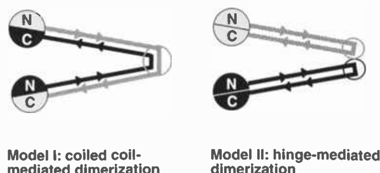

FIGURE 1 Two models for dimerization of SMC proteins. Coiled-coil interactions between two different subunits may mediate dimerization (Model I). Alternatively, two self-folded subunits may dimerize by a hinge-mediated interaction (Model II).

first replaced all cysteine residues present in BsSMC with serines, making a cysteine-less mutant. We are now introducing artificial cysteines into specific sites of this mutant so that intra- and intersubunit cross-linking products can be distinguished by their distinct electophoretic mobility in a gel. As a second approach, we have taken advantage of the heterodimeric nature of eukaryotic SMC proteins. For example, SMC2 and SMC4 form a heterodimer that functions as the core of the condensin complex. If Model I is correct, the amino terminus of SMC4 would interact with the carboxyl terminus of SMC2. Likewise, the amino terminus of SMC2 would associate with the carboxyl terminus of SMC4. If Model II is correct, then the amino terminus of SMC4 (and SMC2) would interact with its own carboxyl terminus. We are expressing a number of truncation fragments of SMC2 and SMC4 in vitro and testing their reciprocal interactions by coimmunoprecipitation.

Single DNA Molecule Manipulation

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

In collaboration with Terence Strick's group here at CSHL, we study the interaction of BsSMC with DNA by an approach involving single DNA molecule manipulation. This powerful technique is also being used to understand the action of the condensin complex at a single molecule resolution.

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EPITHELIAL CELL POLARITY AND CANCER

S. Muthuswamy T. Haire
B. Xiang

EARLY EVENTS IN BREAST CANCER

Almost all malignant breast cancers originate from epithelial cells. Several oncogenes and tumor suppressor genes have been implicated in breast cancer; however, we do not understand the mechanisms by which oncogenes initiate transformation of mammary epithelial cells (MEC) that line the ducts and alveoli in the breast (Fig. 1). Early lesions, characterized by uncontrolled proliferation and disruption of epithelial architecture, are currently defined by histological features, and their prognosis is imprecisely determined. Although lesions within a category look alike, they must possess molecular differences that allow some to become malignant and others to remain benign. Understanding the molecular mechanisms involved in initiation of carcinoma will not only identify early diagnostic markers, but also identify drug targets for treating premalignant disease.

Conventional transformation assays (foci formation, soft agar growth, and tumor formation in nude mice) do not allow us to investigate the cellular and molecular changes that take place in polarized epithelia that line the ducts and alveoli in vivo. We have adapted a cell culture technique where nontransformed MECs form three-dimensional acini-like structures containing a single layer of polarized, growth-arrested epithelial cells when grown within a matrix rich in laminin and collagen (Matrigel) (Fig. 1). The epithelial cells in these structures, unlike cells grown on plastic dishes, share several properties with those that line the ducts and lobules in an adult breast. Interestingly, tumor-derived epithelial cell lines or epithelial cell lines transformed in culture fail to form acini-like structures in three dimensions. They either form filled spheroids or do not growth-arrest, or both, suggesting that acini-like structures formed by nontransformed MEC possess unique properties that are lost during transformation.

Oncogenes in the ErbB family of receptor tyrosine kinases have important roles in breast cancer. Among its four members, ErbB2 overexpression is observed

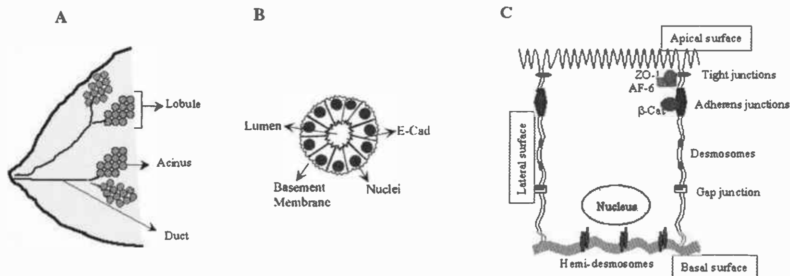

FIGURE 1 (*A*) Epithelial cells in an adult breast. The epithelia are organized as ducts and lobules. The lobules comprise multiple individual units referred to as the acinus. (*B*) An acinus. The acini have a central luminal space surrounded by a single layer of polarized luminal epithelial cells. (*C*) Polarized epithelia. Polarized epithelial cells have an apical surface facing the luminal space, a lateral surface that is in contact with its neighbor, and a basal surface that contacts the basal myoepithelial cells and the basement membrane. Several junctional complexes are spatially separated along the lateral and basal surfaces of polarized epithelia. Tight junctions separate the apical and lateral membranes.

in 25–35% of breast cancers and correlates with poor clinical prognosis. ErbB2 is targeted for therapy using anti-ErbB2 antibodies, i.e., Herceptin. ErbB3 is co-overexpressed with ErbB2. Despite the compelling evidence for the role of the ErbB family in breast cancer, it has been challenging to decipher the signaling and biological specificities of ErbB receptor activation, because MECs express multiple ErbB receptors, and homo- and heterodimerization of ErbB receptors complicate our ability to determine how specific ErbB dimers transform mammary epithelia.

We have developed a novel method to control dimerization and activation of ErbB receptors without contribution from endogenous receptors. This method, for the first time, will allow us to dissect-out the mechanisms by which specific ErbB homo- and heterodimers transform normal mammary epithelial cells. We have successfully integrated the controlled dimerization strategy and the three-dimensional cell culture method. Using this powerful and unique system, we can now activate specific ErbB dimers of choice in three-dimensional epithelial structures that share several properties with cells that line the ducts and alveoli in vivo. We believe that this approach will take us a step closer to conditions in vivo and will allow us to uncover molecular mechanisms involved in initiation of transformation that cannot be readily determined under regular culture conditions or using animal models.

ACTIVATING ErbB RECEPTOR DIMERS IN THREE-DIMENSIONAL ACINI

We have analyzed the differential effects of activating ErbB1 and ErbB2 receptors in preformed, growth-arrested, polarized acinar structures to mimic the conditions in which ErbB receptors are amplified and activated in vivo. Activation of ErbB2 in preformed mammary epithelial acini results in reinitiation of proliferation, loss of polarized organization, and formation of structures containing multiple acinar units. Each acinus within these structures had a filled lumen, surrounded by an intact basement membrane, and did not display any invasive properties. In addition, activation of ErbB2 homodimers disrupted cell polarity in polarized epithelial monolayers. Thus, ErbB2 dimerization in differentiated acini induces a phenotype that displays some properties of a premalignant stage of breast cancer in vivo referred to as carcinoma in situ. In contrast, neither EGF stimulation nor activation of ErbB1 homodimers was sufficient to induce reinitiation of proliferation, alterations in the organization of preformed mammary epithelial acini, or disruption of epithelial cell polarity. These results suggest that ErbB2 homodimers are uniquely able to disrupt normal regulation of mammary epithelial cell proliferation and organization, and that this activity of ErbB2 may contribute to the phenotype of ductal carcinoma in situ under conditions where its expression is significantly amplified. We are investigating (1) the effect of activating different ErbB dimers in three-dimensional acini; (2) identification of the signaling pathways that are required for the ability of ErbB dimers to transform an epithelial acinus; and (3) how growth control is achieved in three-dimensional acinar structures and how activation of ErbB receptors disrupts growth control.

ErbB RECEPTORS AND EPITHELIAL CELL POLARITY

Activation of ErbB2 but not ErbB1 induces disruption of cell polarity as monitored by its ability to induce mislocalization of tight junction-associated proteins, ZO-1 and AF-6, and apical membrane-associated protein gp135 (Fig. 1).

Recent studies have identified a protein complex conserved in *Drosophila*, *Caenorhabditis elegans*, and mammals that regulates establishment of epithelial cell polarity. The PDZ domain containing protein Par-3 (for partitioning defective)/Bazooka/ASIP, associates with atypical PKC (δ and λ), and the amino-terminal regulatory domain of aPKC associates with another PDZ-CRIB domain containing protein, Par-6. Par6 in turn associates with GTP-bound form CDC42, thus forming the Par-3:aPKC:Par-6:CDC42 tetrameric complex referred to as the Par complex. The Par complex is required for establishment of the tight junction complex in epithelial cells. Misexpression of active CDC42 induces mislocalization of the tight junction complex along the lateral membrane, and overexpression or expression of dominant-interfering mutants of Par3 or Par6 inhibits tight junction assembly. Whether the Par complex is regulated during carcinogeneis is not known. We will determine (1) how activation of different ErbB dimers affects epithelial architecture and polarity and (2) the role played by the Par protein complex in ErbB-mediated effects on cell polarity.

REGULATION OF EPITHELIAL POLARITY IN EARLY STAGES OF BREAST CANCER

We are also investigating whether the observed changes in cell polarity markers are seen in the early hyperplastic lesion of breast tissue. For instance, we can immunostain for lesions that are categorized as proliferative lesions without atypia (PDWA); atypical ductal hyperplasia (ADH), and ductal hyperplasia in situ (DCIS) to determine whether there is a relationship between changes in localization and organization of selected polarity markers and patient outcome. Such an analysis will identify early markers for lesions with premalignant or metastatic potential.

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Teresa Haire

CELL BIOLOGY OF THE NUCLEUS

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S. Hearn P. Sacco-Bubulya
S.M. Janicki H. Sunwoo
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Studies in our laboratory are focused on the structural-functional organization of the mammalian cell nucleus with particular emphasis on gene expression. During the past year, we were joined by Edith Heard whose research efforts are focused on chromatin modifications associated with X-inactivation. She has now started her own group at the Curie Institute in Paris. The microscopy core facility has been used extensively during the past year, and numerous collaborations were pursued with the excellent technical expertise of Stephen Hearn. Stephen has moved on to assume a position in Toronto, Canada.

Metabolic Energy-dependent Movement of PML Bodies within the Mammalian Cell Nucleus

M. Muratani, S.M. Janicki [in collaboration with D. Gerlich, M. Gebhard, and R. Eils, German Cancer Research Center, Heidelberg, Germany]

The mammalian cell nucleus contains a large number of nuclear bodies (organelles) that are thought to function in a variety of nuclear processes. PML nuclear bodies have received much attention because they display a more dispersed intranuclear pattern in blast cells from individuals with acute promyelocytic leukemia carrying a t(15,17) translocation involving a fusion of the PML protein and the retinoic acid receptor α. Retinoic acid treatment can induce complete remission of the disease and results in reformation of the PML bodies. In addition, PML bodies are modulated by interferon or heat shock treatments. They are associated with the sites of initial DNA tumor virus transcription/replication in infected cells and are subsequently disrupted at later stages in the infectious cycle. Numerous proteins have been localized to PML bodies, including Sp100, SUMO-1, USP7, CBP, Daxx, pRB, p53, and BLM. A variety of functions have been suggested for PML bodies, including transcriptional activation, repression, protein storage, and interferon-induced antiviral defense (for a review, see Maul, *Bioessays 20:* 660 [1998]; Zhong et al., *Nature Cell Biol. 2:* E85 [2000]). Furthermore, prior work from our laboratory suggested that PML bodies may function to detect and mark local accumulations of proteins or protein nucleic acid complexes that are foreign or "suspicious" (i.e., misfolded or aggregated) to the nucleus. Such a broad range of potential functions is consistent with the possibility of multiple classes of PML bodies and/or that these bodies may be dynamic.

In an effort to examine these possibilities, we examined during the past year PML body dynamics in living BHK cells. Analysis of BHK cells expressing an EYFP-Sp100 fusion protein by time-lapse microscopy allowed us to distinguish between three different classes of PML bodies on the basis of their nuclear dynamics (see movie at www.cshl.org/labs/spector). One class of PML bodies (~25%) was stationary, exhibiting little movement over 12-minute time periods (Fig. 1, arrows) suggesting that these bodies may be restricted in their movement due to either an association with other nuclear components or their location in a region of the nucleus that limits their migration. The lack of dynamic behavior was not size-dependent, however, as immobile bodies ranging in diameter from 0.2 μm to 2.0 μm were observed. A second class of PML bodies (~63%) exhibited very localized movements that were somewhat similar in their ranges to those previously observed for nuclear speckles and Cajal bodies. This type of localized movement was observed in 100% of the cells examined ($n = 56$).

Most interestingly, the third class of PML bodies exhibited rapid and more extended movements in the nucleoplasm that could be seen to start and stop several times over the course of 12-minute observation periods (Fig. 1, arrowheads). On average, one to two PML bodies/nucleus (~12% of the PML bodies) exhibited this type of movement during any given time period, although we did observe nuclei with 10–12

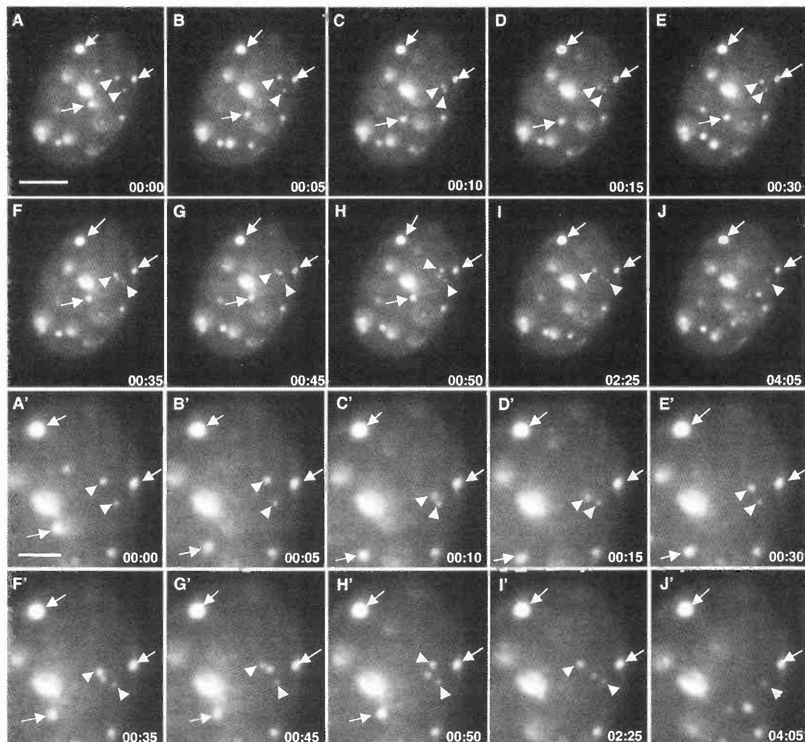

FIGURE 1 PML bodies can be classified into three groups on the basis of their nuclear dynamics. Time-lapse fluorescent images of BHK cells expressing the EYFP-Sp100 protein were taken every 5 seconds for 12 minutes. Selected images are shown to demonstrate the observed dynamics. One population of PML bodies (*white arrows*) exhibited no movements. A second set of PML bodies exhibited limited localized movement. A third set of PML bodies (*white arrowheads*) exhibited rapid nuclear movements. Scale bars: (*a–j*) 5 μm; (*a'–j'*) 2.5 μm.

rapidly moving PML bodies and others where none were observed. Of the cells (n = 56) examined, 55% showed one or more PML bodies exhibiting this rapid movement. The rapidly moving PML bodies traversed the nucleoplasm at an average velocity of 4.0–7.2 μm/minute with a maximum magnitude of ~18.0 μm/minute; this movement was metabolic-energy-dependent (see movie at www.cshl.org/labs/spector). Different PML bodies in the same nucleus appeared to be active during different observation periods. Neither the localized nor the more extended dynamics were sensitive to inhibition of RNA polymerase II activity by α-amanitin. The three classes of PML bodies were also observed in primary mouse embryonic fibroblasts and mouse ES cells. The rapidly moving PML bodies, although small at any given time, may act as nuclear "sensors" traveling within nuclear regions and associating with populations of foreign or "suspect" pro-

teins, including viral proteins. Future studies will examine the biochemical composition of these nuclear bodies.

Methylation of Histone H3 at Lys-9 Is an Early Mark on the X Chromosome during X-inactivation

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In female mammals, one of the two X chromosomes is converted from an active euchromatic state into transcriptionally inert heterochromatin by a process known as X-chromosome inactivation. This results in the transcriptional silencing of several thousand genes and ensures dosage compensation for X-linked gene products between XX females and XY males. When initiation of X inactivation is induced during differentiation, Xist transcript levels are up-regulated from the chromosome that will become the inactive X, and this untranslated RNA coats the inactive X chromosome. Since X inactivation is tightly coupled to cellular differentiation during early embryogenesis, female embryonic stem (ES) cells represent a useful model system with which to study this process.

Nothing is known about the partners, protein or nucleic acid, of the Xist transcript that enable it to coat a chromosome in *cis*, or about the early changes in chromatin structure that lead to the transcriptional shut-down of genes during X inactivation. Covalent modifications of histones such as acetylation, methylation, and phosphorylation represent an important means of epigenetic control in eukaryotes, enabling heritable but reversible changes in gene expression. Different combinations of these modifications may act synergistically or antagonistically to enable finely tuned gene regulation in response to specific developmental or cellular cues. During the past year, we assessed the potential involvement of histone H3 Lys-9 methylation in the establishment of X inactivation by examining its localization on the X chromosome in female ES cells before and after differentiation. In addition, we sought to determine whether histone H3 Lys-9 methylation could be implicated in the initiation and propagation of X inactivation. We have identified a constitutive and uniquely enriched "hot spot" of H3 Lys-9 methylation located upstream of the *Xist* gene, and we have been able to demonstrate that chromosome-wide methylation of H3 Lys-9 is a very early event in the X-inactivation process, occurring simultaneously with or immediately after the coating of the X chromosome by *Xist* RNA. Hypoacetylation of H3 Lys-9 and hypomethylation of H3 Lys-4 occur with the same kinetics as H3 Lys-9 methylation. These three histone H3 modifications therefore represent the earliest known chromatin changes in the X-inactivation process. Importantly, transcriptional inactivation occurs subsequent to X-chromosome-wide H3 Lys-9 methylation. On the basis of these findings, we propose that H3 Lys-9 methylation and the factors that bind to it constitute the "spreading signal" responsible for propagating the inactive state along the X chromosome under the action of the Xist transcript. In this context, the hot spot of H3 Lys-9 methylation identified upstream of *Xist* might provide some insight into the nature of X inactivation relay elements. Given its location at *Xic*, its hypermethylation prior to X inactivation, and the potential role of H3 Lys-9 methylation in spreading heterochromatin, this hot spot may be a nucleation center from which heterochromatin could spread into neighboring regions. The fact that it is present constitutively, but becomes more enriched in H3 Lys-9 methylation during the window of time that *Xist* RNA can induce silencing, suggests that it can only function as a nucleation center when *Xist* RNA becomes stabilized and starts to accumulate on the X chromosome. These findings provide novel insights into the dynamic changes in chromatin structure that are induced by the Xist transcript during X inactivation. In particular, methylation of H3 Lys-9 is likely to be an important epigenetic marker both for the initial silencing process that spreads from the Xic and for the mitotically heritable transmission of the inactive state, once it is established.

Differential Entry of the Gene Expression Machinery into Daughter Cell Nuclei after Mitosis

P. Kannanganattu, P. Sacco-Bubulya

In eukaryotic cells, RNA polymerase II transcription and RNA processing are coordinated events, involving a large number of factors. Our emphasis has been to address how these factors are organized during mito-

sis, when there is global repression of transcription, and how they are recruited to daughter nuclei after mitosis. Immunofluorescence studies using antibodies against several transcription and RNA processing factors in mitotic HeLa cells revealed their differential localization during various stages of mitosis. In prophase cells, transcription and splicing factors showed a homogeneous cytoplasmic staining. In metaphase cells, splicing factors (B′′, SF2-ASF, m3G) and the hyperphosphorylated form (Ser-2 of the CTD) of the large subunit of RNA polymerase II (H5) localized in mitotic interchromatin granules (MIGs), structures analogous to the interphase interchromatin granule clusters (IGCs), along with homogeneous cytoplasmic staining. The number and size of MIGs increased from metaphase to telophase. The staining of MIGs with the above-mentioned antibodies decreased differentially in late telophase cells, the time point when these factors reentered the daughter nuclei. Interestingly, the transcription and RNA processing factors studied were found to have a low turnover rate during mitosis and seemed to be recycled.

To directly visualize the dynamics of these factors in living cells, SR splicing factors SF2/ASF or SC35 tagged with yellow fluorescent protein were transiently expressed in HeLa cells, and their distribution in living mitotic cells was studied. During anaphase, the splicing factor SC35-EYFP was seen in a diffuse cytosolic pool in addition to multiple large MIGs. When cells reached telophase, SC35 entered the nucleus and the MIG signal decreased simultaneously. Similar results were seen with SF2/ASF. This is consistent with recycling of SR splicing factors from MIGs to the nucleus.

Many of the transcription/RNA-processing factors studied were found to enter the daughter nuclei during different time points in telophase, and not as a unitary complex. Their nuclear transport occurred after nuclear lamina and nuclear envelope formation, suggesting that at least some of them enter via an energy-dependent mechanism. Coimmunolocalization studies using an antibody that primarily recognizes the initiation-competent form of the large subunit of RNA polymerase II (H14; hyperphosphorylated at Ser-5 on the CTD) labeled the daughter nuclei prior to RNA processing factors (B′′, SF2/ASF, m3G, hnRNPs, SCAF10, PAB II, etc.). However, the elongation-competent form of the large subunit of RNA polymerase II (H5; hyperphosphorylated at Ser-2 on the CTD) was detected in the daughter nuclei only after the entry of RNA-processing factors.

To visualize the onset of global transcription in the daughter nuclei with respect to the differential entry of the various transcription and RNA-processing factors, in situ bromo-UTP incorporation assays were carried out in mitotic HeLa cells. Prior to entry of RNA polymerase II, transcription was not detectable in situ by bromo-UTP incorporation. At the time that the initiation-competent form of RNA polymerase II (H14 epitope) was first detectable in daughter nuclei during telophase, global transcription was detected by bromo-UTP incorporation as weak, punctate foci throughout the nuclei. However, at the time when the elongation-competent form of RNA polymerase II (H5 epitope) was detected in daughter nuclei, bromo-UTP incorporation dramatically increased, consistent with the elongation of transcripts at this stage. Using a stable HeLa cell line expressing a β-tropomyosin minigene, we have been able to demonstrate that in telophase cells, splicing factors colocalize with the nascent reporter transcripts, suggesting that transcription and splicing occur immediately upon nuclear entry of RNA polymerase II and splicing factors.

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MOLECULAR BIOLOGY OF PAPILLOMAVIRUSES

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The papillomaviruses are a group of viruses that infect and transform the basal epithelium-inducing proliferation of the cells at the site of infection. The resulting tumors (warts) are in most cases benign and will usually regress after some time, but certain types of human papillomaviruses (HPVs) give rise to tumors that are prone to progress toward malignancy, especially frequently, cervical carcinoma. Indeed, HPV infection appears to be a necessary cause of invasive cervical carcinoma and thus represents one of the few firmly established links between viral infections and the development of cancer.

An impediment to the study of papillomaviruses has been the inability to define simple in vitro cell culture systems for analysis of the viral life cycle. These viruses normally require specialized differentiating cells that can be generated in cell culture only with difficulty. However, for a bovine papillomavirus (BPV-1), a convenient cell culture system exists where viral gene expression, oncogenic transformation, and viral DNA replication can be studied. Thus, BPV has become a useful model for these aspects of the viral life cycle. The DNA replication properties of the papillomaviruses show some unique and interesting characteristics. As part of their normal life cycle, these viruses can exist in a state of latency, which is characterized by maintenance of the viral DNA as a multicopy plasmid in infected cells. The copy number of the viral DNA is tightly controlled and the viral DNA is stably inherited under these conditions. Papillomaviruses therefore provide a unique opportunity to study plasmid replication in mammalian cells. In addition, the viral DNA replication machinery represents one of the most promising targets for antiviral therapy.

In previous years, we have reported the characterization of the papillomavirus replicon and the identification of the viral components that are required for viral DNA replication. In recent years, we have directed our attention toward the biochemical events that are associated with initiation of DNA replication. We are studying the biochemical properties of the viral E1 and E2 proteins and how these two proteins interact with the viral origin of DNA replication and with the cellular replication machinery to generate initiation complexes. Our studies demonstrate that the E1 protein has all of the characteristics of an initiator protein, including ori recognition, DNA-dependent ATPase activity, and DNA helicase activity. The transcription factor E2, whose precise function has remained more elusive, appears to serve largely as a loading factor for E1. Through direct physical interactions with both E1 and the ori, E2 provides sequence specificity for the formation of the initiation complex.

We are currently attempting to show how the E1 and E2 proteins orchestrate the precise biochemical events that precede initiation of DNA replication at the viral ori. These events include binding of the initiator to the ori, the initial opening of the DNA duplex, and the assembly and loading of the E1 replicative helicase at the replication fork. Our studies so far indicate that these activities are generated in an ordered process that involves the sequential assembly of E1 molecules on the ori. This sequential assembly generates different complexes with different properties that in turn recognize ori, destabilize the double helix, and function as the replicative DNA helicase.

RECOGNITION OF THE ORI BY E1

The E1-binding site in the ori consists of an 18-bp inverted repeat. We have previously proposed that this E1-binding site actually consists of multiple E1 recognition sequences that are arranged in an overlapping array. To determine what sequence constitutes a binding site for a single E1 molecule, we took advantage of our previous observation which demonstrated that a monomer of the E1 DBD (DNA-binding domain) forms a stable complex when bound cooperatively with the E2 DBD. This complex can be detected by the electrophoretic mobility shift assay (EMSA). Using this assay and extensive mutational analysis, we could demonstrate that a monomer of E1 recognizes the hexanucleotide sequence AACAAT, a sequence which with slight variations occurs six times in the 18 bp. Due

to these variations, these sites have different affinities for E1. Four of the sites are arranged pairwise and are bound pair-wise by dimers of E1 DBD. Thus, two dimers of E1 can bind simultaneously to four overlapping sites, occupying different faces of the DNA helix, separated by approximately 100 degrees. Recent structural studies (in collaboration with E.J. Enemark and L. Joshua-Tor here at CSHL) support this manner of binding and provide a detailed view of how the base sequence is recognized. Interestingly, by utilizing the binding site consensus sequence derived from the mutational analysis, we have been able to identify a similar arrangement of binding sites in many other papillomaviruses, indicating that the arrangement of E1-binding sites is an important conserved feature of the origin of DNA replication.

STRUCTURAL CHANGES IN THE ORI INDUCED BY BINDING OF E1

The mutational analysis of the E1-binding site in the ori demonstrated that six putative overlapping E1-binding sites are present. To determine whether the overlapping sites could bind E1 simultaneously, we examined DNA binding by the E1 DBD utilizing high-resolution OH radical footprinting. The results from these studies demonstrated that binding of the E1 DBD to the E1-binding sites follows a particular sequence. At low concentration, a dimer of E1 DBD binds to sites 2 and 4. At higher concentrations of E1 DBD, the overlapping sites 1 and 3 are also filled, generating a structure where two dimers of the E1 DBD bind to the ori but on different faces of the DNA helix. Two observations indicate that the binding of four molecules of E1 results in significant changes in the DNA structure. First, formation of a stable complex containing four molecules of E1 occurs only on short probes, indicating that the ability to deform the DNA is of importance for complex formation. Second, the binding of four E1 DBD molecules results in significant OH radical hypersensitivity outside the ori. These results indicate that DNA binding by the E1 DBD provides initial structural changes in the template likely related to subsequent melting of the ori. Although full-scale melting depends on ATP hydrolysis by E1, limited base opening can be observed upon DNA binding by the full-length E1 protein even in the absence of ATP.

INITIATOR COMPLEX ASSEMBLY

An intriguing aspect of viral initiator proteins is their ability to perform several different, seemingly unrelated, biochemical functions. Our model to explain how these activities can reside in one single polypeptide is that different oligomeric forms of the protein may have different activities. In agreement with such a model, we have previously demonstrated that a dimeric form of E1 recognizes the ori together with E2 and that the form of E1 which has helicase activity is a hexamer. Our current understanding of the assembly pathway of these complexes is that a sequence-specific complex of a dimer of E1 and a dimer of E2 together bind to the ori and initially recognizes the ori. In an ATP-dependent manner the E2 dimer is displaced and additional E1 molecules are added to the complex. These are bound sequence-specifically, forming a tetramer on the four paired E1-binding sites in the core. The role of E2 in the initiation process thus is limited to the early steps of initiation. An interesting, and possibly important, difference between different types of papillomaviruses is the arrangement of E2-binding sites in the vicinity of the ori. The human genital papillomaviruses that are the cause of widespread disease lack the proximal E2-binding site that is present in the bovine papillomavirus. To determine whether the role of E2 as a loading factor for E1 that we have established for the bovine virus can also be applied to the human papillomaviruses, we tested the ability of the E2 protein to load E1 from a distal E2-binding site. These results demonstrated clearly that E2 functions in a similar manner from the distal E2-binding site, indicating that the function of E2 is the same in both systems. E2 is responsible for the initial recruitment of E1 and also assists in the further assembly of E1 complexes through protein-protein interactions between the E1 and E2 proteins.

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DNA REPLICATION AND CHROMATIN INHERITANCE

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While we continued to study the replication of chromosomal DNA in eukaryotic cells, our research during the past year has been expanding its focus to include the interaction between DNA replication and other cellular events, such as gene silencing and even nutrition and the production of new ribosomes. It seems remarkable that 10 years after the discovery of the origin recognition complex (ORC), we are still learning new functions for this interesting protein, perhaps reflecting its importance to many cellular processes.

INITIATION OF CHROMOSOME REPLICATION

Forming the prereplication complex in yeast and human cells. We reported in these pages last year that Cdc6 in *Saccharomyces cerevisiae* interacts with the ORC in an ATP-dependent manner to increase the DNA sequence specificity of ORC and recruit the minichromosome maintenance (MCM) proteins. Continued studies on this interaction have shown that regions of Cdc6p that are predicted to control ATP hydrolysis are required for the remodeling of ORC on the DNA. In the past year, research from other laboratories using *Schizosaccharomyces pombe* and *Xenopus* egg extracts has shown that a protein called Cdtl is required for loading of the six-protein MCM complex onto chromatin to form a prereplication complex (pre-RC) that allows chromosomes to initiate DNA replication in S phase. Although it was initially reported that there was no obvious ortholog of the Cdt1p in the budding yeast, we (and other workers) found a protein encoded by the YJR046W open reading frame in the yeast genome sequence that had some similarity to the *S. pombe* and *Xenopus* Cdt1p. Normally viable mutations in this open reading frame have been reported to be lethal when strains carrying mutations in the gene encoding topoisomerase 1 are also present. Topoisomerase 1 most likely interacts with the DNA replication fork machinery to relieve torsional stress in the double helix as DNA begins to initiate DNA replication, similar to the interaction between SV40 large-T antigen and topoisom-erase 1 we have reported previously. Subsequent purification of a molecular tagged version of Cdt1p showed that it formed a stable complex with the six MCM proteins. The complex required low levels of Zn^{++} because the MCM proteins require Zn^{++} to form a hexamer containing all of the MCM proteins. The functions of this complex are under investigation.

Yeast Cdc6p is associated with chromatin only in the G_1 phase of the cell cycle, but in human cells, Cdc6p is regulated differently, being degraded as cells pass through late mitosis, the time when the MCM proteins are loaded onto chromatin. In proliferating cells, Cdc6p is then resynthesized and loaded onto chromatin as cells progress through the cell cycle. We also found that the human Orc1 protein, unlike its yeast homolog, is degraded at the G_1- to S-phase boundary and then resynthesized later in the cell cycle and loaded onto chromatin as cells exit mitosis. We showed that Skp2p, an E3 ligase for the ubiquitin-conjugating complex, is in part responsible for adding ubiquitin to human Orc1. Interestingly, the ubiquitylated Orc1 was associated with chromatin. We have also shown that cyclin E/CDK2 and cyclin A/Cdk2 can phosphorylate Orc1, and removal of the region that contains the numerous phosphorylation sites stabilizes the protein on chromatin in human cells. This raises the interesting possibility that ubiquitylation of ORC might have some role in activation of DNA replication, much as Bill Tansey's lab here at CSHL has shown for the transcriptional activation of c-Myc and other transcription factors. The variation in the levels of Orc1 in human cells reflects the pattern seen for Cdc6p in yeast, raising the possibility that these two proteins, which share significant sequence similarity, have partially swapped functions during the time separating yeast and human cells from a common ancestor (Fig. 1).

Possible links between cell proliferation control, DNA replication, and ribosome biosynthesis. It is already known that ORC functions in heterochromatin formation in *Drosophila* and in transcriptional gene silencing in yeast. Although cell cycle regulation of the assembly of the pre-RC has been well studied, we hypothesized that ORC might interact with other proteins that function in either DNA replication or other cellular processes. Therefore, a search for ORC interacting proteins was initiated.

Immunoprecipitation of *S. cerevisiae* ORC using monoclonal antibodies revealed a small number of coprecipitating proteins that were of unknown function. One of these, called Yph1p, was related to a gene

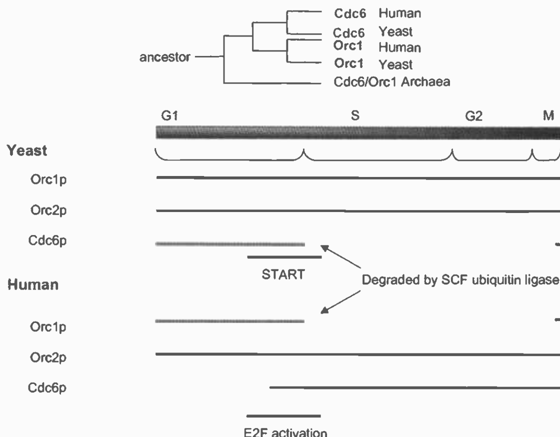

FIGURE 1 (*Top*) Comparison of the sequence similarity between Orc1 and Cdc6 in different species. (*Bottom*) Abundance of different DNA replication proteins throughout the cell cycle in yeast and human cells. Line indicates protein is present.

product in zebrafish called *pescadillo* that was conserved and contained a BRCT motif which is present in many proteins involved in maintaining genome stability or cell cycle checkpoint control. Yph1p bound directly to ORC, and we showed that the protein was predominantly a nucleolar protein, although some Yph1p was found in the nuclear soluble pool. A temperature-sensitive mutant that causes the destruction of Yph1p at the nonpermissive temperature causes cells to arrest in either G_1 or G_2 phase, with no cells in S phase. Furthermore, when cells were blocked in S phase with hydroxyurea and released at the nonpermissive temperature, S phase was greatly prolonged compared to wild-type cells. The data suggested that Yph1p has a role in S-phase progression. Interestingly, the interaction between Yph1p and ORC was increased threefold in a strain that lacked the NAD-dependent histone deacetylase Sir2p, a protein which regulates the rDNA locus that makes ribosomal RNA. Sir2p also provides a potential link between sensing of energy levels in the cell and regulation of rDNA replication.

Because of the location of Yph1p in the nucleolus, we tested its role in ribosome biosynthesis and found that Yph1p mutants were defective in production of 60S pre-ribosomal particles, but not 40S pre-ribosomal particles. Moreover, at the nonpermissive temperature, strains lacking Yph1p accumulate 80S ribosomes that were depleted of polysomes. Thus, we conclude that Yph1p is required for 60S ribosome biosynthesis and possibly for efficient translation of mRNAs on polysomes, or for initiation of translation. Indeed, Yph1p was specifically degraded from an 88-kD protein present on 80S ribosomes to a 70-kD protein associated with polysomes, suggesting that proteolytic cleavage of Yph1p is coupled to translation regulation.

We were concerned that the effect on DNA replication mentioned above was indirect due to defects in ribosome biogenesis or translation. However, cells arrested in HU and released from the HU block in the presence of translation inhibitors or other mutants defective for ribosome biogenesis still progressed through S phase normally. Thus, Yph1p had an effect on DNA replication independent of its role in ribosome biogenesis or translation. We suggest that Yph1p links DNA replication and ribosome biosynthesis, possibly via coupling replication of rDNA genes with active rDNA transcription in proliferating cells.

Yph1p was biochemically purified and shown to form two complexes, a small complex containing three proteins and a much larger complex containing the proteins from the small complex as well as many other proteins. Some of these proteins have recently been shown to be involved in ribosome biosynthesis, but others, like ORC and the MCM proteins, are involved in DNA replication and might be required to replicate rDNA genes.

One of the Yph1p-associated proteins present in

the large and small complexes is a protein of unknown function in yeast, but Lester Laue's lab has shown that a dominant-negative version of its murine ortholog arrests mouse cells in the G_1 phase of the cell cycle. This arrest only occurs in cells containing a wild-type version of the p53 tumor suppressor protein. We suggest, based on the links between Yph1p and ribosome biosynthesis and possibly translation control, that p53 regulates these processes in vivo under conditions of cell stress.

Finally, we have shown that in yeast, Yph1p levels in cells were controlled by the type of carbon source. Furthermore, Yph1p levels drop as yeast cells move from a nutrient environment of high glucose to low glucose, a shift coupled with a switch from glycolysis to gluconeogenesis and respiration. This suggests a fundamental link between energy production and cell proliferation to processes such as DNA replication, ribosome biosynthesis, and possibly translation control.

CHROMATIN ASSEMBLY AND INHERITANCE

ORC functions in establishment and inheritance of the silent mating-type loci by binding to the silencer elements that flank the *HM* loci. We have previously shown that the amino terminus of Orc1p is not essential for the DNA replication functions of ORC, but instead is required for efficient *HM* gene silencing. In collaboration with Rui-Ming Xu's lab here at CSHL, the crystal structure of the *S. cerevisiae* Orc1p amino-terminal domain was determined. This domain contains a conserved BAH (bromo-associated homology domain) and interacts with the yeast silencing protein Sir1p.

The structure shows that the BAH domain, which is also found in Orc1 protein from human cells, the silencing protein Sir3p, and DNA (cytosine) methyl transferase, consists mostly of β sheets that form a surface which might interact with the tails of histone proteins. Mutations in a helical subdomain of the BAH domain (H domain) that is not conserved in sequence among the various BAH-domain-containing proteins failed to bind to Sir1p, suggesting that Sir1p interacts specifically with Orc1p via this domain.

Mutations in the BAH domain had a phenotype similar to the phenotype found in cells lacking Sir1p. In such cells, genetically identical cells in a population had different repression states at the silent mating-type *HM* loci. Approximately 15% of the cells in a population had the *HMLα* genes repressed, whereas the other cells expressed the *HMLα* genes. This demonstrated that the BAH domain contributed to epigenetic inheritance of gene expression in yeast.

Four SIR proteins are essential for efficient silencing of genes in yeast: Sir1p, Sir2p, Sir3p, and Sir4p. We determined whether the BAH domain mutations affected the location of SIR proteins at specific DNA sequences across the two mating-type loci, *HMR* and *HML*. In wild-type cells, Sir1p localized to the E silences at both *HML* and *HMR*, whereas Sir2p, Sir3p, and Sir4p localized across the entire loci. In the absence of the Orc1p BAH domain, Sir1p no longer localized to the silencers, but surprisingly, at the *HML* locus, the pattern of Sir2p, Sir3p, and Sir4p was identical to that found in wild-type cells, except that the levels of the protein present at the locus were lower. The results suggest that in the ~15% of cells that retained *HML* repression, Sir2p, Sir3p, and Sir4p localized normally in the absence of Sir1p and the Orc1p BAH domain.

The fact that a normal distribution of Sir2p, Sir3p, and Sir4p was observed in the absence of Sir1p or the Orc1p BAH domain shows that the latter proteins are not required for the spreading of the silencing complex at *HML*. Sir1p and the Orc1p BAH domain either are required for establishing the repressive complex efficiently or are required for efficiently inheriting the SIR complex over many generations once it is formed. We plan to distinguish between these two possibilities.

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PROTEIN TYROSINE PHOSPHATASES AND THE CONTROL OF CELLULAR SIGNALING RESPONSES

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

During the last year, Yu Shen left the lab to take up a position as a Research Scientist at Abbott Laboratories, and Mike Myers accepted an independent position on the faculty here at Cold Spring Harbor Laboratory. Deirdre Buckley joined the lab as a postdoctoral fellow. Jannik Andersen completed the academic component of his industrial Ph.D. Educational Program in our lab, sponsored by the Danish Academy of Technical Sciences, and is now continuing his studies as a postdoctoral fellow. Josema Torres, a graduate student from Rafael Pulido's lab in Valencia, Spain, spent 4 months working in the lab over the summer as a visiting scientist. Kim Pennino left the lab to enjoy time with her baby son, and her position has been taken by Mianjia (Jennifer) Ye.

PTP1B: A THERAPEUTIC TARGET FOR THE TREATMENT OF DIABETES AND OBESITY

It is now apparent that the insulin receptor (IR) is a major physiological substrate of PTP1B. Interestingly, targeted disruption of the *PTP1B* gene in mice yielded a dramatic phenotype—the animals displayed enhanced sensitivity to insulin and resistance to obesity when placed on a high-fat diet. (Elchelby et al., *Science 283:* 1544 [1999]; Klaman et al., *Mol. Cell. Biol. 20:* 5479 [2000]). These and other data linking PTP1B to the regulation of IR signaling highlight the potential of this PTP as a target for novel strategies for therapeutic intervention on diabetes and obesity, two of the major, and fastest growing, global diseases. In collaboration with David Barford (Institute of Cancer Research, London, United Kingdom), we analyzed the recognition of the activation loop of the IR as a substrate by PTP1B. By integrating crystallographic, kinetic, and peptide-binding studies, we defined the molecular basis of specificity in the interaction between PTP1B and activation loop of the IR. We observed that the sequence E/D-pY-pY-R/K was important for optimal recognition of the IR by PTP1B. Of particular importance is the presence of tandem pY residues; PTP1B displays 70-fold higher affinity for tandem pY-containing peptides derived from the IR activation loop relative to mono-pY derivatives.

In light of this observation, we tested whether the presence of the motif could be used to predict other physiological substrates of PTP1B. We identified all proteins in the SwissProt database that contained two adjacent tyrosine residues flanked by acidic and basic amino acids. This search retrieved more than 800 proteins, but the subset of these that is tyrosine-phosphorylated is not yet annotated. However, we noted that 7 of the 22 subtypes of PTKs contained this motif in their activation loop. We focused our analysis on the JAK subfamily of PTKs and have now shown that

JAK2 and TYK2 are physiological substrates of PTP1B. We observed that a substrate-trapping mutant of PTP1B formed a stable interaction with JAK2 and TYK2 in response to interferon stimulation. Expression of the wild-type or substrate-trapping mutant PTP1B inhibited interferon-dependent transcriptional activation. Finally, mouse embryo fibroblasts deficient in PTP1B displayed subtle changes in tyrosine phosphorylation, including hyperphosphorylation of JAK2. These data illustrate that PTP1B may be an important physiological regulator of cytokine signaling.

Interestingly, the satiety hormone leptin exerts its effects through a receptor in the hypothalamus that displays the hallmarks of a cytokine receptor. Our data, demonstrating recognition of JAK2 and TYK2 as substrates by PTP1B, suggest that the PTP may normally function as a negative regulator of leptin signaling in the brain and that the resistance of PTP1B knockout mice to high-fat-diet-induced obesity may arise from defects in leptin signaling. Thus, aberrant down-regulation of members of the JAK subfamily of PTKs may contribute to the resistance to weight gain displayed by PTP1B-deficient mice. Furthermore, our data illustrate that D/E-pY-pY-K/R is a consensus substrate recognition site for PTP1B. The definition of consensus phosphorylation sites has proven to be an important tool in signal transduction research for the identification of substrates for protein kinases. Our study demonstrates for the first time that this concept may also be applicable to protein phosphatases, such as PTP1B, and that consensus recognition sites may also exist for other PTPs. Perhaps it may be possible in the future to use this approach to expand the repertoire of physiological substrates of other members of the PTP family of enzymes.

In addition to the above, we have been examining PTP1B function through characterization of its promoter. It had been noted that the levels of PTP1B are altered in several human diseases, particularly those associated with dysfunctional tyrosine phosphorylation. We had shown previously that expression of PTP1B was induced specifically by the p210bcr-abl oncoprotein, a PTK that is directly responsible for the initial manifestations of chronic myelogenous leukemia, and that PTP1B antagonizes p210bcr-abl-induced transformation. We have now demonstrated that the effects of p210bcr-abl on expression of the *PTP1B* gene are manifested at the transcriptional level. To define the mechanism by which *PTP1B* gene expression is regulated, we cloned the 5′-flanking region of the human gene. We found two elements that are important for expression from the human *PTP1B* promoter. A sequence motif that possesses features of a site of interaction with GATA-binding proteins was identified at –167 to –151 bp from the transcription start site. Disruption of this site inhibited promoter activity in the presence of p210 bcr-abl, but responsiveness to the oncoprotein PTK was maintained. However, a p210 bcr-abl responsive sequence, termed PRS, which was important for stimulation of activity in response to the PTK, was identified at –49 to –37 bp from the transcription start site. The PRS is contained in a sequence that displays features of a stress response element, a feature originally identified in *Saccharomyces cerevisiae* and which functions as a binding site for C_2H_2 zinc finger proteins. In our study, we showed that three mammalian C_2H_2 zinc finger proteins, Egr-1, Sp1, and Sp3, bind to PRS. Of these, both Sp1 and Sp3 function as positive regulators, whereas Egr-1 represses Sp3-mediated *trans*-activation of the *PTP1B* gene. Furthermore, expression of p210 bcr-abl results in down-regulation of the levels of Egr-1. Our data illustrate that the reciprocal actions of the Sp1/Sp3 and Egr-1 C_2H_2 zinc finger transcription factors are an important aspect of the regulation of PTP1B expression in response to the p210 bcr-abl oncoprotein.

In collaboration with Tony Tiganis, a former postdoctoral fellow in the lab now on the faculty at Monash University, Melbourne, Australia, we have continued to examine the regulation of TCPTP, the closest relative to PTP1B. There are two spliced variants of TCPTP, which share the same catalytic domain but differ at their carboxyl termini and are targeted to different subcellular locations. TC48 is found in the endoplasmic reticulum, whereas TC45 is nuclear in the basal state but exits the nucleus in response to various stimuli including cellular stress. We have shown that nuclear exit of TC45 may occur passively, with cellular stress inducing cytoplasmic accumulation by inhibiting nuclear import. We noted that only those stresses that stimulated the metabolic stress-sensing enzyme AMP-activated protein kinase (AMPK) induced the redistribution of TC45. In addition, specific pharmacological activation of AMPK was sufficient to cause the accumulation of TC45 in the cytoplasm. Thus, specific signaling pathways involving AMPK may regulate the subcellular location, and function, of TC45 in response to cellular stresses.

RECEPTOR PTPS AND CELL ADHESION

Our studies have focused on two receptor PTPs. PTPμ, which displays features of an immunoglobulin (Ig) super family cell adhesion molecule, is expressed at high levels in endothelial cells. In subconfluent cultures, the level of PTPμ is low, found particularly in intracellular membranes, including the Golgi. However, in confluent cells, the level of PTPμ is dramatically upregulated and the protein accumulates on the cell surface, particularly in junctional complexes. PTPμ was the first RPTP for which a physiological ligand was identified, through our observation that the extracellular segment of the enzyme participates in homophilic binding interactions; thus, one molecule of PTPμ recognizes another PTPμ molecule on the surface of an apposing cell. Furthermore, we showed that PTPμ interacts with cadherin-catenin cell adhesion complexes, in tissues and in epithelial cell cultures. These observations suggest that PTPμ may be positioned to regulate cell adhesion by controlling the tyrosine phosphorylation status of junctional proteins. It appears that in endothelial cells, PTPμ can associate with both cell-cell and cell-matrix junctions. We are performing a structure-function analysis to examine the importance of activity and homophilic binding of PTPμ in survival and adhesion of endothelial cells. For example, we examined the effects of expressing a PTPμ mutant in which the Ig domain had been deleted from the extracellular segment and which, therefore, has lost its ability to participate in homophilic binding. This mutant appears to be directed to the leading edge of cells where it disrupts formation of focal contacts and appears to result in enhanced tyrosine phosphorylation. We are currently examining the effects of various forms of PTPμ and trying to link those effects to the dephosphorylation of defined protein substrates.

We originally identified DEP-1 (density-enhanced PTP) as the first PTP to be shown to display enhanced expression in confluent, compared to subconfluent, cell cultures. There are now several lines of evidence to implicate DEP-1 in contact inhibition of cell growth. Therefore, we have been trying to derive insights into the mechanism of action of DEP-1 through the identification of its physiological substrates. We have been using the method developed in our lab involving production of mutant PTPs that maintain a high affinity for substrate but do not catalyze dephosphorylation effectively ("substrate traps"). Following expression in cells or in affinity chromatography in vitro, the mutant PTP binds to its physiological substrates but, because it is unable to dephosphorylate the target efficiently, the mutant and substrate form a complex. Upon isolation of this complex, the substrate(s) can be identified, yielding important insights into the function of the PTP. We have focused on two different human breast tumor lines, MDA-MB-231 and T47D. Sequence data on one of the trapped proteins identified it as p120 catenin. Originally identified as a substrate of Src, p120 catenin is a component of adherens junctions. It interacts with classical cadherins, such as E-cadherin, which are cell-cell adhesion molecules that have been implicated in the inhibition of tumor growth and invasion. Recently, DEP-1 was shown to colocalize with VE-cadherin in endothelial cells (Takashaki et al., *J. Am. Soc. Nephrol. 10:* 2135 [1999]). However, although DEP-1 recognized p120 catenin as a substrate, it did not trap E-cadherin. In addition, although we detected an interaction with the cadherin-associated proteins β-catenin and plakoglobin, this interaction was not phosphorylation-dependent and was observed with both wild-type and trapping mutant forms of DEP-1. Interestingly, our data also suggest that the receptor PTK Met, the receptor for hepatocyte growth factor/scatter factor, is a substrate of DEP-1. Currently, we are characterizing these interactions to obtain insights into the signaling function of DEP-1.

DUAL SPECIFICITY PHOSPHATASES AND THE CONTROL OF CELL SIGNALING

The mitogen-activated protein kinases (MAPKs) are integral to the mechanisms by which cells respond to diverse stimuli from growth factors, hormones, and cytokines to a wide variety of environmental stresses. MAPKs are stimulated by phosphorylation of a TXY motif in their activation loop and are components of signal transduction cascades in which sequential activation of a series of protein kinases culminates in their activation and their subsequent phosphorylation of various effector proteins that mediate the physiological response. Several dual specificity phosphatases (DSPs) have been implicated in the dephosphorylation and inactivation of MAPKs. In a collaborative project with Ralf Luche and Bo Wei at CEPTYR Inc., we have identified and isolated full-length cDNA clones for a variety of novel DSPs. We have made the unprecedented observation that one of these novel DSPs did not inactivate MAPKs, but instead functioned as a

specific activator of the JNK subfamily of MAPKs. We have named the enzyme JSP-1, for JNK-stimulatory phosphatase-1. Our data indicate that JSP-1 induced the activation of MKK4, a MAP kinase kinase (MAPKK) immediately upstream of JNK in the signaling pathway. The effects of the JSP-1 were blocked by a dominant-negative mutant form of MKK4, suggesting that JSP-1 acts upstream of the MAPKKs in the signaling pathway. We have now identified several proteins that have the potential to function as regulators of JSP-1 and are combining the characterization of these proteins with the use of such techniques as RNA interference to define the mechanism of action of this DSP. Aberrant activation of JNK has been linked to several human diseases, raising the possibility that JSP-1 may prove to be a novel therapeutic target.

The production of distinct proteins from alternate reading frames at the same gene locus is unusual in higher eukaryotes. A classic example is the *Ink4a-Arf* locus, encoding the unrelated proteins $p16^{Ink4a}$, which functions as an inhibitor of cyclin-D-dependent kinases, and $p19^{Arf}$, which binds to and inactivates MDM2, thereby potentiating the activity of p53. We have identified a novel example in which the two open reading frames encode closely related, VH1-like DSPs currently termed DSP7 and DSP17. The presence of mRNA for DSP7 and DSP17 has been shown by northern blotting and reverse transcriptase–polymerase chain reaction (RT-PCR), with both of these DSPs expressed specifically in skeletal muscle. Currently, we are characterizing these DSP proteins, including attempts to identify their physiological substrates.

REGULATION OF PTP FUNCTION BY REVERSIBLE OXIDATION

The signature motif, [I/V]HCXXGXXR[S/T], which defines the PTP family of enzymes, contains an invariant cysteine residue that functions as a nucleophile in catalysis. Due to the environment of the active site, this cysteine residue has an unusually low pKα that enhances its nucleophilic properties but renders it susceptible to oxidation. A variety of studies have illustrated the potential for regulation of PTP function by oxidation and its importance for optimal tyrosine-phosphorylation-dependent signaling. To examine this issue further, we have developed a modified "in-gel" PTP assay to visualize the oxidation of PTPs in response to a stimulus in a cellular context. We observed the reversible oxidation of multiple PTPs in response to treatment of Rat 1 cells with H_2O_2 and demonstrated that this oxidation was required for the mitogenic effects of H_2O_2. We also demonstrated that stimulation of Rat 1 fibroblasts with platelet-derived growth factor (PDGF) led to the production of reactive oxygen species (ROS), which induced the rapid and reversible oxidation of the PTP SHP2. Ligand-induced autophosphorylation of the PDGF receptor (PDGF-R) produces docking sites for various signaling proteins, including SHP2. We showed that mutant forms of the PDGF-R that were unable to bind to SHP2 displayed enhanced autophosphorylation and enhanced activation of MAP kinase. Thus, SHP2 appears to be an inhibitor of PDGF-R signaling. Interestingly, it was only the population of SHP2 that was bound to the PDGF-R which was susceptible to reversible oxidation and inhibition. Thus, we propose that the PDGF-induced production of ROS leads to the rapid oxidation of the pool of SHP2 which has been recruited into a complex with the PDGF-R. This augments autophosphorylation of the receptor and the initiation of the signaling response. The transient nature of the oxidation results in reduction and reactivation of the pool of SHP2, which promotes dephosphorylation of the PDGF-R and termination of the signal. These data illustrate how PTK-induced production of ROS may augment tyrosine-phosphorylation-dependent signaling through inactivation of PTPs (Fig. 1) and defines the mechanism by which SHP2 functions as an antagonist of PDGF-R function. We have also observed the production of ROS in response to various other stimuli, including hormones such as insulin and activators of G-protein-coupled receptors, that lead to PTK activation. Currently we are attempting to apply this strategy to utilize stimulus-induced oxidation as a means of "tagging" and identifying those PTPs that are integral to the regulation of the signaling events triggered by that stimulus. Hopefully, this will provide further insights into the physiological function of other members of the PTP family.

A GENOMIC PERSPECTIVE ON THE PTPS

The availability of sequence data on the whole genomes of various organisms, including the first draft of the human genome, offers the potential to expand protein families and study evolutionary relationships. To provide a framework for a genome-wide

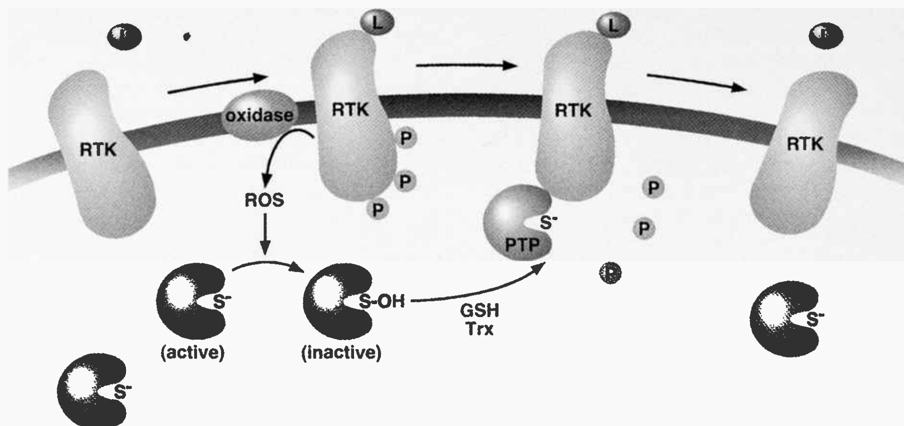

FIGURE 1 Regulation of PTP activity by reversible oxidation. Ligand-dependent activation of a receptor protein tyrosine kinase (RTK) triggers the activity of a Rac-dependent NADPH oxidase leading to production of reactive oxygen species (ROS). ROS oxidizes the active-site cysteine residue of members of the PTP family, converting it from a thiolate ion (the active form) to sulfinic acid. Oxidation results in inhibition of PTP activity, thereby promoting tyrosine phosphorylation. However, due to the action of glutathione or thioredoxin, oxidation of the PTPs is transient. Restoration of PTP activity following reduction back to the thiolate form of the active site cysteine residue terminates the tyrosine-phosphorylation-dependent signal. A variety of growth factors, hormones, and cytokines induce ROS production and stimulate tyrosine phosphorylation. We are developing methods to identify which PTPs become oxidized in response to a physiological stimulus as a way of establishing links between particular PTPs and the regulation of defined signaling pathways.

analysis of the PTPs, we have compiled a comprehensive on-line resource for sequence analysis of the pTyr-specific members of the PTP family. The Web Site includes amino acid sequence alignments, phylogenetic classification of family members into 17 PTP subtypes, and evaluation of amino acid conservation in three dimensions using X-ray crystal structures of PTP domains and low resolution homology modeling. The PTP database is available under CSHL databases at http://intron.cshl.org/biocomp/ or at the Web Site www.science.novonordisk.com/ptp. In the future, we plan to expand this resource to include intron/exon organization, splice variants as well as mutations, polymorphisms, and disease linkages. In addition, we have been conducting a genome-wide analysis of DSPs. In collaboration with Benjamin C. Kirkup at Yale University, we conducted a phylogenetic analysis of all known DSPs, including the novel enzymes we have identified, using parsimony to assess evolutionary relationships. Preliminary analysis indicates a high degree of diversification, with many of the novel DSPs forming distinct subfamilies, suggestive of a wide range of functions. Interestingly, many of the disease linkages established to date for the PTP family involve the DSPs. Currently, we are working in collaboration with Ravi Sachidanadam here at CSHL to collect and organize the data on the DSPs, in conjunction with our analysis of the PTPs, to set up an interactive database.

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Research in my laboratory is focused on the role of the Ras and Rho GTPase family members in signal transduction. Members of these families encode low-molecular-weight guanine nucleotide-binding proteins that function as binary switches by cycling between an active GTP-bound state and an inactive GDP-bound state. It is only in their GTP-bound state that they are able to interact with downstream effector molecules which mediate their effects. The ratio of the two forms is regulated by the opposing effects of guanine nucleotide exchange factors (GEFs) that promote the exchange of bound GDP for GTP, and the GTPase-activating proteins (GAPs) that stimulate hydrolysis of bound GTP. Alterations that affect proper Ras and Rho function have been found to result in the development of several disease processes, including cancer, inflammation, neuropathological disorders, and bacterial infections. Efforts in my laboratory have been focused on defining the role and mechanisms by which these GTPases exert their effects on (1) specific aspects of tumorigenesis and (2) neuronal development.

The cancer studies are focused on three aspects of tumorigenesis. One project involves defining the mechanisms by which Rac1, a Rho family member, contributes to cell growth and invasiveness. The second project aims to gain insights into the molecular basis by which the Ras-related GTPase Rap1 and its effector AF-6 affect epithelial cell shape changes and the coordinated movements of cells, which are pivotal alterations governing tumor progression and wound healing processes. The third project involves the functional characterization of $p62^{dok}$, a Ras-GAP-associated protein, which was found to be constitutively tyrosine-phosphorylated in chronic myelogenous leukemia (CML) progenitor cells. A more recently initiated focus in our lab involves investigating the role of the Ras and Rho GTPases in neuronal development and synaptic transmission. Of particular interest to my lab is to understand how a lack of oligophrenin-1, a potential Rho-GAP and regulator of GTPases, results in mental retardation.

SIGNAL TRANSDUCTION PATHWAYS MEDIATING EFFECTS OF RAC1 ON CELL GROWTH AND INVASIVENESS

Several lines of evidence have indicated the involvement of the Rho GTPases, and in particular Rac1, in cellular events such as proliferation, adhesion, and invasion. However, a major challenge remains to define the signaling pathways mediating these effects. To this end, we have made use of the yeast two-hybrid system to isolate Rac1-interacting proteins. This resulted in the identification of several putative Rac1 effectors (see previous report), as well as a novel class of Rac1 activators, which contain a region of homology with Dock180. The latter protein has previously been shown to activate Rac1 and to have a role in phagocytosis, cell migration, and invasion of tumor cells. We have designated the two novel proteins, *Darc*1 and 2 (for *D*ock180-like *a*ctivator of *Rac*1) and are currently investigating their roles in cell motility and invasion. As an additional approach, we employed cDNA-RDA (representational difference analysis) in combination with microarray analysis to identify target genes of Rac1, whose expression is altered as a result of constitutively active Rac1 (Rac1V12) expression. These experiments were assisted by Dr. R. Lucito here at CSHL. Among the cDNAs isolated were cyclooxygenase 2 (COX-2) and cyclin D1, two genes whose expression has been previously shown to be up-regulated in many tumors. We found that the expression levels of both of these genes are up-regulated in Rac1V12-expressing epithelial and glioblastoma cells. Further characterization of the other cDNAs is likely to identify additional relevant Rac1 targets and is presently ongoing in my lab.

ROLE OF RAP1 AND AF-6 IN EPITHELIAL MORPHOGENESIS

Several studies suggest a relationship between loss of epithelial cell polarity and loss of growth control.

Studies using mammalian epithelial cells have suggested a role for Rap1, a member of the Ras family, and the junctional protein AF-6, which associates with GTP-bound Rap, in the regulation of epithelial cell shape changes and cell adhesion. Due to the finding that AF-6 is largely refractory to both loss-of-function and gain-of-function analyses, we decided to explore this hypothesis in the genetically more tractable *Drosophila* system.

In *Drosophila*, a process called dorsal closure (DC) relies on the elongation and migration of epithelial cells and has proved to be an excellent model system for the study of the molecular basis underlying epithelial cell shape changes. In collaboration with U. Gaul (Rockefeller University), we recently provided evidence that Canoe (*Drosophila* orthologs of AF-6) acts as a *Drosophila* Rap1 effector in the process of dorsal closure. To date, no other protein has been reported to act as a mediator or direct effector of the small GTPase Rap1 in a physiological context. Furthermore, we obtained evidence that Canoe participates in DC through two cellular pathways: One is controlled by DRap1 and is independent of the JNK cascade, whereas the second is not activated by DRap1 and feeds into JNK signaling. We are currently planning to identify additional Canoe-interacting proteins to obtain further insights into Canoe's mode of action in dorsal closure. To complement these studies, we are planning to assess a role for the mammalian counterparts of these *Drosophila* proteins in mammary epithelial polarity and invasive behavior. These studies will contribute to a better understanding of the fundamental mechanisms that drive cell shape changes and migration.

P62^{DOK} IS A NEGATIVE REGULATOR OF GROWTH-FACTOR AND P210$^{BCR-ABL}$-INDUCED CELL PROLIFERATION

p62^{dok} is a protein initially identified as a prominent 62-kD constitutively tyrosine-phosphorylated, RasGAP-associated protein, in p210$^{bcr-abl}$-expressing cells. This protein was termed Dok (*do*wnstream of *k*inases), since it was also found to be a common substrate of many receptor- and membrane-associated tyrosine kinases. To investigate the role of p62^{dok}, we made use of different cell types derived from p62^{dok} null mice, generated by P.P. Pandolfi (Memorial Sloan-Kettering Cancer Center, New York). In collaboration with Pandolfi's group, we obtained evidence that p62^{dok} acts as a negative regulator of growth-factor-induced cell proliferation, since p62^{dok}-deficient cells possess a higher proliferation rate in response to growth factors. The increase in cell proliferation in p62^{dok}–/– cells can be suppressed by ectopic expression of p62^{dok}. Furthermore, p62^{dok} inactivation causes a significant shortening of the latency of the fatal myeloproliferative disease induced by retrovirus-mediated transduction of p210$^{bcr-abl}$ in bone marrow cells. We have also obtained data suggesting that p62^{dok} exerts its negative effect on growth-factor-induced cell proliferation at least in part by negatively influencing the Ras/MAPK (mitogen-activated protein kinase) pathway. We have shown that in order for p62^{dok} to act as a negative regulator of the Ras/MAPK pathway, p62^{dok} must be recruited to the membrane. This platelet-derived growth factor (PDGF)-triggered translocation of p62^{dok} to the plasma membrane involves the activation of phosphatidylinositol-3 kinase (PI3-kinase) and binding of its PH domain to 3′-phosphorylated phosphoinositides. We are presently investigating the underlying mechanism by which p62^{dok} inhibits the Ras/MAPK pathway, and growth factor and p210$^{bcr-abl}$-mediated signaling.

FUNCTIONAL CHARACTERIZATION OF OLIGOPHRENIN IN THE BRAIN

Recent studies revealed that four of the nine genes thus far identified to be involved in nonspecific X-linked mental retardation (MRX) are regulators or effectors of Ras-related small GTPases. These include oligophrenin-1, which is a putative RhoGAP; PAK3, which is a downstream serine/threonine target of Rac and Cdc42; and a novel exchange factor for Rac, called PIX. At present, there is a lack of understanding as to how these proteins contribute to MRX. However, there is compelling evidence that perturbing the activity of the Rho GTPases results in alterations in the formation of neuronal processes. In collaboration with H. Cline here at CSHL, we investigated whether Rho GTPases regulate branch dynamics and branch extensions in optic tectal neurons in live *Xenopus* tadpoles. These studies indicated that the three members of the Rho GTPases have distinct effects on dendritic arbor development: Rac and Cdc42 regulate branch additions and retractions, whereas RhoA regulates the elongation of existing branches. In addition, my lab used organotypic slices of developing hippocampus to demonstrate the role of GTPases in the development of dendritic structures of pyramidal neurons. To study the function of oligo-

phrenin-1 in the developing central nervous system, we have established that oligophrenin-1 is highly expressed in the developing rat hippocampus and is expressed in axons, dendrites, and dendritic spines of pyramidal hippocampal cells. In addition, we have demonstrated that oligophrenin-1 functions as a GAP for RhoA, Rac, and Cdc42 in a cellular context. We are currently testing what effect the ectopic expression or absence of oligophrenin-1 has on the cellular morphology in developing neurons using organotypic hippocampal slices as an assay system. In addition, we recently identified a novel oligophrenin-1-interacting protein. Future studies are geared to address this interaction and its functional significance in determining cell morphology and synaptic plasticity. These studies will provide a better understanding of neuronal development and how disregulation of molecules intimately involved with Rho GTPases contributes to MRX.

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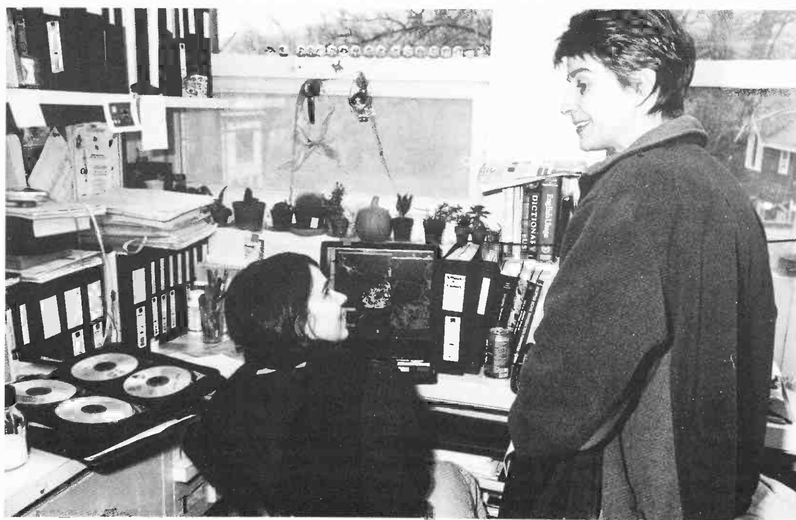


Eve-Ellen Grovek, Linda Van Aelst

BIOINFORMATICS AND GENOMICS

The Bioinformatics and Genomics group is composed of diverse investigators who are addressing many of the crucial problems in modern biology. They interact with large numbers of investigators at both Cold Spring Harbor Laboratory and elsewhere. While diverse in their interests and approaches, they share a common interface: the intersection of biology and computation. These interactions increasingly form an iterative cycle where computational biologists suggest an experiment in the lab, the results of which refine the computational tools.

- The Mishra lab is developing and testing algorithms for mapping genomes. With the completion of reference genome sequences and high-resolution physical maps, the ability to rapidly map changes in the genomes of individuals will become a key to deciphering the genomic changes in cancer and many other genetic diseases.

- The Neuwald lab continues to make progress in their efforts to develop and use computational tools to determine the correlation between protein structure and function. They have continued to enhance the performance of their comparative hierarchical alignment and interaction network (CHAIN). Using CHAIN, they have discovered that certain weak atomic interactions are conserved among members of the Ras-like GTPase family and are crucial to their function.

- The Stein lab has maintained their efforts in genome visualization databases with the SNP database and WormBase (*Caenorhabditis elegans*). They have also extended these efforts to plant genomes with Gramene. Their efforts are leading to next-generation databases such as the Genome-KnowledgeBase which they are developing and the distributed annotation server (DAS) architecture which they had a major role in popularizing.

- The Zhang lab has continued developing algorithms for the analysis of genomic DNA sequence data. They have evolved from developing gene prediction algorithms to focusing largely on developing algorithms for the prediction of promoters and first exons of genes. This is a difficult and important informatics challenge. Their newly published analysis using these tools on the human genome predicts as many as 56,000 genes, nearly doubling some previous estimates. These types of analyses are providing and will continue to provide the focal point for exciting discoveries as computational and "wet lab" scientists interact.

- As important reference genomes are being completed, the McCombie lab is focusing on developing comparative approaches to decipher their meaning. They are using *Brassica* as a model for the plant *Arabidopsis* and the dog as a model for the human genome. They are sequencing from these genomes and have developed interactive, Web-based viewers to look at the comparisons between the pairs of genomes.

Notably, this year also saw the publication of the first draft sequence of the human genome and the first high-resolution polymorphism map of the human genome. The McCombie and Stein labs contributed respectively to these landmarks.

GENOME SEQUENCE ANALYSIS

W.R. McCombie M. de la Bastide, J. Baker, V. Balija, M. Bell, N. Dedhia, S. Dike, K. Ferraro, M. Katari, F. Katzenberger, L. King, K. Kuit, B. Miller, S. Muller, L. Nascimento, A. O'Shaughnessy, L.E. Palmer, R. Preston, L. Santos, L. Spiegel, C. Yang, M. Yu, T. Zutavern

Our work in past years has focused on developing strategies and tools to facilitate the analysis of large genomes. Increasingly, this has led us in the direction of comparative genomics and developing new tools and strategies for use in these analyses. This year, we want to highlight several areas that contribute to those long-term goals: (1) the generation of raw sequence data that we have substantially automated in the past year, (2) our efforts in collaboration with colleagues at SUNY Stony Brook to test a new DNA sequencer, and (3) the use of comparative genomics to analyze the *Arabidopsis* and human genome sequences, which we helped to complete.

Production Sequence Generation

T. Zutavern, B. Miller, S. Muller, F. Katzenberger, L. Nascimento, V. Balija

Upon moving into the Genome Research Facility in August of 2001, we accelerated our efforts to automate much of our production sequencing process. This ongoing process had reached a limit due to space constraints. Since the move, we have automated a number of steps on a Biomek FX robot, including reaction setup, polymerase chain reaction (PCR), archive creation, and reaction precipitation. We have also automated reaction setup, and the rearranging of reactions for loading on a Tomtec Quadra. This provides necessary backup for the Biomek. As a result of this automation, we can now carry out up to about 20,000 reactions per week with a staff of four (including the group leader). In 1996, we were performing about 800 reactions per week with a staff of seven. Moreover, in most cases, the automated steps are more consistent than those carried out manually.

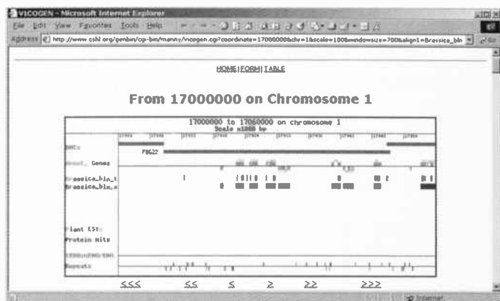
This production sequencing group provides the engine that drives most of our other activities. The raw data produced in the production group in the past year fuel our sequencing efforts in rice and mouse. They also generate the data for our *Brassica* sequencing described below. In years past, the production group has also provided data for human genome sequencing. The results of the human genome sequencing effort, to which we contributed, were published in the past year (The International Human Genome Sequencing Consortium 2001).

Testing of a Novel, Ultrahigh Sensitivity DNA Sequencer

V. Balija, L. King , L. Spiegel [in collaboration with V. Gorfinkel, SUNY Stony Brook]

A group in the electrical engineering department at SUNY Stony Brook has developed a highly sensitive DNA sequencing instrument. The detection capability of the instrument, tentatively named the Stony Brook (SB) Sequencer, far surpasses the two most commonly used DNA sequencers, namely, the ABI 3700 and the Amersham Pharmacia MegaBACE 1000. We have been testing these instruments in collaboration with the SB group. The detection design of the SB Sequencer was tested using dilutions of commercially available DNA sequencing standards. Compared to the ABI and Amersham platforms, which were incapable of producing resolved trace data from dilutions greater than 1:25, the SB Sequencer was able to routinely detect dilutions of greater than 1:100. We also tested the instrument using standard samples generated by our production group. Dilutions of 1:100 were consistently detected, indicating that the amount of sequencing reagent currently used in sequencing reactions could be theoretically reduced by that amount. The quality and reliability of the data were tested by using the instrument to sequence a cDNA clone.

A clone from a canine expressed sequence tag (EST) library was chosen, and the 5′ and 3′ ends were sequenced using universal primers. The trace data produced were then used to choose primers to walk into the sequence. In this manner, the entire gene was



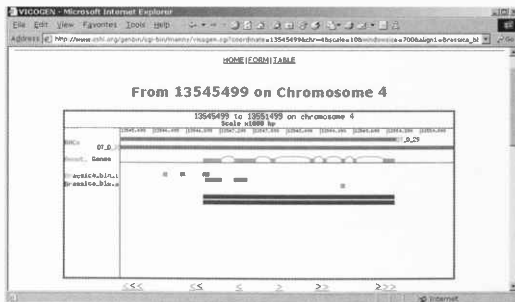


FIGURE 1 The figure shows a detailed view of the alignments to the *Arabidopsis* genome. Here, each alignment track is an individual sequence. The first track contains three regions of similarity from one *Brassica* read. Two of the regions are upstream of an annotated gene. This demonstrates the ability to find regulatory regions and 5′UTR using comparative genomics. The second track shows one read spanning over two exons, but the intron does not show a significant match. This demonstrates the conservation of coding sequence between the *Brassica* and *Arabidopsis*. The last two black tracks are the same reads discussed above, but aligning to the gene at a protein level.

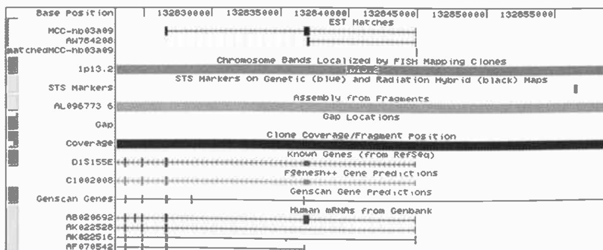


FIGURE 2 Canine ESTs were mapped to the human genome using the Blat server at the University of California, Santa Cruz, and the Sim4 program. Mapped ESTs are uploaded to the Santa Cruz genome browser to visualize the canine ESTs. The above example shows canine ESTs which map to an N-Ras-related protein.

sequenced, which established the efficacy of this instrument. The accuracy of the sequence was confirmed by sequencing the same clone using standard instruments and techniques in our lab. This represents the first real utilization of this potentially exciting new generation sequencing instrument.

Comparative Analyses of the *Brassica* and *Arabidopsis* Genomes

M. Katari, N. Dedhia [in collaboration with Rob Martienssen, Cold Spring Harbor Laboratory; the Washington University Genome Sequencing Center; and The Institute for Genomic Research]

Brassica oleracea and *Arabidopsis thaliana* belong to the mustard family, Brassicacea, and diverged 16–19 million years ago. Sequence identity between the genomes is much higher in the coding regions (85%) than in the noncoding regions. This makes *Brassica* an excellent species to improve the *Arabidopsis* annotation.

In the current annotation of the *Arabidopsis* genome, predicted genes that are not supported by any experimental evidence or sequence similarity are annotated as hypothetical (24% of 25,772). ESTs are useful in determining gene structure; however, only 60% of the predicted genes contain similarity to ESTs. We have suggested that an analysis of the *Brassica* genome could help fill this gap in the analysis of *Arabidopsis*. Our group, which includes the Washington University Genome Sequencing Center in collaboration with The Institute for Genomic Research, has been testing this concept. These reads are useful for improving the structure of predicted genes, suggesting the presence of hypothetical genes, and for discovering overlooked genes. They can also help characterize regulatory elements and discover noncoding genes.

We have created a Web-based *Arabidopsis-Brassica* genome viewer (http://www.cshl.org/genbin/cgi-bin/manny/vicogenta.cgi) to view the current *Arabidopsis* genome annotation and the positions of the alignment of the *Brassica* reads. Read and EST matches can be added to the viewer as separate tracks. This makes the viewer a useful tool for annotating the *Arabidopsis* genome by comparing conserved sequences from other species. It is also useful in identifying reads and ESTs that align to regions of interest in the *Arabidopsis* genome. Screen shots from the viewer are shown in Figure 1.

Sequencing and Analysis of Canine ESTs

L.E. Palmer, T. Zutavern, L. Santos, L. Nascimento, R. Preston, W.R. McCombie [in collaboration with Greg Hannon, Cold Spring Harbor Laboratory]

The completion of the draft for the human genome has presented the scientific community with many biological challenges and opportunities. The human draft sequence enables the use of comparative approaches to further understand the human genome through analysis of other species. Cold Spring Harbor Laboratory's Genome Center has undertaken a project to sequence canine ESTs from a variety of canine tissues and disease models. To date, the CSHL group has sequenced and made available to the public more than 5000 canine ESTs. Currently, an analysis of the sequence is under way with the use of an automated pipeline.

The ESTs have been mapped to both the human genome (using the Blat server at the University of California, Santa Cruz) and the human proteome (using Blastx vs. a nonredundant set of human proteins). Using these data, we have assigned Gene Ontology terms as well as probable names to the ESTs. This information has been stored in a relational database for easy retrieval and analysis. Most ESTs match to the human genome and/or proteome. However, there are a number of ESTs that do not match either. These ESTs will be analyzed further to determine whether they can be used to identify unknown human genes. A Web-based interface has been developed to allow outside users to view the annotations that we have assigned to the ESTs. The interface and the data can be accessed at the Web Site: www.cshl.org/genbin/cgi-bin/golden_retriever.cgi. A screen shot of a portion of the display available on this site is seen in Figure 2.

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COMPUTATIONAL BIOLOGY, GENOMICS, AND BIOINFORMATICS

B. Mishra M. Antoniotti J. McQuown A. Rudra
W. Casey S. Paxia N. Ugel
V. Cherepinsky M. Rejali Y. Zhou
R. Daruwalla

At the interface of mathematics and biology, there have emerged many challenging questions requiring consilience of elegant ideas and concepts from applied mathematics, theoretical computer science, logic, and physical modeling. The impulse has come from a better understanding of processes involved at the molecular level, technology at a meso- and nanoscale, the ability to perform high-throughput experiments, and the amount of genomic and proteomic data that can now be generated and made publicly available for processing. In response to these challenges, a group of scientists and mathematicians from Cold Spring Harbor Laboratory and the Courant Institute, an international leader in applied mathematics, has begun to focus its collective attention on these questions. In the past, this group has developed several sophisticated algorithms and statistical analysis tools to attack biological problems that range from deciphering the genome of pathogens (e.g., *Escherichia coli, Plasmodium falciparum*) to understanding chromosomal aberrations implicated in cancer.

Various projects pursued by this group are described below. These projects fall into four different categories: (1) physical mapping, (2) genomics and proteomics, (3) cell modeling, and (4) genome evolution.

MAPPING

The study of genetics relies on complete nucleotide sequences of the organism, together with a description of their structural organization. Although this information at its finest level is not always available, even a sparse collection of fragmentary data with unrecognized assembly and sequencing errors can be of enormous practical utility when combined with genomic physical maps, as these maps provide a natural structure for organizing, verifying, and deciphering the genetic data about chromosomes.

Our group has developed algorithmic foundations for two physical mapping approaches: (1) array-based correspondence and linear mapping and (2) optical mapping, which are described below in more detail.

ARRAY MAPPING: CORRESPONDENCE AND LINEAR MAPPING

In principle, microarrays are ideal tools for mapping genomes because a large amount of information can be gathered in parallel from a single hybridization. In particular, probes can be assigned to pools of BACS, and the statistical performance of the underlying protocols can be modeled faithfully. On the basis of these models and supporting experiments, our group devised the algorithmic, mathematical, and statistical tools needed to utilize microarray hybridization to bacterial artificial chromosome (BAC) pools to establish "correspondence maps," the assignment of probes to individual BACs. Subsequently, the effectiveness of the algorithms was demonstrated in simulations, and this led to the definition of the experimental parameters required for successful mapping. The applications of correspondence mapping are many and important, as they include finding transcribed regions of the genome, filling gaps in assembled genomic sequence, and integrating the annotations of two probe sets.

The preliminary experiments and simulations are sufficient to indicate that binary partition/hybridization can be used for correspondence mapping, even without reflective representations. Similar ideas can be further generalized to measure distances between every pair of probes (in a statistical sense with accurate error models) and then integrated to assign chromosomal locations to all of the probes. These ideas led to an even more powerful physical mapping algorithm, called linear mapping, and is not discussed here in details (see Casey et al. 2001). The accuracy and resolution of these maps depend on the number of hybridization experiments and the number of probes and can be improved by judiciously increasing these numbers. Other related ideas include statistical models of hybridization needed to measure the copy number fluctuations of various genomic regions of interest

(e.g., genomic amplifications and deletions) (see R. Lucito et al., *Genome Res. 10:* 1726 [2000]).

OPTICAL MAPPING

Optical mapping is another older physical mapping approach that provides an ordered enumeration of the restriction fragments along the genome. The physico-chemical approach underlying optical mapping is based on immobilizing long single DNA molecules on an open glass surface, digesting the molecules on the surface and visualizing the gaps created by restriction activities using fluorescence microscopy. The corrupting effects of many independent sources of errors affect the accuracy of an optical map created from one single DNA molecule but can be tamed by combining the optical maps of many single molecules covering completely or partially the same genomic region and by exploiting accurate statistical models of the error sources. To a rough approximation, the resolution and accuracy of an optical map can be arbitrarily improved by simply increasing the number of enzymes and number of molecules, respectively.

STATISTICAL MODELS AND BAYESIAN ALGORITHMS FOR OPTICAL MAPPING

The main error sources limiting the accuracy of an optical map are either due to incorrect identification of restriction sites or due to incorrect estimation of the restriction fragment lengths. Since these error sources interact in a complex manner and involve resolution of the microscopy, imaging and illumination systems, surface conditions, image processing algorithm, digestion rate of the restriction enzyme, and intensity distribution along the DNA molecule, statistical Bayesian approaches are used to construct an ensemble consensus map from a large number of imperfect maps of single molecules. In the Bayesian approach, the prior conditional pdf models the restriction fragment sizing error in terms of a Gaussian distribution, the missing restriction site event (due to partial digestion) as a Bernoulli trial, and the appearance of false restriction sites as a Poisson process. Using the Bayes' formula, the posterior conditional can be computed and used to provide the means for searching for the best hypothetical model given the set of single-molecule experimental data. Since the underlying hypothesis space is high dimensional and the distributions are multi-modal, a naïve computational search must be avoided. An efficient implementation involves approximating the modes of the posterior distribution of the parameters and accurate local search implemented using dynamic programming (Anantharaman et al., *J. Comp. Biol. 4:* 91 [1997]). The correctness of the constructed map depends crucially on the choice of the experimental parameters (e.g., sizing error, digestion rate, and number of molecules). Thus, the feasibility of the entire method can be ensured only by a proper experimental design, thus guaranteeing successful application of this technology even with low-quality chemical data.

The algorithms developed to create optical maps of clones (cosmids, BACs, and YACs) as well as genome-wide maps of microorganisms (e.g., *Plasmodium falciparum, Escherichia coli, Deinococcus radiodurans*), involve essentially the same Bayesian approach and the same error models, but vary in how they employ various local approximations in controlling the algorithmic complexity (see Aston et al. *Trends Biotechnol. 17:* 297 [1999]; Lin et al., *Science 285:* 1558 [1999]; Lai et al., *Nat. Genet. 23:* 309 [1999]). The current implementation of the Bayesian algorithm "Gentig" and a body of associated software continue to be routinely used by biologists for mapping various pathogens, rice and humans.

BIOINFORMATICS: VALIS AND NYUMAD

Comparative functional genomics relies on the fact that, with advances from modern computation, complex sequences for many different organisms can now be read. Our group's effort is centered around the goal of providing the computational tools to complete this genomics revolution, through the development of computational algorithms and tool kits that will allow biologists to discern, from vast data sets, the very basic genetic similarities and differences among these organisms. Toward this end, our group is constructing and developing a large bioinformatics computer system known as Valis, a unique computing environment consisting of three components: database, hardware, and algorithms. At this point, a pilot hardware component has been built, a detailed database has been designed, and a large software library implementing many crucial sequence analysis algorithms is in place.

Instead of developing each genomic software tool *ab initio*, the Valis team's strategy is to create a base of low-level tools out of which rapid prototyping software can be created. The low-level tools include string

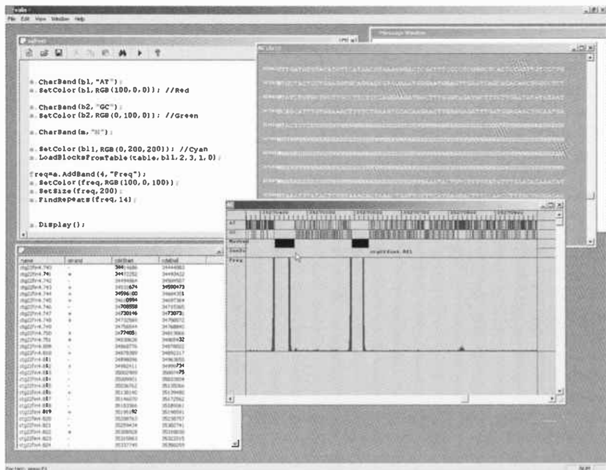

FIGURE 1 A screenshot of the Valis Bioinformatics system being used to analyze repeats. In the top-left corner appear the 12 lines of Valis code (in JavaScript) needed to achieve this task.

algorithms, statistical subroutines, map manipulation routines, image processing routines, visualization widgets, and database tools. A possible high-level application is in large-scale statistical tests to infer the effects of genomic evolutionary processes operating at different scales and with different statistical distributions.

In a related project, this group has also developed a versatile Microarray Database (NYUMAD). Its functionality ranges from the storage of the data in relational database management systems to front-end capabilities for the presentation and maintenance of the data.

The database is a unified platform to understand the microarray-based gene expression data. The data can be output to a wide class of clustering algorithms, based on various "similarity measures" and various approaches to grouping. Particularly, our group has developed a new statistically robust similarity measure based on James-Stein Shrinkage estimators and provided a Bayesian explanation for its superior performance. Additional research is focused on incorporating statistical tests for validation and measuring the significance (e.g., jackknife and bootstrap tests). Finally, the database will be augmented with an experiment design module, which suggests how the future array experiments should be organized, given that one understands how the past experiments have performed. Most of the underlying DB schema design follows closely the specifications put forth by the Microarray Gene Expression Database group (http://cs.nyu.edu/faculty/mishra/ or http://bioinformatics.cat.nyu.edu/), especially when it comes to the XML-based MAML exchange format.

SYSTEMS BIOLOGY

This group has recently initiated a systems biology project with the ultimate aim of providing mathematical and computational tools to study population of cells, individual cells, and intracellular processes. The group's approaches are soundly based on realistic modeling, analysis, simulation, and visualization of these biological processes at multiple scales both temporally and spatially. Their plan is to devise algorithms and implementations that allow both qualitative and

quantitative analyses and combine with the formalisms developed to study hybrid (discrete and continuous time) systems.

Note that although many of the group's approaches are based on well-studied mathematical frameworks arising in the context of logic (modal logic, proof systems, model checkers, etc.), dynamical systems (ODEs, kinetic analysis, control theory, etc.), and computational theory (discrete event systems, hybrid systems, complexity theory, etc.), their main innovations are in effectively integrating these tools to study complex systems at multiple spatiotemporal scales and in automatically designing experiments that can falsify and eventually revise existing models.

The group is currently studying various models of cell-to-cell communication, primarily in the context of cell signaling involved in cancer. The models can then be used to interpret the data generated by cocultivation experiments where cells from two cancer cell lines are cultured separately and then in combination; in each case, the transcriptional states of the cells are measured by microarray experiments in order to generate the data for model-based interpretation.

COLLABORATIONS

The research work described here has benefited from close and productive collaborations with other laboratories: the Wigler lab here at CSHL (array mapping), T.S. Anantharaman and D.C. Schwartz at OpGen and the University of Wisconsin (optical mapping), G. Coruzzi of New York University (NYUMAD project), and the Wigler lab here at CSHL and Weinstein lab at Mr. Sinai School of Medicine (modeling cellular processes).

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PREDICTION OF PROTEIN STRUCTURE AND FUNCTION

A.F. Neuwald A. Poleksic
N. Kannan

Our aim is to better understand cellular processes through computational analysis of the proteins mediating those processes. Because progress in biology is typically driven by technological breakthroughs, we also focus heavily on developing algorithmic and statistical methods that address specific biological questions of interest. This year marked continued progress on two major projects involving the development of such methods. First, we have further developed heuristic procedures relating to hidden Markov models—a mathematical formalism used to represent protein characteristics and thereby predict which sequences are likely to share those characteristics. In addition, we have further developed our CHAIN (*c*omparative *h*ierarchical *a*lignment and *i*nteraction *n*etwork) analysis procedures, which allow us to predict specific structural roles for individual residues within a protein sequence. Progress on the CHAIN analysis procedures has been closely coupled to their application to the study of various protein superfamilies, the most notable of which are Ras-like GTPases. Finally, we have initiated studies of the structural principles underlying protein function, which has helped us both to better understand how specific families of proteins work and to improve our computational methods.

Fast and Sensitive Hidden Markov Model Searches

A. Poleksic, A.F. Neuwald

We are continuing research on incorporating BLAST heuristics into hidden Markov model (HMM) sequence alignment procedures, which is allowing us to speed up various HMM programs by more than an order of magnitude. We recently released a test version of "HMM-BLAST," a program that uses these heuristics to compare a query sequence against a database of HMMs. For an average length sequence, HMM-BLAST is about 20 times faster than comparable non-heuristic programs. Several people affiliated with the Pfam and SMART databases have received copies of this program for testing. We are also performing our own tests on this version of HMM-BLAST, which, because of its speed and its domain architecture visualization capability, allows us to quickly characterize various protein families of interest. At the same time, we are working on further improvements, including the development of a mathematically rigorous heuristic fitting algorithm that will allow us to optimize the search speed and sensitivity for each HMM individually, rather than using standard heuristic settings for every HMM. This heuristic fitting algorithm is also applicable to the standard gapped BLAST/PSI-BLAST program and may therefore lead to significant improvements in this popular sequence analysis tool. We have already found it to be important for HMMs, for which using standard heuristic settings may lead to a loss of sensitivity.

CHAIN Analysis of Ras-like GTPases

A.F. Neuwald, N. Kannan, A. Poleksic [in collaboration with Jun Liu, Department of Statistics, Harvard University]

Another application of the HMM and BLAST heuristic procedures is their incorporation into our CHAIN analysis programs. This is because CHAIN analysis uses the PSI-BLAST search engine, along with other sequence, taxonomic, and structural analysis procedures, to detect, align, and cluster a typically large superfamily of proteins. Most importantly, when structural data are available, it compares conserved patterns in the alignment with corresponding structural features, thereby facilitating prediction of the specific functional roles of individual residues. The CHAIN analysis project is a centerpiece of our research inasmuch as it brings together nearly every developed or acquired tool at our disposal with a view

to fully characterizing specific protein superfamilies. During the last year, for example, we have incorporated a Gibbs sampling multiple alignment procedure into the CHAIN analysis PSI-BLAST algorithm in order to help eliminate sequence alignment errors, which can hinder the analysis. (Gibbs sampling is a powerful statistical optimization method, which, in our applications, helps identify subtle, but biologically significant patterns in sequences.) We have also developed, in collaboration with Dr. Jun Liu, another Gibbs sampling procedure, called Bayesian clustering with column selection, which optimally clusters the aligned sequences into functionally related sets. We have found that both of these Gibbs sampling procedures are important for accurate functional assignment of conserved residues.

A major current application of our CHAIN programs is the analysis of Ras-like GTPases, which function as molecular switches in the regulation of cellular signaling pathways. This analysis reveals that several Ras-like GTPase families, namely, Ras, Rho, Ran, and Rab, share functionally critical sequence and structural features. It also shows that the Rab1 subfamily is most typical of the superfamily, implying that the predecessor of these GTPases was a Rab1-like protein, from which Ras and these other related GTPases were derived via divergent evolution. Interestingly, the conserved structural features shared by these families often involve weak atomic interactions between residues, including CH-O, CH-N, and CH-π hydrogen bonds, whose role in protein structure has only recently been appreciated. This discovery has broad implications inasmuch as this sort of information currently is ignored by structural refinement and homology modeling programs. A similar study, focusing on the Ran family, predicts several specific structural mechanisms underlying Ran's interaction with importin-β, an effector molecule required for its role in nuclear transport, and with its exchange factor RCC1, which functions in Ran activation.

Structural Principles Critical to Protein Function

N. Kannan, A.F. Neuwald

Another focus of our research this year has been the study of basic principles underlying protein structure, which relies heavily on hints contained in multiple sequence alignments. As a starting point in this analysis, we have developed preliminary procedures for searching the sequence and structural databases for conserved patterns that correspond to residues that contact each other in corresponding protein structures. We have also relied on CHAIN analysis to identify structurally conserved features, as was mentioned above regarding weak atomic interactions. These analyses were combined with extensive surveys of the chemical and structural literature. A key discovery is the association of aromatic-assisted proline *cis-trans* isomerization with important protein conformational changes. Aromatic-assisted proline isomerization has been reported for short peptides in the chemical literature in recent years, but it has not yet been reported for proteins, concerning which we now have several striking examples. These include protease inhibitor conformational switching upon binding to their protease targets and Rho-family GTPase effector loop switching in the absence of Mg^{++} and in complex with its exchange factor. As this area of research continues to expand, it should contribute substantially to our protein modeling efforts and, more generally, to our understanding of protein structure and function.

COMMUNITY ACCESS TO GENOME RESOURCES

L.D. Stein A. Arva, P. Carter, K. Chang, N. Chen, A. Day, T. Harris, J. Kakol, L. Lee, E. Nickerson, X. Pan, J. Paolino, R. Sachidanandam, S. Schmidt, L. Teytelman, G. Thorisson, P. Van Buren, D. Ware, G. Wu, D. Wurtz, F. Yen, R. Zhang, W. Zhao

GRAMENE: A COMPARATIVE MAPPING RESOURCE FOR GRAINS

The Gramene database (www.gramene.org) is a comparative mapping resource for rice and other grains. Gramene allows researchers to compare the genetic and physical maps of the major monocot crops, namely, maize, barley, oats, sorghum, and wheat, to the emerging rice genomic sequence. This allows researchers to identify candidate genes in the rice genome that correspond to genetically mapped mutants and quantitative traits in the non-rice crop they are studying. Hence, the resource allows researchers studying traits in maize, barley, and so forth the benefit of genomic sequencing without waiting for the sequencing of these much larger genomes.

In addition to comparative maps, Gramene offers up-to-date genomic annotation of the rice genome, including both predicted and confirmed genes, and the current physical maps of rice and sorghum. We have

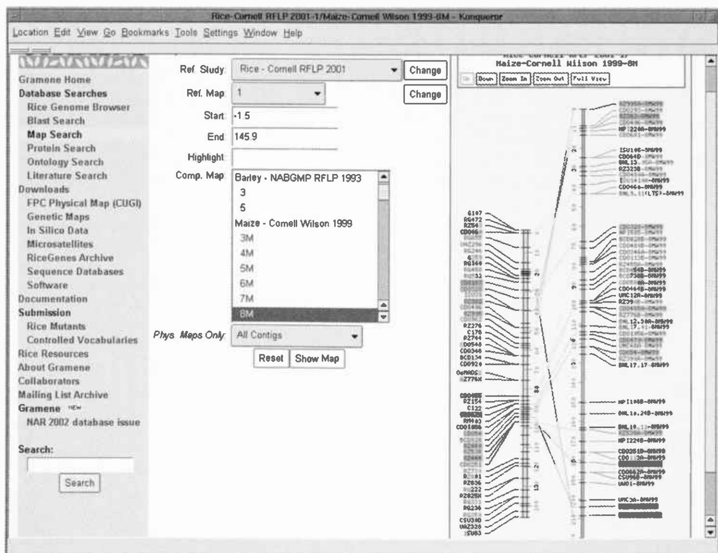

FIGURE 1 The Gramene comparative map viewer relates the genetic map of rice chromosome 1, shown on the left, to the genetic map of maize chromosome 8, shown on the right.

mapped more than 600,000 monocot expressed sequence tags (ESTs) to the rice genome, allowing gene predictions to be further refined based on cross-species comparisons.

Curation is an important component of Gramene. In collaboration with Susan McCouch's laboratory at Cornell, we have curated more than 10,000 rice proteins, assigning them classifications in the Gene Ontology (www.geneontology.org). In addition, we have classified more than 600 rice mutants using a trait ontology that we have developed. We are currently curating the rice biological literature, much of which is in non-English languages, and making this information available via Gramene as well.

Version 1.00 of Gramene went live in December 2001. Much of the software developed for Gramene is novel and includes such useful software modules as a comparative map viewer (Fig. 1), an ontology browser, and a physical map viewer.

The work on Gramene resulted in a publication in *Nucleic Acids Research* in 2001.

A HIGH-DENSITY MAP OF HUMAN SINGLE-NUCLEOTIDE POLYMORPHISMS

Single-nucleotide polymorphisms, or SNPs, are the most common source of genetic diversity in mankind, accounting for more than 90% of the differences between individuals' genomes. These single-base-pair changes occur approximately once every 1300 nucleotides and are usually silent. However, SNPs are of keen medical and research interest because they provide a fast and convenient way of mapping and identifying the genes responsible for genetically linked diseases.

The ability of geneticists to identify the genes responsible for human disease is directly proportional to the density of available genetic maps. The current generation of human genetic maps is based on the older technologies of restriction fragment length polymorphisms (RFLPs) and simple sequence repeats (SSRs). These maps typically contain several thousand mapped markers, providing a resolution of at most 1 Mb. This means that even after establishing tight genetic linkage between a trait and a map marker, the researcher still must search through a million or more bases of DNA sequence data in order to identify candidate genes that might be responsible for the trait.

In contrast, by virtue of the abundance of SNPs, an SNP-based map can contain hundreds of thousands or millions of markers, giving the map a theoretical resolution of a few thousand bases. This means that researchers can skip the tedious search for candidate genes and immediately hone in on the gene of interest. Furthermore, a highly dense genetic map allows researchers to design studies that take advantage of linkage disequilibrium in isolated populations. This technique obviates the need to collect large detailed family pedigrees; instead, it allows geneticists to search for association between an SNP and a genetic trait in the general population.

Other uses for SNPs include the design and development of diagnostic tests for genetically linked disorders, and as a potent tool for studying the evolution and migration of human populations.

Under a grant from The SNP Consortium, our lab is coordinating a large multicenter study to identify SNPs in human sequences. The identified SNPs are verified, archived, and mapped to the human genome using the emerging working draft sequence. After mapping, the data are released into the public domain and to GenBank and other major sequence databases.

To date, we have identified and mapped more than 1.6 million SNPs. These mapped SNPs have been combined with data from other publicly financed SNP-discovery efforts and with gene prediction data from the human working draft, to yield an integrated map containing more than 1,430,000 SNPs. This map contains one SNP every 2.3 kb of DNA on average, and represents a two orders of magnitude increase in resolution over previous maps of the genome. This work resulted in a major publication in the journal *Nature*, "A High-Density SNP Map of the Human Genome" in February 2001.

WORMBASE: A RESOURCE FOR *C. ELEGANS* GENOME AND BIOLOGY

During the past year, our lab received a major grant to develop WormBase, an on-line resource for the small free-living nematode, *Caenorhabditis elegans*. This organism is favored as a simple model animal because of its small genome size, experimental malleability, and well-understood cellular anatomy. WormBase is a curated model organism database developed as part of an international collaboration with the California Institute of Technology, Washington University at St. Louis, and the Sanger Centre. Our lab is responsible for the Web Site, user interface, and software architecture for the project.

The resource, which is available to the public at www.wormbase.org, contains the complete *C. elegans* genome and key annotations, including predicted genes, alternative splicing patterns, oligonucleotide probes, and evolutionarily conserved segments. It also contains many other types of biological information, including the *C. elegans* cell pedigree, the organism's neuroanatomy, its genetic map, and the physical map from which the genomic sequence was derived.

During 2001, we added the following key features to Wormbase:

- An expression pattern browser, which allows researchers to compare the microarray-based expression patterns of genes.
- Information on and the display of transposon-based insertions and single-nucleotide polymorphisms in the *C. elegans* genome.
- A comparative sequence viewer that relates the *C. elegans* genome to the *Caenorhabditis briggsae* genome (Fig. 2). *C. briggsae* diverged from *C. elegans* approximately 100,000 years ago; by comparing the two genomes, one can identify conserved coding and regulatory regions.
- A neuroanatomy browser that allows researchers to explore the synaptic map of the *C. elegans* nervous system.

DISTRIBUTED SEQUENCE ANNOTATION SYSTEM

DAS, the distributed sequence annotation system, is a software architecture that allows researchers from around the world to share and exchange annotations on the genome without the need for the error-prone and time-consuming reformatting that is currently

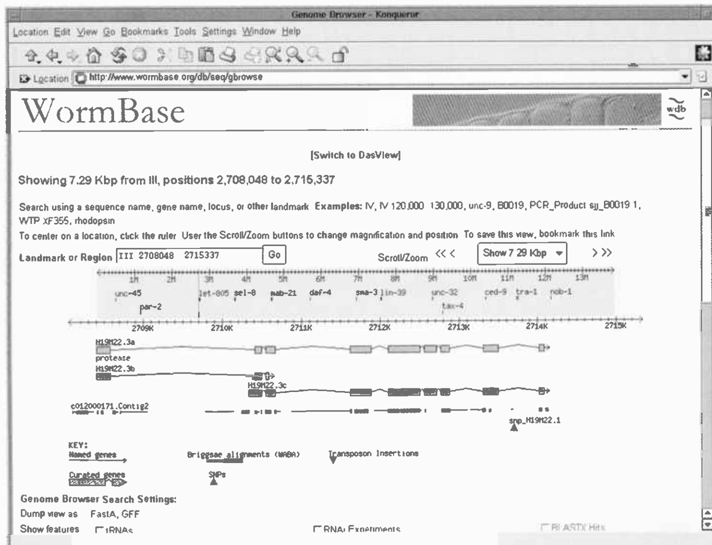

FIGURE 2 WormBase now displays alignments between *C. elegans* and the related nematode *C. briggsae*. The box labeled "c01200171.contig2" shows regions of high and low similarity between the two species and highlights coding regions as well as a potential regulatory region upstream of a gene. Also shown in this view is a single-nucleotide polymorphism (SNP) located in the last intron of the gene.

required. This year saw the widespread adoption of DAS. It is now in use as the data exchange format by the EBI/Sanger EnsEMBL project, the UCSC Genome Browser, TIGR, FlyBase, WormBase, JGI at OakRidge, and the Whitehead Institute.

The year 2001 was marked by the release of LDAS, a lightweight distributed annotation system client, and Omniview (written by our collaborators at Whitehead Institute), a flexible DAS viewer and browser application. The DAS project resulted in a publication in *BMC Bioinformatics*.

GENOME KNOWLEDGEBASE

The Genome KnowledgeBase (GKB) is a new project begun during the summer of 2001. In collaboration with Ewan Birney of the EBI and Suzanna Lewis of the Gene Ontology Consortium, we are developing a Web-accessible resource for curated information about biological processes.

The GKB is organized like a review journal. Practicing biologists are invited to create "summations" that summarize a particular aspect of their field. Early summations that we have worked on are "DNA replication" and "Cell Cycle Checkpoints." Summations are similar to minireviews, except that each paragraph of text is reduced to a series of logical assertions that is entered into a database of processes and macromolecules. The database is then used to drive a Web Site. The Web Site can be browsed like a textbook or searched with queries to discover pathways and connections. The first version of GKB went on-line in September 2001, and it is available at www.genomeknowledge.org.

GENERIC MODEL ORGANISM SYSTEM PROJECT

Another new project is the Generic Model Organism Database Project (GMOD). In collaboration with the model organism system databases FlyBase, SGD, and MGD, the project will be developing a set of database schemas, applications, and interfaces suitable for creating a model organism system database. The hope is to significantly reduce the time and expense required to create new databases to curate genomic information coming out of various model organism system sequencing projects (e.g., rat, *Dictyostelium, Plasmodium*). In 2001, we released the following modules:

- GBrowse, a genome browser and annotation tool.
- Bio::Graphics, a middleware layer for rendering genomic annotations.
- LabDoc, a system for creating and maintaining standard operating procedure documents.

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COMPUTATIONAL GENOMICS

M.Q. Zhang	R.V. Davuluri (Postdoc)	T. Zhang (Postdoc)	Z.Y. Xuan (Postdoc)
	N. Hata (Postdoc)	J.H. Wang (Postdoc)	G.X. Chen (Sci. Prog.)
	N. Banerjee (Grad. Student)	Z.W. Zhu (Grad. Student)	M. Hoffman (URP)
	J. Jaeger (Grad. Student)	E. Shum (PFF Student)	W. Palumbo (PT. Student)
	A. Kel (Visiting Scientist)	I. Grosse	

Dr. T. Zhang left in April for a scientist position at Merck Research Institute and Dr. R.V. Davuluri started as a tenured-track assistant professor at Ohio State University in August. Our research interest continues to be identification and characterization of the genetic elements in nucleic acid sequences by computational means. As the Human Genome Project is about to finish sequencing the human genome, developing efficient computational methods for identification of genes and their control/regulatory elements has become extremely important. Knowing the organization of a gene often becomes the prerequisite for further functional studies. In the past, we studied statistical characteristics of exons and introns in protein-coding regions and developed coding-exon prediction programs by applying multivariate statistical pattern recognition techniques. Recently, we started looking into more difficult problems of finding regulatory *cis* elements in noncoding regions. This year, our main achievement is to develop the FirstEF algorithm. For the descriptions of our collaborative works, see the reports of our colleagues Krainer, Huang, Tully, Wigler, Lowe, and Hannon in this Annual Report.

FirstEF: The First Algorithm for Identification of Human Promoters and the First Exons

R.V. Davuluri, I. Grosse, M.Q. Zhang

Identification of promoters and first exons has been the most difficult problem in gene finding. Here, we present a set of discriminant functions that can recognize structural and compositional features such as CpG islands, promoter regions, and first donor sites. We explain the implementation of the discriminant functions into a decision tree that constitutes a new program called FirstEF. We used different models to predict CpG-related and non-CpG-related first exons. We showed, by cross-validation, that the program could predict 86% of the first exons with 17% false positives. We also demonstrated its prediction accuracy on the genome level by applying it to the finished sequences of human chromosomes 21 and 22 and by comparing the predictions with the experimentally verified first exons. Finally, we presented analysis of the predicted first exons along with the nearest CpG island for all 24 chromosomes of the human genome. Our analysis predicts that there are about 56,000 human genes which almost double the previous estimates.

PEG: A Computer Tool for Automatic Extraction of Promoter Sequences from GenBank

T. Zhang, M.Q. Zhang

Promoter extraction from GenBank (PEG) extracts promoter sequences for large sets of genes using information present in GenBank. For a gene whose promoter sequence is not found, PEG will attempt to extract promoter sequences of the orthologous genes instead.

Use of Chromatin Immunoprecipitation to Clone Novel E2f Target Promoters

T. Zhang, M.Q. Zhang [in collaboration with the P.J. Farnham Lab at the University of Wisconsin Medical School]

Using a ChIP assay, we have cloned nine chromatin fragments that represent both strong and weak in vivo E2F-binding sites. Further characterization of three of the cloned fragments revealed that they are bound in vivo not only by E2Fs, but also by members of the retinoblastoma tumor suppressor protein family and by RNA polymerase II, suggesting that these fragments represent promoters regulated by E2F transcription complexes. In fact, database analysis indicates

that all three fragments correspond to genomic DNA located just upstream of start sites for previously identified mRNAs. One clone, ChET4, corresponds to the promoter region for Beclin1, a candidate tumor suppressor protein. We demonstrated that another of the clones, ChET8, is strongly bound by E2F family members in vivo but does not contain a consensus E2F-binding site. However, this fragment functions as a promoter whose activity can be repressed by E2F1. Finally, we demonstrated that the ChET9 promoter contains a consensus E2F-binding site, can be activated by E2F1, and drives expression of an mRNA that is up-regulated in colon and liver tumors. Interestingly, the characterized ChET promoters do not display regulation patterns typical of known E2F target genes in a U937 differentiation system.

GFScan: A Gene Family Search Tool at the Genomic DNA Level

Z.Y. Xuan, M.Q. Zhang [in collaboration with W.R. McCombie, Cold Spring Harbor Laboratory]

A gene family search tool at the genomic DNA level, GFScan, was developed based on motif and matrix searches. Two human gene families were tested: neurotransmitter-gated ion channels and carbonic anhydrases. All known members of these families with motif regions in genomic DNA were found, whereas some new motif locations were also found that revealed novel members in these families. Compared with other methods, GFScan can find all true positives and with very few false positives at the same time. We also used the human motifs in these two families to search the mouse genome.

Evaluation and Comparison of Clustering Algorithms

G.X. Chen, N. Banerjee, M.Q. Zhang [in collaboration with the Ko Lab, NIA, National Institutes of Health]

Many clustering algorithms have been used to analyze microarray gene expression data. Given the embryonic stem cell gene expression data, we applied several indices to evaluate the performance of several clustering algorithms including hierarchical clustering, k-means, PAM, and SOM. The indices were homogeneity and separation scores, *silhouette width*, redundant score (based on redundant genes), and WADP (testing robustness of clustering results after small perturbation). The results showed that the embryonic stem cell data set posed a challenging situation for the cluster analysis in that the clusters generated by different methods were only partially consistent. Using this real data set, we were able to evaluate the advantages and weaknesses of these algorithms with respect to both internal and external quality measures. This study may provide a guideline on how to select suitable clustering algorithms for a specific data set and can help to raise relevant issues in trying to extract meaningful biological information from microarray expression data.

Large-scale Analysis of Gene Expression Profile in Developmental Human Hippocampus

M.Q. Zhang [in collaboration with the Shen Lab, Chinese Academy of Medical Sciences and PUMC]

The gene expression profile of the developmental human hippocampus is of particular interest and importance to biologists devoted to development of the human brain and development-related diseases. Here we describe an expression profile of 946 different genes in developmental human hippocampus. A comparison of 14 related human neural cells/tissues identified genes highly or specifically expressed in the normal developmental human hippocampus. Some novel findings appeared unique to our results. It is remarkable that *wnt2b* and some disease-related genes (e.g., *stathmin*, *APP*, and *APBP*) are highly expressed; these might be involved in development and differentiation of normal human hippocampus by coordinated effects.

Toward Classification of Tissue-specific Genes and Their Promoters

N. Hata, M.Q. Zhang

One of our primary goals is to understand the gene regulation network in terms of promoter motif interactions. Our approach is to classify gene promoters from genome-wide microarray expression data and then identify promoter motifs common in each gene group by applying a state-of-the-art search algorithm. Since May, we have been working on algorithm development and motif search in tissue-specific gene promoters. Our results include (1) development of statistical tools for exhaustive motif search, (2) rigorous extensive tests of our algorithm with Monte Carlo simulations, and (3) development of graphical tools to visualize

data. We have applied our method to liver- and heart-specific promoters, and our initial results were reported in a poster session of the meeting "Integrating Genome Sequence, Sequence Variation & Gene Expression" (CSHL, September, 2001). Completion of the algorithm and large-scale application to tissue-specific expression data are under way.

On the Origin of Isochores

I. Grosse, M.Q. Zhang [in collaboration with H. Herzel, Humboldt University]

We are working to find the one possible mechanism that could explain the dynamic origin of isochores by using mutation patterns derived from the first draft of the human genome published in Spring 2001. The key element of the dynamic model is the strong G+C dependence of mutation rates. We show that the observed mutation patterns imply an unstable mutational kinetics. This instability together with equilibrium smoothing can induce large-scale patterns of G+C variation. Simulations of the model over a time period of 300 million years show that variations of the G+C content appear on many length scales, resembling the observed heterogeneity in human chromosomes. Moreover, we can simulate the correlations of human and bird isochores by comparing realizations of simulations that diverged 250 million years ago. Finally, we note that the proposed mechanism of isochore origination may explain why G+C variations are much larger in vertebrates than in other organisms: Due to the typically short replication time in nonvertebrate organisms, the G+C dependence of the mutation rate is smaller than that in vertebrates, leading to a stable mutational equilibrium and therefore a lack of large fluctuations of G+C content in nonvertebrate organisms.

DBSD: *Drosophila melanogaster* Binding Site Database

M. Hoffman, Z.W. Zhu, M.Q. Zhang

We generated data from two sources: databases such as those listed above and the primary scientific literature. We wrote Perl programs to extract information from the databases that met our criteria, unify its coordinate system, and verify its correctness. We read about 500 papers from 2000 and 2001 to add new data, although our criteria for inclusion were so strict that the data from only about 60 of those papers were included in the database. We verified data from the primary literature with the same programs we used for databases. We then moved the collected data into a MySQL database. We created an easy-to-use Web interface to the database, allowing others to use the collected data. Information collected in the database for each element includes (1) name of the element, (2) element sequence, (3) gene loci where the element exists and its coordinates relative to the start of the gene, (4) information about the gene and its locus, including links to other Web pages, (5) links to the primary sources of the information in the literature, and (6) links to the secondary source of the information in another database, if we used a secondary source to get the information. The database has many internal links so one can easily find all of the instances of a particular element or all of the elements on a particular gene.

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NEUROSCIENCE

As basic neuroscience research is fueled increasingly by information from the Human Genome Project, we continue to identify the molecular machinery of brain function. At an accelerating pace, this effort is beginning to reveal the biological bases of various forms of cognitive dysfunction and disease. Molecular-genetic studies recently have suggested that four types of hereditary mental retardation—Down's syndrome, Fragile X syndrome, Rubinstein-Taybi syndrome, and Neurofibromatosis—actually may result from biochemical defects fundamental to normal neuronal function. Work here at CSHL continues to contribute to this emergent view of cognitive dysfunction. Dr. Yi Zhong and co-workers have established a link between the neurofibromatosis-1 (NF1) protein and cAMP biochemistry (involved in long-term memory formation), and Dr. R. Malinow's group is investigating a novel role for β-amyloid peptide (associated with Alzheimer's disease) in the regulation of neuronal activity.

To date, we all have assumed that "mental retardation" results from a development defect and, thus, is not treatable. If the emerging "biochemical hypothesis" holds true, however, traditional pharmaceutical intervention in fact may help to ameliorate the cognitive dysfunction associated with these patients.

COMPUTATIONAL SYSTEMS

C.D. Brody

We pursued three research projects during 2001. The first is a theoretical exploration of computational roles for spike time coding in networks of neurons. Neurons mostly communicate through events that are punctate in time (spikes). What implications does this have for computation? The second project focuses on analyzing data from awake behaving monkeys performing a memory and decision task. Our goal is to elucidate the nature and mechanisms of decision-making processes in the brain. A third project, related to the second one, has been initiated. It combines the development of neural network models of short-term memory with experimental tests of these models, using human psychophysics.

SPIKE TIMING, COMPUTATION, AND PLASTICITY

In the past, our ideas regarding the working of the brain have often proceeded by analogy with the most complex man-made systems available at the time: For example, in the nineteenth century, the brain was sometimes referred to as a clock-like mechanism, and, more recently, the brain is often compared to a computer. Yet many prominent aspects of brain function have only few or distant parallels to man-made systems. One of the most notable of these aspects is that neurons mostly communicate with each other following punctate events in time, known as "spikes" or "action potentials." Few man-made systems share this property; as a consequence, our intuitions regarding the computations that can be carried out by large networks of elements communicating in such a fashion is limited. Using computer simulations of networks of spiking neurons, we and John Hopfield (Princeton University) have searched for computations that are easily and robustly implemented using spike timing, but which would be much more difficult to carry out without it. We have described a computation that we referred to as a "many are equals" spike-timing-based computation (Hopfield and Brody, *Proc. Natl. Acad. Sci. 97:* 13919 [2000]; Hopfield and Brody 2001). Connections between model neurons can be easily set up such that the neurons synchronize their spike generation times only when the neurons are driven to similar spiking rates; spike timing synchronization can thus be used as an indicator of similarity across neurons. We illustrated the computational power of this spike-timing-based operation by building a small simple network of neurons that was nevertheless capable of a very complex computation, recognizing the spoken word "one." This network's wiring and connectivity pattern were handcrafted by us. But in real biological systems, networks are automatically wired up, through what are often referred to as "learning rules." These are the mechanisms that would lead a network, when exposed to external stimuli, to appropriately modify its connections into instantiating a desired computation. During 2001, we focused our attention on the question: What biologically plausible learning rules would automatically wire up our network? (Brody and Hopfield 2001). We have again explored our ideas through computer simulations and have found a local, spike-timing-dependent learning rule that allows the right connections to develop automatically, so that repeated exposures to the spoken word "one" are sufficient to generate a network that recognizes this particular word. Our learning rule is illustrated in Figure 1, and it is remarkably similar to an

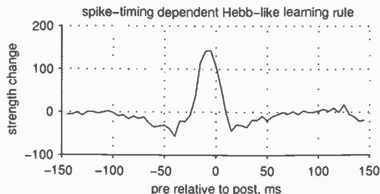

FIGURE 1 Local learning rule for constructing a network that recognizes the spoken word "one" using spike-timing-based computation. This rule is applied to all connections from one neuron ("presynaptic neuron") to another ("postsynaptic neuron"). The graph represents the change in synaptic strength connecting one neuron to another following a pair of spikes, one from each neuron. The two neurons are called the "presynaptic" neuron and the "postsynaptic" neuron, respectively, and the time of the presynaptic spike relative to the postsynaptic spike determines whether the synapse should increase or decrease in strength.

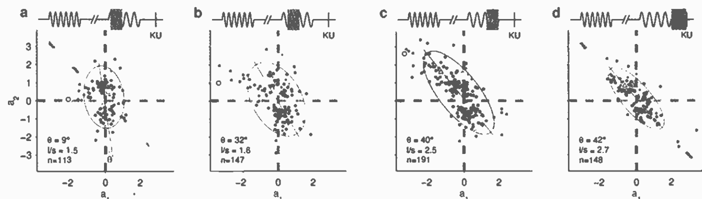

FIGURE 2 Neuronal responses in somatosensory area S2 to the second stimulus of a sequential stimulus comparison task. Two vibrotactile stimuli (f1 and f2) were applied to the tip of a finger. In each panel, each dot represents the response of one neuron to the second stimulus, during the time schematically indicated by the grey box in the inset above the panel. Responses were fit as linear functions of f1 and f2, firing rate = a1*f1 + a2*f2, and the resulting fit coefficients a1 and a2 are shown. As the analysis window moves further in time from panel a to panel d, responses become more closely aligned with the a2 = -a1 axis. See text for further explanation.

experimentally observed learning rule (Zhang et al., *Nature 395:* 37 [1997]). More recently, in collaboration with Zachary Mainen here at CSHL, we have also turned to mapping our "many are equals" computational operation to properties and computations of the olfactory bulb; we are now investigating how different task contexts (word recognition, olfaction) affect the learning rule that we derived.

SENSORY COMPARISON AND DECISION PROCESSES

In collaboration with the awake monkey experimental laboratory of Ranulfo Romo at the National University of Mexico, we have investigated various aspects of a somatosensory discrimination task. Two 500-msec-long mechanical vibrations, separated from each other by 3 seconds, were applied sequentially to the tip of a monkey's finger. The monkeys were trained to compare the two vibrations. They had to decide which one had the higher frequency of vibration, and then indicate their decision by pressing, with their unstimulated free hand, one of two pushbuttons in front of them. Previously, we investigated the short-term memory component of this task (to compare the two stimuli, the monkey must remember the frequency of the first one during the delay between the two; Romo et al., *Nature 399:* 470 [1999]). During 2001, we focused on neuronal responses during the second stimulus period, when the monkey is comparing the two stimuli and deciding which of the two is the higher. How does this comparison and the ensuing decision take place? It has generally been thought that such decision-related processes take place in higher-level areas of cortex. Neurons of the prefrontal cortex (a high-level area) and of the second somatosensory cortical area (S2, a relatively early sensory cortex) were recorded while the monkeys carried out the task. Since S2 is an early somatosensory cortex, we expected neurons in S2 to simply encode the first stimulus (which we call f1) during the first stimulus period and encode the second stimulus (f2) during the second stimulus period. Because of the long delay between the two (3 seconds), we expected no interaction between them. However, we found that many neurons in S2 appeared to encode the comparison between f1 and f2 and, in addition, encoded the monkey's resulting decision. We found this by analyzing the neuronal data by fitting each neuron's firing rate response as a linear function of both f1 and f2. The coefficients of these fits are shown, for different time windows during and after the second stimulus, in Figure 2. As time progressed, neuronal responses gradually became a function of f2-f1 (see Fig. 2c,d, where data points are aligned along the diagonal dashed line that indicates the f2-f1 axis). Note that correct performance in the task depends on the sign of f2-f1. In other words, during the second stimulus period, the responses of S2 neurons gradually became correlated with the comparison between f2 and f1 that was necessary for the monkey to make its decision. Responses similar to those illustrated in Figure 2

were also recorded in the prefrontal cortex. Since such responses depend on f1, and information about f1 is not stored in S2, our results suggest that an important role for feedback pathways to early sensory areas (such as S2) may be to allow sensory areas to participate directly in sensory comparisons and decisions.

MODELING AND PSYCHOPHYSICS OF SHORT-TERM MEMORY

The sensory discrimination task described in the previous section involves a short-term memory component: Subjects that compare two stimuli presented sequentially in time must maintain a memory of the first stimulus during the time delay between the two stimuli. We have begun efforts to develop models of networks of neurons that can actively maintain such information in the neurons' firing rates. Some of the generic properties of such models have implications that can be tested in human psychophysical experiments. For example, when remembering a stimulus presented as a continuous analog variable (such as the frequency of a mechanical vibration), it has been widely assumed that a corresponding continuum of possible memories is possible. Yet networks that allow a continuum of memories are far less robust than networks that allow only a discrete set of possible memories. This has motivated us to search for signatures of discreteness observable in psychophysical experiments; we are setting up to specifically test for such signatures.

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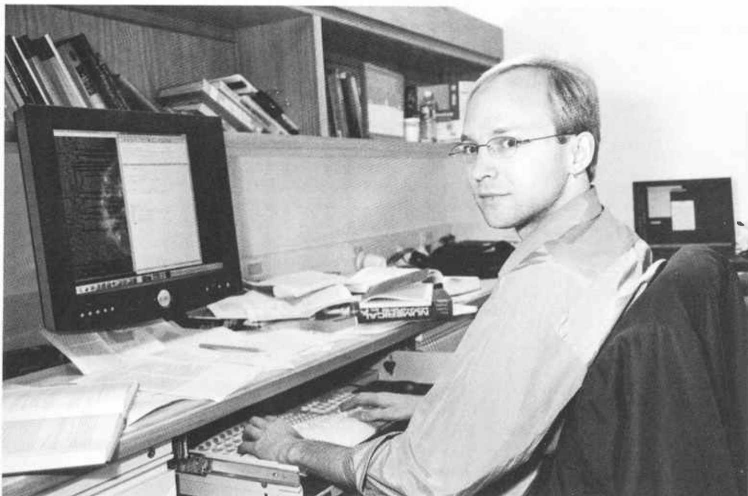

Christian Machens

PRINCIPLES OF BRAIN DESIGN

D.B. Chklovskii A. Stepanyants

Our goal is to understand the function of the human brain. We pursue this goal by building quantitative theories that account for existing observations and make experimentally testable predictions. Our main focus is on the physical constraints of brain design. The idea is that any computational strategy used in the brain must be implemented in biological hardware. Because of the high computational complexity of many brain functions, this hardware is very complex. Therefore, evolution had to solve difficult engineering problems to come up with a working model. By analyzing various constraints on the brain design, we hope to understand which computational strategies the brain is using. One important constraint on the brain design is the space, or the so-called wiring economy principle. By using this principle, we were able to explain many features of brain design: from neuronal arbor morphology to cortical maps. Recently, we started exploring another important factor in brain design: plasticity potential of the neuronal circuits. Because the genes cannot foresee the precise environment that the organism is born into, the organism must be adaptable. Adaptability of the neuronal circuits is usually referred to as plasticity.

Direction Preference Maps in the Visual Cortex: A Wire Length Minimization Approach

D.B. Chklovskii [In collaboration with A.A. Koulakov, University of Utah]

Neurons in the mammalian visual cortex respond to the movement of objects across their receptive field. Direction of movement, which evokes the most vigorous response, determines the direction preference of a neuron. The spatial arrangement of direction of motion preference on the cortical surface is known as the direction preference map. Maps of direction preference vary in appearance between species and cortical areas. We propose that the observed variability in map appearance reflects the variability in intracortical circuits as a consequence of wire length minimization. This hypothesis allows us to bypass a detailed developmental analysis of map formation. We solve the layout optimization problem numerically for various intracortical circuits and obtain direction preference maps that reproduce the observed variability in map appearance. These results allow one to predict intracortical connectivity from the appearance of direction preference maps.

Search for Repeating Motifs in Biological Networks

D.B. Chklovskii [In collaboration with U. Alon, Weizmann Institute]

The functioning of living organisms relies on numerous complex networks: from gene transcription regulation to neuronal circuits in the brain. Although these networks have been mapped out in simpler cases: *Escherichia coli* (transcription regulation) and *Caenorhabditis elegans* (synaptic connectivity), their function is not understood. Identifying functional modules out of subsets of the whole network may help our understanding of these networks. The goal of this project is to search for functional modules including several neurons in *C. elegans*. The hope is that the modules which have a particular functional role are present more often than by chance. To find them, we perform a statistical analysis on a real network and compare the outcome with a randomized network. So far, it seems that some connectivity motifs are statistically significant.

Branching Law for Axons

A. Stepanyants, D.B. Chklovskii

Multicellular organisms have solved the problem of efficient transport of nutrients and communication between their body parts by evolving spectacular networks: branching trees, blood vessels, bronchs, and neuronal arbors. These networks consist of segments bifurcating into thinner and thinner branches. Understanding of branching in transport networks has been advanced through the application of the opti-

mization theory. Here, we apply the optimization theory to explain the caliber of branching segments in communication networks, i.e., neuronal axons. We pursue the hypothesis that the axonal caliber has evolved to minimize signal propagation delays, while keeping arbor volume to a minimum. We show for a general cost function that the optimal diameters of mother (d_0) and daughter (d_1,d_2) branches at a bifurcation obey a branching law: $d_0^{\nu+2} = d_1^{\nu+2} + d_2^{\nu+2}$. The derivation relies on the fact that the conduction speed scales with the axon diameter to the power ν ($\nu = 1$ for myelinated axons and $\nu = 0.5$ for nonmyelinated axons). We tested the branching law on the available experimental data and found a reasonable agreement.

Relationship between Neurite Diameters and Branching Angles in Cultured Neurons

D.B. Chklovskii [In collaboration with A. Ayali, Tel-Aviv University]

The wiring economy principle predicts a relationship between branching angles of neurites and their diameters. This prediction can be derived by balancing virtual forces on neurite branches and is reminiscent of the cosine theorem. However, testing this prediction in vivo is problematic because of the high density of entangled neurite branches. Therefore, we decided to perform the test in a low-density neuronal culture. Neurite diameters can be measured with high precision by scanning electron microscope, whereas angles are easily available from optical microscopy. This allows us to compare the measured angles and diameters with the theoretical predictions.

Structural Plasticity of Dendritic and Axonal Branching

A. Stepanyants, D.B. Chklovskii

Dendritic and axonal arbors come in a dazzling variety of different shapes that had been known for more than a century but remained unexplained. We have shown in the past that the wiring economy principle explains the sizes (diameters) of arbors as well as the typical mesh size. However, the topology of arbors as characterized by the branching structure remains unconstrained. We pursue the hypothesis that the branching structure of neuronal arbors is determined by the plasticity requirements on synaptic connectivity. Indeed, neuronal arbors must accommodate the inevitable variability in possible locations of appropriate presynaptic inputs. Branching of the processes enhances plasticity because branches can be formed or eliminated locally, without global remodeling of the arbor shape. We developed two models describing arbor plasticity: one is potentially more appropriate for developmental plasticity, and the other, for adult plasticity. Our predictions can be tested in the experiments done by the Svoboda lab here at CSHL.

Optimal Stimulus Design

D.B. Chklovskii, K. N'Diaye

Neurons in the brain respond to sensory stimuli by firing action potentials. The firing rate of a particular neuron depends on which stimulus is presented to the animal. Stimulus to which a neuron responds with the highest firing rate is called the optimal stimulus. Therefore, a neuron can be thought of as a detector for an optimal stimulus. Although we know what the optimal stimulus is for some neurons, for the majority of neurons, it remains unknown. Our goal is to design a computer algorithm that would automatically search for an optimal stimulus (Fig. 1). This is a very difficult task because the dimensionality of the stimulus space is extremely high. We are currently designing an appropriate algorithm and testing it against a computer model of a neuron with a known optimal stimulus.

Contribution of the Dendritic Spines to the Structural Plasticity: A Comparative Study

A. Stepanyants, D.B. Chklovskii [In collaboration with P.R. Hof, Mount Sinai School of Medicine]

Learning and memory rely on changes in neuronal circuits, or plasticity. One possible mechanism of plasticity is a dendritic spine retracting from a current presynaptic axon and extending toward another axon. Such switching of presynaptic partners is only possible if within a spine length of a dendrite there are available axons, which do not have synapses with the dendrite. The availability of such adjacent axons relies on their abundance relative to the number of spines on a given dendrite (Fig. 2). If the number of axons within a spine length of a dendrite is equal to the number of spines (Fig. 2B), then there are no available presynaptic partners and spine remodeling cannot contribute to circuit reorganization. If the number of adjacent axons is greater than the number of spines (Fig. 2C), then spine

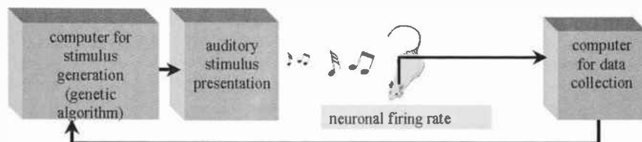

FIGURE 1 Schematic diagram showing procedure for stimulus optimization using a genetic algorithm. A computer generates an ensemble of auditory stimuli (*left box*), which are then presented to a rat (*middle box*) whose neuronal activity is being monitored (*right box*). The neural activity is then fed back into the stimulus generator, where it is used to assess the fitness. Note that although the example described here is auditory, the approach is general.

remodeling can contribute to circuit reorganization (Fig. 2D). To determine which scenario (Fig. 2, B or C) better reflects the real brain, we have derived a mathematical expression for the ratio of actual synapses to adjacent axons, which we call the filling fraction, in terms of anatomical parameters that can be measured by light microscopy. We found that the filling fraction in mammalian cortical structures (mouse neocortex, rat hippocampus, and macaque visual areas) is about 0.2. This means that there are roughly five times more available axons than spines. Therefore, spine remodeling can contribute to reorganization of cortical circuits. In addition, we found significant differences in the filling fraction between different V1 and other visual areas of macaque.

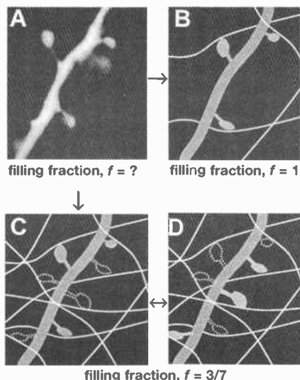

FIGURE 2 Spine remodeling as a mechanism for circuit reorganization. (*A*) Spiny dendrite in macaque neocorten visualized by light microscopy. (*B*) If the number of adjacent axons is equal to the number of spines (filling fraction equal to one), spine remodeling cannot contribute to circuit reorganization. (C) If the number of adjacent axons is much greater than the number of spines (low filling fraction), spine remodeling can contribute to circuit reorganization. Dashed contours show possible spine locations. (*D*) Reorganized circuit obtained from C through spine remodeling.

Spontaneous Monocular Deprivation in the Vicinity of Vertical Meridian Representation in the Visual Cortex

D.B. Chklovskii [In collaboration with M. Rosa, Monash University]

It is hard to overestimate the importance of the Blackford bar for the scientific progress at Cold Spring Harbor Laboratory and the rest of the world. This project was started by the observation of an interesting feature in the ocular dominance pattern, which can be observed in a photograph hanging on the wall of the bar. In particular, the representation of the two eyes in the vicinity of vertical meridian is biased: One eye occupies a greater fraction of the territory than the other. We propose that this bias is a reflection of a decussation asymmetry in the optic chiasm. This project explores whether this asymmetry is a developmental error or a manifestation of a stereopsis strategy.

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ACTIVITY-DEPENDENT NEURONAL DEVELOPMENT

H.T. Cline C.D. Aizenman, C. Akerman, K. Bronson, I. Cantallops, R. Ewald, E. Fingar (URP), L. Foa, K. Haas, A. Javaherian, K. Jensen, Z. Li, E. Rial Verde, E. Ruthazer, W.-C. Sin

We are interested in understanding the function of neuronal activity in brain development. We address this issue by examining the development of the visual system in amphibian tadpoles. These animals are transparent, which allows us to observe brain development in the living animal. Many of the experiments we perform involve in vivo imaging of neuronal structure over periods up to several weeks. In addition, we assess neuronal function using electrophysiological assays of synaptic connectivity and synaptic plasticity. We combine these studies with gene transfer methods that allow us to test the function of genes of interest in brain development. This range of technical approaches allows us to identify key regulatory mechanisms governing the development of brain structure and function.

Our experiments have demonstrated the important role of synaptic activity in promoting the development of the brain. Therefore, we are devoting considerable effort to determine the mechanisms of synapse formation and the role of excitatory and inhibitory inputs in the formation of retinotectal connections. Another potential effect of synaptic activity is to induce gene transcription. The activity-induced genes may then promote the further development of the brain. We have continued our investigations of several activity-induced genes to test their potential function in brain development.

Electroporation-mediated In Vivo Gene Transfer

K. Haas, L. Foa, A. Javaherian, K. Jensen, Z. Li, W.-C. Sin, H.T. Cline

Electroporation is becoming a popular technique for gene delivery into neurons within intact tissue. One advantage of electroporation over other transfection techniques is the ability to target a particular brain region, or even single neurons, for transfection. Several members of the lab have continued our efforts to optimize electroporation-mediated gene transfer. Kurt Haas, working with Ashkan Javaherian, Kendall Jensen, Zheng Li, and Wun Chey Sin, has improved methods for single-cell electroporation into neurons within the optic tectum and spinal cord of living *Xenopus* tadpoles. Kurt Haas has also worked with several scientists visiting Cold Spring Harbor Laboratory in an effort to optimize electroporation into cells in other preparations, including intact chicken embryos and tissue slices from avian and mammalian brain and spinal cord. K. Jensen and L. Foa have developed methods to restrict transfection to clusters of cells, or to include large portions of the brain or retina of the intact *Xenopus* tadpole. Restriction of transfection to individual cells aids in imaging of neuronal morphology, and bulk cell transfection allows examination of the effects of gene expression on populations of cells by biochemical assays, imaging, and electrophysiological recording.

Regulation of RhoA GTPases by Cross-talk and Neuronal Activity In Vivo

Z. Li, C.D. Aizenman, H.T. Cline [in collaboration with L. Van Aelst, Cold Spring Harbor Laboratory]

Proper development of neurons depends on synaptic activity, but the mechanisms of activity-dependent neuronal growth are not well understood. The small GTPases, RhoA, Rac, and Cdc42, regulate neuronal morphogenesis by controlling the assembly and stability of the actin cytoskeleton. Li previously demonstrated that expression of mutant forms of GTPases affected tectal cell dendritic arbor development in vivo. This year, Li developed an in situ method to determine endogenous Rho GTPase activity in intact *Xenopus* brain. She used this method to provide evidence for cross-talk between Rho GTPases in optic tectal cells in vivo. Moreover, Li demonstrated that cross-talk between the Rho GTPases affects dendritic arbor

development in the intact animal. Finally, Li and C. Aizenman demonstrated that optic nerve stimulation regulates Rho GTPase activity in a glutamate receptor-dependent manner. These data suggest a link between glutamate receptor function, Rho GTPase activity, and dendritic arbor growth in the intact animal.

Visual Stimulation Regulates Dendritic Arborization by a Mechanism Requiring NMDA-R Activation and Rho GTPases In Vivo

W.-C. Sin, K. Haas, E. Ruthazer, H.T. Cline
[in collaboration with L. Van Aelst, Cold Spring Harbor Laboratory]

The mechanisms by which sensory input shapes neuronal development are not known. W.-C. Sin demonstrated by in vivo time-lapse imaging of *Xenopus laevis* tadpoles that enhanced visual activity driven by a light stimulus promotes optic tectal cell dendritic arbor growth. K. Haas collected images of optic tectal neurons once an hour over 8 hours with the 2-photon microscope. These images allowed us to analyze arbor dynamics during light-induced dendritic arbor growth. The data show a rapid transition in rates of branch dynamics when animals are exposed to visual stimulation. The data analysis of these four dimensional data sets was carried out using macros conceived and written by E. Ruthazer. The stimulus-induced dendritic arbor growth requires glutamatergic synaptic transmission, decreased RhoA activity, and increased Rac activity. Furthermore, we know from the experiments of Li and Aizenman (see above) that increasing synaptic activity with optic nerve stimulation down-regulates endogenous RhoA activity and increases endogenous Rac activity. The results delineate a role for Rho GTPases in the structural plasticity driven by visual stimulus in vivo.

Competition-based Dynamics of Retinotectal Axon Morphology in *Xenopus* Optic Tectum

E. Ruthazer, H.T. Cline

Coactive retinal input activity is required to maintain the topographic retinal projection into the optic tectum and other sensory projections; however, the requirement for patterned afferent activity in the establishment of topographic projections is not clear. E. Ruthazer has carried out experiments to test the requirement for afferent activity in the formation of organized retinotopic projections. He uses *Xenopus* tadpoles in which both retinas are forced to innervate the same optic tectum. Under these conditions, the inputs from the two eyes gradually segregate out to form eye-specific innervation zones. E. Ruthazer used time-lapse imaging with the 2-photon microscope to observe the morphological rearrangements that occur in retinal axon arbors during competition-based afferent segregation. He finds that axon branches are selectively stabilized in regions innervated by other axons from the same eye, i.e., regions in which there is a relatively high degree of afferent coactivity. In contrast, branches are rapidly retracted from zones in which the dominant input is derived from the other eye, i.e., regions of low coactivity. The selective stabilization is blocked by *N*-methyl-D-aspartate receptor (NMDA-R) antagonists. This indicated that the NMDA type of glutamate receptor is required for the selective stabilization of axon branches during the initial formation of eye-specific zones in the optic tectum.

Visually Driven Modulation of Glutamatergic Synaptic Transmission by Intracellular Polyamines

C.D. Aizenman, G. Muñoz, H.T. Cline

Sensory-driven, activity-dependent mechanisms have been implicated in brain development, yet little is known about short-term effects of enhanced sensory experience. C. Aizenman examined the effects of 4 hours of increased visual stimulation on retino-tectal synapses of developing *Xenopus* tadpoles. Tectal neurons have Ca^{++}-permeable α-amino-3-hydroxy-5-methyl-4-isoxazole receptors (AMPA-Rs), as revealed by kainate-induced Co^{++} labeling. These receptors are inwardly rectifying due to an interaction of intracellular polyamines with negatively charged residues in the channel pore. Whole-cell voltage-clamp recordings of optic tectal neurons revealed varying amounts of inward rectification in AMPA-R-mediated excitatory postsynaptic currents (EPSCs). Inward rectification was increased by adding the polyamine, spermine, to

the recording pipette. This suggests that endogenous polyamine concentrations are not saturating with respect to the AMPA-R block and are a possible mechanism for synaptic modulation. A 4-hour period of enhanced visual stimulation also resulted in an overall increase of inward rectification, an effect that was occluded by recording with spermine in the pipette. Ornithine decarboxylase (ODC), the rate-limiting enzyme in polyamine synthesis, is increased with activity. Treating tadpoles with difluoromethyl ornithine (DFMO), an ODC blocker, during light stimulation also prevented light-induced changes in rectification. Together, the data suggest that light can regulate AMPA-R-mediated transmission via the polyamine synthesis pathway. This may function to compensate for increased levels of activity experienced during visual stimulation and thus maintain a constant level of excitation.

Role of GABAergic Transmission in Visual System Development

C. Akerman, H.T. Cline

We are using the *Xenopus* optic tectum to study the development and interactions of neurotransmitter systems during the construction of a functional neuronal circuit. Previous electrophysiological studies in the laboratory have focused on synapses that use glutamate, the major excitatory neurotransmitter in the brain. These studies showed that glutamatergic synapses follow a developmental sequence in which immature synapses use predominantly one type of receptor called the NMDA-R. As these synapses mature, they acquire a different type of glutamate receptor called the AMPA-R. Using electrophysiological methods, we have now shown that during the same period of development, tectal cells also express receptors for two further neurotransmitters—glycine and GABA (γ-amino-*n*-butyric acid). Classically, when these receptors are activated in the mature brain, they generate an inhibitory effect. However, our preliminary studies indicate that intracellular levels of ions in the immature tectal cells could actually make these receptors excitatory. Consistent with this, our immunohistochemistry has shown that these cells do not yet express the ionic transporters that are believed to make these transmitters inhibitory. We are now in a position to test directly the function of these transmitter systems in the development of the tectal circuit.

CPG15 Regulation of Circuit Development In Vivo

I. Cantallops, M. Davenne, A. Javaherian, K. Bronson, H.T Cline [in collaboration with E. Nedivi, Massachusetts Institute of Technology]

The activity-regulated candidate plasticity gene 15 (*cpg15*, also known as neuritin) encodes a glycosylphosphatidylinositol (GPI)-linked protein. E. Nedivi, G.-Y. Wu. and H.T. Cline previously demonstrated that CPG15 expression in vivo increases the dendritic arbor growth rate of *Xenopus* optic tectal cells. I. Cantallops has shown that CPG15 expression enhances retinal axon arbor elaboration, and K. Haas has demonstrated that CPG15 promotes retinotectal synapse maturation. A thorough understanding of CPG15 function requires knowledge of the spatiotemporal expression of the endogenous protein. A. Javaherian cloned *Xenopus cpg15* and together with E. Nedivi used RNA in situ hybridization and immunohistochemistry to determine the pattern of CPG15 expression. *cpg15* mRNA and CPG15 protein are first detectable in the developing spinal cord and become widespread as development proceeds. CPG15 is expressed in sensory regions of the brain, including the visual, auditory, and olfactory systems. Within the retina, CPG15 is only expressed in retinal ganglion cells. CPG15 protein is concentrated in axon tracts, including retinal axons. These data support a model in which CPG15 expressed in retinal ganglion cells is trafficked to retinal axons, where it modulates postsynaptic dendritic arbor elaboration and synaptic maturation. To pursue the mechanisms by which CPG15 carries out its growth-promoting action, I. Cantallops has been examining the trafficking of CPG15 in retinal axons and M. Davenne has attempted to reduce CPG15 expression in the retina.

Role of Homer Proteins in Axon Pathfinding In Vivo

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

Axon pathfinding during early stages of central nervous system (CNS) development is essential to establish the fundamental connectivity of the brain. Mechanisms of axon pathfinding include receptor-mediated recognition of attractive and repulsive cues

in the environment, which induce changes in the direction of the axonal growth cone. Homer proteins are a family of multidomain cytosolic proteins which have been postulated to serve as scaffold proteins that affect responses to extracellular signals by regulating protein-protein interactions. One isoform of Homer proteins, called Homer1a, is induced by activity in the rodent and *Xenopus*. L. Foa tested whether Homer proteins have a role in axon pathfinding in vivo by expressing both wild-type and mutant isoforms of Homer in *Xenopus* optic tectal neurons. Time-lapse imaging demonstrated that interfering with the ability of endogenous Homer to form protein-protein interactions resulted in axon pathfinding errors at stereotypical choice points.

These data demonstrate a novel function for scaffold proteins such as Homer in axon guidance. Homer may facilitate signal transduction from cell surface receptors to intracellular proteins that govern the establishment of axon trajectories.

Regulation of Cell Proliferation in *Xenopus* Brain by Nitric Oxide

H.T. Cline [in collaboration with N. Peunova, V. Scheinker, and G. Enikolopov, Cold Spring Harbor Laboratory]

The regulation of the progression from proliferation to differentiation in the population of precursor cells has direct bearing on the formation of a functional nervous system. Nitric oxide (NO) may mediate such a transition, since it can suppress DNA synthesis and cell proliferation. We cloned the gene encoding the neuronal isoform of *Xenopus* NO synthase (XNOS) and found that in the developing brain of *Xenopus* tadpoles, a zone of XNOS-expressing cells lies adjacent to the zone of dividing neuronal precursors. Exogenous NO, supplied to the tadpole brain in vivo, decreased the number of proliferating cells and resulted in an overall decrease in the total number of cells in the optic tectum. Conversely, inhibition of NOS activity in vivo increased the number of proliferating cells and the total number of cells in the optic tectum. This NOS inhibition yielded larger brains with grossly perturbed organization. Our results indicate that NO is an essential negative regulator of neuronal precursor proliferation during vertebrate brain development.

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SIGNAL TRANSDUCTION AND DIFFERENTIATION

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T. Michurina
J. Mignone
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N. Nakaya
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N. Peunova
V. Scheinker
Y. Stasiv
A. Vaahtokari
T. Sonbuchner (URP)

Our laboratory is interested in signaling pathways that control transitions through the differentiation cascade. During the last several years, we have demonstrated that nitric oxide (NO), a versatile signaling molecule, is an important regulator of progression of stem and progenitor cells through the cascade. Part of this regulation is due to the antiproliferative properties of NO that we have demonstrated in several developmental contexts. We are continuing to work on *Drosophila* and *Xenopus*, and we are characterizing the role of NO in mammalian development, with a focus on hematopoiesis and neurogenesis. We are now concerned with identifying the molecular and genetic interactions of the NO signals with the major signaling pathways in differentiating cells.

Our studies of signaling during development have led us closer to questions about the biology of stem and progenitor cells, which have now become an important new area of interest for our laboratory. We have generated several mouse models in which selected classes of cells in the brain are marked, and we are using these animals to investigate the origins and plasticity of stem cells.

NO and Mouse Hematopoiesis

T. Michurina, P. Krasnov

The proliferative activity of hematopoietic stem cells in the bone marrow is highly restricted. We wondered if NO has the potential to mediate stem cell quiescence since it can alter gene expression and effectively suppress cell proliferation. We found that the population of Sca-1$^+$ cells in mouse bone marrow is strongly enriched in NO synthase (NOS) mRNA and protein. When mice are exposed to inhibitors of NOS after bone marrow transplantation, there is an increase in the proportion of bone marrow cells carrying markers of stem and early progenitor cells. Retransplantation of bone marrow from animals treated with NOS inhibitors after primary transplantation demonstrates that the fraction of early hematopoietic precursors capable of forming colonies in the spleens of the secondary recipients (pre-CFU-S) is also increased. Upon cessation of treatment with NOS inhibitors, this increase is shortly followed by an increase in the number of neutrophils in the peripheral blood of the animals. Furthermore, exposure to NOS inhibitors also strongly affected erythroid and granulocytic differentiation of bone marrow cells and resulted in the accumulation of less mature blast cells. Our results suggest that NO may act to restrict progression through the cell cycle at both early and late stages of the cascade of hematopoietic differentiation.

NO and Neurogenesis in the Adult Brain

M. Packer [in collaboration with S. Goldman, Cornell Medical School]

A large number of neurons are continuously generated in the postnatal mammalian brain. The signaling messengers that regulate the number of dividing cells in the adult brain are largely unknown. We blocked production of NO by intraventricular infusion of NOS inhibitor and found that the number of new cells in both neurogenic and nonneurogenic areas of the adult brain was greatly increased. Most of these newly generated cells express neuronal markers. Importantly, inhibition of NOS activity induced generation of new cells in the areas of the brain that are not renowned for persistent adult neurogenesis. One of these regions is the striatum, a region affected in many degenerative neurological disorders. Our results indicate that NO acts as an important negative regulator of cell proliferation in the adult brain.

NO and Gene Expression

N. Nakaya, J. Hemish

We have found that NO blocks proliferation of NIH 3T3 cells and murine embryonic fibroblasts at various points of the cell cycle. To understand the mechanisms of NO-induced cell cycle arrest, we studied the changes in the gene expression profiles in NIH 3T3 cells treated with NO donors using microarray analysis. These cells stop dividing after treatment with NO donors without any signs of cell death. The effect of NO was reversible and cells resumed growth 48 hours after the treatment. Using microarray hybridization with about 10,000 genes, in collaboration with V. Mittal here at CSHL, we found that NO induces profound changes in gene expression. We next used quantitative real-time reverse transcriptase–polymerase chain reaction (Q-RT-PCR) to obtain a more precise estimate of the changes and to analyze the signaling pathways that mediate NO-induced gene expression. We found that some genes are induced through NFκB-, guanylylcyclase-, PI3K-, PKC-, and p53-dependent pathways. We are currently analyzing the microarray data to gain insight into the time course of sequential gene induction by NO.

Some of the expressed sequence tag (EST) clones induced by NO were analyzed in more detail. In particular, we focused on a novel gene that we termed *noxin* and whose expression was dramatically increased after exposure to NO. We determined the complete sequence of the gene, its genomic structure, and its expression pattern. The *noxin* gene is expressed in the developing brain and in several adult tissues. The noxin protein is highly serine-rich, has ataxia telangectasia mutated (ATM) and DNA-PK phosphorylation consensus sequences, and carries a potential nNOS-PDZ interaction domain. It accumulates at specific points of the cell cycle, and its expression in fibroblasts is partially dependent on p53. Our results suggest that *noxin* may be an important mediator of NO action in the control of the cell cycle.

NO and *Xenopus* Brain Development

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

Mechanisms controlling the transition from proliferation to differentiation of neural precursor cells during brain development determine the distinct anatomical features of the brain. NO may mediate such a transition, since it can suppress DNA synthesis and cell proliferation. We cloned two genes encoding *Xenopus* NO synthase (XNOS). Both genes code for enzymatically active protein which is analogous to the neuronal NOS isoform of mammals. In the developing brain of *Xenopus* tadpoles, a zone of XNOS-expressing cells lies adjacent to the zone of dividing neuronal precursors. Exogenous NO, supplied to the tadpole brain in vivo, decreased the number of proliferating cells and the total number of cells in the optic tectum. Conversely, inhibition of NOS activity in vivo increased the number of proliferating cells and the total number of cells in the optic tectum. NOS inhibition yielded larger brains with grossly perturbed organization. Our results indicate that NO is an essential negative regulator of neuronal precursor proliferation during vertebrate brain development.

NO and *Drosophila* Development

Y. Stasiv, B. Kuzin [in collaboration with M. Regulski and
T. Tully, Cold Spring Harbor Laboratory]

In the *Drosophila* genome, NO synthase is encoded by a single gene (*dNOS*). Analysis of this gene reveals that it encodes a family of at least ten transcripts of different structures. Alternative promoters and alternative splicing sites are used to produce the observed RNA diversity. Alternative splicing of the primary *dNOS* transcript changes the coding capacity of the resulting isoforms. Most of the splicing alterations in the coding region of the *dNOS* gene lead to premature termination of the open reading frame. As a result, none of the alternative transcripts encode enzymatically active protein. However, some of these shorter DNOS proteins are capable of suppressing enzymatic activity of the full-length active enzyme (DNOS1) when coexpressed in cultured mammalian cells. Thus, these protein isoforms act as dominant-negative regulators of NO synthesis. Using immunoprecipitation, we demonstrated that these truncated DNOS proteins form heterodimers with DNOS1, pointing to a physical basis for the dominant-negative effect. To investigate the biological role of one of the most abundant *dNOS* transcripts, *dNOS4*, we generated transgenic flies expressing this isoform under the control of different promoters (inducible, tissue- and development-specific). Analysis of our transgenic models clearly demonstrates that overproduction of DNOS4 in the

developing *Drosophila* suppresses the antiproliferative action of DNOS1 and causes a hyperproliferative phenotype in adult flies. We detected endogenous DNOS4 expression in fly extracts using antibody raised against its unique carboxyl terminus. Using immunoprecipitation, we were able to detect heterodimeric DNOS1/DNOS4 complexes formed in vivo, indicating that DNOS4 acts as an endogenous dominant-negative regulator of NO synthesis in *Drosophila*.

Neuronal NOS Knockout Mice

Y. Stasiv

The majority of NOS catalytic activity in the mouse brain is accounted for by neuronal NOS (nNOS). To study the role of nNOS in the developing and adult nervous system, we generated nNOS knockout mice. We used homologous recombination to delete exon 6 of the gene. This exon encodes a heme-binding site that is crucial for enzymatic activity. In collaboration with Heiner Westphal's laboratory (National Institute of Child Health and Human Development), we generated homozygous nNOS–/– mutant mice. These animals lack nNOS, and catalytic NOS activity was not detectable in their brains. Although these mice do not show a survival disadvantage as compared with their wild-type or heterozygous littermates, they display a significant increase in premature death shortly after birth. Currently, together with Michael Packer in the lab, we are studying the role of nNOS in neurogenesis by analyzing patterns of bromodeoxyuridine (BrdU) incorporation in embryonic and adult –/– brains. In collaboration with John Mignone in our lab, we are now generating nNOS –/– mice that have various lineages of the brain cells differentially marked using particular fluorescent proteins.

Visualization of Stem, Progenitor, and Differentiated Cells in the Brain

A. Vaahtokari, M. Maletic-Savatic, B. Mish

Our interest in signal transduction during differentiation prompted us to explore more general issues of neural stem cell biology. We have generated several mouse lines in which particular classes of progenitors and differentiated cells are differentially marked by expression of fluorescent proteins. We are now able to identify, track, and isolate subclasses of cells at specific points in the differentiation cascade. We are also using these mice to follow the course of neuronal differentiation in the neurogenic areas of the developing and adult brain.

Neural Stem and Progenitor Cells in the Developing and Adult Brain

J. Mignone

We are interested in neurogenesis in the adult brain and in plasticity of neural stem cells in the adult organism. By placing green fluorescent protein (GFP) under the regulatory control of the nestin promoter, we are able to specifically label a small pool of neural stem cells throughout the central nervous system. They are present in the dentate gyrus, subventricular zone, and rostral migratory stream, areas where adult neurogenesis is known to be supported. These cells can be characterized both in vivo and in vitro for their ability to generate neurons, astrocytes, and oligodendrocytes in the adult brain. We are particularly interested in the question of quiescence of various stem and progenitor populations in the adult brain. To discriminate between various subclasses of neural progenitors, we are studying the changes in nestin-GFP cells following exposure of the brain to antimitotic drugs. This work is further supplemented by the use of transgenic lines that have been generated in our lab and that use differential labeling of various lineages of the brain cells. By using mice with differentially labeled subgroups of cells, we will be able to pursue the fate of neural stem cells. Furthermore, in collaboration with Tatiana Michurina, we have been transplanting the cells from nestin-GFP mice to discern the degree to which these cells possess multipotent properties and their ability to contribute to various lineages in the organism.

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CONSTRUCTION AND PLASTICITY OF NEOCORTICAL GABAergic CIRCUITS

Z.J. Huang H. Higashiyama Q. Wang
G. diCristo T. Pal
B. Chattopadhyaya J. Walls
S. Kulhman

The cellular architecture of the neocortex is exceedingly complex, yet there are well-defined cell types and stereotyped synaptic connectivity patterns that are strikingly conserved between different cortical areas and among different mammalian species. The formation and functional tuning of these circuits are determined by both genetic instructions and epigenetic influences such as experience. It is likely that the genetic programs control the time course for the development of these circuits and constrain the influence of experience in shaping neuronal connectivity during an early postnatal critical period. Characterization of the neocortical microcircuits is essential to any theory of cortical function. For decades, however, the cellular heterogeneity, daunting connectivity, and complexity have made the cortical circuit impenetrable to the neurobiologist due to the lack of appropriate experimental tools. The rules that govern the wiring and communication of neurons within the cortex remain largely unknown. Recent advances in our understanding of neuronal gene expression, in genetic manipulations, and in neuronal imaging provide new opportunities to explore the design of neocortical circuits at unprecedented detail.

The GABAergic inhibitory circuits control neuronal excitability and information processing at precise spatial and temporal domains in neocortex. A fascinating yet puzzling feature of the cortical GABAergic system is the rich array of interneuron cell types. These cell types often display remarkably distinct morphology, intrinsic physiological properties, synaptic input and output patterns, and gene expression profiles. In addition, the functional maturation of the GABAergic system is strongly influenced by experience during early postnatal ages and, as a result, may contribute to the experience-dependent refinement of neocortical circuits. We are using cell-type-specific promoters and bacterial artificial chromosome (BAC) transgenic mice to achieve GABAergic cell-type-specific labeling and manipulations. Combined with physiological and imaging techniques, we are studying the following set of questions in the mouse visual cortex: How do different types of interneurons mature morphologically and physiologically during the critical period of visual cortical development? What are the effects of visual deprivation on the maturation of GABAergic circuits? How do neurotrophic factors regulate the experience-dependent maturation of GABAergic interneurons? How do specific types of GABAergic circuits contribute to critical period plasticity?

BAC Transgenic Labeling of Defined GABAergic Cell Types

H. Higashiyama, J. Walls, Z.J. Huang

In the visual cortex, the fast-spiking (FS) basket-type interneurons selectively innervate the soma of target neurons, are capable of high-frequency nonadaptive firing, and express parvalbumin. On the other hand, the low-threshold spiking (LTS) bitufted cells selectively innervate the distal dendrites of targets, fire broader spikes that show pronounced adaptation of firing frequency, and express somatostatin. How do GABAergic interneurons develop such distinct morphology, synaptic connectivity, and physiologic property? To address these questions, we are using a series of GABAergic transcriptional promoters to systematically label specific GABAergic cell types with green fluorescent protein (GFP) variants using BAC transgenic mice. For example, we have generated BAC transgenic mice expressing GFP driven by the GAD67 promoter (active in all GABAergic neurons) and parvalbumin promoter (active in basket interneurons). In these transgenic lines, the morphology of defined GABAergic interneurons can be visualized at unprecedented detail, in living brain tissue (Fig. 1). We

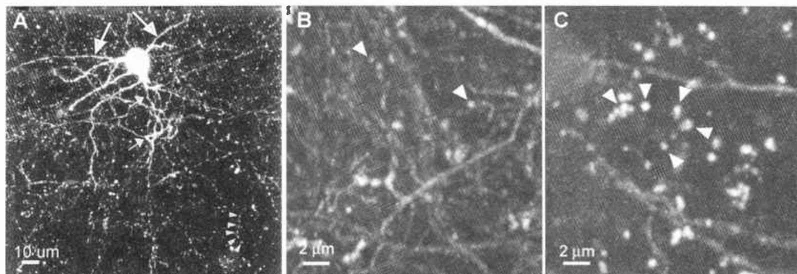

FIGURE 1 (*A*) Neocortical basket interneurons labeled by BAC transgenic expression of GFP, imaged using confocal microscopy. (*Large arrows*) Dendrites; (*small arrows*) axons; (*small arrowheads*) putative presynaptic boutons. (*B,C*) Two-photon imaging of the development of basket interneuron innervation in visual cortical slice cultures prepared from BAC transgenic mice. (*B*) At postnatal day 14 (10 days in vitro), putative basket interneuron boutons (*arrowheads*) were small and diffusely distributed. (*C*) At postnal day 23 (19 days in vitro), basket interneuron boutons were large and frequently formed ring-like (*arrowheads*) structures around the soma of a target neuron.

are also characterizing several other BAC transgenic lines expressing GFP variants in other subtypes of GABAergic neurons expressing a different set of calcium-binding proteins and neuropeptides.

These transgenic mice now allow us (1) to characterize the development of somatic versus distal dendritic patterns of synaptic innervation in basket and bitufted interneurons; (2) to examine the maturation of distinct firing properties of these interneuron cell types; and (3) to examine the effects of visual deprivation (e.g., dark rearing) on the maturation of these interneuron cell types.

Morphological Maturation and Plasticity of Basket Interneurons in the Visual Cortex

G. diCristo, B. Chattopadhyaya, Z.J. Huang [in collaboration with Karel Svoboda, Cold Spring Harbor Laboratory]

Basket interneurons are important components of neorcortical circuits, yet the morphological development of basket interneurons is poorly characterized. The dynamics and structural plasticity of GABAergic synapses are entirely unknown. In Pv-GFP BAC transgenic mice, entire dendrites and presynaptic boutons of basket interneurons are intensely labeled. Using confocal and two-photon laser scanning microscopy, we are characterizing development in dendritic structures and synaptic innervation of basket interneurons during the critical period of visual cortex using acute brain slices. We confirm that the majority of GABAergic synapses made by basket interneurons target the soma rather than the distal dendrites. The size and shape and density of presynaptic boutons mature during the critical period. We have also established brain slice cultures from P2 BAC transgenic mice, which can be grown for 3–4 weeks in vitro. Therefore, we are now in a position to "watch" the entire process of functional differentiation and maturation of basket interneurons in both acute and cultured visual cortical slices. We will develop morphometric measurements to quantify the maturation and dynamics of basket interneuron boutons. We will also study the morphological plasticity of GABAergic boutons in response to changes of neuronal activity.

Physiological Maturation and Plasticity of Basket Interneurons in the Visual Cortex

S. Kuhlman, Z.J. Huang

At the mature state, basket interneurons are capable of high-frequency spike transmission and high-frequency and nonadaptive firing, and they provide strong feed-

forward inhibition. However, the maturation and experience-dependent plasticity in firing properties and synaptic transmission of basket interneurons are poorly characterized. Our Pv-GFP BAC transgenic mice allow, for the first time, reliable and efficient physiological recording of basket interneurons during postnatal development. We are characterizing the maturation of intrinsic firing properties and inhibitory synaptic transmission of basket interneurons during the critical period. We will also examine whether and how experience deprivation such as dark rearing retards such physiological maturations. In parallel, using another transgenic line that labels bitufted interneurons, we are also characterizing the maturation and plasticity of physiological properties of bitufted interneurons in the visual cortex.

Role of Basket Interneurons in Critical Period Plasticity in the Visual Cortex

B. Chattopadhyaya, Z.J. Huang [in collaboration with Lamberto Maffei, Institute of Neurophysiology, Italy]

GABAergic inhibitory circuits have been implicated in the regulation of experience-dependent development of the visual cortex. For example, ocular dominance (OD) plasticity, the capacity of visual cortical neurons to modify their eye preferences following visual deprivation to either eye, was abolished in mice deficient in an isoform of the GABA synthetic enzyme (GAD65). Because GAD65$^{-/-}$ mice show no critical period plasticity, yet are normal in other aspects of visual physiology and function, this mutant strain provides an ideal genetic background to systematically study the role of specific classes of interneurons in OD plasticity using a transgenic "rescue" approach. We have generated BAC transgenic mice expressing GAD65 under the control of the parvalbumin promoter. We will introduce BAC transgenes expressing GAD65 in basket interneurons into the GAD65$^{-/-}$ mice by breeding. We will then test whether expression of GAD65 in a specific class of interneurons can restore critical period plasticity in GAD65$^{-/-}$ mice. In addition, this strategy will allow us to correlate the specific types of inhibitory mechanisms that are restored by cell-type-restricted expression of GAD65 with the rescue of critical period plasticity.

BDNF Overexpression Rescue of Effects of Visual Deprivation of Visual Development

J. Walls, Z.J. Huang [in collaboration with Lamberto Maffei, Institute of Neurophysiology, Italy, and Alfredo Kirkwood, Johns Hopkins University]

Visual deprivation during an early postnatal period profoundly influences the development and function of the visual cortex. For example, dark rearing (DR) decreases visual acuity and prolongs the critical period for ocular dominance plasticity. In addition, dark rearing also retards the maturation of the GABAergic inhibition in the visual cortex. The molecular signals that mediate the effects of dark rearing on the development of the visual cortex are not well defined. The expression of the brain-derived neurotrophic factor (BDNF) in the visual cortex is tightly regulated by visual experience. To test the role of BDNF, we examined the effects of dark rearing in transgenic mice in which BDNF expression in the visual cortex was driven by the calmodulin protein kinase II (CaMKII) promoter, and therefore was uncoupled from visual experience and remained elevated under dark-rearing conditions. In these transgenic mice, the visual acuity and receptive field size of visual cortical neurons developed normally during dark rearing. More importantly, the development of critical period for ocular dominance plasticity was not delayed by dark rearing. Furthermore, intracortical inhibition matured in dark-reared transgenic mice as in light-reared mice. Therefore, BDNF overexpression is sufficient for the development of several properties of the visual cortex in the absence of visual experience. These results suggest that BDNF is a key molecular signal, which links visual deprivation to the retarded maturation of GABAergic inhibition and the delayed development of the visual cortex.

Role of BDNF in Experience-dependent Maturation of GABAergic Circuits

G. diCristo, Z.J. Huang

The maturation of GABAergic inhibitory circuits in the visual cortex contributes to the development of the critical period and is in turn regulated by visual expe-

rience. BDNF has been proposed to link the pattern and level of activity to the maturation of GABAergic interneurons, but the cellular mechanism of BDNF action is poorly defined. We are producing BAC transgenic mice in which a BDNF-GFP fusion protein is expressed under the control of the endogenous BDNF regulatory mechanism. Using organotypic culture and two-photon laser scanning microscopy, we will characterize the dynamics of subcellular localization, transport, and trans-synaptic transfer of BDNF-GFP following different patterns of neuronal activity. Furthermore, we will visualize defined GABAergic neurons and synapses by biolistic transfection of red fluorescent protein (RFP). We will then correlate the mode and dynamics of BDNF transport (anterograde vs. retrograde) with its regulation of identified GABAergic cell types and synapses. These experiments will shed light on the precise cellular mechanisms linking neuronal activity, BDNF expression, and maturation of GABAergic circuitry.

Construction of a BAC Location Database

J. Markson (URP), Z.J. Huang [in collaboration with Z. Xuan and M. Zhang, Cold Spring Harbor Laboratory]

The completion of mouse genome sequencing has greatly accelerated the design and execution of mouse genetic manipulations, including BAC transgenics. In collaboration with Michael Zhang, we have developed bioinformatics programs and databases to quickly identify and map BAC clones containing any genes of interest.

In Press

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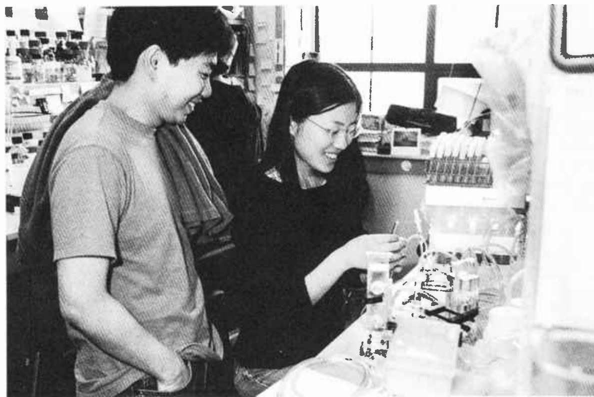


Koichi Iijima, Kanae Iijima

THE NEURAL ORGANIZATION OF OLFACTORY BEHAVIOR

Z. Mainen N. Caporale V. Egger
H. Gurden S. Ranade
S. Edgar R. Gasperini
M. Quirk N. Uchida

How do the functions of the brain make it possible for a creature to find food, mate, and avoid predators, and how does the malfunction of these same functions lead to human mental illnesses such as schizophrenia? With the long-term goal of addressing these questions, we seek to understand cognitive and affective aspects of the mind in terms of neural codes and computations, and these, in turn, in terms of biophysics and biochemistry. Our research program is centered on understanding the sense of smell and olfactory-guided behavior in the rat. To integrate information spanning levels of analysis, we are employing a wide range of experimental approaches, including behavioral, optical, and electrophysiological techniques, as well as theoretical approaches such as computational modeling. Thus, we hope ultimately to understand the nature of social interactions in the workings of single synapses.

Neural Representation of Social Identity

N. Caporale, S. Ranade, Z. Mainen

Recognition and classification of individual members of a species have been shown to be essential to many aspects of mammalian social organization, including selection of mates, communication of diet preference, establishment of dominance hierarchies, and maintenance of kin relationships. Deciphering the neural representation of individual identity is therefore key to understanding the brain systems responsible for the organization of social behavior.

In rodents, social recognition relies largely on the sense of smell. The accessory olfactory (vomeronasal) system is known to mediate a wide range of instinctual behaviors elicited by pheromones (e.g., pregnancy block). The main olfactory system, on the other hand, is known to have a significant role in learned behaviors (e.g., social transmission of food preference). It has been proposed that the main olfactory system is responsible for the formation of social representations of individual identity. This idea is supported by a variety of evidence, including data from our lab showing that rats will use both naturally secreted and non-natural chemicals (e.g., perfume) for social identification (Caporale et al. 2001).

We are interested in how the complex chemical signatures of animals are encoded in the main olfactory bulb and especially in how individual and categorical differences (e.g., kinship) are represented. It is known that the composition of volatile chemicals released in urine and other secretions is unique to each individual and can be discriminated by other members of that species (or other species). Using intrinsic optical imaging (see below), we are studying spatial activation patterns evoked by urine in the main olfactory bulb (Caporale et al. 2001). By comparing activity maps evoked by urine from different individuals at different times, we can investigate the encoding of individual and categorical differences by receptors in the main olfactory bulb. With multi-electrode recordings, we plan to follow the processing of social stimuli into higher-order olfactory structures such as the olfactory cortex, amygdala, and hypothalamus.

Olfactory Maps and Odor Quality

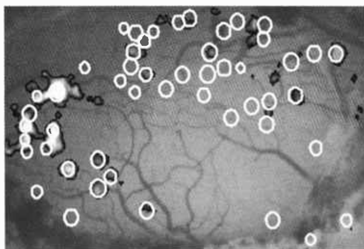
N. Uchida, R. Gasperini, S. Edgar, Z. Mainen
[in collaboration with M. Chklovskii, Cold Spring Harbor Laboratory, and S. Macknik, University College, London]

Volatile chemicals are encoded in neural activity distributed across a set of more than 10,000,000 olfactory receptor neurons, each expressing 1 of 1000 differ-

ent olfactory receptor types. The sensory neurons project to the olfactory bulb, where the axons of cells corresponding to each receptor type converge at stereotyped locations to form dense functional units known as "glomeruli." Consequently, any olfactory stimulus produces a unique spatial pattern in the olfactory bulb expressed by specific activation values across glomeruli.

If the perceptual qualities of olfactory stimuli are derived from the activity associated with each receptor type, then "smells" can be read by measuring activity patterns across these glomerular maps. To read glomerular activation patterns, we are using the technique of intrinsic optical imaging (Fig. 1, top; see below). To assay the perceptual similarity of different chemicals, we are using a two-alternative discrimination task. Because rats are able to discriminate perfectly even the most closely related pure chemicals that we have tested, we have developed a more sensitive task in which rats are trained to report the dominant chemical in binary mixtures of two chemicals at different ratios. This task produces a regular psychometric curve similar to that observed commonly in human and monkey psychophysics experiments. When fit with a sigmoid, the curve is a sensitive measure of the discriminability of a given pair of chemicals (Fig. 1, bottom).

Using these assays, we have demonstrated a correlation between the glomerular spatial representation (optical imaging data) and perceptual odor quality (psychophysical data) (Uchida et al. 2001). With collaborator M. Chklovskii, we are developing computational methods for optimizing the analysis of these correlations. With collaborator S. Macknik, we are performing similar psychophysical tests in humans that will allow us to compare olfactory perception across these species.



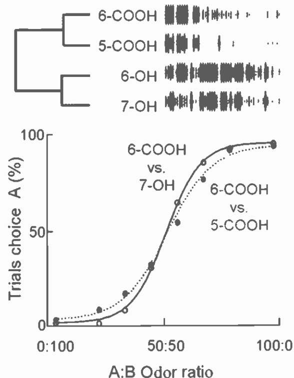


FIGURE 1 (*Top*) Intrinsic signal image of glomerular activity in the rat olfactory bulb produced by the chemical 6-COOH. (*Middle*) Activity patterns of four chemicals extracted as for 6-COOH. Each line corresponds to a white circle in the top image. (*Black*) Mean signal; (*gray*) standard deviation. Cluster plot at left indicates the relative similarity of patterns. (*Bottom*) Psychometric curves for the discrimination of two odor mixtures by a rat.

Neural Population Activity and Olfactory-guided Behavior

M. Quirk, Z. Mainen [in collaboration with C. Brody, Cold Spring Harbor Laboratory, and J. Hopfield, Princeton University]

Sniffing produces coherent oscillations across the olfactory system, particularly at γ and θ frequencies. Thus, olfactory representations not only are spatially distributed, but also may involve the wide-scale temporal coordination of activity across populations of neurons. The distributed nature of these representations also appears to be preserved in more central regions of the brain such as the prefrontal cortex, amygdala, and hippocampus, which are important in the organization of cognitive and emotional behavior.

Information coded collectively in populations of neurons is particularly difficult to study through conventional single-neuron electrophysiology. We are therefore using a tetrode-based multi-electrode recording system that allows us to monitor the activi-

ty of dozens of single cells simultaneously. With this system, we can study the temporal relationships between the activity of neurons within and across brain regions. In parallel, we are developing computational models with collaborators C. Brody and J. Hopfield.

Through this approach, we hope to address two fundamental questions. First, how does temporal coordination contribute to the production of specific olfactory representations? To this end, we will record in anesthetized as well as behaving animals and analyze the relationships between putative collective olfactory codes and sensory and behavioral events. Second, how is activity across brain regions coordinated in the service of olfactory-guided behavior? To this end, we will analyze the temporal relationship between neural activity across various areas of the olfactory system (including amygdala, pyriform, and orbitofrontal cortex).

We are also particularly interested in the temporal relationship of this activity to that of the brain stem nuclei associated with the major diffuse ascending neuromodulatory projections that have been implicated in the control of motivation, attention, learning, and awareness.

Computation and Metabolism in Olfactory Glomeruli

H. Gurden, N. Uchida, Z. Mainen

The first stage of neural processing in the mammalian olfactory system occurs within the glomeruli of the olfactory bulb. A glomerulus is a ball of neuropil about 100 μm in diameter, targeted by more than 10^4 sensory afferents, all carrying signals from just one of the approximately 1000 olfactory receptor types. This massively convergent signal is not simply relayed to the principal neurons (mitral cells), but rather is subject to a complex network of synaptic interactions occurring within the core of the glomerulus. Some of these interactions are mediated by periglomerular (PG) cells, a heterogeneous population of neurons whose cell bodies form dense shells surrounding each glomerulus. It is known that PG cells synthesize dopamine (DA) and γ-amino-*n*-butyric acid (GABA) and that they mainly mediate *intra*-glomerular synaptic interactions (as well as some longer-range inter-glomerular interactions), but the functional role of PG cells is obscure.

Intrinsic optical imaging is a sensitive monitor of odor-evoked responses in individual glomeruli (see Fig. 1). Intrinsic signals are believed to reflect metabolic activity, but, as with related techniques such as functional magnetic resonance imaging (fMRI), the relationship between these signals and underlying neural activity is poorly understood. Our first objective is to describe a quantitative relationship between neural activity parameters such as presynaptic release rate and intrinsic signals. To do so, we are using pharmacology (primarily, specific agonists and antagonists of neurotransmitter receptors) to dissect the synaptic circuitry and biochemistry of the glomerulus. We have found that intrinsic signals are inhibited by GABA-B and DA receptor activation and insensitive to postsynaptic glutamate receptors. Our second objective is to produce a quantitative model of the function of the glomerular circuit in the encoding of olfactory stimuli. To do so, we will combine intrinsic imaging and pharmacology with computational approaches based on detailed biophysical modeling.

Calcium Dynamics in Olfactory Bulb Granule Cells

V. Egger, Z. Mainen [in collaboration with K. Svoboda, Cold Spring Harbor Laboratory]

Granule cells (GCs) are GABAergic interneurons that mediate recurrent coupling between the principal neurons (mitral cells) in the olfactory bulb. Although GCs lack axons, they produce action potentials (APs) that can admit Ca^{++} and hence contribute to synaptic release via the dendrodendritic mitral-granule cell connection. The behavior of these dendritic action potentials and the associated Ca^{++} transients will therefore be a key determinant of lateral inhibition and strongly shape the network properties of olfactory bulb.

To examine the properties of AP propagation within the GC dendritic tree, in collaboration with K. Svoboda, we imaged AP-evoked dendritic Ca^{++} transients in granule cells using 2-photon imaging in rat olfactory bulb slices (Fig. 2) (Egger et al. 2001). Interestingly, as APs propagated into GC dendrites, the size of the evoked Ca^{++} transients increased with distance from the soma, the opposite effect of what is typ-

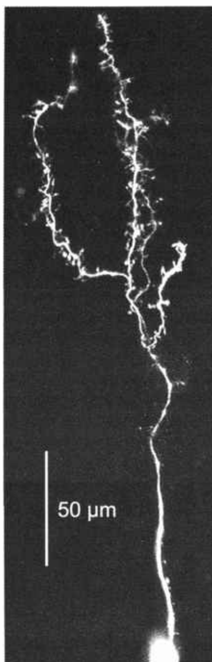


FIGURE 2 Granule cell in a slice from the rat olfactory bulb. The cell was filled with a fluorescent calcium indicator and imaged using a 2-photon microscope.

ically seen in excitatory cells. Moreover, this effect was correlated with the distance of GC somata from the site of dendrodendritic contacts: The longer the distance the AP had to travel, the more amplification. This differential amplification might help to equalize the impact of AP-evoked transients on dendritic release among GCs with different laminar positions within the olfactory bulb.

When GCs were depolarized to near threshold, both AP-evoked Ca^{++} transients and a normally strong afterdepolarization were reduced. Blockers of the low-threshold, fast-inactivating T-type Ca^{++} channel reduced the Ca^{++} transient amplitudes and blocked their voltage dependence. These observations suggest a novel mechanism for regulation of lateral inhibition in the olfactory bulb: Persistent GC depolarization during sustained input from mitral cells or centrifugal afferents may reduce AP-triggered transmitter release and thus restrict GC-mediated lateral inhibition. We are currently examining other mechanisms by which AP propagation in GCs can be regulated, such as by the action of neuromodulators such as norepinephrine.

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TRANSMISSION AND PLASTICITY IN MAMMALIAN CENTRAL SYNAPSES

R. Malinow A. Barria, N. Dawkins, I. Ehrlich, R. Ewald, H. Hsieh, J. Esteban, F. Kamenetz, A. Piccini, K. Seidenman, S. Shi, T. Takahashi, J. Zhu, C. Wilson (URP)

Our laboratory addresses issues directed toward an understanding of learning and memory by studying the physiology of synapses. We study synaptic transmission and plasticity in rodent brain slices. This preparation is sufficiently complex to show glimpses of emergent properties as well as simple enough to allow hard-nosed biophysical scrutiny. To monitor and perturb the function of synapses, we use a combination of electrophysiology, microscopic imaging, and molecular transfection techniques. This allows us to examine the cellular and molecular basis for changes in electrophysiological function. It is our philosophy that synapses have key properties whose understanding will provide insight into phenomena at higher levels of complexity. Below are examples of the projects nearing fruition in our laboratory.

INCREASING MOLECULAR REQUIREMENTS FOR SYNAPTIC PLASTICITY WITH AGE

Synaptic plasticity has important but distinct roles throughout life. Early in development, synaptic plasticity controls the establishment of neural connections. Later in life, plasticity controls learning and memory. Here, we compare the molecular mechanisms controlling synaptic plasticity at different ages. We find that PKA phosphorylation of α-amino-3-hydroxy-5-methyl-4-isoazole (AMPA) receptors (AMPA-Rs) has a key role throughout. Activity-driven PKA phosphorylation of GluR4 is necessary and sufficient for delivery of receptors to synapses during early development, making synapses functional. Phosphorylation relieves a retention interaction that, in the absence of synaptic activity, maintains GluR4-containing receptors away from synapses. In contrast, phosphorylation of GluR1 by PKA is necessary but not sufficient for delivery of receptors to synapses in older animals. Thus, a mechanism that mediates plasticity early in development becomes a gate for plasticity later in life. Increasing requirements may be one way that plasticity becomes more specific and also recalcitrant with age.

CRITICAL PDZ INTERACTIONS BY GLUR1 AND GLUR2 REQUIRED AT DIFFERENT SUBCELLULAR SITES

Interactions between AMPA-R subunits and PDZ domains are critical for the proper delivery of receptors to synapses. Synaptic delivery of GluR1-containing AMPA-Rs can be driven by calcium/calmodulin-dependent protein kinase II (CaMKII) activity or long-term potentiation (LTP) and requires an interaction between GluR1 and a type I PDZ domain. Synaptic delivery of AMPA-Rs with only GluR2 occurs continuously, and this requires an interaction between GluR2 and a type II PDZ domain. We now show that mutations on GluR1 or GluR2 that perturb these critical PDZ domain interactions lead to the accumulation of these receptors at different subcellular sites. GluR1 mutants accumulate in the dendrite, whereas GluR2 mutants accumulate in dendritic spines. This suggests that the critical PDZ-domain interactions are required for entry into spines, for GluR1, and into synapses, for GluR2.

RAS AND RAP CONTROL AMPA RECEPTOR TRAFFICKING DURING SYNAPTIC PLASTICITY

Recent studies have shown that AMPA-R trafficking has an important role in synaptic plasticity. However, the intracellular signaling pathways controlling this trafficking remain largely unknown. Small GTPases can signal diverse neuronal processes, and their perturbation is responsible for several mental disorders. Here, we have examined the role of small GTPases Ras and Rap in the postsynaptic signaling that mediates synaptic plasticity. We show that Ras is the downstream effector for *N*-methyl-D-aspartate (NMDA)

receptor (NMDA-R) and CaMKII signaling during LTP. Ras activity drives synaptic delivery of AMPA-Rs. In contrast, postsynaptic activation of Rap mediates NMDA-R-dependent removal of synaptic AMPA-Rs that occurs during long-term depression. Ras and Rap exert their effects on AMPA-Rs that contain different subunit composition. Thus, Ras and Rap, whose activity can be controlled by postsynaptic enzymes, serve as independent regulators for potentiating and depressing central synapses.

CONTROL OF NMDA RECEPTOR TRAFFICKING TO SYNAPSES BY THE NR2 SUBUNIT

To elucidate mechanisms controlling the number and subunit composition of synaptic NMDA-Rs in hippocampal slice neurons, the NR1, NR2A, and NR2B subunits were optically and electrophysiologically tagged. The NR2 subunit directs delivery of receptors to synapses with different rules controlling NR2A and NR2B. NR2B-containing receptors incorporate into synapses in a manner that is not limited by synaptic transmission nor enhanced by increased subunit expression. NR2A-containing receptors, whose expression normally increases with age, replace synaptic NR2B-containing receptors. Replacement is enhanced by increased NR2A expression, requires synaptic activity, and leads to reduced NMDA-R responses. Surprisingly, spontaneously released transmitter acting on synaptic NMDA-Rs is sufficient for replacement. Thus, as with AMPA-Rs, synaptic trafficking of NMDA-Rs is tightly regulated and has unique rules.

HOMEOSTATIC FEEDBACK BETWEEN NEURONAL ACTIVITY AND APP PROCESSING

A large body of work implicates the accumulation of Aβ peptides and other derivatives of the amyloid precursor protein (APP) as central to the pathogenesis of Alzheimer's disease. However, little is known regarding the relationship between neuronal electrophysiological function and APP processing. Here, we find that neuronal activity controls the formation of Aβ. Furthermore, increased formation of Aβ reversibly depresses synaptic function. These results suggest that APP cleavage products act as negative feedback homeostatic regulators that keep neuronal hyperactivity in check. Pathology that disrupts this homeostatic system could increase the production of Aβ and/or produce neuronal hyperactivity, events that may contribute to Alzheimer's disease.

In conclusion, we are continuing to elucidate the basic mechanisms involved in central synaptic transmission and plasticity.

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PLASTICITY OF CORTICAL NEURONS AND THEIR CIRCUITS

K. Svoboda B. Burbach I. Bureau B. Chen K. Greenwood A. Karpova M. Maravall E. Nimchinsky P. O'Brien T. Oertner T. Pologruto B. Sabatini G. Shepherd R. Yasuda C. Zhang K. Zito

The cortex underlies most cognitive functions in mammals. Cortical tissue is dauntingly complex: 1 mm^3 of tissue contains nearly a million neurons, each of which connects to thousands of other neurons. How does electrical and biochemical dynamics in this awesome network produce our perception of the world? How does this network change in response to experience? Answers to these questions will profoundly change our understanding of the function and diseases of the brain.

To begin to unravel neocortical function, we are studying how the basic units of the network, neurons and their synapses, work within the intact network. For this purpose, we build and use sensitive optical tools. 2-photon laser scanning microscopy (2PLSM) allows us to detect the excitation of single synapses in brain slices and in the intact brain by measuring intracellular calcium accumulation associated with activation of synaptic receptors with single receptor sensitivity. Excitation of neuronal elements by focal uncaging of neurotransmitters allows us to efficiently probe the connectivity of neural networks. We combine these optical methods with electrophysiological measurements of synaptic currents and potentials and molecular manipulations of neurons.

We use both in vivo measurements to address system level questions and in vitro methods to get at detailed mechanisms. For our in vivo measurements, we use the rodent barrel cortex. Similar to other sensory areas in the mammalian cortex, the barrel cortex is arranged in maps. Each whisker is represented by a small cortical region (barrel). Whisker maps are shaped by experience during development and reshaped in the adult. Plasticity in these maps can be induced by trimming specific whiskers for hours to days. The cellular mechanisms underlying sensory map plasticity are likely to share mechanisms with those underlying learning and memory. Because of the precision of the barrel map, it is possible to predict where in the brain experience-dependent changes are likely to occur, simplifying the search for the cellular, synaptic, and molecular basis for plasticity. For our in vitro measurements, we use brain slices from the barrel cortex or hippocampus.

Cellular Basis of Experience-dependent Plasticity in the Developing Cortex In Vivo

I. Bureau

How do the ordered connections of the brain form during development? We have begun to address this issue in layers 2/3 of barrel cortex by recording sensory-evoked synaptic currents as the network is beginning to wire up (PND 8–10). To our surprise, we found that at these early ages, weak but highly ordered sensory maps already exist and that these are supported by synapses dominated by *N*-methyl-D-aspartate receptors (NMDA-Rs). Thus, sensory maps form much earlier than previously thought but are largely electrically silent. We also found that the structure of maps in layers 2/3 is strongly shaped by sensory experience.

Cellular Basis of Experience-dependent Plasticity in the Developing Cortex In Vitro

M. Maravall, G. Shepherd [in collaboration with T. Takahashi and R. Malinow, Cold Spring Harbor Laboratory]

Some experience-dependent changes in cellular properties are difficult to study in the intact brain, requiring measurements in brain slices. We are comparing the properties of neocortical pyramidal neurons and their circuits in brain slices derived from deprived and control animals over developmental ages spanning the barrel cortex critical period. To discover possibly diverse modes of plasticity, we are using a barrage of electrophysiological and imaging approaches to look for effects of sensory experience on neuronal Ca^{++} dynamics, excitability, synaptic currents, dendritic structure, and plasticity potential. For example, we found that in deprived animals, the spatial distribution

of branchpoints in dendritic trees is altered. In addition. we have adopted and improved laser scanning uncaging of glutamate to rapidly and efficiently probe the structure and dynamics of neural circuits. Finally, we are probing the experience-dependent delivery of glutamate receptors into layer 4 to layer 2/3 synapses.

Experience-dependent Plasticity in Adult Cortex In Vivo

B. Chen, J. Trachtenberg

Even in the adult cortex, changes in sensory experience modulate the properties of cortical maps. It has long been debated whether changes in neuronal structure and growth of new synapses underlie such experience-dependent cortical plasticity. We used time-lapse 2PLSM of layer-5 pyramidal neurons in the adult mouse barrel cortex to image the structural dynamics of dendritic spines, the postsynaptic specialization of synapses. We are using transgenic mice that express fluorescent proteins in a sparse subpopulation of neurons. These animals allow us to look at the structure of neurons over chronic time scales at high resolutions. We find that synapses exist with broadly distributed lifetimes, up to our measurement time (~2 weeks). Changes in sensory experience produce increased turnover of synapses, suggesting that novel experiences are encoded in part by growth of synapses. To relate these morphological responses to functional changes at the level of neurons, we are measuring maps of sensory evoked synaptic potentials.

Experience-dependent Gene Expression in the Developing Neocortex

K. Zito, S. Chakraborty, B. Burbach [in collaboration with F. Naef and M. Magnasco, Rockefeller University]

What molecules are involved in the rapid, experience-dependent development of neocortical circuitry? Oligonucleotide arrays offer an opportunity to follow the expression of thousands of genes as the cortex wires up and to potentially discover genes (plasticity genes, *pgs*) that regulate experience-dependent aspects of development. We isolate populations of neurons from a region of the barrel cortex using microsurgery techniques. The procedure is brief (20 minutes per animal), allowing large quantities of tissue (hundreds of milligrams) and mRNA (micrograms) to be harvested, sufficient for expression array studies. mRNA is isolated from deprived and control brains at various developmental ages and used to make fluorescent probes. The probes are then hybridized to microarrays. The expression data are analyzed for "temporal patterns" of expression.

Cellular and Molecular Basis of the Fragile X Syndrome

K. Zito, E. Nimchinsky, B. Burbach, K. Greenwood, C. Zhang [in collaboration with W. O'Donnell and S. Warren, Emory University)

The Fragile X mental retardation syndrome is the most common form of inherited mental retardation. The molecular basis of the syndrome is a large expansion of a triple repeat (CGG) in the 5′ UTR of the *FMR1* gene that renders it transcriptionally silent. The protein coded by this gene, called FMRP (Fragile X mental retardation protein), is an RNA-binding protein of unknown function. One suggested function is the transport of mRNA out of the nucleus and into neuronal dendrites. To determine whether FMRP regulates the expression of other genes, we performed an oligonucleotide array screen to search for genes misexpressed in FMR1 knock-outs. This screen produced several hits, including CCL21, a chemokine not previously shown to be involved in brain development and function. We are currently analyzing the role of CCL21 in neuronal function. In addition, we are analyzing the function of FMRP in brain slices. In these experiments, FMRP, or one of its mutants, is manipulated in pyramidal neurons by transfection, and its effects on neuronal structure and function are analyzed.

Molecular Methods to Reversibly Inactivate Synapses In Vivo

A. Karpova, C. Zhang, K. Svoboda [in collaboration with J. Huang, Cold Spring Harbor Laboratory]

A major obstacle in gaining an understanding of neural networks is our inability to selectively modulate

selected circuit elements in vivo. For example, changes in experience change the amount and quality of activity seen by most neurons in a given pathway. Similarly, pharmacological tools are functionally nonspecific.

We are developing genetically deliverable tools that will allow us to interfere with synaptic function in a rapidly inducible and reversible manner. These tools combine recent insights about the molecules regulating neurotransmitter secretion with chemical genetics. Using cell culture and brain slice assays, we are currently testing candidate systems that are designed to turn synapses off. The ultimate goal is to introduce these systems into particular neuronal populations using BAC (bacterial artificial chromsome) transgenic technology to produce animals in which neuronal subpopulations can be shut off "at will."

[Ca^{++}] Signaling in Single Dendritic Spines

R. Yasuda, E. Nimchinsky, B. Sabatini, T. Oertner

Long-term changes in synaptic efficacy are triggered by increases in [Ca^{++}] in the postsynaptic neuron. We have used whole-cell patch clamp recordings and 2PLSM to measure [Ca^{++}] signals that are evoked in spines and small dendrites of CA1 pyramidal neurons by back-propagating action potentials and synaptic stimuli. We have been able to characterize spines as functionally distinct neuronal compartments for Ca^{++} signaling. We now understand the life cycle of Ca^{++} ions in individual dendritic spines. In addition, we have developed a variety of assays to learn about the molecular pathways of Ca^{++} influx, including the counting of individual Ca^{++} permeable channels and receptors in spines.

We have also discovered that the activation voltage of voltage-gated Ca^{++} channels in single spines can be shifted by neuronal activity. This long-term modulation is produced stochastically in single spines and requires the activity of calcium/calmodulin protein kinase (CaMKII). Furthermore, all of the channels in a spine (~10) are modulated together, suggesting that we are witnessing either the output of a single enzyme or the result of an autophosphorylation cascade involving several copies of CaMKII. This provides strong evidence that individual spines act as functionally isolated chemical compartments.

Optical Studies of Single Synapses

T. Oertner, E. Nimchinsky, R. Yasuda, T. Pologruto

Synapses release vesicles of glutamate stochastically. We can detect successes and failures of release at single synapses by imaging Ca^{++} in postsynaptic spines (Fig. 1). By isolating Ca^{++} entering cells through NMDA-Rs, we can measure the amount of glutamate released at single synapses. This allows us to address old questions in synaptic physiology that have important implications for the nature of information transmission in the brain and the mechanisms of plasticity.

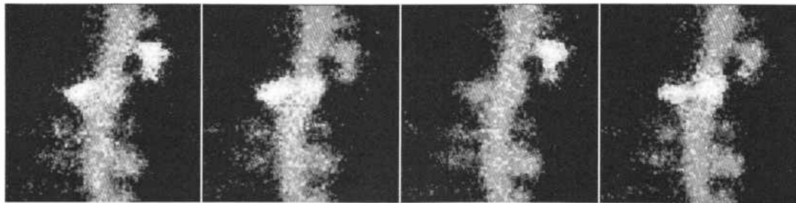

FIGURE 1 Stochastic synaptic transmission. A dendritic branch was filled with Ca^{++} indicator and afferent axons were stimulated. Each frame is the result of a different stimulus trial. Ca^{++} influx associated with synaptic transmission manifests itself as increasing fluorescence in spines. (Image courtesy of Thomas Oertner.)

For example, does a synapse always relcase a single vesicle per action potential? Using single spine imaging we find that synapses release multiple vesicles when strengthened. These measurements show that the dynamic range of synapses is larger than previously thought.

Do synapses function as independent information transmission channels? Or can transmitter released at one synapse activate receptors of several spines, destroying synaptic independence? To address this question, we used NMDA-Rs as "sniffers" of glutamate-monitored Ca^{++} signals in neighboring spines, often less than 1 μm apart. We found that under a wide range of conditions, glutamate activates only single synapses. This is surprising because with the fusion of a single vesicle, a large excess of glutamate is released. Using knock-out animals and pharmacological tools, we have shown that astrocytic glutamate transporters are significant players in limiting the spread of glutamate in the extracellular space.

Instrumentation

K. Svoboda, B. Sabatini, T. Pologruto, P. O'Brien,
G. Shepherd [in collaboration with R. Eifert,
Cold Spring Harbor Laboratory]

Our instrumentation efforts during the last year have focused on getting further microscopy/physiology stations working and others updated. We have developed software tools for image acquisition and microscope control based on Matlab. We have built a new system for laser scanning glutamate uncaging that allows rapid analysis of neural circuits in brain slices, facilitating our analysis-experience-dependent changes of synapses.

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MOLECULAR NEUROBIOLOGY OF LEARNING AND MEMORY IN *DROSOPHILA*

T. Tully J. Barditch J. Dubnau S. Gossweiler L. Grady J. McNeil M. Regulski P. Smith S. Xia

By investing in complementary forward- and reverse-genetic approaches to identify genes involved in memory formation, we have (1) developed a novel statistical method to analyze data from DNA microarrays, (2) identified 141 statistical candidate genes, and (3) validated this approach by assessing memory defects in mutants of some of these candidates. Along with other mutants identified from the "Harford screen," we now have added nearly 200 new genes to the list of those contributing to memory formation.

Identification of New Genes Involved with Olfactory Memory

T. Tully, P. Smith, L. Grady, J. Barditch, S. Gossweiler, J. Dubnau

A behavioral screen (forward genetics) for defects in olfactory memory has yielded 57 new mutant lines. Molecular lesions have been identified for 55 of these 57 mutants, defining 47 new genes. These candidate memory genes (CMGs) include those involved in gene regulation, RNA processing, translational control, trafficking, signal transduction, and cell adhesion. Future experiments will focus on characterizing the expression and cellular function of these genes. DNA microarray chips also have been used to identify CMGs that are transcriptionally regulated during long-term memory formation. This approach has yielded 141 CMGs, showing statistically significant differences in levels of transcription between spaced or massed training at one of three time points after training ($t = 0$, 6, or 24 hr).

Importantly, two memory mutants were identified with independent mutations of the *milord* gene, and this gene also was a CMG from the DNA chip experiments at both $t = 0$ and $t = 6$ hours after training. These convergent results strongly implicate *milord* as a bona fide memory gene. Several other genes are already known to participate in the same biological pathway as *milord*. Three of these also were identified as memory mutants from our behavioral screen, and seven others also were CMGs from the DNA chip experiments. One of the latter CMGs is known in other biological systems to interact with *milord*, and temperature-sensitive alleles of this "*milord* interacting gene" (MIG) exist. Hence, our data strongly predicted that disruption of this gene would block long-term memory formation. It does. Mutant flies were trained as tested at permissive temperature but were shifted to restrictive temperature for the 24-hour retention interval after spaced training. After such treatment, no significant 1-day memory was detected, even though 1-day memory after spaced training was near normal when the experiment was conducted entirely at permissive temperature (Fig. 1).

Behavioral Analyses

T. Tully, S. Xia, J. Dubnau, L. Grady

The *shibire* gene in *Drosophila* encodes a homolog of dynamin, a synaptic protein involved in vesicle recycling. We restricted expression of a *UAS-shi*^{ts1} trans-

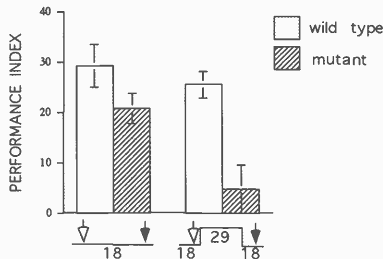

FIGURE 1 One-day memory after spaced training in normal flies (*wild-type*) and temperature-sensitive mutants of a "*milord* interacting gene" (MIG; *mutant*). When MIG is disrupted only during memory formation, long-term memory is blocked. $n = 6$ PIs per group.

gene to the central nervous system and showed that all locomotion ceased within 1 minute at the restrictive temperature, suggesting that synaptic transmission was severely disrupted. When *UAS-shi*^{ts1} expression was further limited to the mushroom body (an anatomical region of the insect brain known to be involved in olfactory learning), acquisition of odor-shock avoidance was unaffected, but subsequent memory retrieval was blocked.

More refined analyses of various temporal stages of memory formation have revealed that different memory phases require synaptic transmission in different anatomical regions of the brain during olfactory memory formation. These data suggest (1) an anatomical dissection of genetically distinct memory phases during memory formation and (2) extend our knowledge of the "memory circuit" in *Drosophila*.

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Gowan Tervo

LONG-TERM MEMORY FORMATION IN *DROSOPHILA*

J. Yin M. Chiarelli J. Horiuchi M. Stebbins
E. Drier K. Iijima M. Tello
R. Filipkowski C. Margulies P. Wu
E. Friedman

Atypical PKC and Memory Formation

E. Drier, P. Wu, M. Tello

We have shown previously that atypical PKC activity is sufficient to enhance memory formation and is necessary for the normal process in flies. Inducing gain-of-function (truncated or PKM) transgenes increases atypical kinase activity twofold and produces more memory, whereas inducing dominant-negative transgenes interferes with memory formation, without affecting learning. Enhancement of memory formation requires persistent kinase activity, since neither a full-length nor a kinase dead point mutant can enhance if they are induced.

How specific is this enhancement? All PKC proteins require phosphorylation of multiple residues to activate the proteins, and atypical PKCs require two. One of these phosphorylations, at the very carboxy-terminal end of the protein, is an autophosphorylation, whereas the second one is in the "activation loop." For atypical PKCs, in fibroblasts, the activation loop phosphorylation is thought to be mediated by a kinase cascade consisting of PI3 kinase and PDK. If the mouse *PKMζ* transgene is mutated at the phosphoacceptor serine residue in the activation loop, the mutant protein is no longer able to enhance memory formation, demonstrating that the enhancing protein has similar requirements in neurons and fibroblasts.

Three subtypes of the PKC genes are distinguished by their cofactor requirements: conventional, novel, and atypical. All PKC proteins require phosphatidylserine for activation. Conventional PKC proteins require calcium and phorbol esters, novel PKC proteins require phorbol esters, and the atypical proteins do not require either. Induction of a truncated, conventional PKC gene (producing a PKM form) does not enhance memory formation, demonstrating subtype specificity in the behavioral enhancement.

The α-calmodulin-dependent protein kinase II (CaMKII) is widely touted as a key protein in synaptic plasticity and memory processes ("the memory molecule"). Truncation of this protein produces a constitutively active isoform, and this reagent has been used extensively in the neurobiology community. When we induce this protein, and increase kinase activity sevenfold, it does not enhance memory formation, demonstrating that enhancement does not result from simple overexpression of any plasticity-related kinase.

In summary, we have demonstrated that the aPKM-mediated enhancement of memory formation is specific. Enhancement requires proper signal transduction that leads to activation loop phosphorylation, and it requires that an atypical PKC protein is truncated to the PKM form. Overexpression of other neuronally important plasticity kinases cannot enhance. Given our belief that aPKM is acting through its role in marking and tagging active synapses, it is not surprising that other partially activated or constitutively active kinases cannot substitute.

Molecular Characterization of the *DaPKC* gene

M. Tello, E. Drier [in collaboration with A. Wodarz, Düsseldorf University, and T. Sacktor, State University of New York Health Science Center, Brooklyn]

Northern blot analysis and genomic sequencing shows that the *DaPKC* gene consists of eight coding exons, two of which are alternatively spliced in a mutually exclusive manner. These exons are spread out over a 30-kb region, which includes a large 12-kb intron located between coding exons 3 and 4. Northern analysis shows that three transcripts are made (A, B, and C). Probes from the 5′ end of the gene recognize transcript C, whereas probes from the 3′ end of the gene identify transcripts A and B. The probe made from exon 7′, one of the two alternatively spliced exons, also recognizes transcripts A and B. Reverse transcriptase–polymerase chain reaction (RT-PCR) analysis using various combinations of primers, and

northern analysis using probes from across the gene show that transcripts A and B contain parts of the large internal intron. Northern blots, RT-PCR, and 5′ RACE suggest the existence of at least one promoter (for transcript B) located about 3 kb from the intron/exon 3 border. A P-element insertion is located about 4 kb from this intron/exon 3 border.

Northern analysis of imprecise excisions from the P-element led to the localization of the promoter for transcript B, although we still cannot definitively distinguish between two of the clusters of 5′ ends of transcripts identified using RACE. These two regions are about 500 bp apart, and in between these two possible start sites is a very good match to a consensus CRE site (5′ TGACGTaA 3′). We speculate that neuronal activity and dCREB2 may regulate transcription initiation from one of these sites or may be involved in synchronizing the 5′ transcription start site. This promoter organization is almost identical to what is found in the rat atypical PKCζ gene (T. Sacktor, pers. comm.). This gene also contains a large intron in the middle of the gene, and 5′ RACE has identified a cluster of transcription start sites within the intron. There are two very good matches to CRE sites located just upstream of and just downstream from the major start sites. In rats, this mRNA codes for a truncated PKMζ protein, suggesting that the short protein can also be produced via transcription initiation.

Multiple antibodies identify two protein bands on western blots of head proteins. One protein corresponds to the full-length protein, whereas the other band is approximately the size of the rat PKM protein. We are characterizing antisera raised from exon-7-specific and exon-7′-specific peptides.

Multiprotein Complexes and the Synaptic Tag

E. Drier, K. Iijima

In early development, the DaPKC protein participates in asymmetric cell division and cell polarity. The involvement in these functions is evolutionarily conserved and appears to be true for a number of different cell types. We hypothesize that postmitotic neurons utilize features of these cellular properties in synaptic tagging, a recently hypothesized neuronal function that is critically important in the process of long-term memory formation. We are testing other genes that are known to participate in asymmetric cell division for their involvement in memory formation. We are also investigating the feasibility of visualizing aspects of the tagging hypothesis in neurons.

Tetracycline-mediated Cell Ablation

M. Stebbins [in collaboration with W. Hillen, University of Erlangen, G. Byrne, Nextran, Inc., and B. Bello, Cambridge University]

Utilizing the improved rtTA/VP16-M2-alt *trans*-activator, we have integrated the tetracycline-inducible system together with the GAL4/UAS system. This tripartite system allows both spatially and temporally regulated gene induction. Using the apoptotic gene *rpr*, we have successfully ablated wing and eye tissue using the *salm*-GAL4 and *sevenless*-GAL4 drivers. When the traditional bipartite *salm*-GAL4/UAS-*rpr* system is used, developmental lethality precludes the analysis of adult phenotypes. However, when a *salm*-GAL4 transgene is combined with UAS-rtTA/VP16-M2-alt and TetO-*rpr* transgenes, doxycycline-dependent ablation of wings occurs in a dosage-dependent manner. Late instar larvae are fed the drug, thus bypassing lethality due to *salm*-mediated expression of *rpr* early in development in another tissue besides the wing disc. Thus, the addition of temporal control allows a late wing phenotype to be revealed. It is likely that many GAL4 drivers express in a number of different tissues throughout development. We will use this system for ablation of various adult structures in the adult brain.

Circadian Genes, Sleep, and Memory Formation

P. Wu, C. Margulies [in collaboration with J. Hendricks and A. Sehgal, University of Pennsylvania]

Because of the circadian regulation of a CRE-responsive reporter fly, we have been interested in the possible involvement of the circadian system, and sleep, in the formation and consolidation of memories. We have

shown that a number of different circadian molecules participate in memory formation, since we can both disrupt and enhance the process. However, we have not yet shown whether these effects are because the proteins participate in the different processes or because the various physiologies affect each other.

Regulation of dCREB2 Activity

J. Horiuchi

We have shown that the dCREB2-b protein is largely cytoplasmic and inhibited from DNA binding. Inhibition results from phosphorylation on multiple casein kinase sites located amino-terminal to the well-characterized Ser-133 site (Ser-230 on the fly protein). To demonstrate more convincingly that phosphorylation occurs in vivo prior to extract preparation, we have developed a tissue solubilization and cell lysis procedure in 100 mM EDTA, followed by subsequent analysis of the samples on a native protein gel. Phosphatase treatment or mutation of the casein kinase sites results in slower mobility of the protein relative to untreated samples, supporting the contention that the protein is phosphorylated in vivo prior to making an extract.

Mouse Behavior

E. Friedman, R. Filipkowski

We have been investigating training parameters for the Morris water maze and contextual fear conditioning. Training procedures have been developed whereby multiple trials are required for the establishment of long-term memories. The number of trials, the inter-trial or interblock intervals, and the complexity of contextual cues all contribute to the duration of the memories that are produced. For contextual discrimination, long-lasting memory requires acute protein synthesis around the time of training.

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NEURAL SUBSTRATE OF SELECTIVE AUDITORY ATTENTION IN THE CORTEX

A. Zador M. DeWeese L.-H. Tai
M. Wehr K. Mantzaris
S. Rumpel T. Hromadka
S. Edgar

We use a combination of theoretical and experimental approaches to study how the brain computes. The brain is able to solve hard computations that remain far beyond the reach of the fastest computers. Our goal is to understand this computation at the synaptic, cellular, behavioral, and algorithmic levels.

One example of such a hard computation is the "cocktail party problem." When we tune in to one voice at a cocktail party and tune out the others—a task that remains beyond the capacity of modern computers—we are engaging in a form of selective auditory attention. Our ability to attend selectively is not limited to the auditory domain: Analogous tasks demand selective attention in the visual and even somatosensory domains. In monkeys, visual attention selectively enhances neural activity even in the most peripheral (i.e., near the retina) areas of the visual cortex. This enhancement is surprising because the areas associated with these first stages of visual processing have traditionally been thought of as representing the sensory world faithfully, in a way that depends only on the properties of the sensory input itself. The discovery of attentional modulation overturns the notion that the peripheral sensory cortex is a passive "TV screen" available for viewing by a "homunculus" buried deep within the cortex.

The specific projects in our laboratory fall into two main categories. First, we are interested in how neurons represent auditory stimuli, and how these representations are computed from the cochlear inputs half a dozen synapses away. To address these questions, we are using electrophysiological and imaging approaches in anesthetized rats, as well as computational approaches to characterize the properties of natural sounds. Second, we are interested in how these representations are modified dynamically—within seconds—in awake behaving rats by the demands imposed by attentional tasks.

Binary Coding in the Auditory Cortex

M. DeWeese

Cortical neurons have been reported to use both rate and temporal codes. We have discovered a novel mode, in which each neuron generates exactly zero or one action potential, but not more, in response to a stimulus. Cortical responses recorded using conventional extracellular techniques typically show responses to be highly variable. However, in our studies, we have been using a technique adapted from in vitro studies, cell-attached recording, which has the advantage of ensuring that the recorded responses all arise from a single neuron. Surprisingly, we have found that most neurons exhibit binary behavior, with few multispike responses; some responses consist of exactly one spike in 100% of the trials, with absolutely no trial-to-trial variability. We are currently investigating the computational implications of this previously undescribed coding scheme.

Variability of Coding in the Auditory Cortex

M. DeWeese

Computers rely on extremely precise, low-noise components to compute, whereas the components that make up neural circuits appear to be very noisy. Nevertheless, brains outperform computers on the kinds of hard computational problems required for survival in the real world. To understand how brains compute in the presence of such high levels of appar-

ent noise, we are characterizing the sources of variability (i.e., noise) in single neurons. Using in vivo whole-cell patch-clamp recording techniques, we are examining the trial-to-trial variability of the postsynaptic potential (PSP) elicited by brief tone pips. In some neurons, trial-to-trial variability in the PSP is small, consistent with "private" sources limited to only the neuron under study; but for other neurons, "shared" sources of variability produce circuit-wide fluctuations in the synaptic drive to the neuron and its neighbors, greatly increasing the apparent noise in the PSP. These stimulus-independent correlations could provide a substrate for feedback underlying cognitive processes, such as attention and motivation.

In Vivo Whole-cell Patch-clamp Recordings of Sound-evoked Synaptic Responses in the Auditory Cortex

M. DeWeese, L-H. Tai

Neurons in the auditory cortex respond to some sounds but not to others. What determines this selectivity? We are using whole-cell patch-clamp recording methods in vivo to measure the synaptic currents elicited by simple and complex auditory stimuli. Patch-clamp recordings provide a much richer source of information than do conventional single-unit extracellular recordings because they allow us to monitor not just the *output* of the neuron—the spike train—but the input as well. These data provide clues about the representations with which the cortex solves hard problems in auditory processing.

Responses to Complex Stimuli

M. Wehr [in collaboration with C. Machens and C. Brody, Cold Spring Harbor Laboratory]

How do neurons in the auditory cortex encode complex stimuli, such as animal calls or the sound of rain falling on leaves? Sensory physiologists often ignore this question, dismissing it as too difficult to approach in a systematic fashion. Instead, they often limit their inquiries to simple stimuli, such as (in the auditory system) brief tone pips or sweeps. We have adopted a hybrid approach, in which we probe neuronal responses systematically with a well-defined ensemble of stimuli ("moving ripple stimuli") and then use these responses to predict the responses to the more complex sounds found in nature.

Inhibitory and Excitatory Contributions Underlying Synaptic Responses

M. Wehr

The cortical responses to sensory stimuli such as sounds consist of a mix of excitation and inhibition. Conventional extracellular recording techniques using tungsten electrodes provide information only about excitatory responses. We have developed an approach using whole-cell patch-clamp recording in vivo in voltage-clamp mode to tease apart the temporal dynamics of the excitatory and inhibitory drive underlying responses to auditory stimuli.

AMPA-receptor Trafficking during Experience-induced Plasticity in the Auditory Cortex

S. Rumpel [in collaboration with R. Malinow, Cold Spring Harbor Laboratory]

Which synapses change during learning? Robert Malinow's laboratory has developed a collection of molecular and physiological techniques for monitoring and localizing the changes that synapses in vitro undergo during long-term potentiation (LTP). LTP is a leading candidate for the synaptic basis for learning and memory. We are now applying these techniques in vivo to look for the synaptic correlates of experience-induced plasticity and learning in the rat auditory cortex.

Behavioral Paradigm for Studying Selective Auditory Attention

S. Edgar, L.-H. Tai, M. DeWeese

More than a decade has passed since the first reports of a neural correlate of selective attention in monkeys, but the mechanism underlying the enhancement of neural activity remains a mystery. We are therefore

developing a simpler preparation within which to study the mechanism. We are training rodents to perform a simple selective auditory attention task. We will then use extracellular recording methods (tetrodes) to look for a neural correlate—a change in the firing rate—of this selective attention in the auditory cortex. This simple rodent preparation will have many advantages over the existing primate preparation, as it will permit us to bring to bear the full cellular and molecular armamentarium available to modern neurobiology.

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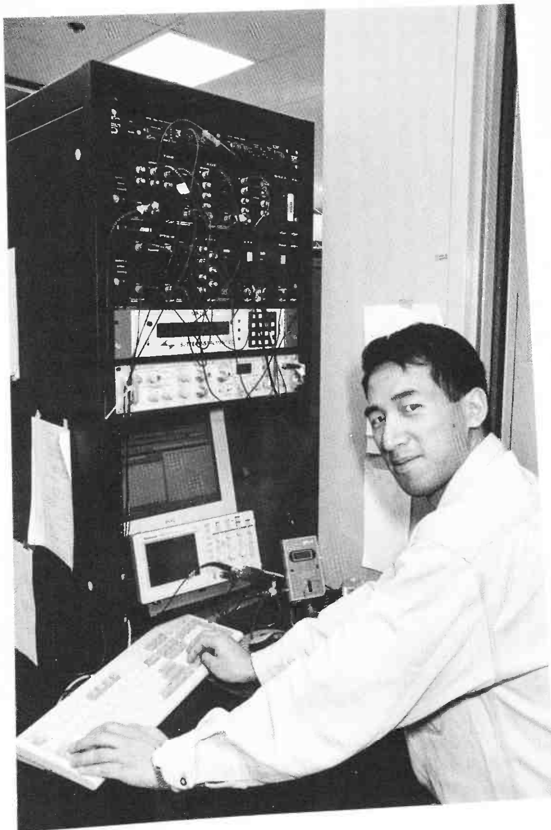

Lung-Hao Tai

NEURAL BASIS OF LEARNING AND MEMORY IN *DROSOPHILA*

Y. Zhong F. Hannan K. Iijima I. Hakker
H.-F. Guo J. Tong I. Ho
Y. Wang

We are interested in the neural basis of learning and memory and are taking an approach of combining functional analyses with genetic manipulation in the study of *Drosophila*. Currently, we are pursuing two major projects. First, we are establishing *Drosophila* models for studying genes involved in human neurodisorders that impair learning and memory. In particular, we are interested in genes known to contribute to neurofibromatosis 1 (*Nf1*) and Alzheimer's disease. *Nf1* patients are identified by neurofibromas and other symptoms including learning defects. We are investigating a hypothesis that the tumor-suppressor gene *Nf1* not only acts as a Ras-specific GTPase-activating protein (GAP), but also is involved in mediating G-protein-stimulated activation of adenylyl cyclase (AC), and this NF1-dependent AC pathway is required for learning. In the case of Alzheimer's disease, our study has been limited to examining how presenilin and accumulation of Aβ affect the age-dependent decline of learning ability. This approach, on the one hand, serves as a new way to identify biochemical cascades underlying learning and memory in *Drosophila*. On the other hand, it may also provide insights into the pathogenesis of diseases. Second, we are investigating how odors are encoded by population neuronal activity in the fly brain and will ultimately study learning and memory at the level of population neural activity. Such study is carried out in living flies via optical recordings. The specific projects are described below.

Site-directed Mutagenesis in Human NF1

F. Hannan, I. Hakker

The tumor-suppressor gene *Nf1* encodes a large protein containing a fragment homologous to Ras-GAPs, which inhibit Ras activity. *Drosophila* NF1 is highly conserved since 60% of its 2803 amino acids are identical to human NF1. Our previous electrophysiological, biochemical, and behavioral analyses of *Drosophila* NF1 mutants have indicated that in addition to functioning as a Ras-GAP, NF1 regulates activation of AC. This NF1-dependent cAMP pathway is crucial for learning and memory. Our recent effort in biochemical assay of AC activity has revealed that G-protein-stimulated AC activity consists of two components, i.e., NF1-dependent and NF1-independent. The NF1-dependent component is largely mediated via the *rutabaga* (*rut*)-encoded AC, which is required for learning in *Drosophila*. To understand how NF1 regulates AC activity, we are investigating how point mutations identified in NF1 patients affect cAMP-pathway-related functions. To do so, we have shown that expression of the human *Nf1* gene was capable of rescuing NF1 mutation-induced phenotype or defects in *Drosophila*, including small body size, reduction in G-protein-stimulated AC activity, and olfactory learning. Moreover, transgenic flies carrying different mutations, two in the GAP domain (directly relating to Ras activity) and one outside of the GAP domain, have been generated. Preliminary data have indicated that expression of human NF1 with the mutation outside of the GAP domain was able to increase learning but failed to rescue the body size phenotype in NF1 mutants. Three further mutations were generated, and we are in the process of testing how expression of the mutated human *Nf1* gene affects its ability to rescue NF1 defects in mutant flies. We are hoping to gain insight into whether Ras activity is required for AC regulation and whether there is a specific domain in the NF1 molecule designated for AC regulation.

Ras-NF1-regulated cAMP Pathway

J. Tong, F. Hannan, I. Ho

We have shown that the NF1 protein is able to regulate G-protein-dependent activation of AC activity in both flies and mice. It remains a concern as to whether Ras has a role in this NF1-dependent AC pathway. In the process of addressing this problem, we found that adding Ha-Ras or Ki-Ras to the membrane fraction extracted from fly heads stimulated AC activity. This

stimulation required the presence of NF1, because Ras failed to increase AC activity in NF1 mutants. Further studies indicated that a reduction of Ras activity resulting from the addition of the purified fragment of NF1 which contains the GAP domain diminished AC activity. These lines of evidence suggest that there is a cross-talk between the Ras and cAMP pathway.

Modeling Alzheimer's Disease in *Drosophila*

K. Iijima, Y. Wang, Y. Zhong

A major hypothesis concerning the pathogenesis of Alzheimer's disease (AD) suggests that accumulation of toxic $A\beta_{42}$ leads to pathologic progression. This is supported by genetic analysis in which all familial AD is caused by mutations of genes encoding either APP or presenilin 1 and 2. APP is the precursor protein of $A\beta_{42}$ peptide, and presenilins 1 and 2 are closely related to γ-secretase activity which is involved in production of $A\beta_{42}$. All familial mutations have been shown to enhance production of $A\beta_{42}$. *Drosophila* has an APP-like gene and a highly conserved *presenilin* gene. By expression of human $A\beta_{42}$ and the *presenilin* gene with mutations that are found in AD patients, we are trying to build a simple *Drosophila* model for studying some aspects of AD. The preliminary results showed that the fly's life span was greatly reduced in flies expressing $A\beta_{42}$ peptides, and learning was also affected in an age-dependent way. We are continuing to examine whether expression of these genes will cause neuronal degeneration.

Notch Neural Plasticity

H.-F. Guo

Another known target of *presenilin* is Notch, which has been shown to be a key signaling molecule in determination of cell lineage across different species. It also expresses in the adult nervous system, but its function in the adult remains to be revealed. As a substrate of *presenilin*, it has been speculated that Notch may have an important role in the AD pathogenesis. However, it has been difficult to investigate Notch's role in the adult nervous system because of the lethality induced by mutations in the gene. In *Drosophila*, the existence of temperature-sensitive mutants and heat-shock-inducible active or dominant negative Notch may facilitate our understanding of its role in the adult. Our studies indicated that Notch is critically involved in mediating activity-dependent nerve terminal plasticity. It has been previously shown that nerve terminal arborization at larval neuromuscular junctions is enhanced by hyperexcitability. This activity-dependent arborization is abolished in temperature-sensitive Notch mutations and by expression of dominant negative Notch. In addition, expression of constitutively active Notch led to enhanced arborization. We are now investigating whether expression of Notch is regulated by neuronal activity.

Stereotyped Representation of Odors in Mushroom Bodies

Y. Wang, H.-F. Guo

The organization and functional logic of the early olfactory system appear to be remarkably similar in *Drosophila* and mammals. An outstanding feature of this system is the topographic map of receptor activity segregated in glomeruli. Yet, how such spatial patterns of activity are transformed to higher centers and then translated into odor perception remains elusive. We are investigating this problem with the relatively simple nervous system of *Drosophila* mushroom bodies. We have developed an optical method that allows us to monitor neuronal activity in the mushroom body with a global view of odor-induced spatially distributed activity over the entire mushroom body, yet with a resolution to single neurons. A green fluorescent protein (GFP)-based Ca^{++} probe is expressed in mushroom bodies specifically. Ca^{++} imaging of the calyx, the dendritic region of the mushroom body, revealed that each odor evoked a specific pattern of spatially distributed activity and that the spatial pattern of activity remained the same among different flies. This stereotyped representation suggests that spatial information at the glomerular level is preserved at higher centers.

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PLANT DEVELOPMENT AND GENETICS

The plant group has made significant progress during the last year, especially in exploring the genetic and epigenetic mechanisms underlying stem cell fate. This has considerable implications for the control of plant architecture (the number and arrangement of flowers, branches, leaves, and shoots), as well as for understanding how potentially totipotent meristematic cells arise and are maintained. Dave Jackson's lab is studying signaling mechanisms, including intercellular protein trafficking. They have shown that specific proteins have different developmental restraints for intercellular trafficking. They also continue to characterize novel meristem mutants and their relationship to crop yields. Highlights of the year include cloning of a cell-surface receptor from maize (*fasciated ear 2*), related to *clv2* in *Arabidopsis*, which controls meristem size and function. Marja Timmermans' lab has identified several proteins that interact with the *myb* domain protein ROUGH SHEATH2 (RS2). This gene is a homolog of *PHANTASTICA* from *Antirrhinum* and is required to repress meristematic genes in the leaf. Interestingly, interacting proteins include histones and chromatin remodeling factors, supporting the conclusion that RS2 acts to epigenetically maintain, rather than establish, the repressed state. They are continuing their studies of leaf polarity in maize, by isolating new mutations as well as candidate genes from *Arabidopsis*.

In my own laboratory, we have isolated several genes required for stem cell fate. *Ramosa1*, which encodes a zinc-finger protein, imposes determinancy on stem cells within the maize inflorescence, preventing the formation of long branches. This may be an important factor in the evolution of the grass inflorescence. *ASYMMETRIC LEAVES1* encodes the *Arabidopsis* homolog of *ROUGH SHEATH2*, and mutually negative interactions with meristematic homeobox genes establish a boundary between stem cells and founder cells that contribute to the leaf. *Argonaute* is another stem cell mutant in *Arabidopsis* and has recently been shown to be required for RNAi (RNA interference). Genetic analysis and microarrays have revealed a number of potential targets, as well as interactions with transcriptional silencing mechanisms.

In fission yeast, we have shown that *argonaute* and other RNAi components are required to silence pericentromeric heterochromatin at the transcriptional level and to maintain its association with modified histones. In *Arabidopsis*, we have used chromosomal microarrays to demonstrate that mutants in the chromatin remodeling factor *DECREASE IN DNA METHYLATION1* also activate heterochromatin by changing the pattern of histone modification and fail to silence transposons. We are pursuing the idea that chromatin remodeling, DNA methylation, and RNA interference cooperate to silence transposable elements in large plant genomes and that these mechanisms have somehow been adopted to regulate stem cell fate.

PLANT DEVELOPMENTAL GENETICS AND FUNCTIONAL GENOMICS

R. Martienssen J.-M. Arroyo, B. Bartelle, M. Byrne, V. Colot, A.-V. Gendrel, C. Kidner, Z. Lippman, B. May, P. Rabinowicz, D. Roh, M. Ronemus, R. Shen, J. Simorowski, T. Singer, J. Tang, R. Umamahsewari, M. Vaughn, E. Vollbrecht, T. Volpe, C. Yordan, E. Head (URP)

We are using genomics and developmental genetics to investigate epigenetic mechanisms of gene regulation, transposon silencing, and stem cell function in plants. In the past year, we have generated genomic microarrays corresponding to a portion of *Arabidopsis* chromosome 4, which was sequenced at the Cold Spring Harbor Genome Research Center. We are using these arrays to investigate the role of chromatin remodeling, DNA methylation, and polyploidy in regulating transposons and heterochromatin in plants. We have also continued genetic studies of *asymmetric leaves1*, *ramosa1*, and *argonaute*, mutations that affect stem cell function in plants. Argonaute has an important role in RNA interference (RNAi), and our studies on argonaute in fission yeast have revealed a role for RNAi in heterochromatin modification. In 2001, we were joined by informatics postdoc Matt Vaughn and Watson School graduate student Zachary Lippman. During the summer, we were joined by our URP, Elizabeth Head, and guest postdoc Andreas Madlung from Seattle. Visiting Scientist Vincent Colot and graduate student Anne-Valerie Gendrel returned to the Institute for Plant Genomics in Evry, near Paris, in August, but they have already returned several times as part of our collaboration on chromosome 4 microarrays. We said goodbye to postdoc Tatjana Singer who left for a staff position at the Torres Mesa Research Institute (Syngenta) in La Jolla, California. Ben Bartelle and Nancy Liu left for graduate school.

DNA Methylation, Genome Organization, and Transposon Mutagenesis in Maize

P. Rabinowicz, B. May, E. Vollbrecht, D. Roh, R. Martienssen [in collaboration with L. Stein, R. Lucito, and W.R. McCombie, Cold Spring Harbor Laboratory]

We have previously shown that the unmethylated portion of the maize genome is highly enriched for genes, and we have extended these findings to several other plant genomes. This suggests a strategy for shotgun genome sequencing using small inserts propagated in McrBC+ bacterial strains. However, a significant concern is that many genes might remain in the methylated portion of the genome. Maize nuclear DNA was digested with McrBC that only restricts methylated DNA, and samples were used as a template for polymerase chain reaction (PCR). Fifty primer pairs from exons, introns, and promoters were randomly chosen from 29 genes and 5 transposons. None of the 5 transposons were amplified efficiently from digested DNA, whereas more than 90% of exons were amplified, including at least one exon from every gene tested. This indicates that all genes will be at least tagged in a methylation-filtered shotgun library. We are applying this method to the maize and sorghum genomes on a pilot scale and are printing selected clones on microarrays. These will be used to anchor genes to the physical map, obtain expression profiles, and perform high-throughput screening of transposon-tagged mutants from the *m*aize *t*argeted *m*utagenesis project (MTM) (http://mtm.cshl.org).

MTM is a large Mutator (Mu) transposon population and screening service constructed by Cold Spring Harbor Laboratory, Syngenta, and the University of Californa, Berkeley, and operated by CSHL. The screening service is open to the public and has been fully operational for 18 months. Insertions into genes of interest are detected by nested PCR on three-dimensionally pooled DNA samples from a collection of more than 44,000 self-pollinated plants. Approximately 40% of genes screened in this way have a heritable insertion, and these are supplied to researchers on request. Insertions predominate in the 5′ portions of genes, but no bias for intron or exon sequences is evident. A description of 8000 mutant lines is available at http://mtm.cshl.org, but most lethal mutations are only observed in families that have retained Mutator activity. This indicates that insertion alleles are suppressed in lines that have lost activity, an epigenetic property that we have previously documented for insertions in promoters and introns. We are currently developing PCR representations of pooled

insertions for use in hybridization mapping on filters and microarrays (see above). In this way, we hope to map many thousands of insertions in parallel.

Transposable Elements and Chromatin Remodeling in *Arabidopsis*

Z. Lippman, A.-V. Gendrel, T. Singer, C. Yordan, V. Colot, R. Martienssen [in collaboration with V. Mittal and W.R. McCombie, Cold Spring Harbor Laboratory]

DDM1 (*Decrease in DNA methylation 1*) was identified several years ago in a screen for *Arabidopsis* mutants resulting in loss of DNA methylation, and it was found to encode a novel member of the SWI/SNF ATPase-chromatin remodeling protein family that is highly conserved in mammals and yeast. We have shown that loss of DNA methylation in *ddm1* mutants results in activation of Robertson's *Mutator* and other families of transposable elements. This may account in part for the "mutator" phenotype of these strains. As part of a collaborative study on the effects of polyploidy in plants, we have constructed genomic microarrays of heterochromatic regions of *Arabidopsis* chromosome IV. We have used these arrays to profile expression, DNA methylation, and chromatin modification in *ddm1*. Several transposons and hypothetical genes are activated, whereas known genes do not alter their expression. To determine methylation patterns, we used the methylation-dependent enzyme McrBC to remove heavily methylated sequences from labeled genomic DNA targets. Two-color microarray hybridization, along with total DNA targets, revealed methylated probes. Transposons in the heterochromatic region were heavily methylated in wild-type plants but unmethylated in *ddm1* mutants. In contrast, known genes lacked methylation in either genotype, consistent with our observations in maize (see above). Hypothetical genes, predicted only by computer, resembled transposons more closely than genes.

In mammalian and fungal systems, histone H3 methylation at Lys-9 (mH3K9) and Lys-4 (mH3K4) correlates with heterochromatic and euchromatic DNAs, respectively. Furthermore, in the fungus *Neurospora*, DNA methylation requires mH3K9. Thus, we set out to test whether there was a corresponding change in histone H3 methylation status in *ddm1* mutants that correlated with loss of DNA methylation. We developed a chromatin immunoprecipitation protocol for *Arabidopsis* and tested which loci were associated with mH3K9 and mH3K4 in wild-type and *ddm1* mutants. PCR amplification of more than 50 features indicated that transposons were associated with mH3K9 in wild-type plants. These same features were associated with mH3K4 in *ddm1* mutants, as were known genes in both genotypes. We are currently pursuing "ChIP on chip" experiments using genomic microarrays to establish the specificity of DNA and histone methylation in heterochromatic domains.

Systematic Investigation of Redundant and Essential Genes in *Arabidopsis* Using Gene Traps

B. May, M. Vaughn, C. Yordan, J. Simorowski, J.-M. Arroyo, R. Shen, B. Bartelle, Y. Liu, R. Martienssen [in collaboration with W.R. McCombie and L. Stein, Cold Spring Harbor Laboratory]

Systematic sequencing of close to 10,000 gene- and enhancer-trap lines has identified multiple insertions into at least 2700 genes (12% of the *Arabidopsis* genome). About 1000 are insertions into unique genes, whereas the remainder are distributed among gene families. Each line is routinely screened for early lethal phenotypes, and a systematic study is under way to determine expression pattern and genetic function during development. The results are entered into a relational database, and a suite of Web-based applications has been written to perform complex searches over the WWW. The Web Site (http://genetrap.cshl.org) is open to the public as a resource for functional genomics; 930 lines have an insertion in the sense orientation and exhibit reporter gene (GUS) expression in specific tissues or cell types. Potential *cis*-acting regulatory motifs in the promoter regions of genes sharing a common expression pattern are being sought using AlignACE, MEME, and GeneSpring. We are also collaborating with The *Arabidopsis* Information Resource (TAIR) to develop the *Arabidopsis* Genetic Trait Ontology, a formal but flexible lexicon for describing morphology and developmental mutations.

Ramosa1 and Grass Inflorescence Architecture

E. Vollbrecht, R. Martienssen

Plant architecture, the overall shape and appearance of a plant, is determined by the number, arrangement, and relative activity of meristems, groups of stem cells found at the growing point of branches, flowers, and shoots. In *ramosa1* (*ra1*) and *ramosa2* (*ra2*) mutants in

maize, meristem activity is prolonged, leading to long branches in the ear and tassel. Last year, we reported on the molecular isolation of the *ra1* gene, and we have now determined by in situ hybridization that *ra1* imposes determinacy on the meristem from an adjacent domain, possibly by specifying the meristem boundary, whereas *ra2* functions by regulating *ra1* transcript levels. Inflorescence architecture is a key character in the morphological classification of wild and domesticated grass species, and we have begun to survey sequence diversity at the *ra1* locus. *ra1* sequence diversity is unusually low among strains of maize and markedly higher in related wild species, consistent with *ra1* being a target of selection during maize domestication. In sorghum, whose branched tassel resembles a *ramosa* mutant, the orthologous *ra1* gene has been duplicated and its transcript is barely detectable. These findings reinforce our fundamental hypothesis that modulation of the *ra1* pathway leads to architectural changes.

Stem Cell Fate and Organogenesis in *Arabidopsis*

M. Byrne, R. Martienssen

The shoot apical meristem comprises undifferentiated stem cells and their derivatives, which include founder cells for lateral organs such as leaves. Meristems are maintained and lateral organs are specified in part by interactions between the myb domain transcription factor *ASYMMETRIC LEAVES1* (*AS1*) and a group of closely related homeobox transcription factors (*knox* genes). The *knox* gene *SHOOT MERISTEMLESS* (*STM*) negatively regulates *AS1* in the meristem which, in turn, negatively regulates other *knox* genes, including *KNAT1* and *KNAT2* in lateral organ primordia. A second gene, *ASYMMETRIC LEAVES2* (*AS2*), acts genetically at the same position in this hierarchy as *AS1*. Thus, *as1* and *as2* suppress the shootmeristemless phenotype in double mutants with *stm*. We used a second-site suppressor screen to isolate mutations that restore the shootmeristemless phenotype in these double mutants. Mutations in *KNAT1* were recovered that correspond to the classic mutation *brevipedicellus*, which alone has a relatively mild inflorescence phenotype. Thus, *KNAT1* is redundant with *STM* in regulating stem cell function, but this redundancy is only revealed in the absence of *AS1*. Mutations in *KNAT2* show no such interaction, but gene-trap insertions in another homeobox gene also restore the shootmeristemless phenotype in *as1stm* double mutants. This gene-trap insertion alone (*quasimodo*) also has a relatively mild phenotype, but is stunted and has lost apical dominance.

Role of RNAi in *Arabidopsis* Development

C. Kidner, M. Ronemus, R. Martienssen

argonaute (*ago1*) was first isolated as a pleiotropic mutation in *Arabidopsis* with defects in organogenesis. We have defined an allelic series of *ago1* with defects in meristem function, organ polarity, and organ identity. Weak *ago1* phenotypes resemble those found in *carpel factory*, a partial loss-of-function mutation in an RNase III helicase required for floral organogenesis. Strong *caf1* alleles are lethal, as are double mutants between *ago1* and the partially redundant homolog, *pinhead/zwille*. Double mutants with *stm*, *wuschel*, and *cup-shaped-cotyledon* (*cuc1 cuc2*) suggest that *AGO1* controls meristem function via *STM*, whereas double mutants with *lfy* and *ufo* show that *AGO1* has a role in floral organ identity. Gene-trap insertions in a third member of the argonaute family (*JASON*) are being characterized.

In *Caenorhabditis elegans* and *Drosophila*, homologs of *argonaute* and *carpel factory* (*rde1* and *dicer*, respectively) were later found to have key roles in RNAi. We have shown that weak alleles of *caf1* strongly enhance weak alleles of *ago1*, indicating a common pathway. Enhancers of weak *ago1* include *as1* and *revoluta* (*rev*), suggesting a possible role for RNAi in communication between meristems and lateral organs. A link between RNAi and transcriptional gene silencing is suggested by the partial rescue of strong *ago1* by loss-of-function mutations in *CURLY LEAF* (*CLF*), a polycomb group gene, and the enhancement of weak *ago1* by a gene-trap insertion in the chromomethylase gene *cmt3*. *ARGONAUTE* regulates transgene methylation during posttranscriptional silencing, and we have found two retrotransposons that are partially demethylated in *ago* and *caf* mutants.

Microarray analysis of weak and strong alleles at two developmental stages has revealed that up to 5% of the genes in *Arabidopsis* are mis-regulated in RNAi mutants. However, the putative transcription factor genes *squamosa promoter binding-protein like10* (*SPBL10*) and *SPBL11* are among a handful that are strongly up-regulated in both *ago1* and *caf1*. These genes form an inverted repeat in the genome and may produce antisense transcripts. A closely related, but unlinked, family member *SPBL2* is also up-regulated.

FIGURE 1 Mutations in the polycomb group gene *CURLY LEAF* (*clf*) partially rescue strong argonaute (*ago1*) alleles. *ago1-9* alone (*left*) forms rods instead of flowers, but in a *clf* background, flowers are formed (*middle*). The flowers (*right*) have fewer organs, carpels are very reduced, but one or two symmetrical ovules are formed. Petals and stamens cannot be clearly distinguished.

Other members of this gene family affect maize leaf development and *Arabidopsis* meristem identity genes, consistent with a role in the *ago* and *caf* phenotypes.

RNAi and Heterochromatin in Fission Yeast

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Argonaute proteins are encoded by multigene families in *Arabidopsis* and other higher eukaryotes. To avoid genetic redundancy, we are studying *argonaute* in the lower eukaryote *Schizosaccharomyces pombe* (fission yeast), which has only a single copy of this gene. With the discovery that *argonaute* homologs are required for RNAi, we have extended these studies to genes encoding a homolog of RNase III helicase (carpel factory/dicer) and a homolog of RNA-dependent RNA polymerase (RdRP), each of which is also unique in the fission yeast genome. *Argonaute* homologs regulate heterochromatic RNA and segregation distortion in *Drosophila*, as well as gene silencing. We have shown that none of the three RNAi genes are essential in fission yeast, but they are required for transgene silencing at the centromere, as well as for normal chromosome segregation. Furthermore, deletion of each of these genes results in the accumulation of transcripts from heterochromatic repeats that are normally silent. Eukaryotic heterochromatin is characterized by a high density of transposons and repeats, as well as by modified chromatin proteins including histones H3 and H4. We have shown that silencing of the repeats attracts histone H3 Lys-9 methylation, which may in turn be responsible for transcriptional silencing at the centromere.

Our results suggest that the sequence specificity of silent heterochromatin is determined in part by double-stranded RNA arising from centromeric repeats. In this sense, these repeats resemble transposons, indicating a role for transposable elements and RNAi in centromere function.

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PLANT CELL SIGNALING AND GENETICS

D. Jackson U. Au, K. Biddle, M. Cilia, K. Aliano, A. Giulini, L.-A. Haller, J. Hsieh, J.Y. Kim, C. Lau, Z. Yuan

We are interested in how the shape or morphology of plants is determined. This is a fundamental question in developmental biology, with downstream applications in plant productivity and crop yields. Specifically, we aim to define the molecular and genetic pathways that control development. Recently, we have been studying cell-to-cell signaling, or how cells talk to each other, to coordinate their development and fate. Our two model organisms are maize, one of the world's most important crops, and *Arabidopsis*, a "lab rat" plant that is ideal for fundamental studies of gene regulation and cell biology. We are also developing new projects to extend what we learn from these model systems to other cereal crop plants, such as wheat, barley, and rice, to learn how developmental genes have contributed to the selection of these different crop species.

Novel Fasciated Mutants

U. Au, L.-A. Haller, M. Muszynski (Dupont-Pioneer Hibred), D. Jackson

Fasciation describes the enlargement of the plant apex by unregulated proliferative growth, analogous to animal tumors. We are systematically identifying, mapping, and characterizing novel fasciated mutants of maize to figure out how plant growth is normally regulated. A particularly rich resource has been the Maize Targeted Mutagenesis collection developed in part by Rob Martienssen's group here at CSHL. During the past three summers, we have screened F_2 families for fasciated mutants that affect the growth of the tassel or ear. We have isolated about ten new mutants that we are introgressing into selected inbred lines for mapping, characterization, and allelism tests. In particular, we will concentrate on mutants that show a genetic interaction with *fasciated ear2* (*fea2*), a gene that we recently found to encode a leucine-rich repeat receptor-like protein. We have already identified one fasciated mutant that shows a dominant genetic interaction with *fea2*, suggesting that it may encode a component of the *fea2* signaling pathway. We are also characterizing the mutant *compact plant2* (*ct2*). This mutant is particularly interesting because it is one of the few that affect both ear and tassel development. *ct2* mutant plants are short and have thick upright tassel branches and fasciated ears. We are characterizing a new mutant from a *Mutator* transposon screen that is similar to *ct2*, and allelism tests are in progress. We also have characterized the development of *ct2* ears by scanning electron microscopy (Fig. 1). In contrast to normal ears (A), at an early stage of development, the apex of *ct2* ears enlarges into a ring-shaped fasciation (B). Abnormal development continues throughout the growth of the *ct2* ear. For example, *ct2* flowers are highly abnormal. Normal maize flowers are borne in pairs, with a forked silk enclosing the conical ovule primordium (C). In *ct2* ears, the ovule is proliferative and spills out like a protruding tongue from the surrounding silk tissue (D).

We conclude that the *ct2* gene is required throughout ear development for the proper growth of different meristem types.

Subcellular Localization of an FEA2-GFP Fusion in Transgenic Rice Plants

D. Jackson [in collaboration with Fumio Taguchi Shiobara, NIAR, Tsukuba, Japan]

The FEA2 protein is predicted to localize to the plasma membrane, but localization of this protein or its orthologs has not been shown. We made a fusion of the green fluorescent protein (GFP) to the carboxyl terminus of FEA2 and introduced the gene fusion into rice

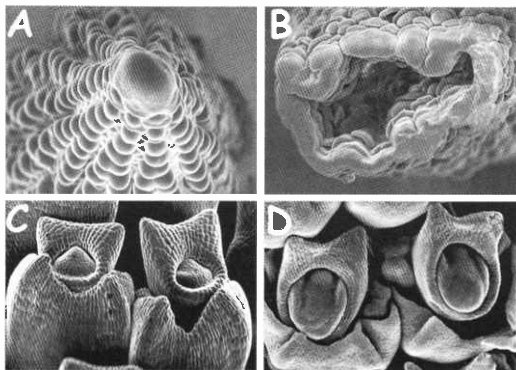

FIGURE 1 Ear development in normal plants (*A,C*) and in *ct2* mutants (*B,D*).

plants using *Agrobacterium*-mediated transformation. Primary transgenics were imaged in the confocal microscope. As seen in Figure 2, FEA2-GFP is localized to the cell periphery and to the endoplasmic reticulum (ER), which forms a characteristic reticulate network in the cytoplasm, and shows perinuclear enrichment. Therefore, these results are consistent with targeting of FEA2-GFP via the ER to the plasma membrane. We are generating FEA2-specific antibodies that will be useful in confirming this localization and for isolating FEA2-interacting proteins.

A Genetic Analysis of Seed Row Number in Maize

U. Au, L.-A. Haller, D. Jackson [in collaboration with T. Rocheford, Illinois]

One of the major factors in the human selection of maize as a crop plant was a massive increase in the number of seeds produced per plant. This was achieved by a combination of factors, one of the major ones being an increase in the number of rows of seed

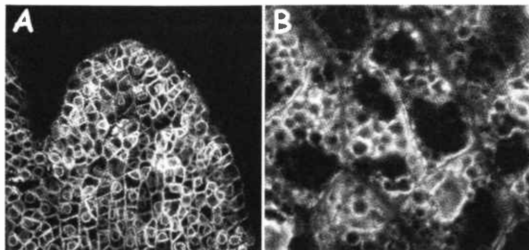

FIGURE 2 Localization of FEA2-GFP in transgenic rice. Image of the shoot meristem (*A*) shows localization of FEA2-GFP fluorescence to the cell periphery and perinuclear sites. In *B*, a close-up image shows in addition that FEA2-GFP localizes to the reticulate ER network.

on the ear. For example, the wild ancestor of maize, teosinte, has only a single row of seed, whereas modern maize cultivars have ears with from 12 to 18 rows. We found that row number correlates with the size of the inflorescence meristem, indicating that regulation of meristem size is an important factor in seed row number.

Our interest was also sparked by the observation that *fea2*, a mutation that controls ear meristem size, maps close to a quantitative trait locus (QTL) for row number. In the past, QTL analysis in maize has been a relatively imprecise method to identify loci controlling a particular trait, but with relatively low genetic resolution. A new mapping population, the integrated B73 Mo17 (IBM) population, has now been developed and is ideal for the fine mapping of quantitative traits. We have begun to utilize this resource by measuring seed row number in the different IBM families. B73 and Mo17 inbred lines differ significantly in row number, and we observed a wide range of row numbers in the IBM families that exceeds the parental range. Our preliminary QTL analysis indicates that row number is under the control of a small number of loci, some of which map to a previously identified row number QTL and some of which are novel. Once we perform the analysis with the full IBM data set, we hope to clarify the correspondence between row number QTL and known mutants affecting row number, such as the *fea* mutants. It may be possible to isolate the corresponding genes if a corresponding mutant is available, or in the future, using a map-based approach.

Tissue-specific Regulation of Protein Trafficking

J.Y. Kim, M. Cilia, Z. Yuan

We previously reported specific intercellular trafficking of GFP fusions to the maize KNOTTED1 protein, as well as to known viral movement proteins (MP). These proteins presumably traffic through plasmodesmata (PD), small channels that connect plant cells, to control development and virus spread, respectively. We have extended this analysis by expressing these and other fusion proteins under the control of tissue-specific promoters, to ask whether there is developmental regulation of PD trafficking. Indeed, we find that there are specific developmentally regulated domains for protein trafficking. For example, the GFP~KN1 fusion protein is able to traffic from perivascular cells through mesophyll and into epidermal cells. However, when expressed in the epidermis, the fusion protein cannot traffic down into mesophyll cells. Remarkably, this implies that there is directional regulation of protein trafficking at the epidermal-mesophyll boundary. This appears to be a specific property of GFP~KN1, since the GFP~MP fusion is able to traffic from epidermal to mesophyll cells. Presumably, this reflects the virus' ability to overcome the normal regulation of PD trafficking in the leaf, which is necessary for the virus to spread and cause infection.

We also confirmed prior observations that the GFP protein itself is able to traffic through PD. We found that this occurs via a passive mechanism, because movement can be blocked by fusion of GFP to yellow fluorescent protein (YFP). The cell-to-cell movement of GFP is relatively easy to image under low-magnification epifluorescence microscopy, and we are using this phenotype to develop genetic screens for mutants affecting PD size exclusion limit. PD are still very poorly characterized, and we envisage that a genetic approach will allow us to identify regulatory or structural components of these elusive channels.

Gal4 Reporters for the Shoot Apical Meristem

K. Biddle, M. Cilia, J.Y. Kim

We are particularly interested in understanding the regulation of protein trafficking in the shoot apical meristem, the developmental command center of the plant. To date, relatively few tools for the analysis of the shoot meristem have been developed. We are therefore making specific reporter constructs that will allow us to drive the expression of any protein in a restricted domain of the meristem. We have made these constructs for an L1 and an L3 layer-specific reporter and have found interesting domain-specific regulation of protein trafficking in the meristem. For example, the GFP~KN1 fusion protein is able to traffic from L3 to the L1 layer; however, a viral movement protein is restricted in its ability to move up from the L3. This observation helps to explain why some plant viruses are unable to invade the shoot apical meristem and provides hope that viral resistance might be

achieved if we can understand how this spatial regulation is achieved. We are extending these studies with different Gal4 drivers and GFP fusions, for example, to a movement protein from a virus that does invade the meristem.

As protein trafficking appears to be an important regulatory mechanism in plant development, we are developing these tools to understand how trafficking is regulated in the meristem. In the future, these tools will also be useful for the characterization of developmental mutants that may affect protein trafficking or cell specification.

Isolation of a Candidate Gene for *abphyl1*

A. Giulini, Z. Yuan

The *abphyl1* mutation affects the geometric pattern of leaf initiation, leading to plants with opposite leaf arrangements instead of the usual alternating pattern found in maize. We have isolated four new alleles of *abph1* using *Mutator* and *Spm* transposon tagging. We found a cosegregating DNA band with one allele, *abph1-181*, and cloned this fragment. The flanking sequence was used to probe Southern blots of DNA from our other *abph1* alleles. We found that the three other new alleles are deleted for the whole of the flanking sequence, which contains a small predicted gene. This gene is expressed in shoot apex tissues. The original allele, *abph1-0*, appears to be rearranged, possibly tandemly duplicated, for this probe. These data support the hypothesis that we have cloned the *abph1* gene, although we do not know the extent of the deletions in our other transposon alleles. We are currently isolating new alleles from the MTM collection, as well as mapping the deletions, to confirm whether we have isolated the correct gene. The way in which leaf arrangements are determined is not well understood, so isolation of the *abph1* gene should provide unique insight into this classical biological question.

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PLANT DEVELOPMENTAL GENETICS

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Plants have the unique property to be able to initiate new organs, such as leaves and flowers, throughout their lifetime, which frequently extends over many years. The growing tips of plants are called meristems, and they consist of a small group of indeterminate stem cells. These cells divide to maintain the stem cell population and indeterminate growth of the plant, and to differentiate into determinate founder cells from which lateral organs arise. Coincident with their initiation, lateral organs are patterned along three developmental axes: (1) the proximodistal axis, which leads to the differentiation of distinct tissues and cell types at the base and the tip of the developing organ; (2) the dorsoventral axis, leading to distinct cell fates in the upper and lower surfaces of the organ; and (3) the mediolateral axis, which distinguishes central and marginal domains of the organ. Early surgical experiments suggested that signals from the meristem have an important role in the patterning of lateral organs. However, the genes involved in the initiation and patterning of lateral organs in plants are still largely unknown.

We are using forward and reverse genetic approaches to generate mutants in maize and *Arabidopsis* that affect these processes. In particular, we are studying the role of *Rough sheath2* in the repression of stem cell fate during organ initiation and growth, and we are analyzing the genetic pathways leading to dorsoventral polarity in leaves and leaf-like lateral organs. A better understanding of the function of genes involved in pattern formation will provide new insights into fundamental concepts of plant development. Variations in the expression patterns of such genes are likely to contribute to the diversity of leaf shapes among many plant species. More importantly, however, the isolation and characterization of genes involved in the patterning of leaves may facilitate the manipulation of plant architecture, which has become increasingly more important for aspects of crop yield such as shade tolerance.

ROLE OF *ROUGH SHEATH2* IN THE REPRESSION OF STEM CELL FATE

The mechanism that distinguishes stem cells from organ founder cells in the shoot apical meristem (SAM) is largely unknown, but the regulation of *knotted1*-like homeobox (*Knox*) gene expression appears to be one of the key determinants. *Knox* genes are expressed in the meristem and are required for indeterminate growth, i.e., stem cell fate. Initiation of determinate lateral organs such as leaves is correlated with the down-regulation of *Knox* gene expression in a subset of cells within the meristem, the organ founder cells.

We have previously shown that the *Rough sheath2* (*Rs2*) gene from maize encodes a MYB-domain protein that acts as a negative regulator of *Knox* gene expression. Recessive mutations in *rs2* cause the misexpression of KNOX proteins in developing leaf primordia. This past year, we determined that expression of at least three of the nine *Knox* genes in maize is regulated by *Rs2*. These include *Knotted1, Rough sheath1*, and *Liguleless3*. Expression of another *Knox* gene, *Gnarly1*, appeared to be regulated independently of *Rs2*. Interestingly, KNOX protein accumulation in leaves of *rs2* null mutants occurs in just a subset of cells that express *Rs2* in wild type. KNOX proteins accumulate in patches with sharp lateral boundaries, suggesting that *rs2* leaves are clonal mosaics of *Knox*$^+$ and *Knox*$^-$ sectors. The numbers and sizes of such sectors vary among leaves and do not correlate with normal developmental domains. Furthermore, the down-regulation of *Knox* gene expression during leaf initiation precedes the onset of *Rs2* expression, suggesting that *Rs2* is required for the maintenance of *Knox* gene repression. On the basis of these observations, we proposed that *Rs2* acts as an epigenetic regulator to keep *Knox* genes in an "off" state. *Rs2* may thus prevent differentiated cells within lateral organs from reverting to indeterminate stem cells.

We are studying the mechanism by which *Rs2* represses *Knox* gene expression by identifying genes that act together with *Rs2*. We have constructed and screened a two-hybrid library made from young maize leaf cDNAs. Results from this screen indicate that RS2 can homodimerize, but it remains to be determined whether RS2 acts as a dimer during *Knox* gene repression in vivo. In addition, we identified six other proteins that interact with the non-MYB domain of

RS2 in this screen. These RS2 interacting proteins (RS2-IPs) have homology with either the protein phosphatase 2A regulatory subunit A (PP2A-A), PP2A regulatory subunit B′ (PP2A-B′), a zinc-finger transcription factor, histone 2B (H2B), a known chromatin remodeling protein, or a hypothetical protein from *Arabidopsis*.

This past year, we have initiated several experiments to confirm our two-hybrid results and to further characterize the RS2-IPs. To confirm the interactions between RS2 and the RS2-IPs in vitro in the absence of other yeast proteins, we are using glutathione-*S*-transferase (GST) pull-down assays. We have generated fusion proteins between GST and several of the RS2-IPs. These fusion proteins were purified by affinity chromatography over a glutathione-Sepharose column and subsequently tested for their ability to interact with RS2. Results from these experiments indicated that all RS2-IPs tested so far were indeed able to physically interact with the non-MYB domain of RS2.

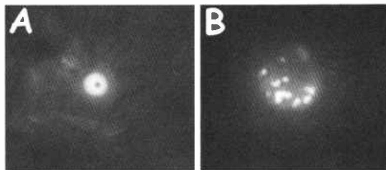

FIGURE 1 The RS2 complex is required to maintain *Knox* gene silencing in the leaf. Proteins that interact with RS2 are therefore predicted to localize to the nucleus of the cell. To test this hypothesis, we transiently expressed fusion proteins of RS2-IPs to GFP in leaf cells and observed GFP localization by fluorescence microscopy. One of the RS2-IPs is highly conserved between yeast, plants, and mammals; however, its function is unknown. The GFP fusion to this particular RS2-IP resulted in fluorescence throughout most of the nucleus of nondividing cells, but fluorescence was excluded from the nucleolus (*A*). In dividing cells, fluorescence marked the condensed chromosomes (*B*). These results suggest that this RS2-IP may be associated with chromatin, consistent with the predicted role for RS2.

On the basis of the amino acid sequence and the function of RS2, we would predict that the RS2 complex is localized within the nucleus. Several of the RS2-IPs, including H2B, the transcription factor, and the chromatin remodeling protein, are known to be localized to the nucleus. Amino acid sequence analysis of the remaining RS2-IPs predicts that all three proteins contain nuclear localization signals. We have tested the subcellular localization of two of these RS2-IPs by transiently expressing chimeric proteins consisting of the RS2-IP fused to the green fluorescent protein (GFP) in leaf tissue. As predicted, fluorescence resulting from the GFP was limited to the nucleus of expressing cells, indicating that the RS2-IPs normally function in the nucleus. Interestingly, the fusion protein between GFP and the RS2-IP with unknown function was excluded from the nucleolus but was localized specifically to chromosomes in dividing cells (Fig. 1). This result together with the confirmed interaction between RS2, H2B, and the chromatin remodeling factor would suggest that RS2 maintains *Knox* genes silenced by altering the organization of chromatin at these loci.

To test this model and to determine the roles of the RS2-IPs in plant development, particularly in stem cell function, we are screening for mutations in the genes encoding RS2-IPs. We have obtained the complete sequence of full-length cDNA clones encoding the different RS2-IPs. Using the reverse genetic tools available in *Arabidopsis*, we have obtained mutations in several histone genes, in the zinc-finger transcription factor, and in the chromatin remodeling protein. We have also begun to generate transgenic lines overexpressing the RS2-IPs, but these experiments are still in a preliminary stage. The outcome of this research will broaden our understanding of the process that distinguishes determinate, differentiating cells from stem cells in plants. It will also contribute to our understanding of the role of gene silencing mechanisms in development. Both of these processes are currently poorly understood.

DORSOVENTRAL PATTERNING OF LATERAL ORGANS IN MAIZE

Normal maize leaves develop as flattened dorsoventral organs with distinct cell types on the upper/dorsal and lower/ventral sides. We previously reported that *Leafbladeless1* (*Lbl1*) is required for the specification of dorsal identity in leaves and leaf-like lateral organs and that dorsoventral polarity in the developing organ is needed to grow out laterally into a flattened leaf. Partial loss of *Lbl1* gene function results in the formation of ventralized, radially symmetric leaves or in ectopic laminar outgrowth surrounding ventralized sectors on the dorsal leaf surface.

We have identified three new alleles of *lbl1* from ethylmethanesulfonate (EMS)- and transposon-mutagenized populations. The most severe allele of *lbl1*, *ragged seedling1* (*lbl1-rgd1*), results in embryo lethality. We are analyzing the embryo lethal phenotype in more detail. Scanning electron microscope analysis of developing maize embryos suggests that homozygous *lbl1-rgd1* embryos develop a distorted and more rounded scutellum (comparable to a cotyledon) and fail to develop any shoot structures. In situ hybridization with a *knotted1*-specific probe indicated that development of the root axis is delayed in the *lbl1-rgd1* mutant, but the organization of the root remains normal. In contrast, no SAM was present in immature mutant embryos. These results suggest that establishment of dorsoventral polarity in the scutellum may be required to initiate or maintain a SAM. Early surgical experiments have shown that signals from the SAM are required to specify the dorsal leaf domain. Therefore, a mutual relationship may exist between the dorsal leaf domain and the SAM. Signals from the dorsal domain of the scutellum or leaf are required to initiate or maintain normal meristem function, and signals from the SAM are required to establish dorsal identity in the next new leaf.

We have recently identified three additional loci required for dorsoventral patterning. To characterize *lbl1* and the new dorsoventral patterning mutants in more detail, we have cloned the maize homologs of several *Arabidopsis* genes that are expressed specifically in either the dorsal or ventral domains of the leaf. The *Arabidopsis YABBY* genes act in the ventral domain of lateral organs and are thought to be required for the specification of ventral cell types in the leaf. We have isolated cDNAs for several *Yabby* homologs from maize and are analyzing their expression patterns in wild-type maize and in the different dorsoventral polarity mutants. As in *Arabidopsis*, the maize *Yabby* genes are expressed throughout the incipient primordium, but interestingly, expression becomes rapidly restricted to the dorsal side and to the margins of the leaf. The basis for and the consequence of the opposite *Yabby* expression patterns in *Arabidopsis* and maize are currently under investigation. However, the observation that *Yabby* expression is restricted to the margins of young leaf primordia suggests that in maize, these genes may not function to specify cell fates, since this would require continual expression until differentiation occurs later in leaf development. Our results suggest that the *Yabby* genes may mediate dorsoventral polarity signals to direct lateral growth at the leaf margins. The establishment of *Yabby*-expressing cells next to nonexpressing cells could be required for lateral growth. If so, there would be no evolutionary constraints to limit *Yabby* expression to, for instance, the ventral side of the leaf.

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In Press

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COLD SPRING HARBOR LABORATORY FELLOWS

In 1986, Cold Spring Harbor Laboratory began a Fellows program to encourage independent research by outstanding young scientists who, during their graduate studies, displayed exceptional promise of becoming leading scientists of the future. The purpose of this program is to provide an opportunity for these young scientists to work independently at the Laboratory for a period of up to 3 years on projects of their own choosing. Fellows are provided with a salary, research support, and technical assistance so that they can accomplish their goals free of distraction. The interaction among research groups at the Laboratory and the program of courses and meetings on diverse topics in biology contribute to a research environment that is ideal for innovative science by these Fellows.

Previous Cold Spring Harbor Laboratory Fellows Adrian Krainer (1987) and Scott Lowe (1995) are currently members of the faculty at the Laboratory. After 9 years at the Laboratory, Carol Greider (1988) left to join the Department of Molecular Biology and Genetics at Johns Hopkins University School of Medicine. Eric Richards (1989) is currently in the Department of Biology at Washington University. After finishing his fellowship, David Barford (1991) returned to the Laboratory of Molecular Biophysics at Oxford University. Ueli Grossniklaus (1994) was a member of our faculty before leaving to join the Friedrich Miescher Institut in Basel, Switzerland in 1998. Marja Timmermans, who joined us from Yale in 1998, ended her fellowship in June of this year, when she became an assistant professor at the Laboratory.

The two current CSHL Fellows, Gilbert (Lee) Henry and Terence Strick, joined the Laboratory in 2000. Their reports are listed below. Lee joined us from Doug Melton's laboratory at Harvard University where he earned his Ph.D. for studies on *Xenopus* development. Lee is studying taste bud development and the role of innervation in this process. Terence joined us after earning his Ph.D. in molecular and cellular biology at École Normale Supérieure in Paris with David Bensimon and Vincent Croquette. Terence is using single-molecule biophysics to study the mechanical response of DNA to stretching and twisting by enzymes that alter DNA topology, thus elucidating the properties of these enzymes.

L. Henry
T. Strick

Structural and Functional Studies of the Vertebrate Taste Bud

L. Henry, A. Samuel, Y. Zhu

The goal of our research is to understand the molecular mechanisms that are required for the formation of taste buds during embryogenesis and the maintenance of their structure and function in the adult. The sensory cells of gustation are housed within ovoid structures called taste buds, which are embedded in the epithelium of the tongue, and to a lesser extent, the epithelium of the palate and upper pharynx. Consisting of 70–100 cells, the vertebrate taste bud is a highly dynamic structure that possesses both epithelial and neuronal qualities. Like all other sensory cell types, the taste receptor cell membrane depolarizes in the presence of a suitable stimulus. Similar to the epithelial cells that line the intestine and other areas of the gut, cells within the bud turn over at a rapid rate (~8–10 days in rodents). Unlike the olfactory system, where the axons of newly formed receptor cells project for some distance back toward and synapse on to neurons in the glomeruli of the olfactory bulb, newly formed taste receptor cells lack classical projections and synapse on to sensory afferents that are associated with the taste bud.

Mammalian taste buds are embedded in epithelial specializations called papillae. On the surface of the tongue, three classes of papillae are easily distinguished. Toward the back of the tongue, there is a cen-

trally located circumvallate papillae; two groups of foliate papillae are found to the lateral extremes of the posterior tongue, and scattered over the anterior tongue are reddish colored fungiform papillae. The papillae form late during embryogenesis in the mouse (E13–E16) as the tongue begins to bud away from the pharynx. Soon after their formation, sensory afferents enter the papillae, and approximately 8–10 days after birth (P8–P10), taste buds form. In rodents, there is a single bud per fungiform papillae, tens of buds within the two foliate papillae, and hundreds of buds in the single circumvallate. An intimate relationship between both the developing papillae and the mature taste bud with innervating sensory afferents has been established through a number of denervation studies. Papilla formation does not require innervation; however, the maintenance of these structures does in some way require contact with sensory afferents. Similarly, in adult animals, denervation of the tongue leads to the loss of taste buds.

We are currently employing a number of different strategies to address three basic issues in taste bud development and function. How do papillae form in the appropriate pattern during late gestation, and what role do innervating sensory afferents have in this process? How diverse is the cellular environment of the adult taste bud and what mechanisms are required to maintain this diversity? How can a dynamic sensory structure maintain a constant level of function? Although these questions at first seem to be loosely connected, we expect to learn something about taste bud function by understanding how taste buds are put together and vice versa.

HOW MANY TYPES OF CELLS ARE CONTAINED WITHIN THE DEVELOPING PAPILLAE?

To understand how papillae are formed and innervated, it is imperative that we first ask what are papillae, at the molecular level. The papillae of the tongue are very similar in structure to the various ectodermal placodes that cover the epidermis and give rise to hair, feathers, and teeth. The secreted signaling factor sonic hedgehog (*shh*) is expressed in cells that will form these structures and that expression is maintained after the papillae or placode has formed. We plan to exploit this finding by using the *shh* locus to ectopically express green fluorescent protein (GFP) in developing papillae. Bacterial artificial chromosome (BAC) clones carrying the *shh* gene have been isolated, and using a recently described recombination technique, a GFP cassette has been inserted into this locus. Transgenic mice will be generated using modified BACs. Single GFP-positive cells from the lingual epithelium of E12–E17 embryos will be isolated and transcriptionally profiled using a single-cell cDNA synthesis method (see below). Profiles will be obtained by screening microarrays and direct sequencing of cDNAs. From this work, we hope to determine the molecular diversity of the papilla during its formation and innervation. Once we can "fingerprint" the cells of the papillae in this manner, it should be possible to analyze the mechanisms that regulate this diversity, in particular, the contribution of innervation to the development of papillae.

HOW DIVERSE ARE BOTH THE MATURE RECEPTOR CELL POPULATION AND THE PROGENITOR POOL FROM WHICH IT IS DERIVED?

Morphologically, there are three types of cells within the adult taste bud. Highly elongated differentiated receptor cells are found at the apex of the bud. Mitotic progenitor cells are found almost entirely at the bud's base. Packed in between these two cell types are less elongated immature receptor cells. The recent identification of taste-specific seven-transmembrane receptors and heterotrimeric G-proteins has demonstrated that within the mature receptor cell population, there is a great deal of cellular diversity, at the level of expression of these genes.

We would like to analyze the molecular diversity of both mature receptor and mitotic basal cells in detail, without a bias toward a particular gene family. In an approach that is similar to what is planned for the developing papillae (see above), we will isolate murine taste buds and generate transcriptional profiles from single cells.

To produce meaningful profiles from single taste bud cells, it is first necessary to sort these cells into a series of meaningful groups or bins. To do this, we have designed and are in the process of generating a small microarray that contains approximately 100 clones or units. The selection of each unit was based on known cellular and molecular biological properties of taste buds. Progenitor cells will be identified by a series of clones that are known to be transcriptionally active in cells that are actively dividing. The mature receptor cell possesses four basic molecular properties: quiescence, a highly polarized cytoarchitecture, an ability to detect external stimuli or tastants, and a mature synaptic arrangement with both other receptor cells and sensory afferents. The vast majority of units in the microarray are dedicated to marking these events. For example, 29 seven-transmembrane receptors are known to be involved in the detection of both bitter and sweet tas-

tants. All of these receptors and the second-messenger systems that they utilize are represented on our microarray. Additionally, a number of clones have been included that mark a cell's ability to produce both synaptic vesicles and particular neurotransmitters. During the next year, we hope to screen this array with probes generated from single taste bud cells.

DEVELOPMENT OF A QUANTITATIVE METHOD FOR THE ANALYSIS OF mRNA IN SINGLE CELLS?

We are currently developing the means to quantitatively profile mRNA in single cells or small groups of cells. Our approach involves the use of solid-phase-based cDNA libraries. By anchoring cDNA to a magnetic bead, it is possible to perform a variety of manipulations without having to rely on inefficient precipitation and/or column chromatography between the steps of interest. Additionally, immobilizing the cDNA to a surface provides a convenient means to produce a library, from which functional copies of the library can be generated by either polymerase chain reaction (PCR) or in vitro transcription, multiple times. We have succeeded in producing libraries of this sort from single taste buds and are in the process of performing similar experiments on single cells from both developing lingual epithelium and adult taste buds. Using currently available microarrays, we are assessing the quantitative abilities of our system.

Single Molecule DNA Analysis

T.R. Strick, T. Kawaguchi

During the past year, our lab has been using single-molecule techniques to quantitatively study DNA supercoiling and its interactions with proteins involved in transcription and replication. Supercoiling describes the wrapping of the two strands of DNA one about the other, as well as the writhing of the double helix through space (a twisted phone cord is a somewhat useful analogy). Controlled in vivo by the topoisomerase enzymes, it appears to be both a regulator and a by-product of DNA transcription, replication, and packaging. It is now experimentally possible to mechanically control the supercoiling of an individual DNA molecule. The major advantage of performing such experiments at the level of single molecules is that it allows observation in real time of the behavior of the molecule, eventually giving the research access to the time course of a reaction between the DNA and a protein. In this report, we briefly introduce the method used and describe some of our recent results on the direct observation of denaturation fluctuations (or "breathing") in DNA and its possible role in transcription initiation.

The single-molecule experiment we have implemented is depicted in Figure 1. An ~10-kbp linear DNA molecule is shown anchored at one end to a glass surface and at the other end to a 1-μm-diameter magnetic bead. The field generated by magnets located above the sample is used to pull on and rotate the magnetic bead, thus stretching and twisting the tethered DNA. The stretching force applied to the DNA depends solely on the distance between the magnets and the sample (the closer the magnets, the higher the force), and the torsion imparted to the DNA is exactly equal to the number of clockwise or counterclockwise turns performed by the magnets. The double helix is thus quantitatively and reversibly supercoiled. By determining the position of the magnetic bead, one measures the end-to-end extension of the DNA molecule, and thus its mechanical response to stretching and twisting.

One topic we pursued in the last year was the study of the onset of denaturation in DNA. In denatured DNA, the double-helical structure of the molecule is

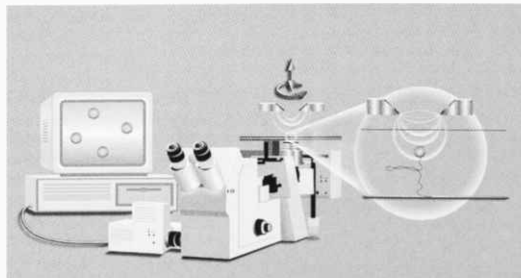

FIGURE 1 Sketch of the experimental setup. A glass capillary tube is mounted on an inverted microscope whose focus is controlled by a computer. The computer also controls the displacements (translation and rotation) of the magnets used to manipulate the bead. A CCD camera connected to the microscope relays video images of the magnetic bead to the computer. The computer extracts from these images the mean position and the Brownian fluctuations of the bead, which can be used to determine the applied stretching force and the DNA's end-to-end extension.

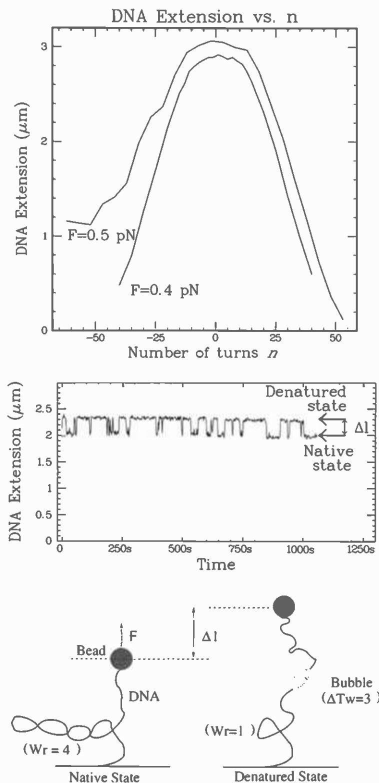

FIGURE 2 (*Top*) Extension vs. supercoiling curve obtained on a single DNA molecule for F = 0.4 *pN* and F = 0.5 *pN* (1 *pN* = 10^{-12} N). Although the extension decreases regularly with winding (at a rate of about 80 nm/turn) for the lower force, symmetry breaking is observed at the higher force. (*Middle*) Discrete changes in the DNA's extension (Δl = 250 nm) are observed as a function of time for the negatively supercoiled DNA subjected to a 0.5 *pN* force analyzed to extract the free-energy difference between the two states. (*Bottom*) The low-extension state corresponds to that where no denaturation bubble is present, but contains supercoils (for the sake of the sketch, here we draw four). The high-extension state occurs when an ~30-bp bubble topologically cancels ~3 supercoils, causing the large change in extension we observe.

destroyed, and the bases on the opposite strands are no longer paired. This process is fundamental to the initiation of transcription and replication, as it gives DNA and RNA polymerases access to the core of the DNA molecule, which is where the genetic information encoded by the bases can be most reliably read.

In Figure 2 (top), we show how the end-to-end extension of a DNA varies with supercoiling for two different forces. At a force of 0.4 *pN*, the system contracts regularly for both over- and underwinding. At a force of 0.5 *pN*, however, contraction is regular for overwinding but irregular for underwinding: This symmetry breaking indicates that the underwound DNA is undergoing local denaturation.

If the extension of the DNA is measured as a function of time (see Fig. 2, middle), the system's extension is seen to alternate between a low-extension state and a high-extension state. In Figure 2 (bottom), we provide a schematic explanation of this phenomenon: The low-extension state is characterized by the existence of supercoils, whereas in the high-extension state, three supercoils have been topologically annihilated by the appearance of an ~300-bp denaturation bubble. As one continues to unwind the DNA, the size of the bubble slowly increases and transitions to the high-extension (denatured) state become more frequent as well as longer-lived. We also measured the rates of denaturation and renaturation as a function of the applied stretching force and the temperature. This allows us to build a quantitative model which unifies the effects of torsion, force, and temperature on the appearance of a denaturation bubble. In essence, thermal agitation is causing the DNA to hop between two states—one with the bubble present, one without—and the energy barrier between the two states, as well as the difference in energy between the two states, can be adjusted by tuning the mechanical and thermal constraints on the DNA.

This quantitative understanding of the physical parameters relevant to DNA denaturation forms the basis for the study of protein-induced DNA melting. As an example, we are currently using this knowledge to directly observe and measure the melting of a transcription promoter sequence under binding of RNA polymerase.

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WATSON SCHOOL OF
BIOLOGICAL SCIENCES

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Lilian Gann, Ph.D., M.B.A., *Assistant Dean*
Janet Duffy, B.A., *Admissions and Academic Records Administrator*
Janet Silver, A.S., *Administrative Assistant*

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Professor, Department of Molecular Biology, Princeton University

WATSON SCHOOL OF BIOLOGICAL SCIENCES DEAN'S REPORT

To say the least, 2001 was an eventful year for the Watson School. It represented our second full year of academic instruction in which we welcomed a third entering class of doctoral students. It was an important year for the School as its young and developing curriculum demonstrated its flexibility by adapting to changes in instruction. The School also expanded to take over responsibility for long-established Laboratory educational programs that relate to the Laboratory's research endeavors. These programs extend from the youngest of students in Nature Study to postdoctoral fellows. Finally, it was a year of successful appraisal as the Watson School excelled in two external reviews, one by the National Institutes of Health for a doctoral training program and the second by the New York State Education Department for renewed accreditation. I am pleased to report that the School ended the year stronger than ever.

The Watson School: A Home for the Nature Study, Partners for the Future, Undergraduate Research, and Postdoctoral Programs

Although Cold Spring Harbor Laboratory only became a degree-granting institution in 1998, it has had educational programs since its origins in 1890. Today, the educational programs of the Laboratory can be divided into two broad categories: those that attract participants from around the world to the renowned Meetings and Courses program, including the Banbury Center, and those that pertain more specifically to the Laboratory's in-house research program. This latter set includes the Nature Study, Partners for the Future, Undergraduate Research, and Postdoctoral programs. The first of these—the Nature Study Program—began in 1925 under the auspices of the Long Island Biological Association, the predecessor to the Cold Spring Harbor Laboratory Association, to offer young students an appreciation of nature. Although not directly related to today's on-going research programs, the Nature Study program is an important investment in tomorrow as many of today's biologists first became interested in biology by studying nature as children. It certainly brings me fond memories when I see the Nature Study students each summer walking down Bungtown Road with knee-high boots and nets in hand eager to see what they might discover in the harbor.

The three of the aforementioned programs that do relate specifically to the Laboratory's ongoing research programs result in students and trainees working in the research laboratories. The Partners for the Future program, now in its 12th year, offers a select group of six to eight students from local high schools a hands-on research experience from October to March. Since its inception in 1990 under the direction of Susan Cooper, the Public Affairs Department has overseen this program. Each year, Lynn Hardin of the Public Affairs Department reaches out to Long Island high school students to identify possible participants and coordinates student participation in the program. In 1999, the Watson School began an initiative to better integrate the Partners for the Future program with the Laboratory's research program. One of the first steps was to identify a leader from among the faculty who could work with the Public Affairs Department to provide programmatic oversight—Yuri Lazebnik has risen to the occasion. Together with Jeff Picarello, the director of Public Affairs, and Lynn Hardin, Yuri Lazebnik has initiated important changes to the program. These changes will enable more outstanding students to participate and, through increased interactions among the students, will provide these students with a greater sense of the excitement of doing research first hand.

The Undergraduate Research Program was established in 1959 and boasts Nobel Laureate David Baltimore's participation in its first year. It hosts up to 25—and sometimes more! —undergraduate students each summer for a 10-week sojourn in a research laboratory. This program may be the most sought-after of all of the Laboratory's programs, as more than 500 students from around the world

apply to the program each year, and the large majority of those to whom offers are made choose to join the program. Direction of this program changed hands this year with Michael Hengartner's departure from the Laboratory. Michael became director of the program in 1995. Fortunately, Michael was astute enough during his tenure as director to identify a co-director—Leemor Joshua-Tor—to help him with the program. Thus, upon Michael's departure, we had a new director in Leemor, one with experience to take over the reins. To help her direct the program, Leemor chose Gregory Hannon to become associate director. Together, Leemor and Greg did a wonderful job directing the program last summer. An important ingredient to this success has been and continues to be the devotion to the program of Jane Reader, the program's administrator. This program's success is important to the Watson School, as the Undergraduate Research Program has been an outstanding source of students for the School's doctoral program.

Among the oldest traditions at the Laboratory is postdoctoral research, in which trainees who have recently been awarded the Ph.D. degree join faculty at the Laboratory to address questions that have piqued their interest as graduate students. The Laboratory offers these researchers an opportunity to hone their skills in new areas as a prelude to future career opportunities. Traditionally, postdoctoral education has been largely the purview of the individual researcher whose laboratory the postdoctoral fellow joins. It is becoming increasingly evident nationwide, however, that a postdoctoral education involves more than simply research experience in a laboratory—it also involves educating postdoctoral fellows in survival skills as a scientist. The Watson School's innovative 4-year Ph.D. program, which emphasizes teaching students the skills of the research scientist and the identification of an important problem to attack after the Ph.D., places more importance on the role of the postdoctoral years in the development of the biologist. The Watson School is therefore eager to participate in discussions of postdoctoral education. As a first step, Lilian Gann, the School's assistant dean, is chair of a working group consisting of faculty members Zachary Mainen, Nicholas Tonks, and Linda Van Aelst; postdoctoral fellows Frances Hannon, Alyson Kass-Eisler, Michael Packer, and Karen Zito; and Denise Roberts, the Laboratory research administrator. This postdoc working group will provide recommendations on ways to enhance the postdoctoral experience at the Laboratory. I also hope to take the opportunity in the upcoming year to engage faculty in discussions on how best to develop a postdoctoral training program. This goal is important, as the largest contingent of researchers at the Laboratory comprises the postdoctoral fellows.

The Spring Curriculum

In general, the Watson School's curriculum for first-year students has been met with acclaim. Nevertheless, as the School grows up, there have been new courses developed and new students to educate. In 2001, the year 2000 entering class of nine students participated in the spring curriculum of a Topics in Biology course, teaching at the Dolan DNA Learning Center, and laboratory rotations.

Masafumi Muratani (foreground) and Marco Mango (left) with Ahmet Denli (right) during the Topics in Biolo course on Evolution.

Topics in Biology Course: Evolution

This year saw our second Topics in Biology course. The Topics in Biology courses held at the Banbury Center and organized by Lilian Gann and Jan Witkowski are rapidly becoming one of the signature courses of the Watson School. These courses provide our students with exposure to areas of science that are beyond the spectrum of research at the Laboratory. Thus, the students spend a weeklong retreat at the Banbury Center

with invited instructors to learn a new topic in biology. This year, the 15 first- and second-year students attended a course on "Evolution" offered by Nipam Patel from the University of Chicago. Nipam provided an outstanding program as well as a special treat when he arranged for the students to spend a day at the Museum of Natural History for a behind-the-scenes tour and a lecture on *Drosophila* speciation by Rob DeSalle. The course was very well received, and we hope to have Nipam back in 2003 for our entering classes of 2001 and 2002.

Teaching at the Dolan DNA Learning Center

One of the goals of the Watson School's graduate program is to educate biologists who can communicate effectively with nonscientists. An important part of achieving this goal is the student participation in the Dolan DNA Learning Center during the spring of their first year. The DNA Learning Center provides students hands-on experience in communication to the layperson, and pioneered the teaching of DNA science with laboratory instruction to high school and middle school students. The Watson School students are given the opportunity to lead classes at the Center as part of a teaching program developed especially for them. In the first week of January, DNA Learning Center director David Micklos met with all of the students and described how the Center's instructors, led by Scott Bronson and Tricia Maskiell and with help from Veronique Bourdeau, would teach the students how to lead the high school and middle school classes. The students worked in pairs and began by observing the instructors before progressing to co-teaching with the instructors and eventually teaching on their own. Once on their own, the students developed individual strategies to explain the lessons being taught to the young students and were prepared to answer the questions of the inquisitive minds of young students. As in the first year, this teaching experience was very popular with the Watson School students.

Laboratory Rotations and Selection of a Research Mentor

Throughout the spring term, students of the entering class of 2000 participated in laboratory rotations and attended building-wide group meetings. The goals of the 6-week rotations continue to be to provide students with hands-on laboratory experience, and to give students and faculty opportunities to get to know each other and to explore possibilities for doctoral thesis research. These rotations are specifically short, but because the students can devote nearly full-time effort due to the separation of course work in the fall term and research in the spring term, they have the opportunity to get a good feel for different laboratory environments and the biological questions being addressed. Although some of the students chose to perform an additional fourth rotation in the summer, the length of rotation generally provided students and faculty alike sufficient information to make well-informed choices concerning the selection of a research mentor.

Watson School student Claudia Feierstein (right) with Postdoctoral researcher Antonella Piccini during a rotation in Roberto Malinow's lab.

ENTERING CLASS OF 2000 DOCTORAL THESIS RESEARCH

Student	Academic Mentor	Research Mentor	Thesis Research
Santanu Chakraborty *George A. and Marjorie H. Anderson Fellow*	David Helfman Michael Wigler	Karel Svoboda Carlos D. Brody	To be determined
Elena S. Ejkova *Engelhorn Scholar*	Jan Witkowski	William Tansey	Role of the proteasome in transcription
Rebecca C. Ewald *Engelhorn Scholar*	Bruce Stillman	Hollis Cline	NMDA receptor trafficking and its impact on neuronal functionality and morphology
Ira Hall *Beckman Graduate Student*	Alexander A.F. Gann	Shiv Grewal	Initiation, assembly, transfer, and genome-wide distribution of heterochromatin in *Schizosaccharomyces pombe*
Zachary Bela Lippman *Beckman Graduate Student*	William Tansey	Robert Martienssen	Comprehensive analysis of chromatin status on *Arabidopsis* chromosome 4
Marco Mangone *Charles A. Dana Foundation Fellow*	Linda Van Aelst	Winship Herr	The role of HCF-1 in cell proliferation
Masafumi Muratani *George A. and Marjorie H. Anderson Fellow*	Nouria Hernandez	William Tansey	Gene regulation by ubiquitin-mediated proteolysis
Patrick J. Paddison *Beckman Graduate Student*	Adrian R. Krainer	Gregory Hannon	An RNAi-based screen in mouse embryo fibroblasts for transformation-lethal gene targets

Qualifying Exam

At the end of June, the entering class of 2000 took the School's oral qualifying exam. This exam tests the ability of the students to acquire and articulate in-depth scientific information by defending their knowledge of two assigned topics. The aim of the exam is to assess whether students have learned how, on their own, to acquire and critically synthesize scientific information on a new topic. With this goal in mind, as in the previous year, students orally defended their knowledge in two individually assigned topics over the course of two hours in front of three examiners. One of the assigned topics was in the area of the student's planned thesis research, whereas the second topic lay outside the thesis research area. Students were encouraged to meet with their examiners to discuss the limits and expectations for the designated topics.

This year, we continued with the approach established in the first year to ensure that the oral exams, which by their very nature will differ in content and style from one student to the next, are fair and equitable to the students. To deal with the larger number of students (nine compared to six the first year), the Qualifying Exam Committee (QEC), which serves as a parent committee to oversee the process, was expanded to six members: Gregory Hannon, David Jackson, W. Richard McCombie, Linda Van Aelst, and Anthony Zador, with me as its chair. The QEC established the list of topics offered to the stu-

dents for examination and made the final topic selections. The three examiners at each individual qualifying exam were all members of the Watson School faculty, and a QEC member chaired the examining committees. I sat in on each exam as a nonparticipating observer to ensure parity in the examining process. This exam process continues to be viewed as a success. Strengths and weaknesses of students were revealed through the course of the qualifying exam process, but in the end, all of the students successfully completed this element of their curriculum and were able to begin focusing on their doctoral research in earnest.

The Watson School Loses Its First Student

Although regrettable, it is perhaps a mark of increasing maturity that we lost our first student at the end of the summer. Guillaume Lettre, who came to us from the Université de Sherbrooke in Québec, Canada, decided to carry out his thesis research with Michael Hengartner, who—also regrettably—had left Cold Spring Harbor Laboratory for the University of Zurich in the winter of this year. Guillaume was an outstanding student and he will be sorely missed. We wish him every success.

A Coordinated Fall Term Curriculum

A special feature of the fall term of the Watson School Ph.D. curriculum is intimate instruction in a series of well-integrated and cohesive courses. By design, this curriculum does not require all the faculty to participate in lecture courses each year. This design allows for exceptional flexibility in the structure of the teaching faculty, allowing the faculty to involve themselves at different levels in the instruction of the first-year students. Thus, it is only a self-selected core group of the faculty—the teaching faculty—that is intimately involved in the design and organization of the courses. Each course is taught by a team of faculty whose members work closely together to develop and present a well-organized and cohesive course. Members of the remainder of the faculty are recruited by the core teaching faculty to lecture in the Scientific Reasoning and Logic (SRL) and Scientific Exposition and Ethics (SEE) core courses and the Specialized Discipline courses.

This strategy requires considerable coordination, as courses change from year to year. Thus, the teaching faculty and School administration devote much effort to curriculum coordination, integration, and evaluation. Much of the coordination is provided by a "Core-Course Coordinating Working Group" affectionately termed the C3WG. Core-course instruction is the major didactic effort in the fall course term. These courses are central to teaching the new students the processes of critical and logical biological thought, communication, and ethical behavior. The importance of these courses to the School curriculum is emphasized by the presence of the lead instructors of each core course (David Helfman, Scott Lowe, and William Tansey) on the School's Executive Committee. Together with the dean and assistant dean, these three core-course instructors form the C3WG. This working group meets during the spring and summer months to prepare for the fall course curriculum and to integrate the course instruction. For example, to provide synergy between the SEE and SRL core courses, papers assigned by the SRL course are also assigned in the SEE core course to study exposition or ethics. The C3WG also meets with the instructors of the Specialized Disciplines courses to integrate those courses into the core course instruction and to provide advice on how to structure their course, adjust student workload, and what to expect from the students. For example, the teaching of genetic principles to the students during the fall term is shared by the SRL core course and The Genome Specialized Disciplines course. Coordination of this instruction occurs at meetings of the C3WG.

This course coordination was put to the test this year, when, for the first time, a Specialized Disciplines course was changed—the course on transcription taught by William Tansey and me was replaced by a course on cellular structure and function taught by David Helfman and David Spector. The C3WG worked out how to adjust the curriculum of the SRL core course to accommodate this change. I was very pleased to see that the course coordination was a great success, as both the SRL and Cellular Structure and Function courses were highly rated.

Knowing that the courses were well integrated relied on student feedback concerning course instruction, and the School depends heavily on this student feedback. Under the aegis of Lilian Gann, students are asked to complete evaluation forms for every course and to evaluate each weekly module of the SRL core course. These evaluations are used to improve course lectures and the structure of the modules. They are also used by the External Advisory Committee to provide the Watson School faculty with advice on changes to course content and format.

A Guiding Executive Committee

A very important ingredient to the smooth sailing that the Watson School has enjoyed to date is the School's Executive Committee. Unfortunately, owing to his departure to the University of Zurich in March, Michael Hengartner, a founding member of the Committee and the first lead instructor of the SRL course, had to step down from the Committee. The five other faculty members on the Committee—Hollis Cline, David Helfman, Scott Lowe, William Tansey, and Jan Witkowski—were thus joined by Lincoln Stein, a three-time instructor of the Bioinformatics/Genome Specialized Disciplines course and an expert in the role of the computer in today's biological investigations. He has proven to be an outstanding selection and has provided much sage advice and counsel. Fortunately for the School, we did not lose Michael Hengartner entirely, as he returned in November to teach a module of the SRL course on his favorite topics, apoptosis and genetics.

Ajit Janardhan, the Stony Brook student representative on the Watson School Executive Committee

The School also benefited greatly from the two student members on the Executive Committee—Watson School student Amy Caudy from the entering class of 1999 and SUNY Stony Brook student Ajit Janardhan. Ajit is an M.D./Ph.D. student who is performing his doctoral studies with the Stony Brook Genetics Program. Ajit's and Amy's contributions, along with those of the previous student representatives Michelle Cilia and John Mignone, have been invaluable. In recognition of the value of their input, this year the Executive Committee decided that student representatives should receive voting rights. Along the same vein, the Committee decided that Lilian Gann, the assistant dean, should also have voting rights as she represents the all-important School administration. Fortunately, the increase in voting members has not changed the usual unanimity in Executive Committee decisions!

Two-tier Mentoring

Another innovative feature of the Watson School that is proving successful is its two-tier mentoring program championed by William Tansey. This program promotes a high level of student achievement by having faculty and administration take an active role in mentoring and supervising the students. The two-tier mentoring program involves an academic and research mentor for each student. At matriculation, each student selects a faculty member as an academic mentor. The main roles of the academic mentor are

- to aid and enrich the student's academic progress during the fall course term and beyond,
- to provide advice about laboratory selection,
- to serve on the thesis committee, and
- to provide informed letters of recommendation for postdoctoral pursuits.

The academic mentor follows the student's academic and research progress and provides advice for the duration of the student's tenure in the graduate program. The academic mentors have been invaluable in providing students with advice on (1) course instruction (sometimes leading to additional instruction at other academic institutions), (2) interactions with research mentors, (3) involvement in extracurricular educational activities such as at the Dolan DNA Learning Center, and (4) personal concerns. After the laboratory rotations, each student chooses a research mentor. The research mentor is the doctoral thesis research advisor, who supervises the student's independent laboratory research. By providing both academic and research mentors, the Watson School provides each student with advice from faculty who hold different views and can then offer unique and in-depth evaluations of the student. In particular, the academic mentors can provide advice that is not influenced by their own research goals. The following are the academic mentors for the entering class of 2001:

Catherine Cormier	David Stewart
Claudia Feiersten	Linda Van Aelst
Tomáŝ Hromádka	William Tansey
Charles Kopec	Anthony Zador
Ji-Joon Song	Scott Lowe
Gowan Tervo	Carlos Brody

Research Progress: Can a Ph.D. Degree Be Done in Four Years?

One of the key challenges that the Watson School faces is demonstrating that, in today's scientific environment, it is possible for a graduate student to perform sufficient research in a four-year program to be competitive with graduates of other, longer graduate programs. The third-year students who entered the School in the fall of 1999 have now been in the program for two and a quarter years, and therefore, it is possible to begin determining whether students will have time to obtain publications from their accelerated research program.

I was therefore very pleased when the beginning of this year saw a milestone in the School's history: the publication of the first article with a Watson School student as one of its authors. In the January 18, 2001 issue of *Nature*, Amy Caudy is a co-author of an article on the discovery of an enzyme, called "Dicer," that is involved in a recently uncovered form of gene silencing called "RNA interference" or RNAi. Amy, a member of the Fall 1999 class, is performing her studies with Gregory Hannon, who studies both RNAi and the oncogenic transformation of human cells (see box on next page). This first publication listing the Watson School was particularly pleasing to me because it represents a collaboration between two graduate students, one of whom, Emily Bernstein, is now a fourth-year student with the Graduate Program in Genetics at SUNY Stony Brook. Emily is the lead author of this article. I hope we see much more of such inter-programmatic synergy in the future.

By the end of this year, our students had first-author publications in press. Masafumi Muratani from the entering class of 2000 had a manuscript accepted for publication in the journal *Nature Cell Biology*, and Patrick Paddison, also from the entering class of 2000, and Amy Caudy were co-first authors on a manuscript in press in the *Proceedings of the National Academy of Sciences*.

These early successes bode well for the School. I believe one of the main reasons that the School will be successful in its four-year degree program is the attention to mentoring that our program provides. Thus, as I described in last year's report, the School has developed a detailed thesis proposal process that gets the students and research advisors to focus on a research direction before the student is a year and a half into the program. All members of the first entering class passed their thesis proposal defense with flying colors last year, and in the fall already had a second meeting with their respective thesis committees to go over their research progress. These meetings, organized by Lilian Gann and the Watson School administration, are thus a very important element of the student's research progress. I am very grateful to the Lita Annenberg Hazen Chair for endowing the support of the Watson School administration that makes this organization and mentoring possible.

ENTERING CLASS OF 1999
DOCTORAL THESIS RESEARCH

Student	Academic Mentor	Research Mentor	Thesis Research
Amy Anne Caudy *George A. and Marjorie H. Anderson Fellow* *Howard Hughes Medical Institute Predoctoral Fellow*	Hollis Cline	Gregory Hannon	The biological function of RNA interference
Michelle Lynn Cilia *William R. Miller Fellow* *Beckman Graduate Student*	Nouria Hernandez	David Jackson	Mechanisms of intercellular trafficking via plasmodesmata
Ahmet M. Denli *David Koch Fellow*	Adrian R. Krainer	Gregory Hannon	Biochemical analysis of RNA-induced gene silencing
Emiliano M. Rial Verde *David and Fanny Luke Fellow* *Howard Hughes Medical Institute Predoctoral Fellow*	Jan Witkowski	Hollis Cline	Arc role in synaptic function
Elizabeth Ellen Thomas *Farish-Gerry Fellow* *Howard Hughes Medical Institute Predoctoral Fellow*	William Tansey	Michael Wigler	A *de novo* approach to identifying repetitive elements in genomic sequences
Niraj H. Tolia *Leslie C. Quick, Jr. Fellow*	David Helfman	Leemor Joshua-Tor	Structural framework and molecular mechanism of caspase-9 activation

A Third Entering Class

On August 27, 2001, the Watson School opened its doors for the third time to welcome a new class of students. Six students—Catherine Cormier, Claudia Feierstein, Tomáŝ Hromádka, Charles Kopec, Ji-Joon Song, and Gowan Tervo—all began their adventure of a graduate education in the biological sciences. James Watson welcomed them by sharing his view of what is important about a graduate education. His comments can be distilled to one main aim: learn what the important problems of the day are so that each student can go off to perform postdoctoral studies on his or her chosen topic. While Jim spoke, I wondered whether any would identify a problem as important as the one Jim came up with for his doctoral studies: to understand the structure of the gene—DNA.

A new Admissions Committee selected this class. I stepped down from the Committee this year. My position as chair was ably taken over by Nouria Hernandez and Adrian Krainer joined the Committee. I stepped down none too soon, because the School had a surprising 67% increase in the number of applications (a total of 250). With traditional excellence, Janet Duffy organized all of the applications both for the Admissions Committee and for our co-application program with four departments and graduate programs with the State University of New York (SUNY) at Stony Brook. Additionally, she greatly aided Nouria Hernandez and the remainder of the Admissions Committee in reviewing the applications.

The Watson School has developed the tradition of interviewing all candidates before making decisions on acceptance into its doctoral program. The Committee decided to invite 25 candidates for interviews. With heroic effort, Janet Duffy made all of the travel arrangements and Lilian Gann organized more than 300 individual meetings with faculty members as part of the interview process! Jim Watson and Bruce Stillman met with almost all of the candidates, which was greatly appreciated by candidates and faculty. The Admissions Committee assessed the candidates and placed them in rank

ENTERING CLASS OF 2001

Catherine Y. Cormier, Boston University, Boston, Massachusetts
Beckman Graduate Student

Claudia E. Feierstein,, University of Buenos Aires, Argentina
George A. and Marjorie H. Anderson Fellow

Tomáŝ Hromádka, Charles University in Prague, Czech Republic
Engelhorn Scholar

Ji-Joon Song, Kwangju Institute of Science & Technology, Kwangju, Republic of Korea
Bristol-Myers Squibb Fellow

Dougal G.R. (Gowan) Tervo, Oxford University, United Kingdom
George A. and Marjorie H. Anderson Fellow
Howard Hughes Medical Institute Predoctoral Fellow

Charles D. Kopec, Rutgers University, New Jersey
Lindsay-Goldberg Fellow

(*Left to right*) Charles Kopec, Ji-Joon Song, Catherine Cormier, Gowan Tervo, Claudia Feierstein, and Tomáŝ Hromádka.

order. Offers of acceptance were extended to 15 candidates, 7 of whom accepted (although we lost one of those students for personal reasons over the summer); we lost students to the University of California at Berkeley and San Francisco, The Rockefeller University, and elsewhere.

As in the two previous years, the entering students hail from around the globe and have provided cultural diversity and academic excellence to the Watson School. Two of the entering students, Charles Kopec and Dougal (Gowan) Tervo, are former participants in the Undergraduate Research Program, continuing the developing tradition of recruiting students to the Watson School from this outstanding program. Indeed, Gowan was successful in obtaining a Howard Hughes Medical Institute (HHMI) Predoctoral Fellowship. This is the third year in a row that one or more Watson School students have been awarded HHMI predoctoral fellowships. This means that 4 of the 20 (or 20%) Watson School students are HHMI fellows! We are also very pleased that Gowan was awarded the prestigious Gibbs prize for his studies at Oxford University.

Tomáŝ Hromádka, who hails from Slovakia, became our third Engelhorn Scholar, joining Rebecca Ewald and Elena Ejkova. The Engelhorn Scholars Program is dedicated to supporting European nationals in the Watson School. This program enriches the School student body by supporting student diversity.

Holiday Party. (Left to right) Ashish Saxena, Farida Emran, Laureen Connell, Anitra Auster, Michelle Cilia

This year also saw the arrival of two families: Soon after his arrival, Tomáŝ was joined by his wife Eva and their two children Samo and Timo, while Ji-Joon was joined by his wife Ah Ran and his newborn daughter Yeonvee. The two families each moved into the De Forrest Stable apartments, appropriately situated over the Mary D. Lindsay Child Care Center.

Perhaps spurred on by Jim's discussion, the six first-year students all finished the intensive fall term in good standing. After the term was over, they and the rest of Cold Spring Harbor Laboratory could partake in the second annual Graduate Student Holiday Party!

The Watson School Is Reviewed—Twice!

This year, the Watson School was reviewed twice—once on October 24 in conjunction with our request for a training grant from the National Institutes of Health (NIH) and again in November 5 for renewal of our accreditation.

Watson School Is Awarded an NIH Training Grant

The Watson School has been fortunate to have many private benefactors who have established an endowment to support the students, faculty, and administration. It was important, however, to seek additional support from other sources, particularly the NIH. Such support would serve not only to increase financial support for the School, but also, through the review process, to provide an outside measure of how the School is progressing. Thus, in May, the Watson School applied for a training grant from the NIH to support two new students each year in the doctoral program. We had a site visit on October 24 by a team of dedicated educators in the biological sciences to review our proposal. The site visit team comprised:

- Susan K. Dutcher, Chair (Washington University)
- Victor G. Corces (Johns Hopkins University)
- Emilie F. Rissman (University of Virginia)
- Rebecca H. Johnson (National Institutes of Health)
- Marion Zatz (National Institutes of Health)

They met with many of the faculty and with all of the Watson School students. I was very impressed with how the faculty and students alike dedicated themselves to presenting our innovative curriculum. The site visitors must have enjoyed what they saw, because the proposal received a score of 132. (The NIH has an unusual scoring system with a range from 100 to 500 in which a score of 100 is the best!) I was told that training grant scores do not get any better. Of course, now the pressure is on to ensure that our innovative curriculum is a long-term success.

Watson School Accreditation Is Renewed

This year, following our initial accreditation as a degree-granting institution in September 1998, the School and Laboratory underwent a second accreditation process. Lilian Gann worked closely with the State Education Department to prepare a "self study," which served as the basis for the review. The self study touched on many elements of the Laboratory's organization and administration. Lilian did a spectacular job getting all the materials together.

On November 4 and 5, a New York State accreditation team visited for a comprehensive review of the Laboratory's application for renewal of its accreditation. The team comprised:

- Jacqueline Joseph-Silverstein, Chair (University of Connecticut at Stamford)
- Maurice S. Fox (Massachusetts Institute of Technology)
- Elizabeth R. Gavis (Princeton University)
- Phillip A. Sharp (Massachusetts Institute of Technology)
- Byron P. Connell (NY State Education Department)

We were very pleased to be reviewed by such an august group that included a Nobel Laureate in the person of Phillip Sharp! The team gave unanimous and enthusiastic support for renewal of our accreditation without conditions. During the site visit and in the report itself, the site visit team offered many helpful suggestions for improving our graduate program and the Laboratory, particularly in how to transform the Laboratory into an academic institution. On December 6, I attended a meeting of the Regents Advisory Council on Institutional Accreditation where our case for accreditation renewal was presented. There was overwhelming support for Cold Spring Harbor Laboratory and the Watson School, resulting in renewal of our accreditation by the Board of Regents of the State of New York for a ten-year period.

NEW STUDENTS FROM SHARED GRADUATE PROGRAMS

Student	CSHL Research Mentor	Affiliation
Elvin Garcia	Alea A. Mills	SUNY Stony Brook, Genetics
Ivan Ho	Yi Zhong	SUNY Stony Brook, Genetics
Asra Malikzay	Shiv Grewal	SUNY Stony Brook, Molecular and Cell Biology
Anagha Matapurkar	Yuri Lazebnik	SUNY Stony Brook, Molecular and Cell Biology
Sabrina Nuñez	Scott Lowe	SUNY Stony Brook, Genetics
Sachin Ranade	Zachary Mainen	SUNY Stony Brook, Neurobiology
Khalid Siddiqui	Bruce Stillman	SUNY Stony Brook, Genetics
Hongjae Sunwoo	David L. Spector	SUNY Stony Brook, Molecular and Cell Biology
Lung-Hao Tai	Anthony Zador	SUNY Stony Brook, Neurobiology
Xioarong Wang	David Helfman	SUNY Stony Brook, Molecular and Cell Biology
Min Yu	W. Richard McCombie	SUNY Stony Brook, Genetics
Chih-Chi Yuan	Nouria Hernandez	SUNY Stony Brook, Molecular and Cell Biology
Zhengwei Zhu	Michael Q. Zhang	SUNY Stony Brook, Biomedical Engineering

Graduate Education in the Biological Sciences on Long Island: The Shared Genetics Program Is Honored

Cold Spring Harbor Laboratory has been involved in graduate education for more than 25 years, largely through shared programs with SUNY Stony Brook. This year, the long-standing Genetics Program was honored by the award of the 2001 Amersham Biosciences & *Science* Grand Prize to Song-Hai Shi. Song-Hai was a Genetics Program graduate student who performed his doctoral studies with Roberto Malinow on mechanisms of long-term potentiation in synaptic transmission. Following a world-wide search, the Amersham Biosciences & *Science* Grand Prize is awarded each year for the most outstanding Ph.D. thesis among graduate students in molecular biology. This award re-emphasizes the high quality of students who participate in our shared programs with SUNY Stony Brook.

SUNY Stony Brook Genetics student Michelle Juárez

Among the shared graduate programs with SUNY Stony Brook, the Genetics Program is unique. It is the only program that was established specifically to integrate the three academic biological research institutions on Long Island. The Genetics Program at Stony Brook was created in 1980 as an inter-institutional program to focus and combine the existing strengths in genetics at SUNY Stony Brook, Brookhaven National Laboratory, and Cold Spring Harbor Laboratory. In so doing, it brought together three very complementary institutions: a premiere university and medical center, a government laboratory with outstanding instrumentation, and the Laboratory which now has a century of experience in genetics. The Genetics Program, currently led by Peter Gergen, a *Drosophila* geneticist at SUNY Stony Brook, has been a major force on Long Island in integrating these three institutions, and students flourish in the environment it creates, as evidenced by Song-Hai's success. This year, we welcomed 13 new students to Cold Spring Harbor Laboratory (see box)—one more than last year.

Graduate Student and Postdoctoral Fellow Departures

With new arrivals come departures. This year, we saw the following graduate students and postdocs depart:

Postdocs

Harsh Bal
Joseph Donovan
Gerardo Ferbeyre
Francisco Ferrezuelo
Lisa Foa
Michael Greenberg
Hiroyuki Higashiyama
Sangeet Honey
Jianping Jin
Wen-Chuan Lin
David MacCallum
Stephen Macknik
Olaf Merkel
Jun-ichi Nakayama
Takashi Odawara
Shannon Pendergrast
Bernardo Sabatini
Noriko Saitoh
Cyril Sanders
Clemens Schmitt
Arndt Schmitz
Lidia Serina
Wun Chey Sin
Tatjana Singer
Yu Shen
Guanming Wu
TongTong Zhang
Jun Julius Zhu

Graduate Students

Andrea Calixto
Jeffrey Christensen
Lavina Faleiro
Jennia Hizver
Jason Kinchen
Ying Ying Koh
Soyoung Lee
Zheng Li
Stuart Milstein
Irina Pugach
Songhai Shi
Michael Stebbins
Josema Torres
Jun Zhu

Career Opportunities in the Biological Sciences

The profusion of genetic sequence and other information currently being uncovered is leading to a wealth of career opportunities for graduate students and postdoctoral fellows in the biological sciences. No longer is it the norm to become a professor in academia. This year, we have again been very fortunate in being able to take advantage of the excellent series of seminars on "Alternative Careers in the Bioscience Industry" arranged by Angeline Judex of the Center for Biotechnology at SUNY Stony Brook. In addition, we were able to attend the first Annual Bioscience Careers Conference: Visions for the Future, arranged by the Center for Biotechnology in partnership with the CUNY Office of Academic Affairs. The conference was specifically designed to help graduate students and postdoctoral fellows explore career opportunities in the commercial sector in New York State. The event was very well attended and provided much useful information—we look forward to the next one!

Lilian Gann to Become Associate Dean

With the beginning of the new year, Lilian Gann will become associate dean of the Watson School. Lilian joined the Laboratory in March 1999 as assistant dean of the School. She received the Ph.D. degree in molecular virology from the University of St. Andrews, Scotland, in 1988 for studies on transcriptional regulation using animal viruses as models. After her postdoctoral studies, Lilian became interested in scientific administration at the Imperial Cancer Research Fund in London and earned an M.B.A. degree in 1996 from the University of Westminster, London. Her dual training has made Lilian unusually well-qualified to help direct a school administration.

Lilian Gann

Since her arrival at the Laboratory, Lilian has played a crucial role in the development of the School's innovative Ph.D. program, while also enhancing

the educational and training environment of the Laboratory for students and postdoctoral fellows generally. She is the secretary for the Executive and Qualifying Exam Committees. She also co-organizes the Research Topics core course and the Topics in Biology Banbury course, participates in the direction of the Scientific Reasoning and Logic core course, and is a member of the C3WG. Last but not least, she is the chair of the postdoctoral fellow working group. The position of associate dean will more properly reflect Lilian's critical role in the development of the Watson School.

External Advisory Committee

The Watson School has been blessed with an outstanding External Advisory Committee composed of individuals—Keith Yamamoto, the chair of the committee, Gail Mandel, Marguerite Mangin, Barbara Meyer, Frank Solomon, and Shirley Tilghman—who have thought deeply about graduate education. Although we may have occasional healthy differences of opinion, their advice is always extremely thoughtful and has had a profound influence on the development of the School's curriculum. Thus, it was a great pleasure to learn that two of its members were elevated to new positions of considerable influence. Keith Yamamoto became Vice Dean of Research at the University of California San Francisco School of Medicine, and Shirley Tilghman was appointed President of Princeton University. We are fortunate that Keith will remain on our Advisory Committee; unfortunately, Shirley had to curtail the scope of her outside activities and therefore resigned from the Committee. We regret losing her, but we congratulate her on her success and take solace in the fact that her promotion only emphasizes how truly outstanding our Advisory Committee is.

The Watson School Grows: Urey and Janet Silver

In March, the School administration relocated from its two-bedroom suite in Dolan Hall to an apartment in Williams. This enabled the School's staff to all be accommodated in a single location, greatly increasing communication and workflow—in other words, the Dean now had an official School office! A further move took place in November to a completely renovated Urey Cottage. This move brought first-year students and the School administration together, where they all have office space and can share a conference room. We are indebted to Art Brings and his staff for working so hard on this new home for the School.

In November, the School staff also welcomed a new member. Janet Silver—called Jan in the office to distinguish her from Janet Duffy—hails from California. Very quickly she has added to the School's welcoming face with her charm and good nature. The School's dedicated, friendly staff and elegant new home make students, postdocs, faculty, and visitors alike feel welcome.

The Watson School Honors David Luke III

A very special element of the Watson School is the support private donors have provided so that students can be supported from a dedicated School endowment. The School is indebted to David Luke III for making the endowment possible. Indeed, as the year came to a close, David was close to achieving his goal of $32 million to support 20 students, the faculty, and the administration. David had much support from numerous friends of the Laboratory and Jim Watson in raising this endowment. I have learned a great deal from working with David during this campaign, particularly that his success comes largely from his exquisite attention to detail. I always knew with David what he expected of me and what I could expect from him.

It was therefore a deep honor for me to be selected to read David's citation at the School's convocation on November 2 when he was bestowed an honorary degree for his outstanding service to science. I am thrilled that the School could bestow such a well-deserved award on someone without whom the School would not exist.

April, 2002 **Winship Herr**

SPRING CURRICULUM

Topics in Biology: Evolution

ARRANGED BY	**Lilian Gann** **Jan Witkowski**
FUNDED IN PART BY	**The Daniel E. Koshland, Jr. Visiting Lectureship** **The Fairchild Martindale Visiting Lectureship** **The Lucy and Mark Ptashne Visiting Lectureship** **The Michel David-Weill Visiting Lectureship**
INSTRUCTOR	**Nipam H. Patel,** University of Chicago
GUEST LECTURERS	**Rob DeSalle,** American Museum of Natural History **Neil Shubin,** University of Chicago **Michael Palopoli,** Bowdoin College
TEACHING FELLOWS	**Greg Davis,** University of Chicago **Casey Bergman,** University of Chicago **Allison Beck,** University of Chicago

Each year, one or a team of invited instructors offer a 7-day course at the Banbury Conference Center to explore specialized topics outside the expertise of the Cold Spring Harbor Laboratory faculty. The course includes morning or evening lectures as well as afternoon sessions during which students read assigned papers. For Spring 2001, the topic was evolution. This course discussed our understanding of the mechanisms of evolution and explored how evolutionary data can be used to further our understanding of various biological problems. The course ran from Sunday to Saturday, March 25–31, and was organized and largely taught by Nipam Patel. Three guests—Rob DeSalle, Neil Shubin, and Michael Palopoli—also lectured in the course and three teaching fellows participated in all aspects of the course. The course was highly rated by all of the student participants, including course auditors.

Teaching Experience at the DNA Learning Center

DIRECTOR **David A. Micklos**

INSTRUCTORS **Scott Bronson**
Veronique Bourdeau
Tricia Maskiell
Amanda McBrien
Danielle Sixsmith

As science plays an increasing role in society, there is an increasing need for biologists to educate nonscientists of all ages about biology. The Watson School of Biological Sciences doctoral program offers its students unique teaching experiences through the Laboratory's Dolan DNA Learning Center, where students teach laboratory courses. From these teaching experiences, they learn how to communicate with nonbiologists and to inspire and educate creative young minds. The teaching module entailed pairs of students teaching one morning a week for 12 weeks. During this time the students had the opportunity to teach both high school and middle school students. In the initial weeks the course instructors taught the students the didactic process—it was not until the fifth week that the students taught on their own. At the end of the 12 weeks, the students were very excited about their teaching experience.

Laboratory Rotations

The most important element of a doctoral education is learning to perform independent research that leads to a unique contribution to human knowledge. After the fall course term, students participate in laboratory rotations. These rotations provide students and faculty with the opportunity to get to know each other and to explore possibilities for doctoral thesis research. At the end of each rotation, students make short presentations of their studies to the other students and their rotation advisors. These talks give students an opportunity to share their laboratory experiences and to learn how to give a scientific presentation. With this latter goal in mind, the instructors of the Scientific Exposition and Ethics core course attend the talks and give individual feedback to students on their presentations. This year, 21 faculty members served as rotation mentors.

ROTATION MENTORS

Hollis Cline
Grigori Enikolopov
Shiv Grewal
Masaaki Hamaguchi
Gregory Hannon
Winship Herr
Z. Josh Huang
David Jackson
Yuri Lazebnik
Scott Lowe
Roberto Malinow
Robert Martienssen
Alea A. Mills
David L. Spector
Arne Stenlund
Bruce Stillman
Karel Svoboda
William Tansey
Marja Timmermans
Linda Van Aelst
Anthony Zador

FALL COURSE CURRICULUM

CORE COURSES

The Leslie C. Quick, Jr. Core Course on Scientific Reasoning and Logic

FUNDED IN PART BY **The David Pall Visiting Lectureship**

INSTRUCTORS
Scott Lowe (Lead)
Grigori Enikolopov
Gregory Hannon
Winship Herr
Leemor Joshua-Tor
Arne Stenlund

GUEST LECTURERS
Alexander A.F. Gann
Shiv Grewal
Nouria Hernandez
David Jackson
Adrian R. Krainer
Robert Martienssen
Alea A. Mills
Jacek Skowronski
Bruce Stillman
William Tansey
Marja Timmermans
Linda Van Aelst

VISITING LECTURERS
Bruce Futcher, SUNY Stony Brook
Michael Hengartner, University of Zurich

A fundamental aspect of earning the Ph.D. is training in the pursuit of knowledge. In this core course, which forms the heart of the Fall Course curriculum, students (1) acquired a broad base of knowledge in the biological sciences, (2) learned the scientific method, and (3) learned how to think critically. This course consisted of 12 weekly segments, each of which had a different theme. Each week, students read an assigned set of research articles (generally four articles) and provided written answers to a problem set that guided them through two (or, on occasion, one) of the articles. Twice weekly, students attended lectures related to the week's topic, which included concepts and experimental methods. During the week, the students met to discuss among themselves the assigned papers not covered by the problem set. At the end of each weekly segment, the students submitted their problem sets and spent the evening discussing with faculty the articles not covered by the problem set. The course culminated in the 13th week with a final exam. Studying for the final exam gave the students the opportunity to synthesize and integrate what they had learned over the course of the fall term. The weekly topics were:

Week 1 Macromolecular Structure
Week 2 DNA Replication
Week 3 Transcriptional Regulation
Week 4 Chromosome Structure and Function
Week 5 mRNA Processing
Week 6 Signal Transduction
Week 7 Apoptosis
Week 8 Cell Cycle
Week 9 Virus-Host Cell Interactions
Week 10 Cancer Genes
Week 11 Mobile Genetic Elements
Week 12 Development
Week 13 Final Exam

The Norris and Henriette Darrell Core Course on Scientific Exposition and Ethics

FUNDED IN PART BY	**The Edward H. Gerry Visiting Lectureship** **The John P. and Rita M. Cleary Visiting Lectureship** **The Martha F. Gerry Visiting Lectureship** **The Seraph Foundation Visiting Lectureship** **The Susan T. and Charles E. Harris Visiting Lectureship**
INSTRUCTORS	**William Tansey (Lead)** **Adrian R. Krainer** **Jan Witkowski**
GUEST LECTURERS	**Terri Grodzicker** **Winship Herr**
VISITING LECTURERS	**Robert P. Charrow, Esq.,** Crowell & Moring LLP **David Kremers,** Distinguished Conceptual Artist, Caltech **Steve Ferguson,** Office of Technology Transfer, NIH **Nicholas Wade,** *New York Times* **Philip Reilly,** J.D., M.D., C.E.O. of Interleukin Genetics

This core course offered instruction about the fundamental elements of scientific exposition—writing skills and public speaking—and ethics. The ability to communicate effectively and to appreciate the intricacies of ethical issues are essential skills for biologists; both subjects were taught in a series of example-based lectures and discussion groups. This year, the course continued a novel format established in 2000 in which the course was organized around the scientific process starting with how the ideas for an experiment develop and covering execution of the experiment, presentation of the results at seminars and in publication, funding, and the implications of the experimental results on scientists and society. As a part of learning how to make oral presentations, together with the instructors, the students also critiqued formal seminar presentations at the Laboratory. A primary objective of the course was for students to consider exposition and ethics as an integral part of scientific research.

Research Topics

ARRANGED BY	**Lilian Gann** **David Helfman**

This core course provided students with an in-depth introduction to the fields of research that the Laboratory scientists investigate. Students and faculty attended a weekly seminar at which faculty members presented their current research topics and methods of investigation each Wednesday evening. The students learned how to approach important problems in biology. These seminars, together with the annual fall in-house symposium, provide students with a basis for selecting laboratories in which to do rotations. The weekly speakers were:

Week 1	Eli Hatchwell, Rui-Ming Xu, Marja Timmermans
Week 2	Nouria Hernandez, Jacek Skowronski, Arne Stenlund

Week 3	Winship Herr, Bruce Stillman, William Tansey
Week 4	Shiv Grewal, Tatsuya Hirano, Michael P. Myers
Week 5	Andrew Neuwald, David L. Spector, Michael Q. Zhang
Week 6	Adrian R. Krainer, Alea A. Mills, Tim Tully
Week 7	Leemor Joshua-Tor, Yi Zhong, Jerry Yin
Week 8	Hollis Cline, Yuri Lazebnik, Nicholas Tonks
Week 9	Masaaki Hamaguchi, Gregory Hannon, Linda Van Aelst
Week 10	Robert Lucito, Vivek Mittal, Senthil K. Muthuswamy
Week 11	Scott Lowe, Robert Martienssen, Michael Wigler
Week 12	Dmitri Chklovskii, David Jackson, Lincoln Stein
Week 13	Carlos D. Brody, Z. Josh Huang, W. Richard McCombie
Week 14	Grigori Enikolopov, David Helfman, Roberto Malinow

SPECIALIZED DISCIPLINES COURSES

The Genome

FUNDED BY **The Edward H. and Martha F. Gerry Lectureship**
The Pfizer Lectureship
The George B. Rathmann Lectureship

INSTRUCTORS **Robert Martienssen**
W. Richard McCombie
Lincoln Stein

GUEST LECTURERS **Andrew Neuwald**
Jan Witkowski
Michael Q. Zhang

The first draft of the human genome sequence was completed in 2000, and the new science of genomics promises to revolutionize biological concepts and approaches. With computers, scientists can digest the enormous amount of genetic information produced by the genome project and answer questions about evolution, model complex processes such as signal transduction and gene regulation, and manage and organize experiments. This course provided essential background as to how sequence and mapping information is generated and interpreted, including the principles of genetics, the principles of microarray and other functional strategies, and the history of the genome project. Topics covered included genetic and physical mapping, DNA sequence acquisition and interpretation, Web-based resources for genome data, gene modeling and protein and DNA sequence analysis, phylogenetic analysis, and functional genomics.

Cellular Structure and Function

FUNDED BY **The Mary D. Lindsay Lectureship**
The Sigi Ziering Lectureship

INSTRUCTORS **David Helfman**
David L. Spector

GUEST LECTURERS **Tatsuya Hirano**

With the complete set of instructions available for many organisms, i.e., their genome sequence, there is now much emphasis on understanding the function of the gene products. This understanding will require an increasing appreciation of the structure and function of the cell. This course provided a basic overview of the structural and functional organization of cells with particular emphasis on cellular compartmentalization and communication. In addition, it provided insight into the basic toolbox of the cell biologist of the 21st century.

Systems Neuroscience

FUNDED IN PART BY **The George W. Cutting Lectureship**
The Klingenstein Lectureship

INSTRUCTORS **Carlos D. Brody**
Zachary Mainen
Anthony Zador

Cognition and behavior arise from complex interactions between billions of neurons. "Systems Neuroscience" examined the fundamental properties of neurons (including synaptic transmission and plasticity) and how they gave rise to higher-level (systems) brain functions such as visual perception.

WATSON SCHOOL OF BIOLOGICAL SCIENCES 2001 CONVOCATION

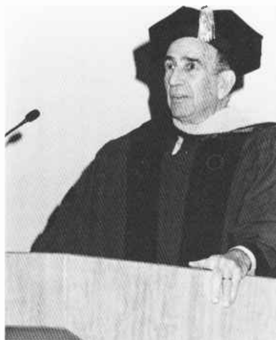
On November 2 of this year, the Watson School held its second convocation. Gail Mandel, a member of the Watson School External Advisory Committee, was the keynote speaker and spoke of individuals who give of themselves to serve science. At the occasion, the Watson School honored three individuals—William Maxwell Cowan, Robert J. Glaser, and David L. Luke III—for their service to science by awarding them honorary doctoral degrees. Below are reproduced the citations that were read at the convocation for each of these great benefactors to science.

William Maxwell Cowan

William Maxwell Cowan was born in Johannesburg, South Africa on September 27, 1931. He received Bachelor's degrees from the University of Witwatersrand and both medical and doctoral degrees from Oxford University. Dr. Cowan's first neurobiology studies mapped an unusual group of connections in the brain between the thalamus and the corpus striatum. This work explained a long-standing mystery in the field and would lay the foundation for a career that includes more than 200 published articles. Later, Dr. Cowan established principles that control the number of cells in the brain, and he uncovered an "activity-dependent" mechanism that prunes erroneous connections between brain cells. Dr. Cowan's association with Cold Spring Harbor Laboratory began in 1971, when he was invited to lecture in the Laboratory's newly created summer neurobiology course, "Basic Principles of Neurobiology." He returned for the next ten summers to lend his expertise to neurobiology education at the Laboratory. Dr. Cowan is a member of the U.S. National Academy of Sciences and a Fellow of the Royal Society. As Vice President and Chief Scientific Officer of the Howard Hughes Medical Institute, Dr. Cowan helped establish the Institute as a premier supporter of biomedical research and education in the United States and throughout the world.

Robert J. Glaser

Robert J. Glaser was born in St. Louis, Missouri, on September 11, 1918. He received a Bachelor's degree from Harvard University and an M.D. from Harvard University Medical School. After completing clinical training in internal medicine, Dr. Glaser was appointed a National Research Council Fellow in the Medical Sciences at Washington University. Dr. Glaser's medical research explored the relationship between Group A streptococcal infections and rheumatic fever and led him to develop an animal model for the study of rheumatic fever. He served as Vice President for Medical Affairs, Dean of the Medical School, and Professor of Medicine, first at the University of Colorado and later at Stanford University. Dr. Glaser is a member of the American Academy of Arts and Sciences, the American Philosophical Society, and the Institute of Medicine of the U.S. National Academy of Sciences. As Director for Medical Science of the Lucille P. Markey Charitable Trust, Dr. Glaser recommended that the Trust support structural biology research at several institutions, including Cold Spring Harbor Laboratory. This support helped launch the structural biology program here, which continues to flourish today. With Dr. Glaser's leadership, the Markey Charitable Trust also supported major new initiatives in neuroscience research at Cold Spring Harbor Laboratory and elsewhere.



David L. Luke III

David L. Luke III was born in Tyrone, Pennsylvania, on July 25, 1923. He is the great-grandson of William Luke, founder of Westvāco Corporation, an industry leader in paper and packaging products and in the development of tree breeding and sustainable forestry technologies. After serving in the United States Marine Corps during World War II, Mr. Luke received a Bachelor's degree from Yale University in 1948. Mr. Luke's career as CEO of Westvāco sparked his interest in plant molecular biology and genetics. As a result, he joined the Cold Spring Harbor Laboratory Board of Trustees in 1985. As co-creator of the Laboratory's Infrastructure Fund and as chair of its Second Century Fund, Mr. Luke helped expand and improve Laboratory facilities, endow chaired professorships, and launch a major new initiative in neuroscience research. To support plant biology research, Mr. Luke provided seed money for the international *Arabidopsis* genome sequencing project. The project generated the first complete DNA sequence of plant genome. As Chairman of the Board of Trustees, Mr. Luke spearheaded the establishment of the Watson School of Biological Sciences and initiated an endowment entirely devoted to the Watson School. Mr. and Mrs. Luke have established a graduate student fellowship that provides, in perpetuity, full support for one Watson School student. Mr. Luke continues today to head the successful endowment campaign as Chairman of the Watson School of Biological Sciences Campaign Committee. Mr. Luke is also Director of the Josiah Macy, Jr. Foundation's "Ventures in Education" program which links academically promising high school students with participating medical schools. In 1998, Mr. Luke retired as Chairman of the Board of Cold Spring Harbor Laboratory, and he was elected a lifetime Honorary Trustee.

UNDERGRADUATE RESEARCH PROGRAM

Program Director: Leemor Joshua-Tor

Associate Program Director: Gregory Hannon

Program Administrator: Jane Reader

An important aspect of the summer program at the Laboratory is the participation of college undergraduate students in active research projects under the supervision of full-time Laboratory staff members. The program was initiated in 1959. Since that year, 586 students have participated in the program, and many have gone on to productive careers in biological science.

The objectives of the program are to provide (1) a greater understanding of the fundamental principles of biology; (2) an increased awareness of experimental approaches to science; (3) a deeper understanding of the major issues in the fields of biochemistry, genetics, and molecular and cellular biology; and (4) a personal acquaintance with research, research workers, and centers for study. The following students, selected from 640 applicants, took part in the program:

Brian J. Adkins, Tuskegee University
Advisor: **Dr. Masaaki Hamaguchi**
Sponsor: National Science Foundation
A study of the *DBC2* gene: Tumor suppressor candidate in breast cancer.

Gautam Agarwal, University of Texas, Austin
Advisor: **Dr. Zachary Mainen**
Sponsor: Jephson Educational Trust
Discriminability and coding of odors in the olfactory bulb.

Sitting: Elizabeth Head, Lakshmi Swamy, Yao Chen, Allison Carey, Lindsay Hufton, Carolyn Dong
Left to right: Kelly Biddle, Raymond Chen, Benjamin de Bivort, Jovana Drinjakovic, Alicia Berger, Timothy Sonbuchner, Lindzy Friend, Joe Markson, Michael Hoffman, Shantanu Jadhav, Seth Bechis, Christopher Wilson, Lauri Friesenhahn, Laura Burrack, Meelis Kadaja, Elizabeth Fingar, Gautam Agarwal, Julie Plocher
Not pictured: Brian Adkins

Seth Kenneth Bechis, Harvard University
Advisor: **Dr. Leemor Joshua-Tor**
Sponsor: The Read Fund
Purification and crystallization of the replication initiation protein of the human papillomavirus high-risk strains.

Alicia Berger, University of Colorado
Advisor: **Dr. Gregory Hannon**
Sponsor: National Science Foundation
Creation of a phenotype array using RNA interference in *Drosophila* S2 cells.

Kelly Biddle, Rice University
Advisor: **Dr. David Jackson**
Sponsor: National Science Foundation
Intercellular trafficking of transcription factors in *Arabidopsis*.

Laura Burrack, Macalester College
Advisor: **Dr. Bruce Stillman**
Sponsor: Libby Fund
Complex formation and function of scMCM proteins in initiation of DNA replication.

Allison Carey, Pennsylvania State University
Advisor: **Dr. Jerry Yin**
Sponsor: National Science Foundation
Identification of molecular partners for the memory protein DaPKCζ.

Raymond Chen, Harvard University
Advisor: **Dr. William Tansey**
Sponsor: Burroughs Wellcome
Myc: The unphosphorylated, the phosphorylated, and the imposters.

Yao Chen, Cambridge University
Advisors: **Dr. Karel Svoboda**
Sponsors: The Olney Fund and Howard Hughes Medical Institute
Visualizing mRNA trafficking in living neurons.

Benjamin de Bivort, Duke University
Advisor: **Dr. Yi Zhong**
Sponsor: Burroughs Wellcome
Roles of Notch and NF1 proteins in activity-dependent synaptic plasticity.

Carolyn Dong, University of Massachusetts, Amherst
Advisor: **Dr. David L. Spector**
Sponsor: National Science Foundation
Modification of transcriptional activity by nuclear positioning.

Jovana Drinjakovic, Oxford University
Advisor: **Dr. Yuri Lazebnik**
Sponsor: The Emanuel Ax Fund
Oncogenes induce cell fusion.

Elizabeth Fingar, Ohio University
Advisor: **Dr. Hollis Cline**
Sponsor: Burroughs Wellcome
Homer constructs in the *Xenopus* visual system.

Lindzy Friend, University of Evansville
Advisor: **Dr. Adrian R. Krainer**
Sponsor: Jephson Educational Trust
Investigating the relationship between UP1 and telomeric DNA using footprinting techniques.

Laurie B. Friesenhahn, Texas A&M University
Advisor: **Dr. Shiv Grewal**
Sponsor: National Science Foundation
Histone H3 Lys9 methylation and epigenetic silencing in *S. pombe*.

Elizabeth Head, University of Minnesota
Advisor: **Dr. Robert Martienssen**
Sponsor: National Science Foundation
Characterizing three putative RNAi genes in *S. pombe*.

Michael Hoffman, University of Texas, Austin
Advisor: **Dr. Michael Q. Zhang**
Sponsor: Garfield Trust
AtProbe: *Arabidopsis thaliana* promoter binding element database.

Lindsay Hufton, Cambridge University
Advisor: **Dr. Linda Van Aelst**
Sponsor: Burroughs Wellcome
Identification of oligophrenin-1 binding partners in brain.

Shantanu P. Jadhav, Indian Institute of Technology, Bombay
Advisor: **Dr. Anthony Zador**
Sponsor: Glass Fund
A psychophysical investigation of the effect of attention on auditory stream segregation, and a statistical analysis of sounds.

Meelis Kadaja, University Tartu
Advisor: **Dr. Arne Stenlund**
Sponsor: Shakespear Fund
The effect of tumor suppressor protein p53 on Bev-1 replication in vitro.

Joseph Markson, Harvard University
Advisor: **Dr. Z. Josh Huang**
Sponsor: Burroughs Wellcome
Bioinformatic approach to mechanisms of GABAergic cell-type-specific gene expression.

Julie Plocher, University of Illinois, Urbana-Champaign
Advisor: **Dr. Marja Timmermans**
Sponsor: Burroughs Wellcome
Rough sheath2: How to keep hormones under control.

Timothy Sonbuchner, Gustavus Adolphus College
Advisor: **Dr. Grigori Enikolopov**
Sponsor: Frederica von Stade Fund
Expression of nitric oxide isoforms in hematopoietic stem cells.

Lakshmi Swamy, University of Georgia
Advisor: **Dr. Lincoln Stein**
Sponsor: Bliss Fund
The mining of miniature inverted-repeat transposable elements in rice.

Christopher G. Wilson, Kalamazoo College
Advisor: **Dr. Robert Malinow**
Sponsor: National Science Foundation
Involvement of spontaneous activity in the phosphorylation of GluR1/4 by PKA.

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 2001, a total of 383 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, the Nature Conservancy, the Cold Spring Harbor Fish Hatchery and Aquarium, and other local preserves and sanctuaries.

In addition to the three 2-week sessions, the *Adventure Education* course met on Friday July 27, 2001 for a 6-mile canoe trip on the Nissequogue River in Smithtown to navigate and explore the waters of Long Island. The course emphasizes the plant and animal life indigenous to the area as well as historic points of interest.

PROGRAM DIRECTOR: William M. Payoski, M.A., Adjunct Professor, Nassau Community College

REGISTRAR: Sharon Bense, Cold Spring Harbor Laboratory

INSTRUCTORS: Alison Smith, B.S., Marine Science, University of Rhode Island
Michael Zarzicki, B.A., English, Adelphi University
Margot Gallowitsch, B.S., Biology, Fairfield University
Amy Friedank, B.S., Marine Science, Long Island University/Southampton College
Marlena Emmons, M.S., Art Education, Long Island University/C.W. Post Campus; BFA, Photography, SUNY New Paltz
Patricia Grimaldi, B.S., Biology, SUNY at Stony Brook

COURSES

Nature Bugs (Kindergarten): Exploration, games, stories, and dramatics are used to introduce the young child to a variety of natural habitats.

Nature Detectives (Grades 1–2): An introductory course in nature study, stressing interrelationships between plants and animals. A variety of habitats are thoroughly explored.

Nature Discovery (Grades 1–2): Students continue their discovery of nature through activities and concepts.

Ecology Explorers (Grades 3–4): Natural communities, food webs, and the succession of communities are studied in this course. Students study the diversity of plant and animal forms native to the Cold Spring Harbor Laboratory area.

Frogs, Flippers, and Fins (Grades 3–4): Designed for younger students as an introduction to aquatic ecosystems. Fresh water and marine habitats are explored.

Pebble Pups (Grades 3–4): Elementary geology for students interested in making a basic study of rocks and minerals available on Long Island. Each student completes a rock and mineral collection. Dinosaurs and fossils are featured themes. Some of the highlights of this course include field trips to local museums.

Freshwater Life (Grades 5–7): Students study the vertebrate, invertebrate, and plant life found in area bogs, ponds, lakes, and streams.

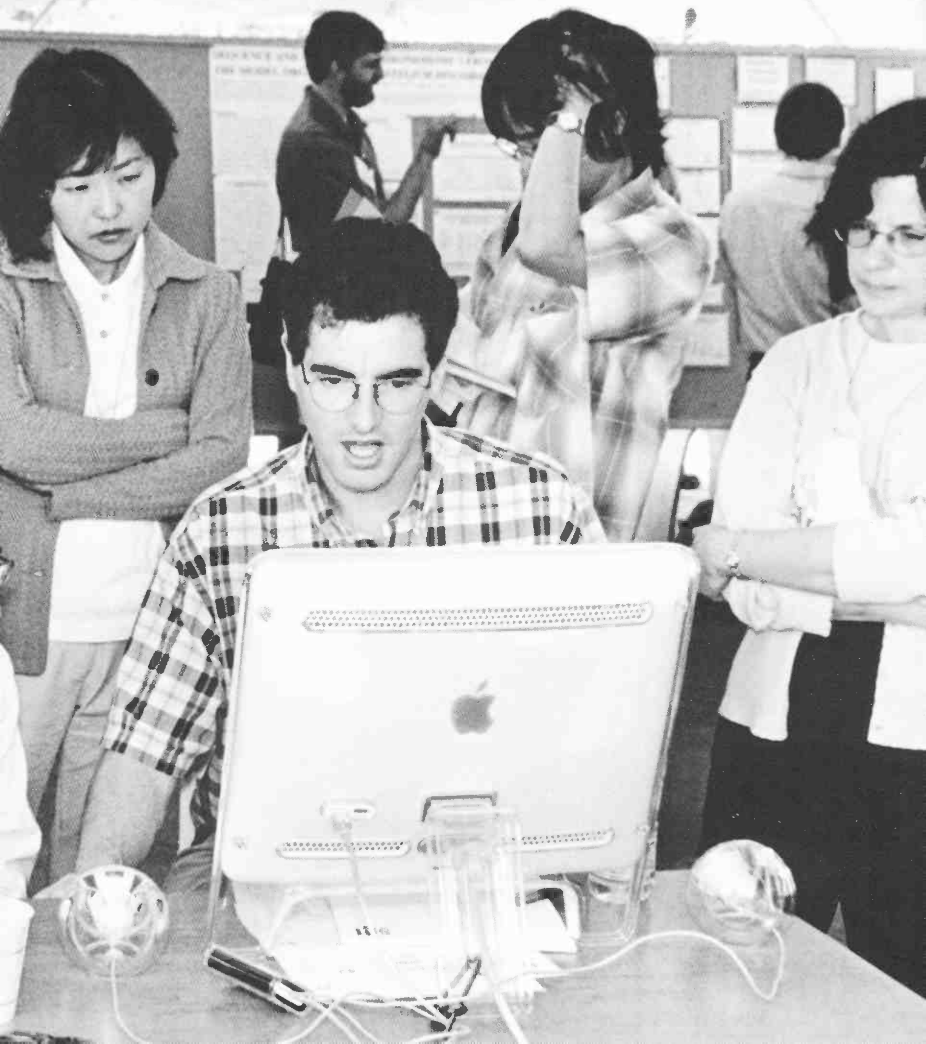
Seashore Life (Grades 5–7): Children examine plant and animal life found below the tidemark. Fish, marine worms, algae, shellfish, beach plants, and shore birds are studied.

Adventure Education (Grades 6–10): This course is a 6-mile canoe trip up and down the Nissequogue River, exploring the flora and fauna of the waterway.

Marine Biology (Grades 8–10): This course offers a more sophisticated study of plants and animals native to the inner and outer harbors. This course provides field trips, dissection, use of the microscope, and laboratory experiments.

Nature Photography (Grades 8–10): Students take pictures and use the darkroom to learn techniques of printing and developing. Photographic techniques relating to nature photography are emphasized. Students supply their own camera and film. Darkroom supplies are provided.

COLD SPRING HARBOR LABORATORY MEETINGS AND COURSES



ACADEMIC AFFAIRS

The rapid pace of advances in cell and molecular biology as well as neurobiology has made the communication and dissemination of new information and technologies critical. The academic program at Cold Spring Harbor Laboratory is designed to address the needs of the broader scientific community. The program comprises a wide-ranging series of postgraduate laboratory and lecture courses, workshops, and large meetings. The program extends from early in March through mid-December, starting with a short program of winter biotechnology conferences; followed by spring courses and meetings, summer courses, fall meetings and courses; and ending with several late fall biotechnology conferences. This year, scientists ranging from graduate students to senior faculty participated in the courses and meetings. These scientists represent an international group who come from universities, medical schools, research institutes, and industry.

The academic program includes a series of 3-week laboratory courses that are held in the summer, as well as 1- and 2-week laboratory courses that are given in the spring and fall. They are held in the Howard Hughes Medical Institute (HHMI) teaching laboratories in the Beckman Neuroscience Center, the Marks Imaging Laboratory, and in Delbrück Laboratory. The bioinformatics courses take place in the Bush Auditorium, which is adapted for the needed technology, and the neurobiology lecture courses are held at the Laboratory's Banbury Center. In total, 26 courses were held this year, including two new neurobiology lecture courses: *Cellular Biology of Addiction* and *Biology of Developmental Disabilities*. All of the courses had more applicants than could be accommodated, which is a reflection of their timeliness and the expertise of the instructors.

The instructors included a few Cold Spring Harbor faculty, but most came from universities and research institutes from around the world. Their energy, enthusiasm, and very hard work were the key to the success of the program. Many of them return for several years to teach at the Laboratory, for which we continue to be very grateful. Course instructors, assistants, lecturers, and students are listed in the pages following Meetings. We must make note this year of several heroic instructors who have been teaching courses for at least 10 years. These include Dick Burgess, an instructor in the *Protein Purification and Characterization* course; Carlos Barbas, who teaches in the *Phage Display of Combinatorial Antibody Libraries* course; and the entire teaching faculty of the *Macromolecular Crystallography* course: Bill Furey, Gary Gilliland, Alex McPherson, and Jim Pflugrath. Jim Boulter, who started as an instructor in the *Molecular Cloning of Neural Genes* course, is retiring with honor after this year. All of the course instructors, assistants, and lecturers are listed in the pages that follow.

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. A grant from the National Institute of Mental Health supports several of the neurobiology courses. A large education grant from HHMI has provided stable support for the neurobiology program and has allowed the Laboratory to begin and to expand its series of spring and fall courses. The Laboratory also had a grant from the John Merck Fund to support the *Biology of Developmental Disabilities* course.

A series of 19 meetings and biotechnology workshops were held this year, including the Annual Symposium on Quantitative Biology. This year's exciting and timely symposium on the ribosome was organized by Bruce Stillman and David Stewart and is discussed on the following pages. The other meetings, which are often held in alternate years, covered topics ranging from *Telomeres and Telomerase* to *Eukaryotic mRNA Processing*. Many meetings filled Grace Auditorium to capacity, including those on *Genome Mapping, Sequence, and Biology; Retroviruses*; and *Mechanisms of Eukaryotic Transcription*. Two meetings—*Programmed Cell Death* and *Microbial Pathogenesis and Host Response*—had to be postponed after the tragic events of September 11. However, due to much

work and resourcefulness on the part of the organizers and Meetings office staff, the meetings were rescheduled and held several weeks later. The organizers and session chairs are listed in the pages that follow.

The success of the very large program of courses and meetings is also due to the skilled efforts of many people at Cold Spring Harbor Laboratory. They include Andrea Stephenson, the Course Registrar; Ed Campodonico, Manager of Audiovisual Services; and Barbara Zane, the Course Coordinator. Staff of the Meetings and Courses office handle an ever-increasing and complex workload with skill and good humor. They include those listed above, as well as Michael Glaessgen, Drew Mendelsohn, Mary Smith, Marge Stellabotte, and Jenna Williams. Housing and front-desk services are provided by Nancy Weeks, Operations Supervisor, Andrea Newell and Donna Dykeman (to whom we say goodbye this year). Audiovisual services are ably provided by Ed Campodonico, William Dickerson, John Parsons, and the part-time audiovisual staff. Staff from several other departments also do crucial work for the courses and meetings, including Wendy Crowley, the Senior Education Grants Administrator; Cliff Sutkevich and his staff, who set up and maintain course equipment; and Leigh Johnson in the Library, who handles the book and journal needs of the courses. The Information Services department, headed by Jerry Latter for much of 2001 and subsequently by Mark Kilarjian, provides ever-increasing computer support for all of the courses and meetings. We are grateful to them all.

Terri Grodzicker
Assistant Director for Academic Affairs

David Stewart
Director of Meetings and Courses

T. Grodzicker, H. Gesteland, R. Gesteland

66TH COLD SPRING HARBOR SYMPOSIUM ON QUANTITATIVE BIOLOGY

The Ribosome

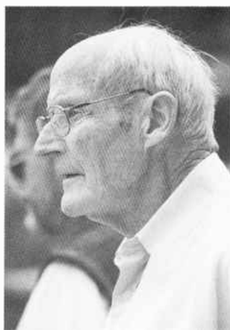
May 31–June 5 295 participants

ARRANGED BY **Bruce Stillman,** Cold Spring Harbor Laboratory
David Stewart, Cold Spring Harbor Laboratory

How proteins are made became a major focus following the discovery of the double-helical structure of DNA in 1953. Once it was realized that the genetic code existed within the order of base pairs of DNA, it became of immediate importance to understand how that information was converted into the sequences of amino acids that defined individual proteins. The solution of this problem, which includes deciphering the genetic code, is one of the great achievements in the science that is now known as molecular biology. Many of these achievements have been recorded and celebrated at previous Symposia in this series.

Protein synthesis occurs within the ribosome, a molecular machine comprising both protein and RNA that in eukaryotes is first assembled in the nucleus of a cell. RNA also functions as the adaptor in the form of tRNA that brings the individual amino acids to the ribosome. Ribosomes themselves associate with other molecular machineries, such as the machinery that directs proteins to the cell surface. It was once thought that figuring out the molecular details of how protein synthesis occurs would be an insurmountable problem. But dramatic events in structural biology in recent years have changed this view, and underscored the decision to select the ribosome as the topic for this year's Symposium.

High-resolution images of the structure of a ribosome not only tell us how beautiful this machine is, but also reveal the surprising finding that peptide bond formation is directed by RNA. Although this has been hinted at for some time, it was difficult to envision without seeing the ribosome structure. The structure of the ribosome, either as its individual parts or as a whole, has also allowed views of how the newly minted peptide chain emerges and, in the case of some proteins, how it interacts with other molecular machinery that exports proteins to the cell surface.


A. Tissières

The meeting started with a talk from Jim Watson about his early and important role in the ribosome field while at Harvard. Later followed outstanding introductory presentations by Tom Steitz, Venki Ramakrishnan, Nahum Sonenberg, and Roland Beckmann. The Friday evening Reginald Harris Lecture was masterfully presented by Harry Noller, and the Dorcas Cummings Memorial Lecture to our friends and neighbors was beautifully presented by Venki Ramakrishnan. There were 60 oral presentations and 136 poster presentations over the course of the meeting, which was this year held from May 31 to June 5. On the final day, Peter Moore presented us with a very thoughtful and thought-provoking summary of the Symposium.

Particularly pleasing at this meeting was the return to Cold Spring Harbor Laboratory of many of the principal contributors to the early days of ribosome research who reminisced about experiments that contributed to understanding, at a most fundamental level, the central dogma of biology. They included Alfred Tissières, Donald Caspar, and Alex Rich.

Essential funds to run this meeting were obtained from the National Cancer Institute, a branch of the National Institutes of Health. In addition, financial help from the corporate benefactors, sponsors,

affiliates, and contributors of our meetings program is essential for these Symposia to remain a success, and we are most grateful for their continued support. *Corporate Benefactor:* Pfizer, Inc. *Corporate Sponsors:* Abbott Bioresearch Center, Inc.; Amgen Inc.; Aventis Pharma AG; BioVentures, Inc.; Bristol-Myers Squibb Corporation; Chiron Corporation; Chugai Research Institute for Molecular Medicine, Inc.; Cogene BioTech Ventures, Ltd.; Diagnostic Products Corporation; DuPont Pharmaceuticals Company; Forest Laboratories; Genentech, Inc.; Genetics Institute; GlaxoSmithKline; Hoffmann-LaRoche Inc.; Johnson & Johnson; Kyowa Hakko Kogyo Co., Ltd; Eli Lilly and Company; Merck Research Laboratories; New England BioLabs, Inc.; Novartis Pharma Research; OSI Pharmaceuticals, Inc.; Pall Corporation; Pharmacia & Upjohn, Inc.; Research Genetics, Inc.; Schering-Plough Corporation. *Plant Corporate Associates:* Monsanto Company; Pioneer Hi-Bred International, Inc.; Torrey Mesa Research Institute; Westvaco Corporation. *Corporate Affiliates:* Affymetrix, Inc.; Ceptyr, Inc. *Corporate Contributors:* Alexis Corporation; Biogen, Inc.; Digital Gene Technologies, Inc.; DoubleTwist, Inc.; Epicentre Technology; Genomica Corporation; Incyte Genomics; InforMax; KeyGene; LabBook, Inc.; Lexicon Genetics, Inc.; Proteome, Inc.; Pyrosequencing; Qiagen; Silicon Genetics; Transgenomics, Inc.; ZymoGenetics, Inc. *Foundations:* Albert B. Sabin Vaccine Institute, Inc.

P. Moore, V. Ramakrishnan, A Dahlberg

M. Mathews, T. Pe'ery, B. Stillman

J. Frank, D. Stewart, J. Warner, A. Hinnebusch

Introduction
B. Stillman, *Cold Spring Harbor Laboratory*

James D. Watson: Introductory Comments on the Ribosome

Ribosome Structure
Chairperson: D. Caspar, *Florida State University, Tallahassee*

Reginald G. Harris Lecture
Speaker: H.F. Noller, *University of California, Santa Cruz*

The Ribosome Enzyme
Chairperson: A. Rich, *Cold Spring Harbor Laboratory*

Initiation
Chairperson: M. Mathews, *UMDNJ-New Jersey Medical School, Newark*

Alternative Initiation
Chairperson: S. Gerbi, *Brown University, Providence, Rhode Island*

Elongation and Termination
Chairperson: R. Gesteland, *Howard Hughes Medical Institute, University of Utah, Salt Lake City*

Evolution of the Code/aatRNA Synthetases
Chairperson: A. Yonath, *Weizmann Institute of Science, Rehovot, Israel*

Dorcas Cummings Lecture: Protein Factories and Antibiotics
Speaker: V. Ramakrishnan, *Medical Research Council, United Kingdom*

Regulation of mRNA
Chairperson: J. Doudna, *HHMI, Yale University, New Haven, Connecticut*

Regulation of Protein Synthesis
Chairperson: T. Kinzy, *UMDNJ-Robert Wood Johnson Medical School, Piscataway*

Ribosome Biogenesis
Chairperson: L. Maquat, *University of Rochester Medical School, New York*

Summary: Peter Moore, *Yale University*

Noller, L. Maquat, A. Yonath, D. Ayres, R. Miller, Mrs. Miller

Rich, H. Noller, A. Spirin, D. Caspar

T. Steitz, O. Uhlenbeck, J.D. Watson

MEETINGS

Genetic Basis of Neurological and Behavioral Disorders

March 7–11 92 participants

ARRANGED BY **Charles Gilbert,** The Rockefeller University
Eric R. Kandel, Columbia University/HHMI

A private meeting on the topic of the Genetic Basis of Neurological and Behavioral Disorders was held here in the spring on behalf of three funds: The Esther A. & Joseph Klingenstein Fund, The Ruth & Milton Steinbach Fund, and The Klingenstein Third Generation Fund. For 18 years, the Klingenstein Fund has awarded fellowships in neuroscience related to epilepsy, and for 8 years, The Steinbach Fund has awarded research grants for the study of macular degeneration. The Third Generation Fund supports research on childhood and adolescent depression and attention deficit hyperactive disorder. In addition to an eminent list of invited speakers, the meeting brought together current and former fellows and investigators supported by the foundations, as well as members of the foundation advisory boards. The meeting included a busy schedule of talks on the Human Genome Project, genomic studies in various animal models, analytic techniques for studying gene expression, and tools for manipulating gene expression. Speakers included Cori Bargmann, James Battey, Jr., Thomas Bouchard, Aravinda Chakravarti, Dennis Drayna, Gerald Fischbach, Wayne Frankel, Nelson Freimer, Fred (Rusty) Gage, Barry Ganetzky, Elliot Gershon, Eric Green, Steven Hyman, Maria Karayiorgou, Kathleen Merikangas, Emmanuel Mignot, Jeremy Nathans, Joseph Takahashi, Stephen Warren, Huda Zoghbi, and Charles Zuker. The meeting was open to all interested Cold Spring Harbor scientists.

E. Kandel, C. Gilbert

J. Battey, W. Frankel, D. Vollrath

A. Ribero, E. Levitan

P. Klingenstein, J.D. Watson, J. Klingenstein

BIOTECHNOLOGY CONFERENCE
Vector Targeting Strategies for Therapeutic Gene Delivery

March 15–18 114 participants

ARRANGED BY **David Curiel,** University of Alabama, Birmingham
Wayne Marasco, Dana Farber Cancer Institute
Stephen Russell, Mayo Foundation

The paramount requirement for advancement of gene therapy is the development of vector systems possessing the capacities for efficient and cell-specific gene delivery. The achievement of these goals requires that the vector system recognize specific cell signatures. In the first regard, both nonviral and viral vectors have been engineered to address this goal. In addition, a variety of high-throughput methods have been advanced for identification of cell-specific markers. Investigators at this meeting provided an update of key technologies relevant to these goals. Since the last conference in 1999, significant progress has been noted in this field. Especially noteworthy was the progress achieved in the content of viral vector tropism modifications. This progress included the translation of novel, advanced generation vectors into the human clinical context. Future work will be required to define the range of cell-specific signatures of relevance in the clinical context. Nevertheless, the linkage of these two technologies—target definition and vector targeting—is already yielding important advances in outcomes achievable via gene therapy methods.

This meeting was funded in part by Amersham Pharmacia Biotech; Berlex Biosciences; Canji, Inc.; Merck & Co., Inc., and VectorLogics, Inc. Contributions from our Corporate Sponsors, Plant Corporate Associates, Corporate Affiliates, Corporate Contributors, and Foundations also provided core support for this meeting.

D. Curiel, D. Larocca

S. Oqueta, W. Marasco

PROGRAM

Target Definition I
Chairperson: D.T. Curiel, *University of Alabama, Birmingham*

Target Definition II
Chairperson: R. Pasqualini, *University of Texas M.D. Anderson Cancer Center, Houston*

Target Definition III
Chairperson: S. Hoffman, *Celera Genomics, Rockville, Maryland*

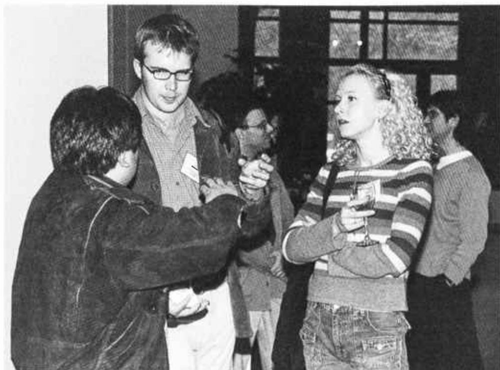
Transcriptional Targeting
Chairperson: R. Vile, *Mayo Foundation, Rochester, Minnesota*

RNA Viruses
Chairperson: W. Marasco, *Dana-Farber Cancer Institute, Boston, Massachusetts*

DNA Viruses
Chairperson: P.W. Roelvink, *GenVec, Inc., Gaithersburg, Maryland*

New Technology
Chairperson: R. Cattaneo, *Mayo Foundation, Rochester, Minnesota*

V. Khuvana, S. Russell, J. Bartlett



M. Yamamoto, A. Hemminki, A. Kanerva

BIOTECHNOLOGY CONFERENCE
Stem and Progenitor Cells: Biology and Applications

March 22–25 195 participants

ARRANGED BY **David Anderson,** California Institute of Technology/HHMI
Richard Gardner, University of Oxford, United Kingdom
Daniel Marshak, Cambrex Corporation/Johns Hopkins University

Cells that give rise to the differentiated tissues of an organism are referred to as stem and progenitor cells. Our understanding of the biology of development has focused recently on stem cells derived from early embryos, although there is now evidence that pluripotent stem cells exist in postnatal organisms. Isolated stem and progenitor cells are being developed as therapeutic agents in cell-based therapies. In addition, genomic and proteomic information derived from the differentiation of stem cells in vitro can add to our ability to select targets for pharmaceutical development. Investigators at this conference provided overview discussions as well as new findings in the field of stem cells. Explosive progress had been noted in this field during the last 4 years, and this conference was timely. Especially noteworthy were new discoveries in understanding how stem cell nuclei might be programmed for development. This reaches to the heart of biological diversity: How the regulation of gene expression in somatic cells and germ cells differs at a molecular level. Applications of stem cells derived from postnatal tissues were discussed at length. It appears that many adult tissues have multipotent, and perhaps pluripotent, stem cells available for the maintenance of tissue integrity, normal cellular turnover, and wound repair. The molecular mechanisms controlling differentiation were discussed, and the applications of stem cells to gene discovery and gene therapy were presented. The conference paved the way for new development in one of the most dynamic fields of this year.

This meeting was funded in part by Osiris Therapeutics, Inc. Contributions from our Corporate Sponsors, Plant Corporate Associates, Corporate Affiliates, Corporate Contributors, and Foundations also provided core support for this meeting.

R. Gardner and meeting participant

PROGRAM

Historical Perspectives and Overview Topics
Chairperson: J. Witkowski, *Cold Spring Harbor Laboratory*

Early Stem Cells
Chairperson: R. Gardner, *University of Oxford, United Kingdom*

BBC Reith Lecture: The End of Age
Speaker: Tom Kirkwood, *University of Newcastle, United Kingdom*
Moderator: Sue Lawley, *British Broadcasting Corporation, United Kingdom*

Mesenchymal Tissues
Chairperson: D. Marshak, *Cambrex Corporation and Johns Hopkins University School of Medicine, Baltimore, Maryland*

Neural Tissues
Chairperson: D. Gottlieb, *Washington University, St. Louis, Missouri*

Signal Transduction
Chairperson: D. Anderson, *California Institute of Technology, Pasadena*

Hematopoiesis
Chairperson: G. Keller, *Mount Sinai School of Medicine, New York*

S. Hall, G. Keller, D. Marshak

Tom Kirkwood answering questions after delivering the BBC Reith Lecture.

Telomeres and Telomerase

March 28–April 1 305 participants

ARRANGED BY **Elizabeth Blackburn,** University of California, San Francisco
Titia deLange, The Rockefeller University
Carol Greider, Johns Hopkins University School of Medicine

The conference consisted of seven sessions of talks and three poster sessions. As in 1999, the format chosen was to invite two investigators to chair each session. These scientists are established in the field, and they not only chaired the session (one chair for each half-session), but also gave a scientific presentation. The rest of the presentations were chosen from openly submitted abstracts, allowing as many younger scientists to present as possible, including graduate students and postdoctoral fellows. Attendance exceeded 300 participants, a large fraction of whom presented in the total of 118 posters and 73 talks.

The topics covered by the talk sessions and posters were all aspects of telomere and telomerase biology, in the following groupings based on talk session: telomere length regulation and replication; telomere structure and function; end-binding proteins; telomere-associated proteins; DNA-damage response factors; consequences of telomere dysfunction; and telomerase-independent telomere maintenance.

The quality of the scientific content was very high throughout the conference in both the talks and the posters, with significant amounts of new unpublished data being presented and extensively discussed. Discussion after each talk was lively and informative. The conference was verbally judged to be highly successful by all who attended. There was generally strong enthusiasm for another meeting on the same topic in 2003.

This meeting was funded in part by the National Institutes of Health. Contributions from our Corporate Sponsors, Plant Corporate Associates, Corporate Affiliates, Corporate Contributors, and Foundations also provided core support for this meeting.

C. Greider, T. deLange, E. Blackburn

PROGRAM

Telomere Length Regulation and Replication
Chairpersons: C. Price, *University of Cincinnati College of Medicine, Ohio;* R. Wellinger, *University of Sherbrooke, Canada*

Telomerase Structure and Function
Chairpersons: J. Lingner, *Swiss Institute for Experimental Cancer Research, Epalinges;* C. Autexier, *McGill University, Montreal, Canada*

End-binding Proteins
Chairpersons: M. McEachern, *University of Georgia, Athens;* J. Cooper, *University of Colorado Health Sciences Center, Denver*

Telomere-associated Proteins
Chairpersons: S. Gasser, *ISREC, Epalinges, Switzerland;* K. Runge, *Cleveland Clinic Foundation Research Institute, Ohio*

DNA Damage Response Factors
Chairpersons: M. Blasco, *National Centre of Biotechnology, Madrid, Spain;* L. Harrington, *University of Toronto, Canada*

Consequences of Telomere Dysfunction
Chairpersons: D. Shippen, *Texas A&M University, College Station;* K. Collins, *University of California, Berkeley*

Telomerase-independent Telomere Maintenance
Chairpersons: V. Lundblad, *Baylor College of Medicine, Houston;* F. Ishikawa, *Tokyo Institute of Technology, Japan*

E. Lewis, R. Wellinger

P. Baumann, A. Eggleston

M. Deault-Bonin, J. Parenteau

FIRST LEHRMAN-WATSON ANNUAL BIOMEDICAL CONFERENCE
Pathways to Alzheimer's Disease

April 19 107 participants

ARRANGED BY **David Stewart,** Cold Spring Harbor Laboratory

The Lehrman-Watson Annual Biomedical Conference Series is targeted at a professional lay audience primarily drawn from the financial and analyst sectors. The first one-day conference focused on Pathways to Alzheimer's Disease, a disease that afflicts approximately 4 million Americans. Recent advances have brought us closer to an understanding of the biological basis of the disease, and a variety of treatments and therapeutic approaches are currently being tested in the clinic or developed in the laboratory. Furthermore, the promise of genomics can be assessed against the backdrop of this major disease, in which people with differing genetic makeup may be more or less likely to develop Alzheimer's disease. These developments in turn drive the design of clinical trials and the evaluation of novel drugs.

The conference opened with a Keynote Address by Marcelle Morrison-Bogorad, deputy director of the National Institute of Aging, and then featured four panel sessions devoted to Understanding, Probing, Treating, and Conquering Alzheimer's Disease. Each panel was moderated by leading scientists, including Peter St. George-Hyslop, John Hardy, Rachelle Doody, and Dennis Selkoe; featured talks by three experts in the field; and was followed by extensive discussion. James D. Watson presented a fascinating lunchtime talk to more than 100 meeting attendees. This conference brought together leading investigators from academia and industry with leading life science investors, health care and biotech venture capitalists, analysts, and business development executives.

The conference was made possible through the generous support of the Lehrman Institute, which underwrote the entire cost of the meeting.

D. Stewart, M. Hargraves

Meeting participant, T. Lehrman, L. Lehrman

PROGRAM

Opening Keynote Address: Marcelle Morrison-Bogorad, *National Institute of Aging*

PANEL I Understanding Alzheimer's Disease: Current Status and Therapeutic Opportunities

Moderator
Peter St. George-Hyslop, University of Toronto

Panelists
Peter Davies, *Albert Einstein College of Medicine*
Rich Mayeux, *Columbia University*
Dennis Selkoe, *Harvard Medical School*

PANEL II Probing Alzheimer's Disease: Impact of Genomics, Bioinformatics, and Enabling Technologies

Moderator
John Hardy, *The Mayo Clinic, Jacksonville*

Panelists
Mark Adams, *Celera Genomics*
Karen Duff, *Nathan Kline Institute*
Jeanne Loring, *Incyte Pharmaceuticals*

PANEL III Treating Alzheimer's Disease: Advances in Clinical Trial Design

Moderator
Rachelle Doody, *Baylor College of Medicine*

Panelists
Steve Dekosky, *University of Pittsburgh*
Mary Sano, *Columbia University*

PANEL IV Conquering Alzheimer's Disease: Novel Therapies in Clinical Development

Moderator
Dennis Selkoe, *Harvard Medical School*

Panelists
Martin Citron, *Amgen*
Perry Molinoff, *Bristol-Myers Squibb*
Dale Schenk, *Elan Pharmaceuticals*

S. Chan, J. Britton

D. Selkoe, J.D. Watson

Learning and Memory

April 25–29 122 participants

ARRANGED BY **John Byrne,** University of Texas–Houston Medical School
Joseph LeDoux, New York University
Leslie Ungerleider, National Institute of Mental Health

The fifth biennial meeting on Learning and Memory consisted of platform and poster presentations and discussions of current research and concepts in the biological basis of memory. Study of the biological bases of learning and memory is a most exciting field in neuroscience. Progress is currently very rapid, particularly at molecular, cellular, neural system, and computational levels. Commonalities of mechanisms appear to be emerging from studies of gene expression, synaptic plasticity (LTP and LTD), and processes of memory storage in invertebrate systems; in particular, similar changes in the properties of membrane channels and intracellular messenger systems were identified in a number of different neural systems in both vertebrate and invertebrate preparations. Discussions ranged from brain systems of memory in humans through brain circuits and systems of memory, to brain plasticity and development, basic processes of learning and memory, mechanisms of synaptic plasticity, e.g., long-term potentiation (LTP) and long-term depression (LTD), biophysical and molecular substrates of synaptic plasticity, to genetic approaches to mechanisms of memory.

The meeting focused on several areas of learning and memory. One was a strong representation of the neural systems underlying memory. In the areas of human memory, world leaders reported on their recent findings which show that rapid advances have been made in using imaging techniques such as PET and fMRI to probe the formation and retrieval of human memories in real time. Furthermore, systems level research in a variety of animal models revealed new insights into memory, including the new and exciting phenomenon of reconsolidation. A second focus of the meeting was on the use of mutant and transgenic mice to identify the roles of gene expression of particular enzyme systems in synaptic plasticity and memory. The "gene knockout" approach is new and very promising. There was much discussion about the possibility of developing localized, inducible, reverse knockout preparations.

The meeting was funded in part by the National Institute of Neurological Disorders and Stroke and the National Institute on Aging, branches of the National Institutes of Health; and the National Science Foundation. Contributions from our Corporate Sponsors, Plant Corporate Associates, Corporate Affiliates, Corporate Contributors, and Foundations also provided core support for this meeting.

J. Byrne, L. Ungerleider, J. LeDoux

PROGRAM

Human Memory
Chairperson: M. Mishkin, *NIMH, National Institutes of Health, Bethesda, Maryland*

Spines and Dendritic Processing
Chairperson: E. Schuman, *California Institute of Technology, Pasadena*

Encoding
Chairperson: H. Eichenbaum, *Boston University, Massachusetts*

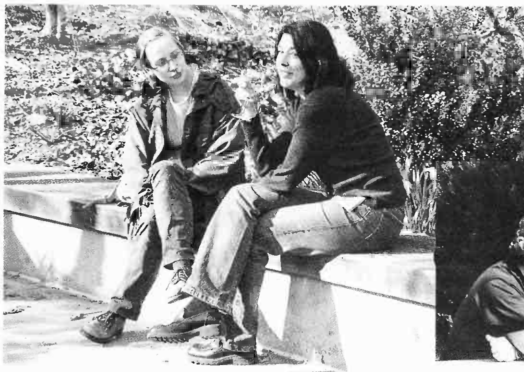
Consolidation
Chairperson: Y. Dudai, *Weizmann Institute of Science, Rehovot, Israel*

Pre- and Postsynaptic Plasticity
Chairperson: J. Byrne, *University of Texas–Houston Medical School*

Kinases and Second Messengers
Chairperson: D. Sweatt, *Baylor College of Medicine, Houston, Texas*

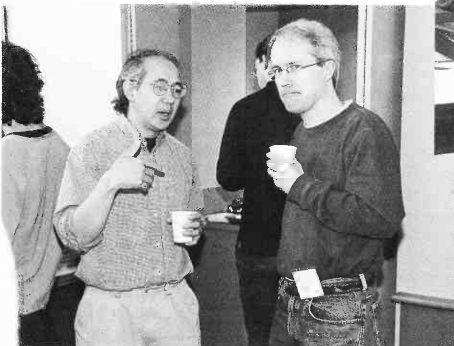
Reward
Chairperson: R. Wise, *NIDA, National Institutes of Health, Baltimore, Maryland*

Genes and Proteins
Chairperson: T. Tully, *Cold Spring Harbor Laboratory*



O. Svarnik, S. Tousey

R. Morris, M. Mishkin



J. Yin, S. Grant

PROGRAM

Signal Transduction
Chairperson: S. Coughlin, *University of California, San Francisco*

Cell Cycle/Cell Death
Chairpersons: G. Salvesen, *Burnham Institute, San Diego, California;* M. Hochstrasser, *Yale University, New Haven, Connecticut*

Development
Chairpersons: C. Hashimoto, *Yale University School of Medicine, New Haven, Connecticut;* J.M. White, *University of Virginia, Charlottesville*

Neurodegeneration
Chairpersons: D.J. Selkoe, *Harvard Medical School, Brigham and Women's Hospital, Boston, Massachusetts;* S. Sinha, *Elan Pharmaceuticals, South San Francisco, California*

Neoplasia/Angiogenesis
Chairpersons: W. Bode, *Max-Planck Institute for Biochemistry, Martinsried, Germany;* D. Hanahan, *University of California, San Francisco*

Immunology/Inflammation
Chairpersons: H. Ploegh, *Harvard Medical School, Boston, Massachusetts;* A. Rosen, *Johns Hopkins University School of Medicine, Baltimore, Maryland*

Bone and Cartilage Metabolism
Chairpersons: D. Prockop, *Tulane University School of Medicine, New Orleans, Louisiana;* D. Brömme, *Mt. Sinai School of Medicine, New York*

Infectious Diseases
Chairpersons: J.M. Musser, *Rocky Mountain Laboratories, National Institutes of Health, Hamilton, Montana;* J. Kay, *Cardiff University, United Kingdom*

L. Dick, B. Dunn

S. Waugh, D. Dauber

C. Craik, W. Bode

Proteolysis and Biological Control

May 2–6 203 participants

ARRANGED BY **Roy Black,** Immunex Corporation
Charles S. Craik, University of California, San Francisco
Ben Dunn, University of Florida
Nancy Thornberry, Merck Research Laboratories

Proteases are key regulators in a wide range of biological processes, from cell cycle and development to cell death. This biennial meeting provides a forum that fertilizes cross-talk between experts on these processes and experts on the proteases as enzymes.

This third meeting attracted more than 200 scientists who discussed the role of proteolysis in signal transduction, development, cell cycle, neoplasia, immune control, cell death, angiogenesis, inflammation, and bone and cartilage metabolism. The reported experimental systems ranged from prokaryotes to humans, whereas the interests of the speakers varied from dissection of protease structure to human neurodegenerative diseases. Despite such a diversity, the participants commented that the meeting was focused, informative, and very exciting. The cross-talk was made possible in large part by the chairs who presented extensive overviews at the beginning of each session. In summary, the Proteolysis and Biological Control meeting was a unifying forum that is helping us to understand how proteases control life and death.

This meeting was funded in part by the National Institutes of Health and the National Science Foundation. The organizers are indebted to W. Clarke Swanson, Jr. and the staff of Swanson Vineyards for generously providing a wonderful venue for planning this meeting. Contributions from our Corporate Sponsors, Plant Corporate Associates, Corporate Affiliates, Corporate Contributors, and Foundations also provided core support for this meeting.

P. Dempsey, S. Higashiyama

H. Ploegh, N. Thornberry

Genome Sequencing and Biology

May 9–13 525 participants

ARRANGED BY **Pui-Yan Kwok,** Washington University
Jane Rogers, The Sanger Centre
Edward Rubin, Lawrence Berkeley National Laboratoy

The annual Genome Sequencing and Biology meeting marked the 14th gathering of genome scientists in this setting. The past decade has seen remarkable progress in the mapping and sequencing of the genomes of many "model organisms" and the publication of a "working draft" of the human genome sequence in February 2001. Phrases such as "functional genomics" and "postgenome biology" have become common terms. More than 500 people from around the world attended the meeting, with 298 abstracts presented describing a broad array of topics relating to the analysis of genomes from a number of different organisms.

The session topics included areas such as biological insights from phylo-genomics, mapping methods and technologies, functional genomics, computational genomics, and 21st century genetics. This year's poster symposium featured the complete sequencing of human chromosomes 21 and 22, in addition to the announcement by the public Human Genome Project that 80% of the human genome had been sequenced. Once again, projection-style, interactive computer demonstrations in Grace Auditorium effectively highlighted the critical new bioinformatics tools being developed for storing, organizing, and analyzing genomic maps and sequences. There was also an ELSI (Ethical, Legal, and Social Implications) panel discussion, chaired by Charmaine Royal of Howard University, which focused on issues surrounding genetic enhancement.

The major themes of the meeting included the analysis of the draft human genome sequence and the smallest human chromosomes, including the Y chromosome, with the surprising observation that the number of genes in the human might be as small as 30,000. Specific presentations continued to report major achievements in the sequencing of model organisms and the human being, the development of new technologies for genetic analysis, and the use of microarrays for performing genome analysis. The now traditional Saturday afternoon keynote talks were delivered by Eric Lander, Director of the Whitehead Institute/MIT Center for Genome Research, and Paul Nurse, Head of the Imperial Cancer Research Fund and winner of this year's Nobel Prize for Physiology or Medicine.

This meeting was funded in part by the National Human Genome Research Institute, a branch of the National Institutes of Health. Contributions from our Corporate Sponsors, Plant Corporate Associates, Corporate Affiliates, Corporate Contributors, and Foundations also provided core support for this meeting.

Computer demo under canvas.

PROGRAM

Large-Scale Sequencing and Analysis
Chairpersons: S. Beck, *Sanger Centre, Cambridge, United Kingdom;* J. Bouck, *Baylor College of Medicine, Houston, Texas*

Polymorphisms I: Discovery and Technology
Chairpersons: D. Altshuler, *Whitehead Center for Genome Research, Cambridge, Massachusetts;* S. Tyagi, *Public Health Research Institute, New York*

Comparative Genomics I: Model Organisms
Chairpersons: J. Ecker, *Salk Institute for Biological Studies, La Jolla, California;* S. Johnson, *Washington University School of Medicine, St. Louis, Missouri*

Functional Genomics
Chairpersons: M. Eisen, *Lawrence Berkeley National Laboratory, California;* Y. Hayashizaki, *RIKEN, Tsukuba, Japan*

ELSI Panel Discussion: Through the Past Darkly: A Window on the American Eugenics Movement

Bioinformatics
Chairpersons: E. Birney, *European Bioinformatics Institute, Hinxton, United Kingdom;* S. Eddy, *Washington University School of Medicine, St. Louis, Missouri*

Polymorphisms II: Mapping and Analysis
Chairpersons: T. Long, *University of California, Irvine;* M. Shriver, *Pennsylvania State University, University Park*

Keynote Speakers
Eric Lander, *Whitehead Institute/MIT Center for Genome Research;* Paul Nurse, *Imperial Cancer Research Fund*

Comparative Genomics II: Mammalian Genetics
Chairpersons: S. Scherer, *Hospital for Sick Children, Toronto, Canada;* H.A. Lewin, *University of Illinois, Urbana*

F. Collins, E. Lander, D. Bentley

P. Kitts, J. Kent

M. Stoll, A. Kwitek-Black

P.-Y. Kwok, E. Rubin, J. Rogers

Tyrosine Phosphorylation and Cell Signaling

May 16–20 243 participants

ARRANGED BY **Nick Tonks,** Cold Spring Harbor Laboratory
Sara Courtneidge, Van Andle Research Institute, Grand Rapids, Michigan
Ben Neel, Beth Israel Hospital, Harvard Medical School

This fourth meeting on Tyrosine Phosphorylation and Cell Signaling opened with Keynote Addresses by Jossie Schlessinger and Jack Dixon. Jossie Schlessinger introduced the field of tyrosine-phosphorylation-dependent signal transduction from the perspective of the protein tyrosine kinases, focusing on signaling through the FGF receptor. Jack Dixon discussed the complementary perpective and the signaling function of protein tyrosine phosphatases and highlighted the potential RNA interference as a tool to delineate signaling pathways. 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 largely based around physiological processes and cellular functions so as to try to provide biological context to the data. A variety of systems were described, with great progress reported in genetic and biochemical approaches to the characterization of physiological functions for tyrosine phosphorylation. In particular, exciting insights were provided into how signaling pathways may be abrogated in a variety of human disease states and to the identity of novel targets for therapeutic intervention. The meeting continues to be successful and alternates with a conference with the same format at The Salk Institute, with the result that there is an annual meeting on tyrosine phosphorylation.

The meeting was supported by grants from the National Institutes of Health and the National Science Foundation. Contributions from our Corporate Sponsors, Plant Corporate Associates, Corporate Affiliates, Corporate Contributors, and Foundations also provided core support for this meeting.

N. Tonks, S. Courtneidge, B. Neel

PROGRAM

Keynote Speakers
Chairpersons: J.E. Dixon, *University of Michigan Medical School, Ann Arbor;* J. Schlessinger, *New York University School of Medicine*

Metabolism and Growth Control
Chairperson: M. White, *Harvard Medical School, Boston, Massachusetts*

Hematopoietic Cell Signaling
Chairperson: D. Cantrell, *Imperial Cancer Research Fund, London, United Kingdom*

Cytoskeleton and Cell Adhesion
Chairperson: L. Van Aelst, *Cold Spring Harbor Laboratory*

Development
Chairperson: R. Klein, *European Molecular Biology Laboratory, Heidelberg, Germany*

Growth Control
Chairperson: J. Wang, *University of California, San Diego*

Signaling Abnormalities and Disease
Chairperson: B. Druker, *Oregon Health Sciences University, Portland*

Immune Cell Signaling
Chairperson: G. Koretzky, *University of Pennsylvania School of Medicine, Philadelphia*

R. Lin, L. Ballou, T. Leeuw

J. Nunes, K. Morgenstern

I. Sansal, S. Ramaswamy, P. Vazquez

Retroviruses

May 22–27 464 participants

ARRANGED BY **Monica Roth,** UMDNJ–Robert Wood Johnson Medical School
Ronald Swanstrom, University of North Carolina Center of AIDS Research

The 2001 meeting represented the 26th offering of the annual Cold Spring Harbor meeting, which began life in 1975 as a meeting on RNA Tumor Viruses and evolved into its current focus on Retroviruses in 1993. In addition to the abstracts presentation of the subscribed attendees, the 26th meeting highlighted current approaches to HIV treatment.

The two keynote speakers addressed different aspects of retroviral therapy: Harriet Robinson (Emory University) spoke on "Shots in the Light: Working toward an AIDS Vaccine" and David Ho (Aaron Diamond AIDS Research Center) spoke on "Virus and Lymphocyte Dynamics in HIV Infection." In addition, Maxine Linial presented the new nomenclature for retroviruses, which was included within the abstract book. The sessions followed the general life cycle of the retrovirus. The opening and closing sessions focused on the retroviral assembly process, where linkages with the host ubiquitination system have been identified.

Contributions from our Corporate Sponsors, Plant Corporate Associates, Corporate Affiliates, Corporate Contributors, and Foundations provided core support for this meeting.

M. Roth

F. Yoshimura, S. Chattopadhqay

PROGRAM

Assembly
Chairpersons: E. Freed, *NIAID, National Institutes of Health, Bethesda, Maryland;* L. Parent, *Pennsylvania State University College of Medicine, Hershey*

Entry
Chairpersons: P. Cannon, *University of Southern California, Los Angeles;* J. Cunningham, *Harvard Medical School, Boston, Massachusetts*

Pathogenesis/Evolution/Endogenous Viruses
Chairpersons: P. Bates, *University of Pennsylvania, Philadelphia;* J. Young, *University of Wisconsin, Madison*

RT
Chairpersons: A. Telesnitsky, *University of Michigan, Ann Arbor;* M. Georgiadis, *Rutgers University, Piscataway, New Jersey*

Preintegration/Integration
Chairpersons: A. Engelman, *Dana-Farber Cancer Institute, Boston, Massachusetts;* J. Leis, *Northwestern University School of Medicine, Chicago, Illinois*

Keynote Speaker
Harriet Robinson, *Emory University*
Shots in the Light: Working toward an AIDS Vaccine

Transcription/Tax/Tat
Chairpersons: H. Fan, *University of California, Irvine;* J. Coffin, *Tufts University School of Medicine, Boston, Massachusetts*

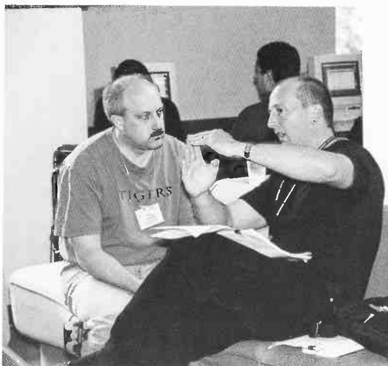
Keynote Speaker
David Ho, *Aaron Diamond AIDS Research Center, New York:*
Virus and Lymphocyte Dynamics in HIV Infection

HIV Biology/Entry
Chairpersons: D. Ho, *Aaron Diamond AIDS Research Center, New York;* B. Berkhout, *University of Amsterdam, The Netherlands*

Assorted Accessory Proteins
Chairpersons: D. Trono, *University of Geneva, Switzerland;* V. Garcia, *University of Texas Southwestern Medical Center, Dallas*

Processing: RNA/Protease
Chairpersons: W. Sundquist, *University of Utah, Salt Lake City;* K. Beemon, *Johns Hopkins University, Baltimore, Maryland*

NC/Packaging
Chairpersons: J. Luban, *Columbia University, New York;* A. Rein, *National Cancer Institute, Bethesda, Maryland*



M. Federspiel, J. Young

K.-T. Jeang, P. Jolicoeur

New York Structural Biology Group

August 8 261 participants

ARRANGED BY **Larry Shapiro,** Mount Sinai School of Medicine
Leemor Joshua-Tor, Cold Spring Harbor Laboratory

The summer meeting of the New York Structural Biology Discussion Group was the fourth in day-long meetings allowing structural biologists from all over the region to meet and discuss their latest results. The meeting was open to structural biologists from different disciplines, including crystallographers, spectroscopists, computational biologists, and biochemists, with more than 261 participants from academia and industry from the tri-state area. The program featured nine talks and a poster session and concluded with a beach barbecue allowing a wonderful opportunity for informal interactions. This meeting complements the bimonthly evening meetings of the group held at The Rockefeller University. No registration was required and participants were encouraged to set up posters.

Financial support was provided by Hampton Research Inc., Molecular Structure Corporation, Bruker AXS Inc., Gilson Inc., and Hoffmann-La Roche Inc.

SESSION I

Chairperson: Steve Almo, *Mount Sinai School of Medicine*

Larry Shapiro, *Mount Sinai School of Medicine:* Signal transduction by Tubby proteins.
David Cowburn, *The Rockefeller University:* Probing multi-domain interactions with NMR.
Rui-Ming Xu, *Cold Spring Harbor Laboratory:* Crystal structure of a SIR2 homolog-NAD complex.

SESSION II

Chairperson: Hao Wu, *Cornell Medical School*

Aneel Aggarwal, *Mount Sinai School of Medicine:* Crystal structure of DNA polymerase η.
Brian Chait, *The Rockefeller University:* Proteomic tools for dissecting cellular function.
Peter Moore, *Yale University:* Structure and function in the large ribosomal subunit.

SESSION III

Chairperson: Barry Honig, *Columbia University*

Chris Jacobsen, *SUNY Stony Brook:* X-ray imaging, with and without optics.
Helen Berman, *Rutgers University:* The Protein Data Bank and the challenges of structural genomics.
John Kuriyan, *The Rockefeller University:* Structural analysis of DNA polymerase clamp loading.

C. Lima, C. Kisker, L. Joshua-Tor, A. Aggarwal

FIRST CSHL/WELLCOME TRUST CONFERENCE
Genome Informatics

August 8–12 256 participants

ARRANGED BY **Ewan Birney,** European Bioinformatics Institute
Suzanna Lewis, University of California, Berkeley
Lincoln Stein, Cold Spring Harbor Laboratory

The first Cold Spring Harbor Laboratory/Wellcome Trust conference on Genome Informatics focused on large-scale genome annotation and utilization. The rationale behind organizing a trial joint conference in Europe derives from the increasingly competitive academic conference market, with many institutions in the United States and Europe openly planning to emulate the success of the Cold Spring Harbor meetings' model. The conference was held at the Hinxton Hall Conference Centre, located several miles south of Cambridge in the United Kingdom, which forms part of the Wellcome Trust Genome Campus, together with the Sanger Centre, the European Bioinformatics Institute, and the HGMP-MRC Resource Centre. The conference followed a format similar to that of traditional Cold Spring Harbor meetings, in that the majority of oral presentations were drawn from openly submitted abstracts.

The explosion of biological data requires a concomitant increase in the scale and sophistication of information technology, ranging from the storage of data and their associated data models, to the design of effective algorithms to uncover nonobvious aspects of these data sets, to ontologies to concisely describe biological information, and finally to software systems to support curation, visualization, and exploration. The conference brought together some of the leading scientists in this growing field, and researchers from other large-scale information-handling disciplines were also invited to attend. In all, 256 participants attended, with more than 50% of delegates coming from North America, a highly unusual statistic for a European meeting, and the meeting hosted 108 scientific presentations in talks and posters.

PROGRAM

Annotation Pipelines
Chairpersons: J. Richardson, *Jackson Laboratory, Bar Harbor, Maine;* C. Mungall, *Lawrence Berkeley National Laboratory, California;* J. Quackenbush, *The Institute for Genome Research, Rockville, Maryland*

Genome Assembly
Chairpersons: J. Kent, *University of California, Santa Cruz;* J. Mullikin, *Sanger Centre, Wellcome Trust Genome Campus, Hinxton, United Kingdom*

Gene Prediction
Chairpersons: R. Durbin, *Sanger Centre, Wellcome Trust Genome Campus, Hinxton, United Kingdom;* A. Krogh, *Technical University of Denmark, Lyngby*

Functional Genomics
Chairperson: A. Bateman, *Sanger Centre, Wellcome Trust Genome Campus, Hinxton, United Kingdom*

Curation and Ontologies
Chairperson: M. Ashburner, *EMBL-EBI, Wellcome Trust Genome Campus, Hinxton, United Kingdom*

Genome Visualization
Chairpersons: M. Clamp, *Sanger Centre, Wellcome Trust Genome Campus, Hinxton, United Kingdom;* J. Richter, *Lawrence Berkeley National Laboratory, California*

The Hinxton Hall Conference Center

Yeast Cell Biology

AUGUST 14–19 393 participants

ARRANGED BY **Brenda Andrews,** University of Toronto, Canada
Chris Kaiser, Massachusetts Institute of Technology
Mark Winey, University of Colorado, Boulder

The conference on Yeast Cell Biology was the eighth biannual international meeting devoted to major aspects of cell biology in yeast. This conference is unusual in that the study of all major areas of cell biology are represented at a single meeting organized around a simple eukaryotic organism, the budding yeast *Saccharomyces cerevisiae*.

A common interest in one organism, instead of one topic in cell biology, encourages extensive cross-fertilization of ideas, insights, and methodologies, ultimately leading to a more integrated view of eukaryotic cell structure and function. Important insights were further gained by studies in the fission yeast *Schizosaccharomyces pombe* as well as other yeasts. The meeting included a session on genome-wide expression analysis in yeast during critical cellular processes and at different stages of the life cycle. Major areas of interest included the functions of the actin and microtubule cytoskeleton, and the targeting and sorting of proteins in the secretory, endocytotic, and nuclear localization pathways. The coordination of several cellular processes was discussed in the context of the response to mating pheromone, meiosis, and the cell cycle, with the exit from mitosis being particularly important. All told, it was a very rich and exciting meeting with more than 390 scientists in attendance presenting some 277 scientific reports in 111 talks and 166 posters.

Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Affiliates, Corporate Contributors, and Foundations provided core support for this meeting.

C. Kaiser, B. Andrews. M. Winey

PROGRAM

Actin and Budding
Chairperson: J. Thorner, *University of California, Berkeley*

Cell Cycle and Checkpoints
Chairperson: P. Meluh, *Memorial Sloan-Kettering Cancer Center, New York*

Endocytosis and Vacuolar Turnover
Chairperson: M. Cyert, *Stanford University, California*

Microtubules and Chromosomes
Chairperson: O. Cohen-Fix, *NIDDK, National Institutes of Health, Bethesda, Maryland*

Functional Genomics and Proteomics
Chairperson: M. Tyers, *Samuel Lunenfeld Research Institute, Toronto, Canada*

ER and Organelle Biogenesis
Chairperson: R. Hampton, *University of California, San Diego*

Meiosis
Chairperson: R. Rothstein, *Columbia University College of Physicians & Surgeons, New York*

Signaling and Cell Regulation
Chairperson: M. Solomon, *Yale University, New Haven, Connecticut*

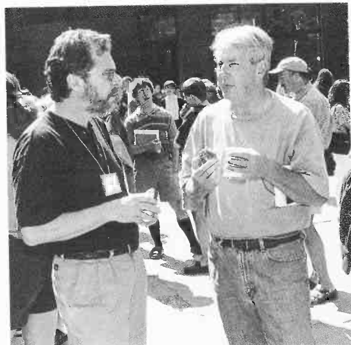
Vesicular Traffic
Chairperson: L. Weisman, *University of Iowa, Iowa City*

Septins and Metabolism
Chairperson: T. Fox, *Cornell University, Ithaca, New York*

E. Swain, H. Watson, J. Baggett

T. Fox, P. Meluh, M. Rose, D. Botstein

J. Blanchette, C. Reinke, P. Connerly, J. Soderholm, B. Bevis



M. Yaffe, G. Sprague

Eukaryotic mRNA Processing

August 22–26 299 participants

ARRANGED BY **Christine Guthrie,** University of California, San Francisco
Timothy Nilsen, Case Western Reserve University

The third Eukaryotic mRNA Processing Meeting was held this year, and it focused on the mechanism and regulation of pre-mRNA splicing, 3′-end formation, transport, editing, and turnover in both yeast and multicellular eukaryotes. Catalysis of a reaction similar to splicing by protein-free snRNAs was demonstrated. These results provide the strongest evidence to date that the spliceosome is a ribozyme. In addition, structural characterization of the spliceosome has progressed beyond individual components; the first cryo-electron micrographs of intact spliceosomes and subcomplexes were presented. There was continued interest in RNA interference from both a mechanistic standpoint and its link to the production of developmentally regulated micro-RNAs. RNA interference has also been used successfully to dissect nuclear transport pathways as well as the mechanisms that control alternative processing.

The functional links between transcription and posttranscriptional events continue to be explored, as are links between processing steps and transport. A major theme was the integration of all steps in gene expression. Nuclear surveillance of mRNAs appears to occur at several steps, but the mechanisms remain largely obscure. Several different mRNA decay pathways were described, many of which target aberrant mRNAs.

Completion of various genome projects has led to an increase in array-based methodologies to detect alternative splicing. It now appears than a substantial majority of genes generate multiple proteins via alternative processing, and the mechanism of regulation of these pathways is beginning to be understood. Other presentations at the meeting described genetic, biochemical, and cell biological approaches applied to the study of diverse aspects of mRNA metabolism, including 3′-end processing mechanisms and regulation, splicing, editing, turnover, and transport in yeast, metazoans, plants, and viruses.

This meeting was funded in part by the National Cancer Institute and the National Institute of Child Health and Human Development, branches of the National Institutes of Health, and by the National Science Foundation. The Laboratory would in addition like to thank the RNA Society for its support of this meeting. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Affiliates, Corporate Contributors, and Foundations also provided core support for this meeting.

T. Nilsen, C. Guthrie

PROGRAM

RNA Trafficking and Localization
Chairperson: I. Mattaj, *European Molecular Biology Laboratory, Heidelberg, Germany*

Splicing Mechanisms
Chairperson: M. Green, *Howard Hughes Medical Institute/ University of Massachusetts Medical School, Worcester*

3´ Ends
Chairperson: M. Wickens, *University of Wisconsin, Madison*

Spliceosome Assembly
Chairperson: M. Ares, *University of California, Santa Cruz*

RNA Turnover
Chairperson: L. Maquat, *University of Rochester School of Medicine, New York*

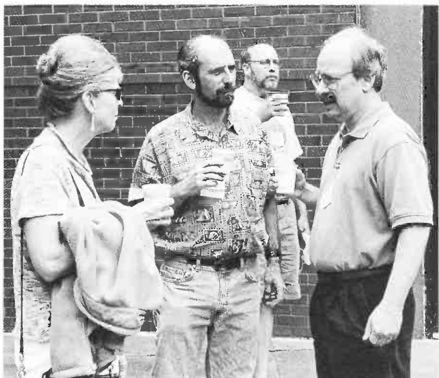
snRNP Biogenesis and Function
Chairperson: J. Steitz, *Yale University School of Medicine, New Haven, Connecticut*

Regulation
Chairperson: S. Berget, *Baylor College of Medicine, Houston, Texas*

N. Villegas, M. De LaRosa, J. Valdés

D. Black, B. Chabot, N. Hernandez

L. Penalva, J. Xie



J. Steitz, M. Green, J. Manley

Mechanisms of Eukaryotic Transcription

August 29–September 2 467 participants

ARRANGED BY **Nouria Hernandez,** Cold Spring Harbor Laboratory
Robert Kingston, Massachusetts General Hospital
Richard Treisman, Imperial Cancer Research Fund, London

A large proportion of the regulation occurring in biological systems is at the level of gene transcription. Accordingly, the transcription field is rapidly evolving and continuously expanding. Mechanisms of Eukaryotic Transcription was the seventh biennial Cold Spring Harbor meeting devoted to mechanisms of transcriptional regulation in eukaryotes, and it allowed scientists working in different areas of this field to hear, discuss, and critically evaluate the latest results, and to incorporate them into a global picture. The conference consisted of eight plenary sessions and two poster sessions. The sessions focused on the structure and function of RNA polymerase, on the role of the TATA-box-binding protein TBP, TBP-related factors and TBP-associated factors, and on advances in our understanding of initiation and elongation mechanisms. They also addressed the structure and function of acetylases, methylases, and their role in epigenetic regulation, interactions between transcription factors, chromatin remodeling mechanisms, regulatory circuits, and mechanisms of activation.

This meeting was funded in part by the National Cancer Institute and National Institute of Child Health and Human Development, branches of the National Institutes of Health. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Affiliates, Corporate Contributors, and Foundations also provided core support for this meeting.

N. Hernandez, R. Kingston, R. Treisman

PROGRAM

RNA Polymerase
Chairperson: P.A. Weil, *Vanderbilt University, Nashville, Tennessee*

TBP and Friends
Chairperson: S. Buratowski, *Harvard Medical School, Boston, Massachusetts*

Initiation and Elongation Mechanisms
Chairperson: K.M. Arndt, *University of Pittsburgh, Pennsylvania*

Acetylases, Methylases, and Epigenetics
Chairperson: C.L. Peterson, *University of Massachusetts Medical Center, Worcester*

Transcription Factor Interactions
Chairperson: W.P. Tansey, *Cold Spring Harbor Laboratory*

Chromatin Remodeling
Chairperson: J.L. Workman, *Pennsylvania State University, University Park*

Regulatory Circuits and Activation I
Chairperson: B.J. Graves, *Huntsman Cancer Institute, University of Utah, Salt Lake City*

Activation II
Chairperson: K.A. Jones, *SAIC, NCI-Frederick, Maryland*

Watching the meeting on TV.

R. Chapman, D. Gregory

M. Garey, K. Yamamoto

Eukaryotic DNA Replication

September 5–9 408 participants

ARRANGED BY **Thomas Kelly,** Johns Hopkins University School of Medicine
Bruce Stillman, Cold Spring Harbor Laboratory

This was the eighth biannual meeting on Eukaryotic DNA Replication held at Cold Spring Harbor. Studies of eukaryotic DNA replication are advancing rapidly on many fronts, and this meeting is now established as the most important in the field. More than 400 investigators participated in the 10 scientific sessions, and there were nearly 275 platform and poster presentations. Thus, interest in the mechanisms and regulation of DNA replication in eukaryotic cells remains extremely high, and the meeting is playing a key role in fostering the exchange of new ideas and experimental approaches. The meeting included eight scientific presentation sessions and two poster sessions.

Essential funding for the meeting was provided by the National Cancer Institute and the National Institute of Environmental Health Sciences, branches of National Institutes of Health, and the National Science Foundation. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Affiliates, Corporate Contributors, and Foundations also provided core support for this meeting.

B. Stillman, T. Kelly

L. Frappier, T. Melendy, K. Fien, D. Jeruzalmi

PROGRAM

Replication Fork Proteins
Chairperson: L. Frappier, *University of Toronto, Canada*

Formation and Control of Initiation Complexes
Chairperson: T. Kelly, *Johns Hopkins University, Baltimore, Maryland*

Control of DNA Replication
Chairperson: O. Aparicio, *University of Southern California, Los Angeles*

Replication and the DNA Damage Response
Chairperson: E. Fanning, *Vanderbilt University, Nashville, Tennessee*

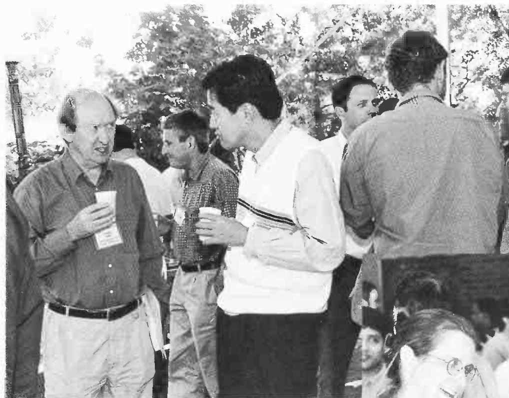
Aspects of Chromosome Replication
Chairperson: J. Li, *University of California, San Francisco*

Origin-binding Proteins
Chairperson: E. Boye, *Institute for Cancer Research, Oslo, Norway*

Initiation
Chairperson: S. Gerbi, *Brown University, Providence, Rhode Island*

Defining Origins
Chairperson: R. Bambara, *University of Rochester, New York*

J. Diffley



R. Laskey, Y. Ishimi

S. Weller, D. Wigley

SECOND CSHL WORKSHOP ON COMPUTATIONAL BIOLOGY
Integrating Genome Sequence, Sequence Variation, and Gene Expression

September 28–30 176 participants

ARRANGED BY **Michael Q. Zhang,** Cold Spring Harbor Laboratory
Aravinda Chakravarti, Johns Hopkins Hospital
Michael B. Eisen, Lawrence Berkeley National Laboratories and University of California, Berkeley

This second workshop on Computational Biology focused primarily on Integrating Genome Sequence, Sequence Variation, and Gene Expression. As the Human Genome Project picks up speed to deliver the finished human genome, functional genomics has started to bear fruit in many areas of biology and medicine. Vast DNA sequence information enables investigators to simultaneously monitor the expression of large numbers of genes in vivo or to closely examine genome variation at the nucleotide resolution. Computational biology and bioinformatics have increasingly significant roles in these large-scale investigations. To further promote interaction between computational and experimental researchers, the 2001 meeting has addressed the following questions:

- What have we already learned from studies of large-scale gene expression, sequence structure, and variation?
- At what stage (and how) should computational methods be introduced into the overall experimental design, beyond their practical application in data analysis?
- What limitations do we have to overcome in order to obtain real and reliable functional interpretations from large amounts of complex and noisy data?

M. Zhang, A. Chakravarti

The workshop concentrated primarily on biological and biomedical principles, rather than algorithmic aspects of computational biology. The topics covered a wide range of structural and functional issues in genomics/proteomics. Despite its brevity, the workshop attracted substantial international participation from laboratories in Japan, as well as from Europe and Canada (originally 230 registrants planned to attend). Due to the terrorist attack on September 11, several international and west-coast people were not able to attend. Among many distinguished session chairs and speakers, two very popular lectures were given by Ron Davis (Stanford University) and Partha Mitra (Bell Laboratories). Attendants praised this workshop for its quality and unique setting and expressed strong interest in continuing this format in the future.

Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Affiliates, Corporate Contributors, and Foundations provided core support for this meeting.

PROGRAM

State of the Art in Analysis of Sequence Variation and Gene Expression
Chairpersons: A. Chakravarti, *Johns Hopkins University School of Medicine, Baltimore, Maryland;* L. Stein, *Cold Spring Harbor Laboratory*

Modeling Global Gene Expression
Chairpersons: M. Eisen, *Lawrence Berkeley National Laboratory, California;* E. Siggia, *The Rockefeller University, New York*

Modeling Whole-genome Variation
Chairpersons: R. Davis, *Stanford DNA Sequencing & Technology Center, Palo Alto, California;* E. Eichler, *Case Western Reserve University, Cleveland, Ohio*

Integrating Genomics and Biological Function
Chairpersons: M. Zhang, *Cold Spring Harbor Laboratory;* G. Rubin, *Howard Hughes Medical Institute, University of California, Berkeley*

R. McCombie, J. McPherson

N. Pavy, N. Banerjee

M. McAlear, M. Liu

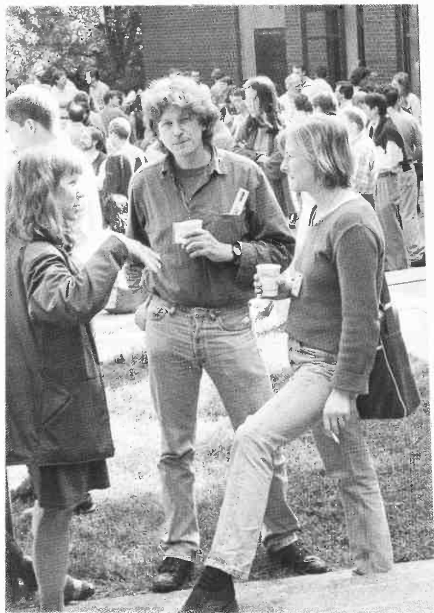
Neurobiology of *Drosophila*

October 3–7 307 participants

ARRANGED BY **Hugo Bellen,** HHMI/Baylor College of Medicine
Barbara Taylor, Oregon State University

The goal of the 2001 Neurobiology of *Drosophila* meeting, as it has been since its inception, was to foster communication of ideas, techniques, and new discoveries within the field of *Drosophila* neurobiology. The meeting was structured with platform and poster presentations by a variety of researchers, including graduate students, postdoctoral fellows, and junior and senior faculty. The topics for the platform sessions were chosen from areas where exciting advances are being made in the molecular and cellular mechanisms underlying neurophysiology, axon guidance and synaptic plasticity, sensory systems, embryonic and adult nervous system development and organization, behavior, neuronal and glial determination and differentiation. The research reported relies on a wide range of techniques, including genetic, molecular, cellular, neurophysiological, and behavioral and genomic approaches to basic questions of nervous system development and function. The highlights of the meeting included presentations of new and exciting developments, including identification of new growth factors involved in synaptic plasticity, a new noncircadian role for clock genes in sleep and rest behavior, and identification of topographic olfactory maps in higher brain centers. At the Elkins plenary lecture, which is presented by a recent Ph.D. graduate, exciting work was introduced on how receptors mediate a repulsive signal act in concert to channel growing axons into separate pathways. The environment of the

B. Taylor, H. Bellen



R. Kraut, U. Thomas, U. Kuchinke

meeting allowed many opportunities for informal discussions among all participants. The high quality of the presentations, novel findings, and new techniques discussed at the meeting demonstrated the vitality of *Drosophila* research. The character of the discussions leads to the cross-fostering of ideas that is valuable to everyone in the field.

This meeting was funded in part by the National Institute of Child Health and Human Development and the National Institute of Neurological Disorders and Stroke, branches of the National Institutes of Health, and the National Science Foundation. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Affiliates, Corporate Contributors, and Foundations also provided core support for this meeting.

PROGRAM

Sensory Systems and Eye Development
Chairperson: J. Treisman, *New York University School of Medicine, New York*

Neuronal and Glial Determination
Chairperson: J. Skeath, *Washington University School of Medicine, St. Louis, Missouri*

Genome and Techniques
Chairperson: E. Bier, *University of California, San Diego*

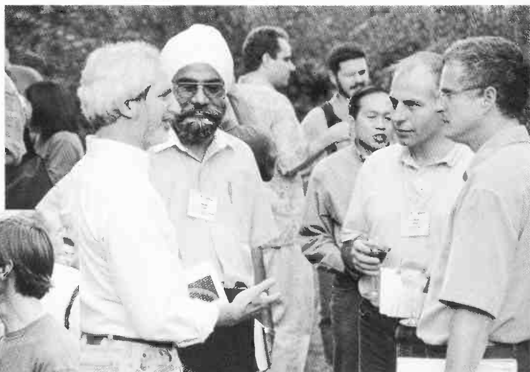
Elkins Memorial Lecture

Axon Guidance Synapse Formation and Synapse Plasticity
Chairperson: V. Budnik, *University of Massachusetts, Amherst*

Adult CNS Development and Neuronal Degeneration
Chairperson: K. Ito, *National Institute of Basic Biology, Okazaki, Japan*

Behavior
Chairperson: J. Ewer, *Cornell University, Ithaca, New York*

Neurophysiology
Chairperson: J.T. Littleton, *Massachusetts Institute of Technology, Cambridge*



B. Ganetzky, S. Singh, D. Ebert, N. Atkinson

U. Heberlein, G. Marden

Microbial Pathogenesis and Host Response

October 10–14 170 participants

ARRANGED BY **Stanley Maloy,** University of Illinois
Paula Sundstrom, Ohio State University
Ronald Taylor, Dartmouth Medical School

Throughout recorded history, microbial pathogens have been a major cause of human disease and mortality. However, with the advent of effective antibiotics, it seemed like the war on microbes had been won. Hence, for several decades, health-related research shifted to topics such as cancer, heart disease, and genetic diseases. Although research in microbial pathogenesis slowed, the microbes continued to evolve. Microbial resistance to antibiotics developed faster than new antibiotics could be made available, and the resistance spread throughout the microbial world. The global expansion of food distribution networks increased the rapid dissemination of microbial pathogens. Simultaneously, emerging microbial pathogens filled new ecological niches, such as indwelling medical devices that provide a surface for biofilms and the growing population of patients who are immunocompromised due to primary infections such as HIV or due to therapies for chronic diseases. Furthermore, recent discoveries have demonstrated that some diseases (e.g., ulcers and coronary heart disease), previously believed to be caused by a genetic predisposition or environmental conditions, are actually caused by or are strongly associated with microbes. Finally, humans have facilitated the development of microbial pathogens as agents of bioterrorism.

P. Sundstrom

This microbial offensive has summoned a renewed counterattack on microbial pathogens that has intensified during the last several years. A variety of new tools have become available that make it possible to dissect the molecular basis of pathogenesis from both the microbial and host perspectives. This has yielded exciting rapid advances in understanding the basis of pathogenesis for several important infectious diseases. Insight into the molecular mechanisms of pathogenesis has predicted new ways to control infection, including the identification of novel targets for antimicrobials and novel approaches for vaccine development. Nevertheless, many more questions remain unanswered and many pathogens are still poorly understood. Understanding microbial pathogenesis demands a detailed knowledge of the host response as well as the pathogen itself. Both of these perspectives provide potential strategies for solving important clinical problems.

M. Neely

To elucidate these distinct aspects of microbial pathogenesis requires an interdisciplinary approach, integrating the fields of microbiology, eukaryotic cell biology, and immunology. To facilitate such interactions, the third meeting on Microbial Pathogenesis and Host Response was planned to begin on September 12–16, 2001. Due to the events of September 11, the meeting was rescheduled for October 10–14. Rescheduling the meeting on such short notice had a major impact on the meeting. Many of the speakers and participants who had planned to attend in September were unable to attend the meeting in October. Nevertheless, a revised program was rapidly prepared and the rescheduled meeting was well attended. The revised program included a Keynote Address by Ron Atlas, an international expert on bioterrorism, presented to an overflow audience that included news media. The timeliness of the Keynote Address and the need for new approaches to combat microbial pathogens was sadly highlighted by the distribution of anthrax spores in the U.S. mail.

The meeting attracted a diverse group of international scientists who approach the study of bacterial and fungal pathogens from a broad range of perspectives. Sessions focused on how genomic DNA sequences and functional genomic approaches can be used to identify gene products involved in pathogenesis; how environmental reservoirs of pathogens, genetic exchange between pathogens, and

mutation allow pathogens to rapidly evolve new traits; how pathogens enter eukaryotic hosts; how pathogens manipulate the host response; how the host responds to ward off infections (or sometimes to inadvertently promote infections); and how recent insights into pathogenesis are being used to facilitate the development of novel antibiotics and vaccines. The talks and poster sessions generated lively, interactive discussions. Many presentations describing the use of a new method to solve a complex problem led to animated discourse about how the approach could be applied to answer recalcitrant questions about other host-pathogen interactions. Some of these interactions have already produced fruitful scientific collaborations. Despite the active scientific research on microbial pathogenesis and the impressive progress in this field, it is clear that as one problem is solved, another microbial pathogen will rapidly take its place. Hence, there will be a continual need for the free, interactive exchange of ideas like that stimulated by this meeting.

This meeting was partially supported by funds from the National Institute of Allergy and Infectious Diseases, the National Institute of Dental Research, branches of the National Institutes of Health, and the U.S. Army. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Affiliates, Corporate Contributors, and Foundations also provided core support for this meeting.

PROGRAM

Genomic Analysis of Pathogens
Chairperson: S. Maloy, *University of Illinois, Urbana*

Evolution of Pathogens
Chairperson: M. Neely, *Wayne State University School of Medicine, Detroit, Michigan*

Adhesion and Entry
Chairperson: J. Puente, *Universidad Nacional Autónoma de México*

Export
Chairperson: R. DeVinney, *University of Calgary Health Science Center, Canada*

Host Responses
Chairperson: B. Cookson, *University of Washington, Seattle*

Toxins
Chairperson: N. Freitag, *Seattle Biomedical Research Institute, Washington*

Antibiotics and Vaccines
Chairperson: L. Miesel, *Schering Plough Research Institute, Kenilworth, New Jersey*

Keynote Speaker
J.W. Murphy, *University of Oklahoma Health Science Center, Oklahoma City*

Reservoirs of Pathogens in the Environment
Chairperson: P. Small, *University of Tennessee, Knoxville*

S. Maloy, J. Gogren, R. Taylor

D. Lee, H. Winther-Larsen, J. Vogel

Programmed Cell Death

November 9–13 312 participants

ARRANGED BY **Hermann Steller,** HHMI/Massachusetts Institute of Technology
Craig Thompson, HHMI/University of Chicago
Eileen White, HHMI/Rutgers University

This fourth meeting on Programmed Cell Death, originally scheduled for September 19–23, was after much discussion rescheduled for mid-November. As a result, a large proportion of senior session chairs had to cancel, but the majority of presenters rearranged their schedules to attend, a testament to the vitality of this meeting. The meeting included eight oral sessions and two poster sessions. The opening Keynote Address was delivered by Gerry Adams of the Walter & Eliza Hall Institute of Medical Research, Australia. In all, 312 international participants attended this fascinating forum on the current state of apoptosis research, with much heated debate at posters, over meals, and in the bar.

This meeting was funded in part by the National Institute on Aging, the National Cancer Institute, and the National Institute of Child Health and Human Development, branches of the National Institutes of Health, and the National Science Foundation. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Affiliates, Corporate Contributors, and Foundations also provided core support for this meeting.

C. Thompson, E. White, K. Tomaselli

H. Steller, Y. Lazebnik, S. Larisch

PROGRAM

Developmental Apoptosis
Chairpersons: H.R. Horvitz, *Massachusetts Institute of Technology, Cambridge;* B. Hay, *California Institute of Technology, Pasadena*

Keynote Speaker
S. Cory, *Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia*

Animal Models
Chairpersons: J.M. Hardwick, *Johns Hopkins University, Baltimore, Maryland;* M. Lenardo, *NIAID, National Institutes of Health, Bethesda, Maryland*

Mitochondrial Involvement in Apoptosis
Chairpersons: Y. Tsujimoto, *Osaka University Graduate School of Medicine, Japan;* J. Reed, *Burnham Institute, La Jolla, California*

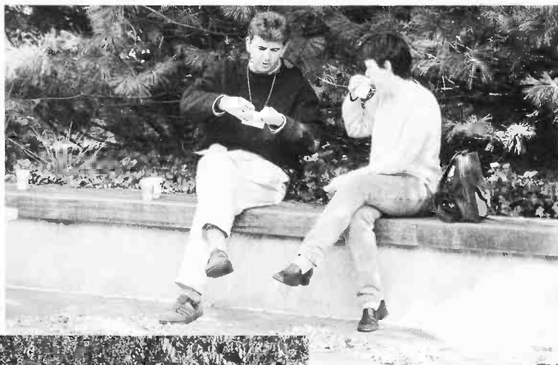
BCL-2 Regulation
Chairpersons: S. Korsmeyer, *Dana Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts;* S. Kornbluth, *Duke University Medical Center, Durham, North Carolina*

Oncogenes/Tumor Suppressor Regulation of Apoptosis
Chairpersons: S. Lowe, *Cold Spring Harbor Laboratory;* G. Evan, *University of California Comprehensive Cancer Center, San Francisco*

Signal Transduction I
Chairperson: M. Greenberg, *Children's Hospital, Harvard Medical School, Boston, Massachusetts*

Signal Transduction II
Chairperson: J. Yuan, *Harvard Medical School, Boston, Massachusetts*

Caspase Activation/Inactivation
Chairpersons: X. Wang, *University of Texas Southwestern Medical Center, Dallas;* D. Vaux, *University of Oxford, United Kingdom*



G. MacGregor, B. McConnell

M. Poukkula, S. Tran, P. Peri

BIOTECHNOLOGY CONFERENCE
Molecular Approaches to Vaccine Design

Nov. 29–Dec. 2 196 participants

ARRANGED BY **Rafi Ahmed,** Emory University School of Medicine
Dennis Burton, Scripps Institute
Emilio Emini, Merck Research Laboratories

This second winter biotechnology conference on Molecular Approaches to Vaccine Design emphasized an understanding of the basic concepts underlying immune responses to infectious agents and how this knowledge could be applied to design new and more effective vaccines. The meeting opened with a fascinating introductory session entitled Perspectives in Vaccine Development, which included talks by Gary Nabel (government), Alan Shaw (industry), Margaret Liu (private foundation), and Michael Parker (vaccines and bioterrorism). Five platform sessions focused on HIV vaccine design; malaria vaccine design; viral immunity and vaccines; bacterial immunity and vaccines; and immunological memory. Despite the events of September 11, the meeting featured 56 scientific presentations in all, and attracted more than 100 participants. The meeting proved to be a unique forum in which to discuss how modern molecular and structural biology and immunology are beginning to contribute to an understanding of how vaccines work and how new vaccines can be designed.

This meeting was funded in part by the Chiron Corporation. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Affiliates, Corporate Contributors, and Foundations also provided core support for this meeting.

PROGRAM

Perspectives on Vaccine Development
Chairperson: F. Brown, *USDA/Plum Island Animal Disease Center, Greenport, New York*

HIV Vaccine Design
Chairperson: J. Moore, *Cornell University Weill Medical College, New York*

Malaria Vaccine Design
Chairperson: L. Miller, *NIAID, National Institutes of Health, Bethesda, Maryland*

Viral Immunity and Vaccines
Chairperson: E. Emini, *Merck Research Laboratories, West Point, Pennsylvania*

Bacterial Immunity and Vaccines
Chairperson: D. Burton, *Scripps Research Institute, La Jolla, California*

Immunological Memory
Chairperson: R. Ahmed, *Emory University School of Medicine, Atlanta, Georgia*

E. Emini, R. Ahmed, D. Burton

P. Colman, L. Miller

BIOTECHNOLOGY CONFERENCE
Physiological Genomics and Rat Models

December 5–8 125 participants

ARRANGED BY **Howard Jacob,** Medical College of Wisconsin
Douglas Vollrath, Stanford University School of Medicine

This second winter biotechnology conference on Physiological Genomics and Rat Models followed from the successful inaugural meeting in December 1999. This conference series is the first to focus exclusively on the rat as a model organism for biological research, and seeks to bring together the vast body of research that continues to be generated in the area of rat physiology, pathophysiology, toxicology, neuroscience, etc., with new genetic resources and genomic tools now being developed for the rat. Topics at the meeting included Genomics: Rat Genome Project; Bioinformatics: Rat Genome Database and Rat Map; Expression Profiling; Comparative Mapping: Radiation Hybrid Mapping; Complex Trait Analysis: Statistical Analysis and High Throughput Phenotyping; Model Systems: Autoimmunity, Behavior, Diabetes, Retinal Degeneration/Gene Therapy, Cancer, Obesity, Heart Disease; Transgenics: Cardiovascular Models, Cancer Models, Rat Transgenic Model for AIDS, Nuclear Transfer, Embryonic Stem Cells; Pharmacogenomics/Risk Assessment: Drugs of Addiction, Ethanol, and Obesity. The meeting also included a special workshop on DNA microarrays and a special pre-meeting on Physiological Genomics: From Phenotype to Genotype and Back Again. Invited speakers included John Critzer, Richard Gibbs, Michael Gould, Goran Levan, Norman Lee, Barry Levin, Mike Menaker, Alan Pack, Eddy Rubin, Jacqueline Schien, and Peter Tonellato. In all, the meeting featured 91 scientific presentations and attracted 125 participants from around the world.

Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Affiliates, Corporate Contributors, and Foundations provided core support for this meeting.

D. Vollrath, H. Jacob

PROGRAM

Functional Genomics
Chairpersons: H. Jacob, *Medical College of Wisconsin, Milwaukee;* D. Vollrath, *Stanford University School of Medicine, California*

Bioinformatics
Chairpersons: F. Stahl, *Göteborg University, Germany;* M. Lundberg, *NHBLI, National Institutes of Health, Bethesda, Maryland*

Models I
Chairpersons: E. Blankenhorn, *MCP Hahnemann University, Philadelphia, Pennsylvania;* M. Menaker, *University of Virginia, Charlottesville*

Models II
Chairpersons: L. Carr, *Indiana University School of Medicine, Indianapolis;* M. Gould, *University of Wisconsin, Madison*

Genomic Resources and Tools
Chairperson: B. Rall, *National Institutes of Health, Bethesda, Maryland*

Models III
Chairpersons: J. Rapp, *Medical College of Ohio, Toledo;* S. Mockrin, *NHLBI, National Institutes of Health, Bethesda, Maryland*

Microarray Minicourse and Expression Study Examples
Chairpersons: N. Lee, *The Institute for Genomic Research, Rockville, Maryland;* P. Tonellato, *Medical College of Wisconsin, Milwaukee*

Sequencing
Chairpersons: J. Peterson, *NHGRI, National Institutes of Health, Bethesda, Maryland;* R. Gibbs, *Baylor College of Medicine, Houston, Texas*

Models IV
Chairpersons: A. Pack, *University of Pennsylvania, Philadelphia;* H. Markholst, *Hagedorn Research Institute, Gentofte, Denmark*

D. Vollrath, M. Menaker, D. Stewart

E. Remmers, P. Gulko, J. Shull

A. Provoost, D. Brown

POSTGRADUATE COURSES

The Postgraduate Courses program at Cold Spring Harbor Laboratory is aimed at meeting the special need for training in interdisciplinary subjects that are either so new or so specialized that they are not adequately treated by universities. Our aim is to provide intensive study in the most recent developments and techniques in these subjects and to prepare students to enter directly into research in a particular area. To ensure up-to-date coverage of current research work, we bring together course faculty from many laboratories around the world and supplement this staff with a series of seminar speakers.

Advanced Genome Sequence Analysis

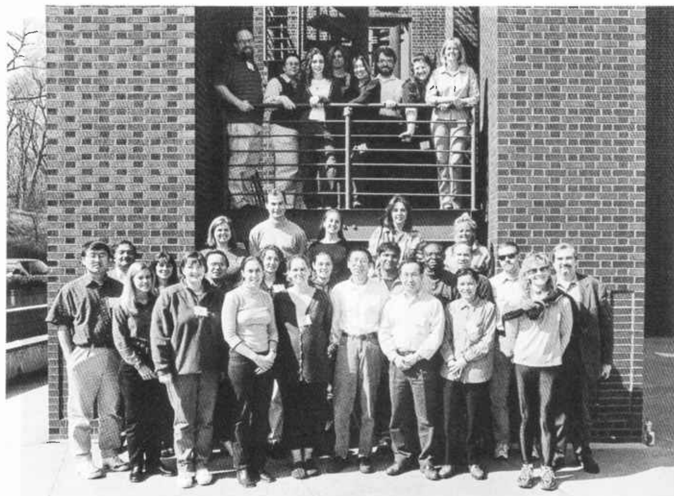
March 14–27

INSTRUCTORS

E. Mardis, Washington University School of Medicine, St. Louis, Missouri
W.R. McCombie, Cold Spring Harbor Laboratory
J. McPherson, Washington University School of Medicine, St. Louis, Missouri
T. Wood, Bryan College, Dayton, Tennessee

ASSISTANTS

A. Bahret, Cold Spring Harbor Laboratory
V. Balija, Cold Spring Harbor Laboratory
M. De la Bastide, Cold Spring Harbor Laboratory
L. Gnoj, Cold Spring Harbor Laboratory
L. King, Cold Spring Harbor Laboratory
K. Kirchoff, Cold Spring Harbor Laboratory
B. Miller, Cold Spring Harbor Laboratory
C. Nusbaum, Whitehead Institute/MIT, Cambridge, Massachusetts
A. O'Shaughnessy, Cold Spring Harbor Laboratory
L. Palmer, Cold Spring Harbor Laboratory
T. Rohlfing, Washington University School of Medicine, St. Louis, Missouri
L. Spiegel, Cold Spring Harbor Laboratory
T. Zutavern, Cold Spring Harbor Laboratory



This course focused on obtaining and analyzing genomic DNA sequence data and on large-scale sequencing, presenting students with important information on the technical and project management aspects of genomic DNA sequencing projects as well as the computational analysis of the sequence. This was achieved by carrying out a large-scale sequencing project during the class, with a special emphasis on the technical nuances of large-scale sequencing. Increases in sequencing efficiency now permit us to carry out the production phase of the student's sequencing project in less time. As a result, the course increased its emphasis on two areas: the finishing process and the analysis of DNA sequence (both computational and experimental). Last year's course included a module on sequence-based polymorphism analysis. This year, the course expanded the polymorphism analysis to include sequencing of regions from multiple individuals and additional SNP analysis. This curriculum allowed students to learn advanced techniques and principles of genomic DNA sequence analysis, from data generation to analysis of sequence variations in populations.

PARTICIPANTS

Bacanamwo, M., B.S., Ph.D., University of Missouri, Columbia
Chen, E., M.D., Ph.D., Morehouse School of Medicine, Atlanta, Georgia
Dudley, P., B.S., Ph.D., National Eye Institute, NIH, Bethesda, Maryland
Glazier, A., B.Sc., Ph.D., MRC Clinical Sciences Centre, London, United Kingdom
Grimsley, C., M.S., Ph.D., University of Chicago, Illinois
Kim, H.R., B.S., M.S., Clemson University Genomics Institute, Clemson, South Carolina
Lee, K., M.S., Ph.D., Fox Chase Cancer Center, Philadelphia, Pennsylvania
Liu, T.-T., B.S., Ph.D., National Yang Ming University, Taipei, Taiwan
Munro, M., B.Sc., Ph.D., University of Leicester, United Kingdom
Parimoo, S., B.S., M.S., Ph.D., Johnson & Johnson, Skillman, New Jersey
Riba, L., B.S., M.S., Biomedical Institute National University of Mexico, Mexico City
Song, Q., Ph.D., Morehouse School of Medicine, Atlanta, Georgia
Stankiewicz, R., B.S., Ph.D., United States Department of Agriculture, Stoneville, Mississippi
Walsh, E., B.S., Ph.D., University of Texas, El Paso, Texas
Willour, V., B.S., Ph.D., Johns Hopkins University, Baltimore, Maryland
Xu, P., B.S., Ph.D., Virginia Commonwealth University, Richmond, Virginia

SEMINARS

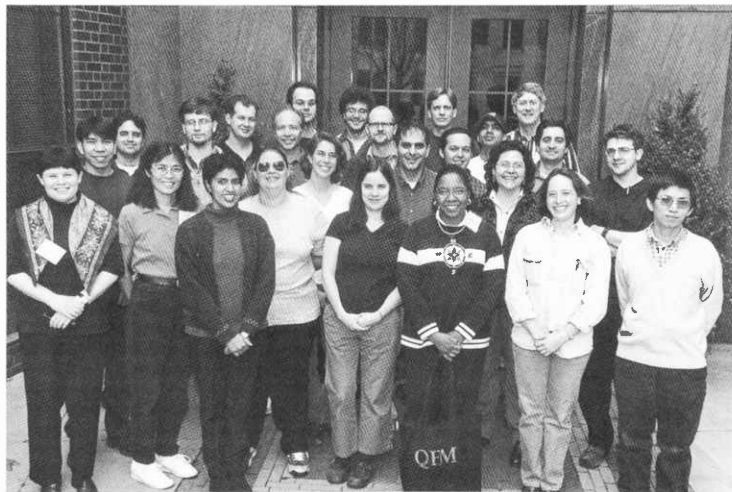
Altschuler, D., Whitehead/MGH/Harvard, Cambridge, Massachusetts: SNPs: Associative.
Birney, E., Europe Bioinformatics Institute, Hinxton, United Kingdom: Gene prediction.
Eichler, E., Case Western Reserve University, Cleveland, Ohio: Segmental duplication of the human genome.
Lai, E., Glaxo-Wellcome, Reseach Triangle Park, North Carolina: SNPs: Pharmacogenetics.
Martienssen, R., Cold Spring Harbor Laboratory: Functional genomics in *Arabidopsis thaliana*.
McKernan, K., Whitehead Institute/MIT Center for Genome Research, Cambridge, Massachusetts: High-throughout automation for genome sequencing.
Metzker, M., Baylor College of Medicine, Houston, Texas: Sequencing technologies.
Stein, L., Cold Spring Harbor Laboratory: WormBase.
Watson, J., Cold Spring Harbor Laboratory: Overview and history.
Wilson, R., Washington University, St. Louis, Missouri: Human Genome Project.

Protein Purification and Characterization

April 18–May 1

INSTRUCTORS	**R. Burgess,** University of Wisconsin, Madison **A. Courey,** University of California, Los Angeles **S.-H. Lin,** University of Texas, Houston **K. Severinov,** Waksman Institute, Rutgers University, Piscataway, New Jersey
ASSISTANTS	**L. Anthony,** University of Wisconsin, Madison **V. Bhaskar,** University of California, Los Angeles **K. Earley,** M.D. Anderson Cancer Center/University of Texas, Houston **F. Gharahdaghi,** Boehringer Ingelheim, Ridgefield, Connecticut **S. Jia,** University of California, Los Angeles **A. Manogaran,** Marquette University, Milwaukee, Wisconsin **D. Markov,** Waksman Institute of Microbiology, Piscataway, New Jersey **S. Nechaev,** Rutgers State University, Waksman Institute, Piscataway, New Jersey **N. Thompson,** University of Wisconsin, Madison

This course was intended for scientists who are not familiar with techniques of protein isolation and characterization. It was a rigorous program that included laboratory work all day and a lecture with a discussion session every evening. Each student became familiar with each of the major techniques in protein purification by actually performing four separate isolations including (1) a regulatory protein from muscle tissue; (1) a sequence-specific, DNA-binding protein; (3) a recombinant protein overexpressed



in *E. coli*; and (4) a membrane-bound receptor. A variety of bulk fractionation, electrophoretic, and chromatographic techniques were employed including precipitation by salts, pH, and ionic polymers; ion exchange, gel filtration, hydrophobic interaction, and reversed-phase chromatography; lectin affinity, ligand affinity, oligonucleotide affinity, and immunoaffinity chromatography; polyacrylamide gel electrophoresis, and electroblotting; and high-performance liquid chromatography. Procedures were presented for solubilizing proteins from inclusion bodies and refolding them into active monomeric forms. Methods of protein characterization were utilized, including immunological and biochemical assays, protein-protein interaction studies by far-western analysis, peptide mapping, amino acid analysis, protein sequencing, and mass spectrometry. Emphasis was placed on strategies of protein purification and characterization. Guest lecturers discussed protein structure, modification of proteins, methodologies for protein purification and characterization, and applications of protein biochemistry to cell and molecular biology.

PARTICIPANTS

Barker, M., B.S., Ph.D., University of Wisconsin, Madison
Bertwistle, D., B.Sc., Ph.D., St. Jude Children's Research Hospital, Memphis, Tennessee
Brodhagen, M., B.S., M.S., Oregon State University, Corvallis
Camacho Carvajal, M., B.S., Ph.D., Max-Planck Institute for Immunology, Freiburg, Germany
Cuajungco, M., B.S., Ph.D., Harvard Medical School/MGH, Boston, Massachusetts
DiTullio, G., B.S., Ph.D., University of South Carolina, Charleston
Godoy, V., Ph.D., Massachusetts Institute of Technology, Cambridge
Karpf, A., B.S., Ph.D., University of Utah, Salt Lake City
Naested, H., B.S., M.S., Copenhagen University, Denmark
Preiss, J., B.A., Ph.D., Fred Hutchinson Cancer Research Center, Seattle, Washington
Saito, M., B.A., Ph.D., Princeton University, New Jersey
Stefani, G., M.D., The Rockefeller University, New York
Tachedjian, G., B.S., Ph.D., Columbia University, New York
Verlade, J., B.S., Ph.D., University of Maryland, Baltimore
Wang, X., B.S., Ph.D., National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina
Wilson, B., B.S., Ph.D., Jackson State University, Mississippi

SEMINARS

Burgess, R., University of Wisconsin, Madison: Overview of protein purification, immunoaffinity purification.
Burgess, R., University of Wisconsin, Madison: Biochemical studies of RNA polymerase/σ factor interactions.
Courey, A., University of California, Los Angeles: Mechanisms of activation and repression by the dorsal morphogen.
Fenyo, D., President, ProteoMetrics, New York: Mass spectrometry and the proteome/automatic analysis of proteomic information.
Joshua-Tor, L., Cold Spring Harbor Laboratory: Proteins in 3-D.
Lesley, S., Genomics Institute of the Novartis Foundation, La Jolla, California: High through-put protein expression, purification, and crystallization.
Lin, S.-H., M.D. Anderson Cancer Center, Houston, Texas: Regulation of angiogenesis by C-CAM tumor suppressor/isolation of bone-response factors in advanced prostrate cancer.
Segal, D., Scripps Research Institute, La Jolla, California: Novel zinc finger proteins for the therapeutic regulation and modification of genomes.
Severinov, K., Waksman Institute, Rutgers, Piscataway, New Jersey: Viral proteins that inhibit host RNA polymerase function.
Stillman, B., Director, Cold Spring Harbor Laboratory: Biochemical approach for understanding chromosome inheritance.

Cell and Developmental Biology of *Xenopus*

April 21–May 1

INSTRUCTORS	**K. Cho,** University of California, Irvine **J. Christian,** Oregon Health Sciences, Portland
ASSISTANTS	**I. Blitz,** University of California, Irvine **N. Hirsh,** University of Virginia, Charlottesville **M. Yuge,** Fukuoka Women's University, Fukuoka, Japan

The frog *Xenopus* is an important vertebrate model for studies of maternal factors, regulation and molecular mechanisms of tissue inductions, and regulation of cell fate decisions. In addition, *Xenopus* oocytes and embryos provide a powerful system in which to conduct a number of cell biological and gene regulation assays. This course provided extensive laboratory exposure to the biology, manipulation, and use of oocytes and embryos of *Xenopus*. The course consisted of intensive laboratory sessions, supplemented by daily lectures and demonstrations from experts in cellular, experimental, and molecular development. Areas covered included (1) care of adults; (2) oocyte isolation and embryo production; (3) stages of embryonic development and anatomy; (4) whole-mount in situ hybridization and immunocytochemistry; (5) microinjection of eggs and oocytes with lineage tracers, DNA constructs, mRNA, and antisense oligonucleotides; (6) micromanipulation of embryos, including explant and transplantation assays; (7) preparation of transgenic embryos; and (8) use of *Xenopus tropicalis* for genetic

analyses. This course was suited both for investigators who had no experience with *Xenopus* and for those who work with *Xenopus* and wish to learn new and cutting edge techniques. All applicants had current training in molecular biology and some knowledge of developmental biology. The 2001 course guest lecturers were Robert Grainger (University of Virginia, Charlottesville), Janet Heasman (Children's Hospital Medical Center, Cincinnati, Ohio), Raymond Keller (University of Virginia, Charlottesville), Kristen Kroll (Washington University School of Medicine, St. Louis, Missouri), Sally Moody (George Washington University Medical Center, Washington, D.C.), Randall Moon (University of Washington, Seattle), Jonathan Slack (University of Bath, Bath, United Kingdom).

PARTICIPANTS

Brugmann, S., B.S., Ph.D., The George Washington University, Washington, D.C.

Coleman, T., B.A., Ph.D., Fox Chase Cancer Center, Philadelphia, Pennsylvania

Costa, R., Ph.D., Wellcome/CRC Institute, Cambridge, United Kingdom

Deignan, K., B.Sc., Ph.D., University College Dublin, Ireland

Deroo, T., B.S., Ph.D., University of Ghent, Belgium

Kaelin, R., B.S., Swiss Federal Institute of Technology, Zurich, Switzerland

Larrain, J., B.S., Ph.D., University of California, Los Angeles

Lee, M., B.S., Hong Kong University of Science and Technology, Clearwater Bay, Hong Kong

Lindsay, H., B.Sc., University of Sussex, Brighton, United Kingdom

Novo, D., B.S., Ph.D., University of California, Los Angeles

Psychoyos, D., B.Sc., Ph.D., The Salk Institute, La Jolla, California

Ruzov, A., Ph.D., Institute of Gene Biology, Moscow, Russia

Schweickert, A., B.S., Ph.D., Institute of Toxicology and Genetics, Karlsruhe, Germany

Simon, K., M.S., Universitatsklinikum Essen, Germany

Tseng, H.-T., B.S., Ph.D., Baylor College of Medicine, Houston, Texas

Wong, L., B.S., Ph.D., University of California, San Diego

Wu, C., B.S., Washington University, St. Louis, Missouri

SEMINARS

Cho, K., University of California, Irvine: DNA microarray analyses in *Xenopus*.

Christian, J., Oregon Health Sciences University, Portland: Cross-talk between CaM KIV and BMP pathways regulates survival and lineage commitment of hematopoietic progenitors.

Grainger, R., University of Virginia, Charlottesville: *Xenopus tropicalis:* A new model for vertebrate developmental genetics.

Heasman, J., Children's Hospital Medical Center, Cincinnati, Ohio: Maternal control of embryonic patterning: loss of function approaches.

Keller, R., University of Virginia, Charlottesville: Early *Xenopus* morphogenic movements.

Kroll, K., Washington University School of Medicine, St. Louis, Missouri: Formation of the vertebrate neural plate.

Moody, S., George Washington University Medical Center, Washington, D.C.: Building the retina from scratch.

Moon, R., University of Washington, Seattle: How the dorsoventral axis is specified by two distinct Wnt signaling pathways.

Slack, J., University of Bath, United Kingdom: Studies on tail development and limb regeneration.

Developmental Neurobiology

June 6–19

INSTRUCTORS **B. Barres,** Stanford Medical School, Stanford, California
S. Burden, New York University Medical Center, New York
H. Cline, Cold Spring Harbor Laboratory

The aim of this lecture course was to discuss principles and recent advances in developmental neurobiology. Major topics considered included determination, proliferation, and differentiation of neural cells; trophic interactions in neural development; gradients and compartments; guidance of axons to targets; and the formation of synapses. These topics were considered within the context of the development of both invertebrate and vertebrate neural systems. Prospective students had a background in neurobiology or molecular biology.

PARTICIPANTS

Cayouette, M., B.S., Ph.D., University College London, United Kingdom
Chrast, R., M.S., Ph.D., The Salk Institute, San Diego, California
DeSilva, T., B.S., Ph.D., Harvard Medical School, Boston, Massachusetts
Dino, M., B.S., Ph.D., Yale University, New Haven, Connecticut
Dugas, J., B.S., Ph.D., Stanford University, California
Freitas, A., B.S., Ph.D., Ludwig Institute for Cancer Research, Sao Paulo, Mexico
Hammond, R., B.A., Ph.D., King's College London,United Kingdom
Hermanson, E., B.S., M.S., Ludwig Institute for Cancer Research, Stockholm, Sweden
Hughes, C., B.A., Ph.D., Indiana University, Bloomington

Ireland, J., B.Sc., Ph.D., University of Cambridge, United Kingdom
Jensen, J., B.A., Yale University, New Haven, Connecticut
Kaneshiro, K., B.S., University of Tokyo, Japan
Liu, L., B.S., Ph.D., Genomics Institute of the Novartis Research Foundation, San Diego, California
McEwen, D., B.A., University of Michigan, Ann Arbor
Page, D., B.S., MRC Laboratory of Molecular Biology, Cambridge, United Kingdom
Parmar, M., B.Sc., Lund University, Sweden
Shao, Y., B.S., Ph.D., Harvard Medical School, Boston, Massachusetts
VanSaun, M., B.S., Ph.D., University of Kansas Medical Center, Kansas City
Vasconcelos, M.L., B.S., Ph.D., HHMI/University of California, Los Angeles
Wilson, P., B.S., Ph.D., The Rockefeller University, New York

SEMINARS

Bargmann, C., Howard Hughes Medical Institute/University of California, San Francisco: Neuronal diversification and neuronal function in *C. elegans*.
Berg, D., University of California, San Diego, La Jolla: Synapse formation on central and peripheral neurons. Calcium transients and gene regulation by nictinic receptors on somatic spines.
Burden, S., New York University Medical Center, New York: Neuromuscular synapse formation.
Dickson, B., IMP, Vienna, Austria: Wiring up the fly: Molecular mechanisms of axon guidance in *Drosophila*.
Huang, J., Cold Spring Harbor Laboratory: GABAergic inhibitory circuits and experience-dependent plasticity.
Jan, Y.N., HHMI/University of California, San Francisco: Neuronal cell fate specification.
Jessell, T., Columbia University, New York: Spinal cord development.
Kinter, C., The Salk Institute, La Jolla, California: Early steps in vertebrate neural development.
Krumlauf, R., Stowers Institute for Medical Research, Kansas City, Missouri: Patterning the hindbrain.
O'Leary, D., The Salk Institute, La Jolla, California
Scheiffele, P., University of California, Berkeley: Target recognition and synapse formation in the cerebellar system.
Shatz, C., Harvard Medical School, Boston, Massachusetts: Role of neural activity in brain wiring during visual system development.
Smith, S., Stanford University, California: Synaptogenic contact events.
Stern, C., University College London, United Kingdom: Molecular dissection of neural induction in the chick embryo.

Advanced Bacterial Genetics

June 6–26

INSTRUCTORS	**K. Hughes,** University of Washington, Seattle **H. Kaplan,** University of Texas Medical School, Houston **K. Pogliano,** University of California, San Diego, La Jolla
ASSISTANTS	**H. Bonifield,** University of Washington, Seattle **A. Perez,** University of California, San Diego, La Jolla **J. Rivera,** University of Texas Medical School, Houston

This laboratory course presented logic and methods used in the genetic dissection of complex biological processes in bacteria. The methods presented include mutagenesis using transposons, mutator strains, and chemical and physical mutagens; mapping mutations using genetic and physical techniques; generation and analysis of gene fusions; molecular cloning; polymerase chain reaction; Southern blot analysis; epitope insertion mutagenesis; and site-directed mutagenesis. A key component of the course was the use of sophisticated genetic methods in the analaysis of pathogenic and "undomesticated" bacteria. Invited lecturers described the use of genetic aproaches to study biological processes in a variety of bacteria.

PARTICIPANTS

Berg, A.A., M.S., Stockholm University, Sweden
Ammendola, S., B.S., M.A., University of Rome, Italy
Carlsson, F., B.S., Ph.D., Lund University, Sweden
Deng, S., B.A., Ph.D., University of Alabama, Birmingham
El Amin, N., M.D., Karolinska Institute, Huddinge, Sweden
Fleckenstein, J., B.S., M.D., University of Tennessee, Memphis
Illingworth, R., B.Sc., Ph.D., AstraZeneca R&D Boston, Waltham, Massachusetts
Karlsson, A., B.S., M.S., Karolinska Institutet, Stockholm, Sweden
Karlsson, S., M.Sc., Karolinska Institutet, Stockholm, Sweden
Komarova, A., B.S., Ph.D., Russian Academy of Science, Moscow, Russia
Magnusson, L., M.S., Goteborg University, Sweden
Mathew, S., B.S., Ph.D., Novartis Pharmaceutical Corporation, Summit, New Jersey
Mattison, K., B.S., Oregon Health Sciences University, Portland
Mylonakis, E., Ph.D., Massachusetts General Hospital, Boston
Pena, J.R., B.S., M.S., Massachusetts General Hospital, Boston
Stefan, A., B.S., Ph.D., University of Bologna, Italy

SEMINARS

Beckwith, J., Harvard University, Cambridge, Massachusetts: Casacades of thiol/disulfide exchange reactions in *E. coli* redox pathways.
Botstein, D., Stanford University School of Medicine, California: Microarray analysis of the yeast genome.
Hatzell, P., University of Idaho, Moscow: Control of differentiation and cell cycle progression in *Caulobacter crescentus*.
Hofmeister, A., University of California, Berkeley: Chromosomal position-dependent temporal control of a sporulation transcription factor.
Jacobs, C., Yale University, New Haven, Connecticut: Control of differentiation and cell cycle progression in *Caulobacter crescentus*.
Kaiser, D., Stanford University School of Medicine, California: The social intelligence of *Myxobacteria*.
Losick, R., Harvard University, Cambridge, Massachusetts: Asymmetric division and cell fate.
Maloy, S., University of Illinois, Urbana: Genetic barriers that limit exchange between closely related bacteria.
Roth, J., University of Utah, Salt Lake City: Adaptive mutagenesis: Growth under selection explains the miracles of adapative mutation and temporary hypermutability.
Silhavy, T., Princeton University, New Jersey: Coping with envelope stress.
Willey, J., Hofstra University, Hempstead, New York: Morphological differentiation in the filamentous bacterium *Streptomyces coelicolor*.

Molecular Embryology of the Mouse

June 6–26

INSTRUCTORS	**S.-L. Ang,** Institut de Genetique et de Biologie Moleculaire et Cellulaire, Strasbourg, France **A. Gossler,** Institut fur Molekularbiologie, Hannover, Germany
CO-INSTRUCTORS	**T. Lufkin,** Mount Sinai School of Medicine, New York **C. Stewart,** National Cancer Institute/FCRDC, Frederick, Maryland
ASSISTANTS	**G. Mellitzer,** Institut de Genetique et de Biologie Moleculaire et Cellulaire, Strasbourg, France **K. Schuster-Gossler,** Institut fur Molekularbiologie, Hannover, Germany

This intensive laboratory and lecture course was designed for biologists interested in applying their expertise to the study of mouse embryonic development. Laboratory components provided an introduction into the technical aspects of working with and analyzing mouse embryos, and lectures provided the conceptual basis for contemporary research in mouse development. Experimental techniques that were covered in the practicals included in vitro culture and manipulation of pre- and postimplantation embryos, transgenesis by DNA microinjection, embryo transfer, establishment, culture, and genetic manipulation of embryonic stem cells, production of chimeras by aggregation with and injec-

tion of embryonic stem cells, and the analysis of development by whole-mount in situ hybridization, skeletal preparation, and transgene expression. Last year's speakers were Arturo Alvarez-Buylia, Ang Siew-Lan, Marisa Bartholomei, Richard Behringer, Charles Emerson, Richard Gardner, Achim Gossler, Tom Gridley, Francois Guillemot, Brigid Hogan, Tom Jessell, Alex Joyner, Robin Lovell-Badge, Tom Lufkin, Gail Martin, Anne McLaren, Andras Nagy, Janet Rossant, John Schimenti, Michael Shen, Austin Smith, Davor Solter, Colin Stewart, and Patrick Tam.

PARTICIPANTS

Basson, M., Ph.D., Mount Sinai School of Medicine, New York
Billon, N., Ph.D., MRC/MCB, London, United Kingdom
Branda, C., B.A., Ph.D., Harvard Medical School, Boston, Massachusetts
Dawson, K., B.Sc., Ottawa Hospital Research Institute, Canada
Dodge, J., B.A., M.S., University of Vermont, Burlington
Gidekel, S., M.S., Ph.D., The Hebrew University–Hadassah Medical School, Jerusalem, Israel
Harboe, T., B.Sc., M.Sc., University of Copenhagen, Denmark
Huh, G., B.Sc., Ph.D., Harvard Medical School, Boston, Massachusetts
Jacquemin, P., B.Sc., Ph.D., Universite Catholique de Louvain, Brussels, Belgium
Kim, S., B.S., M.S., University of Minnesota, Minneapolis
Meletis, K., M.Sc., Karolinska Institutet, Stockholm, Sweden
Reddy, S., B.S., Ph.D., University of Pennsylvania, Philadelphia
Rodrigo-Blomqvist, S., M.Sc., Ph.D., Goteborg University, Sweden
Zhang, M., B.S., Ph.D., Baylor College of Medicine, Houston, Texas

SEMINARS

Alvarez-Buylia, A., University of California, San Francisco: Unified hypothesis of neural stem cell origin.
Ang, S.-L., IGBMC, Strasbourg, France: Anterior-posterior patterning of the mouse embryo.
Bartholomei, M., University of Pennsylvania School of Medicine, Philadelphia: Regulation of genomic imprinting.
Behringer, R., University of Texas/M.D. Anderson Cancer Center, Houston: Transgenesis.
Emerson, C., University of Pennsylvania, Philadelphia: Signaling and transcriptional mechanisms controlling skeletal myogenesis.
Gardner, R., University of Oxford, United Kingdom: Analysis of cell lineage and pattern formation in early mouse development.
Gossler, A., Institut für Molekularbiologie, Hannover, Germany: Somitogenesis.
Gridley, T., The Jackson Laboratory, Bar Harbor, Maine: Notch signaling in development and disease.
Guillemot, F., IGBMC, France: Proneural genes and the regulation of neurogenesis.
Hogan, B., Vanderbilt University Medical Center/HHMI, Nashville, Tennessee: BMP signaling, Forkhead genes, and morphogenesis.
Jessell, T., Columbia University, New York: Regionalization and neuronal specification in the spinal cord.
Joyner, A., New York Medical Center/Skirball Institute of Biology, New York: Organizing regions play critical roles in patterning of the neural tube.
Lovell-Badge, R., MRC National Institute for Medical Research, London, United Kingdom: Sox genes and cell fate decision. Sex determination.
Lufkin, T., Mount Sinai School of Medicine, New York: Homeobox genes and embryonic patterning.
Martin, G., University of California, San Francisco: Genetic approaches to studying the function of FGF signaling in embryonic development.
McLaren, A., Wellcome/CRC Institute, Cambridge, United Kingdom: Germ cells.
Nagy, A., Mount Sinai Hospital, Toronto, Canada: Chimeras and mosaics for dissecting complex phenotypes of induced mutations.
Rossant, J., Samuel Lunenfeld Research Institute, Toronto, Canada: Development of extraembryonic lineages.
Schimenti, J., The Jackson Laboratory, Bar Harbor, Maine: Mouse genetics (Part 1). Mouse genetics (Part II).
Shen, M., UMNDJ–Robert Wood Johns Medical School, Piscataway, New Jersey: Molecular pathway for left-right axis specification.
Smith, A., University of Edinburgh, United Kingdom: Embryonic stem cells: Pluripotency and differentiation.
Solter, D., Max-Planck Institut für Immunobiologie, Freiburg, Germany: Control of gene expression during oocyte to embryo transition: Reprogramming and cloning.
Stewart, C., NCI-FCRDC, Frederick, Maryland: Signaling during embryo implantation and early postimplantation development. Genome engineering using site-specific and homologous recombination.
Tam, P., Childen's Medical Research Institute, Australia: Establishment of tissue patterns during embryogenesis.

Phyiological Approaches to Ion Channel Biology

June 6–26

INSTRUCTORS	**R. Heidelberger,** University of Texas Houston Medical School, Houston **T. Otis,** University of California, Los Angeles **N. Spruston,** Northwestern University, Evanston, Illinois **L. Trussell,** Oregon Health Sciences University, Portland
ASSISTANTS	**J. Dzubay,** Oregon Health Sciences University, Portland **A. Klug,** Oregon Health Sciences University, Portland

This intensive laboratory/lecture course introduced students to electrophysiological approaches for the study of ion channels in their native environments. Hands-on exercises included patch-clamp recording of ion channel activity in acutely isolated or cultured cells or neurons in brain slice preparations. Different recording configurations (e.g., extracellular, whole-cell, cell-free patches, and single channel) were used. The advantages and disadvantages of each method and preparation were considered in order to match experimental approaches to scientific questions. Similarly, various methods for ligand/drug application were demonstrated. A primary goal was to identify properties of ion channels that allow neurons to carry out their unique physiological or systems-level roles. These properties were related to information previously gained from molecular cloning and heterologous expression of ion chan-

nel genes. Areas of particular interest included channels that (1) were activated by the neurotransmitter at central and peripheral synapses, (2) interacted with other channels to shape the response properties of neurons, (3) respond to neuromodulators with changes in functional properties, or (4) were developmentally required and regulated. The research interests of guest lecturers complemented and expanded this list. This course was intended for students with specific plans in applying these techniques to a defined problem. Guest speakers included Chinfei Chen, Steve DeVries, Paul Fuchs, Michael Hausser, Ruth Heidelberger, Dick Horn, John Huguenard, Lori Isom, Wu Ling-Gang, Roberto Malinow, Tom Otis, Indira Raman, Angie Ribera, Nelson Spruston, and Larry Trussell.

PARTICIPANTS

Al-Hallaq, R., B.A., Ph.D., Georgetown University Medical Center, Washington, D.C.
Asamoah, O., B.A., University of California School of Medicine, Los Angeles
Burger, R.M., B.A., Ph.D., University of Washington, Seattle
Calakos, N., B.S., Ph.D., Stanford University, Palo Alto, California
Davis, T., B.S., University of Michigan, Ann Arbor
Dean, C., B.S., University of California, Berkeley
Fall, C., B.S., Ph.D., New York University, New York
Gael, H., B.S., M.S., Swiss Federal Institute of Technology, Zurich, Switzerland
Ji, Q., M.D., Wyeth-Ayerst Research, Monmouth Jct., New Jersey
Kelliher, K., B.S., Ph.D., University of Maryland, Baltimore
Muschol, M., B.A., Ph.D., University of Pennsylvania, Philadelphia
Palfreyman, M., B.S., B.A., University of Utah, Salt Lake City

SEMINARS

Chen, C., Harvard Medical School, Boston, Massachusetts: Synaptic transmission at retinogeniculate synapses.
DeVries, S., University of Texas, Houston Health Science Center: Synaptic transmission in the retina.
Fuchs, P., Johns Hopkins University Medical School, Baltimore, Maryland: Auditory hair cells.
Hausser, M., University College London, United Kingdom: Dendritic coincidence detection.
Heidelberger, R., University of Texas, Houston Medical School: Synaptic transmission in the retina.
Horn, D., Jefferson Medical College, Philadelphia, Pennsylvania: Ion channel gating.
Huguenard, J., Stanford University Medical School, California: Differential ion channel expression: Cellular and circuit consequences.
Isom, L., University of Michigan School of Medicine, Ann Arbor: Accessory subunits and Na^+ channel function.
Ling-Gang, W., Washington University, St. Louis, Missouri: Presynaptic terminal physiology.
Malinow, R., Cold Spring Harbor Laboratory: LTP.
Otis, T., University of California, Los Angeles: Glutamate transport and excitatory synaptic signaling.
Raman, I., Northwestern University, Evanston, Illinois: Ionic mechanisms of spontaneous activity of central neurons.
Ribera, A., University of Colorado, Denver: Ion channel function in zebrafish behavioral mutants.
Spruston, N., Northwestern University, Evanston, Illinois: Dendritic excitability in CA1 pyramidal neurons. Action potential bursting in hippocampal pyramidal neurons.
Trussell, L., Oregon Health Sciences University, Portland: Presynaptic modulation by ionotropic receptors.

DNA Microarrays

June 19–26

INSTRUCTORS	**J. DeRisi,** University of California, San Francisco **V. Iyer,** University of Texas, Austin
ASSISTANTS	**T. Basarsky,** Axon Instruments, Union City, California **A. Carroll,** University of California, San Francisco **M. Diehn,** Stanford University School of Medicine, California **J. Gerton,** University of California, San Francisco **M. Llinas,** University of California, San Francisco **B. Pulliam,** University of California, San Francisco **C. Seidel,** University of California, Berkeley **D. Wang,** University of California, San Francisco

A DNA microarray is a simple, inexpensive and versatile tool for experimental explorations of genome structure, gene expression programs, gene function, and cell and organismal biology. In this hands-on course, students were guided through the process of building a robot for printing DNA microarrays, preparing DNA samples and slides to be used for printing microarrays, printing DNA microarrays, designing and conducting experiments for analysis by DNA microarray hybridization, data analysis, display, and interpretation. Experimental applications covered in the course included systematic studies

of global gene expression programs, inferring gene function using microarrays, genotyping, and measuring changes in gene copy number. Students who completed this course were able to set up their own independent facility for printing and experimental use of DNA microarrays. Guest lecturers presented the state of the art in new technology, experimental applications, and interpretation of large genomic data sets.

PARTICIPANTS

Bonds, W., B.S., Ph.D., Yale University School of Medicine, New Haven, Connecticut
Burchett, S., B.S., Ph.D., Yale University School of Medicine, New Haven, Connecticut
Chowdhary, B., Ph.D., Texas A&M University, College Station
Gopas, J., B.Sc., Ph.D., Ben Gurion University, Beer-Sheva, Israel
Lear, T., B.A., Ph.D., University of Kentucky, Lexington
Legare, D., B.S., Ph.D., Centre de Recherche en Infectiologie, Quebec, Canada
Lopes, U., B.S., Ph.D., Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil
Manduchi, E., B.S., Ph.D., Penn Center for Bioinformatics, Philadelphia, Pennsylvania
Melake-Berhan, A., B.S., Ph.D., International Institute of Tropical Agriculture, Croydon, United Kingdom
Melville, K., B.S., National Research Council of Canada, Nova Scotia
Ott, I., B.S., M.D., Deutsches Herzzentrum Munchen, Germany
Rabinowicz, P., B.S., M.S., Ph.D., Cold Spring Harbor Laboratory
Robinson, K., B.S., Ph.D., North Carolina State University, Raleigh
Rogers, A., B.Sc., Ph.D., University of Southampton, United Kingdom
Sims, T., B.S., Ph.D., Northern Illinois University, DeKalb
Smith, E., B.S., M.S., Virginia Tech., Blacksburg
Teng Teng, L., M.S., Ph.D., University of Alabama, Birmingham
Wagner, F., Ph.D., Free University Berlin, Germany
Wilson, R., Ph.D., Washington University School of Medicine, St. Louis, Missouri
Zack, D., Ph.D., Johns Hopkins University School of Medicine, Baltimore, Maryland

SEMINARS

Basarsky, T., Axon Instruments, Union City, California: Practical and technical considerations when scanning microarrays.
Berka, R., Novozymes Biotech, Inc., Davis, California: Microarray analysis of the *Bacillus subtilis* competence regulation.
Botstein, D., Stanford University School of Medicine, California: Genome-wide gene expression in cancer.
Brown, P., Stanford University, California: Using microarrays to explore human physiology and disease.
DeRisi, J., University of California, San Francisco: Progress update.
Diaz, E., University of California, Berkeley: Spatial and temporal maps of gene expression in the developing nervous system.
Fero, M., Stanford University, California: Low-cost cDNA microarrays: The core facility approach.
Li, H., University of California, San Francisco: Building a dictionary for DNA: Decoding the regulatory regions of a genome.
O'Shea, E., University of California, San Francisco: Signaling phosphate starvation.
Pulliam, B., University of California, San Francisco: NOMAD—A microarray database.

Structure, Function, and Development of the Visual System

June 22–July 5

INSTRUCTORS **K. McAllister,** University of California, Davis
W.M. Usrey, University of California, Davis

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; the role of patterned neuronal activity in the development of central visual pathways; and molecular mechanisms of development and plasticity in the visual system.

PARTICIPANTS

Alitto, H., B.S., University of California, Davis
Basole, A, B.Sc., M.Sc., Duke University Medical Center, Durham, North Carolina
Biederlack, J., Diplom., Max-Planck Institute for Brain, Frankfurt, Germany
Cancedda, L., B.S., Scuola Normale Superiore, Pisa, Italy
Carroll, J., B.S., Medical College of Wisconsin, Milwaukee
Chien, S.H.-L., B.S., M.S., Ph.D., University of Washington, Seattle
Firth, S., B. Pharm, B.Sc., University of Texas, Houston

Harness, P., B.S., University of California, Davis
Hosoya, T., Ph.D., Harvard University, Cambridge, Massachusetts
Huberman, A., B.A., M.S., University of California, Davis
Hunter, C., Ph.D., National Institutes of Health, Bethesda, Maryland
Kaldy, Z., M.A., M.S., Rutgers University, Piscataway, New Jersey
Liu, C.-H., B.S., M.S., Brown University, Providence, Rhode Island
McKay, S., B.Sc., M.Sc., University of Oxford, United Kingdom
Montoro, R., B.S., Ph.D., Columbia University, New York
Saunoriute Kerbeliene, S., B.S., M.S., Vilnius University, Lithuania
Schmid, A., Diplom., ETH Zurich, Switzerland
Sigala, N., B.S., M.S., Max-Planck Institute for Biological Cybernetics, Tüebingen, Germany
Slutsky, D., B.S., University of California, Davis
Wong, E., B.S., University of Houston, Texas

SEMINARS

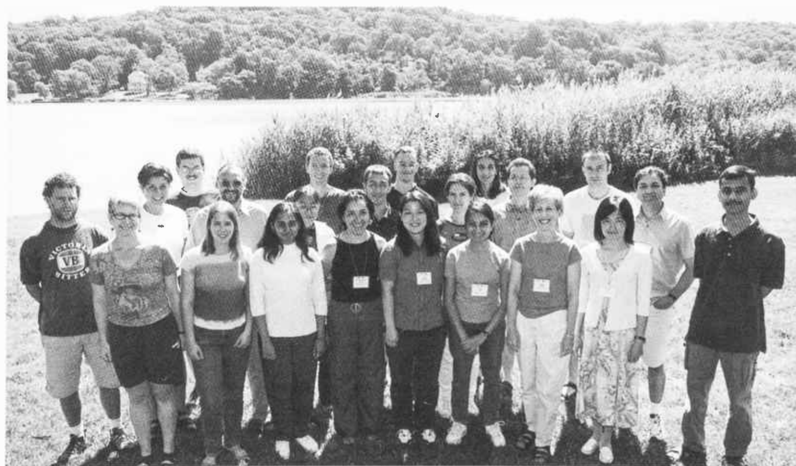
Bonhoeffer, T., Max-Planck Institute of Neurobiology, Muenchen-Martinsried, Germany: Spatial relationships among three columnar systems in cat area 17.
Britten, K., University of California, Davis: Neural mechanisms for processing of optic flow.
Chapman, B., University of California, Davis: How the visual system got its stripes.
Cline, H., Cold Spring Harbor Laboratory: Cellular mechanisms of visual system development II.
Constantine-Paton, M., Yale University, New Haven, Connecticut: Cellular mechanisms of visual system development I.
Dacey, D., University of Washington, Seattle: Parallel pathways for spectral coding in primate retina.
Feller, M., University of California, San Diego: Retinal waves and neural development.
Fitzpatrick, D., Duke University, Durham, North Carolina: Functional properties of neural circuits in the visual system.
Hubel, D., Harvard Medical School, Boston, Massachusetts: Functional architecture of macaque monkey visual cortex.
Katz, L., Duke University Medical Center/HHMI, Durham, North Carolina: What's critical for the critical period in visual cortex?
Martin, K., University ETH Zurich, Switzerland: From single cells to simple circuits in the cerebral cortex.
Masland, D., Harvard University/MGH, Boston, Massachusetts: Neuronal diversity in the retina.
McAllister, K., University of California, Davis: Neurotrophins and visual cortex development.
Movshon, T., New York University, New York: Behavioral choice and the visual responses of neurons in macaque MT.
Paradiso, M., Brown University, Providence, Rhode Island: Neural correlates of brightness perception.
Reynolds, J., The Salk Institute, San Diego, California: The effects of attention on visual processing.
Roelfsema, P., University of Amsterdam, The Netherlands: Object-based attention in the primary visual cortex of the macaque monkey.
Schnapf, J., University of California, San Francisco: Functional properties of vertebrate photoreceptors.
Shadlen, M., University of Washington, Seattle: Neural mechanisms that underlie decisions about sensory stimuli.
Sherman, M., University of Oxford, United Kingdom: The functional organization of thalamocortical relays.
Usrey, M., University of California, Davis: Functional properties of neural circuits in the visual system.

Arabidopsis Molecular Genetics

June 29–July 19

INSTRUCTORS	**J. Bowman,** University of California, Davis **U. Grossniklaus,** University of Zurich, Switzerland **R. Puritt,** Purdue University, West Lafayette, Indiana
ASSISTANTS	**S. Floyd,** University of California, Davis **D. Schurmann,** Friedrich Miescher Institute, Basel, Switzerland

This course provided an intensive overview of topics in plant growth, physiology, and development, focusing on molecular genetic approaches to understanding plant biology. It emphasized recent results from *Arabidopsis thaliana* and other model plants and provided an introduction to current methods used in *Arabidopsis* research. It was designed for scientists with experience in molecular techniques or in plant biology who wish to work with *Arabidopsis*. The course consisted of a vigorous lecture series, a hands-on laboratory, and informal discussions. Discussions of important topics in plant research was presented by the instructors and by invited speakers. These seminars included plant morphology and anatomy; gene dosage effects and epigenetic phenomena; cell biology (including calcium signaling, cell polarity, and centromere behavior); plant development (including development of flowers, roots, meristems, and leaves, male and female gametophytes, and embryos); floral induction; synthesis, function, and perception of hormones; bioinformatics tools available to the *Arabidopsis* community; and the potential of the *Arabidopsis* genome project for accelerating *Arabidopsis* research. Speakers provided overviews of their fields, followed by in-depth discussions of their own work. The laboratory sessions



provided an introduction to important techniques currently used in *Arabidopsis* research. These included studies of *Arabidopsis* development, mutant analysis, methods in plant pathology and phylogenetic analysis, histochemical staining, transient gene expression, applications of green fluorescent protein fusions, protein isolation and detection; mass spectrometry, gene chip technology, QTL analysis and techniques commonly used in genetic and physical mapping, and map-based cloning. The course also included several short workshops on important themes in genetics. This year's speakers included Gethyn Allen, Roger Beachy, Andrew Bent, John Bowman, Winslow Briggs, Clint Chapple, Nancy Dengler, Liam Dolan, Hugo Dooner, Dick Flavell, Ueli Grossniklaus, David Jackson, Toby Kellogg, Rob Martienssen, Peter McCourt, Tom Mitchell-Olds, June Nasrallah, Scott Peck, Craig, Pikaard, Bob Pruitt, Ralph Quatrano, Eric Richards, Eric Schaller, Dave Spector, Ian Sussex, Sakis Theologis, and Tong Zhu.

PARTICIPANTS

Alvarez-Venega, R,, B.S., Ph.D., Purdue University, West Lafayette, Indiana
Boavida, L., LIC., Gulbenkian Institute of Science, Oeiras, Portugal
Carmona Dominguez, E., B.S., Ph.D., Purdue University, West Lafayette, Indiana
Deshpande, A., B.Sc., Ph.D., Purdue University, West Lafayette, Indiana
Hoedemaekers, K., B.S., University of Nijmegen, The Netherlands
Krishnaswamy, L., B.Sc., M.Sc., Iowa State University, Ames
Lall, S., B.Sc., Ph.D., Iowa State University, Ames
Lin, B.-L., B.S., Ph.D., Academia Sinica, Taiwan, Republic of China
Maizel, A., B.S., Ph.D., CNRS, Paris, France
Nakanishi, S., B.E., M.S., Washington State University, Pullman
Raizada, M., B.Sc., Ph.D., University of Guelph, Canada
Royackers, K, , B.S., M.S., Catholic University, Leuven, Belgium
Shippen, D., B.S., Ph.D., Texas A&M University, College Station
Suarez, M.-C., B.S., M.S., California State University, Fresno
Ueda, M., B.S., M.S., Kyoto University, Japan
Wrzaczek, M., M.S., University of Vienna, Austria

SEMINARS

Allen, G., University of California, San Diego: Cellular physiology.
Beachy, R., Donald Danforth Plant Science Center, St. Louis, Missouri: Plant viruses.
Bent, A., University of Wisconsin, Madison: Plant pathogen.
Bowman, J., University of California, Davis: Flowers. Genetics workshop II: Mutagenesis.
Briggs, W., Carnegie Institution of Washington, Stanford, California: Light responses.
Chapple, C., Purdue University, West Lafayette, Indiana: Secondary metabolism.
Dengler, N., University of Toronto, Ontario, Canada: Plant anatomy.
Dolan, L., John Innes Centre, Norfolk, United Kingdom: Roots.
Dooner, H., Rutgers University, Piscataway, New Jersey: Transposons.
Flavell, D., Ceres, Inc, Malibu, California: Past and future of plant molecular biology.
Grossniklaus, U., University of Zurich, Switzerland: Female gametophyte. Genomic imprinting. Genetics workshop III: Epiztasis.
Jackson, D., Cold Spring Harbor Laboratory: Meristem and leaf.
Kellogg, T., University of Missouri, St. Louis: Evolution.
Martienssen, R., Cold Spring Harbor Laboratory: Enhancer traps.
McCourt, P., University of Toronto, Canada: Humane networks.
Mitchell-Olds, T., Max-Planck Institute of Chemical Ecology, Jena, Germany: Natural variation.
Nasrallah, J., Cornell University, Ithaca, New York: Self incompatibility.
Peck, S., John Innes Centre, Norwich, United Kingdom: Proteomics.
Pikaard, C., Washington University, St. Louis, Missouri: Epigenetics I.
Pruitt, B., Purdue University, West Lafayette, Indiana: Male gametophyte and signaling. Genetics workshop I: Mapping.
Quatrano, R., Washington University, St. Louis, Missouri: Cell biology.
Richards, E., Washington University, St. Louis, Missouri: Epigenetics II.
Schaller, E., University of New Hampshire, Durham: Ethylene.
Spector, D., Cold Spring Harbor Laboratory: Microscopy.
Sussex, I., Yale University, New Haven, Connecticut: Plant morphology.
Theologis, S., Plant Gene Expression Center, Albany, California: Auxin and genomics.
Zhu, T., Torrey Mesa Research Institute, San Diego, California: Affimetrix chips.

Molecular Cloning of Neural Genes

June 29–July 19

INSTRUCTORS
J. Boulter, University of California, Los Angeles
K. Jensen, The Rockefeller University, New York
D. Lavery, Glaxo Wellcome Experimental Research, Lausanne, Switzerland
C. Lai, Scripps Research Institute, La Jolla, California

CO-INSTRUCTOR
W. Walwyn, University of California, Los Angeles

ASSISTANTS
J. Arjomand, University of California Neuropsychiatric Institute, Los Angeles
C. Cottiny, GlaxoSmithKline, S.A., Lausanne, Switzerland
A. Klaassen, University of California, Los Angeles
J. LeMieux, Harvard Medical School, Boston, Massachusetts
E. Ruchti, GlaxoSmithKline, S.A., Lausanne, Switzerland
M. Samson, Harvard Medical School, Boston, Massachusetts

This intensive laboratory and lecture course taught neuroscientists current approaches to molecular neurobiology. The course consisted of daily lectures and laboratory exercises on the practice of molecular neurobiology, with an emphasis on modern approaches to cloning and analyzing the expression of neural genes. A series of evening research seminars by invited speakers focused on the ways in which these molecular techniques have been successfully applied. In the past, evening seminar topics have included expression cloning, single cell cloning, subtractive cDNA cloning strategies, and genet-

ic and mechanistic studies of neurologic disease, aquisition of cell identity, and axon guidance in the developing nervous system. The laboratory portion of the course began with instruction in a series of basic molecular biological techniques and rapidly advanced to more sophisticated methodologies. Students learned to prepare genomic, phage, and plasmid DNAs and total and poly(A)$^+$ RNA and to generate and screen cDNA libraries. Additional topics and methods covered included restriction mapping, agarose and polyacrylamide gel electrophoresis, northern and Southern blotting, subcloning, oligonucleotide primer design, a selection of PCR-based techniques, and the use of nucleotide and protein sequence databases. Gene expression studies included the production of fusion proteins in bacteria and mammalian cell transfection. An introduction to gene profiling was presented through the use of cDNA arrays and Affymetrix gene chips. The advanced techniques featured the construction of cDNA libraries from single cells and the use of subtractive cDNA methods to clone genes expressed in limited populations of cells.

PARTICIPANTS

Allen, N., B.Sc, Ph.D., University College London, United Kingdom
Berman, D., B.S., Ph.D., The Weizmann Institute of Science, Rehovot, Israel
Bhandawat, V., M.Sc., Ph.D., Johns Hopkins School of Medicine, Baltimore, Maryland
Bolotina, V., B.S., Ph.D., Boston University School of Medicine, Massachusetts
Caric, D., M.D., Ph.D., Pittsburgh University, Pennsylvania
Drew, L., B.Sc., Ph.D., University College London, United Kingdom
Golovina, V., M.S., Ph.D., University of Maryland School of Medicine, Baltimore
Kanold, P., M.S., Ph.D., Harvard Medical School, Boston, Massachusetts
Kaplan, M., B.A., Ph.D., University of California, San Francisco
Lamprecht, R., B.S., Ph.D., New York University, New York
Linkenhoker, B., B.A., Ph.D., Stanford University, California
Morcos, Y., B.Sc., Ph.D., University of Tennessee, Memphis
Polleux, F., B.S., Ph.D., INSERM, Bron, France
Robert, A., M.D., Ph.D., Yale University, New Haven, Connecticut
Robles, E., B.S., University of Wisconsin, Madison
Xu, J., B.S., Ph.D., University of California, Los Angeles

SEMINARS

Barres, B., Stanford University School of Medicine, California: Neuron-glial interactions in the developing brain.
Bettler, B., Novartis Pharma A/G, Basel, Switzerland: Molecular insights into GABA receptor physiology.
Cline, H., Cold Spring Harbor Laboratory: Functional analysis of activity-regulated genes in visual system development.
Lemke, G., The Salk Institute for Biological Studies, La Jolla, California: Axial polarization of the eye and retino-collicular mapping.
Mayford, M., Scripps Research Institute, La Jolla, California: Genetic approaches to memory in the mouse.
Walsh, C., Harvard Medical School, Boston, Massachusetts: Positional cloning of genes required for cerebral cortical development in mice and humans.
Weinmaster, G., University of California School of Medicine, Los Angeles: Notch signal transduction in mammalian cells.

Neurobiology of *Drosophila*

June 29–July 19

INSTRUCTORS	**K. Broadie,** University of Utah, Salt Lake City **S. deBelle,** University of Nevada, Las Vegas **G. Tear,** King's College, London, United Kingdom
ASSISTANTS	**L. Flintoft,** King's College, London, United Kingdom **R. Renden,** University of Utah, Salt Lake City **C. Riedl,** University of Toronto, Mississauga, Ontario, Canada

This laboratory/lecture course was intended for researchers at all levels from beginning graduate students through established primary investigators who want to use *Drosophila* as an experimental system for nervous system investigation. The 3-week course was divided into the study of development, physiology/function, and behavior. Daily seminars introduced the students to a variety of research topics and developed those topics by including recent experimental contributions and outstanding questions in the field. Guest lecturers brought original preparations for viewing and discussion and direct laboratory exercises and experiments in their area of interest. The course provided students with hands-on experience using a variety of experimental preparations that are utilized in the investigation

of current neurobiological questions. The lectures and laboratories focused on both the development of the nervous system and its role in controlling larval and adult behaviors. In addition to an exposure to the molecular genetic approaches available in *Drosophila*, students learned a variety of techniques including labeling of identified neurons, electrophysiological recording from nerves and muscles, and the analysis of larval and adult behavior. Collectively, the course provided a comprehensive and practical introduction to modern experimental methods for studying the *Drosophila* nervous system. The specific topics from this year's course included neurogenesis, axon pathfinding, synaptogenesis, membrane excitability, synaptic function and plasticity, photoreception, mechanoreception, neural circuits, biological rhythms, courtship, learning and memory, and locomotory and flight behaviors. This year's lecturers included Justin Blau, Kendal Broadie, Steven deBelle, Aaron DiAntonio, Alicia Hidaglo, Matthias Landgraff, Maurice Kernan, Rod Murphey, Kevin O'Dell, Diane O'Dowd, William Pak, David Shepherd, Jim Skeath, Roland Strauss, Guy Tear, Jessica Treisman, Tim Tully, and David Van Vactor.

PARTICIPANTS

Ben-Shahar, Y., B.Sc., M.S., University of Illinois, Urbana
Collins, C., B.A., Ph.D., University of California, Berkeley
Dickman, D., B.A., Harvard Medical School, Boston, Massachusetts
Dunkelberger, B., B.S., University of Nevada, Las Vegas
Forni, J., B.Sc., Ph.D., King's College London, United Kingdom
Jafar-Nejad, H., M.D., Baylor College of Medicine, Houston, Texas
Loren, C, , B.A., M.A., Umea Universitet, Sweden
Palanker, L., B.S., University of Utah, Salt Lake City
Rikhy, R., B.Sc., M.Sc., Tata Institute of Fundamental Research, Colaba, Mumbai, India
Rollmann, S., B.S., Ph.D., North Carolina State University, Raleigh
Wickline, L., B.A., Washington University, St. Louis, Missouri
Ye, B., B.S., Ph.D., University of California, San Francisco

SEMINARS

Blau, J., New York University, New York: Circadian rhythms.
Broadie, K., University of Utah, Salt Lake City: Introduction to *Drosophila* physiology: Synaptogenesis and synaptic structure-function.
De Belle, S., University of Nevada, Las Vegas: Introduction to behavior. Foraging behavior.
Tully, T., Cold Spring Harbor Laboratory: Learning and memory.
DiAntonio, A., Washington University School of Medicine, St. Louis, Missouri: Synaptic plasicity and synaptic modulation.
Hidalgo, A., University of Cambridge, United Kingdom: The role of glia in the nervous system.
Kernan, M., SUNY, Stony Brook, New York: Mechanoreception.
Landgraff, M., University of Cambridge, United Kingdom: Acquisition of neuronal identity in the embryo.
Murphey, R., University of Massachusetts, Amherst: Neural circuits in *Drosophila*.
O'Dell, K., University of Glasgow, United Kingdom: Courtship behavior.
O'Dowd, D., University of California, Irvine: Ion channels and neuronal culture.
Pak, W., Purdue University, West Lafayette, Indiana: Phototransduction.
Shepherd, D., University of Southampton, United Kingdom: Development of the adult CNS.
Skeath, J., Washington University School of Medicine, St. Louis, Missouri: Early events of neurogenesis in the embryo.
Strauss, R., Theodor-Boveri Institut fuer Biowissenschaften, Wuerzberg, Germany: Motor/visual behavior.
Tear, G., Kings College, London, United Kingdom: Introduction to *Drosophila* development and experimental techniques.
Treisman, J., New York University School of Medicine, New York: Development of the visual system in the larva and adult.
Van Vactor, D., Harvard Medical School, Boston, Massachusetts: Axon guidance mechanisms in the embryo.

Mouse Behavioral Analysis

July 6–19

INSTRUCTORS	**M. Fanselow,** University of California, Los Angeles **M. Mayford,** The Scripps Research Institute, La Jolla, California
ASSISTANTS	**B. Godsil,** University of California, Los Angeles **A. Matynia,** University of California, Los Angeles **L. Reijmers,** The Scripps Research Institute, La Jolla, California **B. Wiltgen,** University of California, Los Angeles

This course provided a theoretical and experimental introduction to behavioral analysis in the mouse, with a focus on learning and memory. It is specially designed for geneticists, molecular biologists, pharmacologists, and electrophysiologists with a need for a hands-on introduction to behavioral analysis of the mouse. Additionally, the course covered the principles of using mutant mice in behavioral studies, as well as the issues involved in integrating behavioral, neuroanatomical, neurophysiological, and molecular findings. Among the methods presented were the water maze, cued and contextual fear conditioning, natural/ethologically relevant learning, open field behavior, the rotor-rod, and other activity tests. In addition, there were demonstrations of several aspects of in vitro electrophysiology (fields and whole-cell recordings of synaptic plasticity).

PARTICIPANTS

Berghard, A., B.S., Ph.D., Umea University, Switzerland
Delorey, T., B.S., Ph.D., Molecular Research Institute, Mountain View, California
Garcia, A.D., B.S., University of California, Los Angeles
Lerchner, W., M.Sc., Ph.D., Caltech/Human Frontiers Science Program, Organization, Pasadena, California
Lo, L.-T., B.M., Washington University School of Medicine, St. Louis, Missouri
Offenhaeuser, N., B.S., Ph.D., FIRG Institute of Molecular Oncology, Milan, Italy
Panda, S., M.S., Ph.D., The Scripps Research Institute, La Jolla, California
Till, S., B.S., M.S., Columbia University, New York
Wada, Y., B.A., Ph.D., RIKEN Genomic Sciences Center, Yokohama, Japan
Wilquet, V., B.S., Ph.D., University of Leuven, Belgium
Wood, M., B.S., Ph.D., University of Pennsylvania, Philadelphia

SEMINARS

Balleine, B., University of California, Los Angeles; Holland, P.M., Duke University, Durham, North Carolina; Fanselow, M., University of California, Los Angeles: What are the essentials of learning and behavior?" Introduction to behavioral psychology II.
Blanchard, C., John A. Burns School of Medicine, Honolulu, Hawaii: Crosspieces. Generality of aggression and defense.
Blanchard, R., University of Hawaii: Aggressive and defensive behavior in the rat and mouse.
Fanselow, M., University of California, Los Angeles: Introduction to behavioral psychology I. Functional basis of Pavlovian conditioning. Stats for molecular biologists. What can mouse genetics offer to psychology?
Hen, R., Columbia University, New York: *Roundtable:* Psychiatric genetics in the mouse? Genetics of anxiety.
Holland, P., Duke University, Durham, North Carolina: Introduction to learning theory II. Introduction to learning theory II.
Knowlton, B., University of California, Los Angeles: Neural systems. Neural systems of behavior.
Mayford, M., The Scripps Research Institute, La Jolla, California: Genetic approaches to memory. Genetic background issues. What can mouse genetics offer to psychology? How to combine ethological and genetic approaches.
Takahashi, J., Northwestern University, Evanston, Illinois: ENU, QTLs, transgenics, knockouts, utility of each and future directions. Molecular understanding of a complex behavior through forward genetics.
Thompson, R., University of Southern California, Los Angeles: Classical conditioning of discrete behavioral resposes. *Roundtable:* Cerebellar learning.
Wilson, M., Massachusetts Institute of Technology, Cambridge: Systems neurophysiology.

Biology of Memory: From Molecules to Behavior

July 7–20

INSTRUCTORS
J. Byrne, University of Texas, Houston
H. Eichenbaum, Boston University, Massachusetts
K. Martin, University of California, Los Angeles
L. Squire, University of California, San Diego

This lecture course provided an introduction to cell, molecular, and systems approaches to learning and memory. It was suited for graduate students in molecular biology, neurobiology, and psychology as well as research workers 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) regulation of gene expression; (4) cellular and molecular mechanisms of simple forms of learning and memory in invertebrates and vertebrates; (5) cellular and molecular mechanisms of long-term potentiation and depression in various regions of the mammalian brain; and (6) systems approaches to learning in vertebrates and humans.

PARTICIPANTS

Antzoulatos, E., B.A., University of Texas, Houston
Baldassa, S., B.S., Ph.D., University of Milan, Italy
Bengtsson, F., M.S., Ph.D., University of Lund, Sweden
Boyden, E., B.S., M.S., Stanford University, California
Colombo, J., B.S., M.S., University of Fribourg, Switzerland
Fioravante, D., B.A., Ph.D., University of Texas, Houston
Fraidakis, M., M.D., M.S., Karolinska Institute, Stockholm, Sweden
Frenkel, D., M.S., Ph.D., Tel Aviv University, Israel
Galvez, R., B.A., University of Illinois, Urbana-Champaign
Johnson, A., B.S., University of Texas–Houston HSC Medical School
Jones, S., B.S., East Carolina University, Greenville, North Carolina
Ke, M., B.S., M.D., Stanford University Medical School, California
McGaughy, J., B.A., Ph.D., Boston University, Massachusetts
Nery, S., B.S., New York University Medical Center, New York
Robitsek, J., B.A., Boston University, Massachusetts
Spors, H., M.D., Max-Planck Institute for Medical Research, Heidelberg, Germany
Vale-Martinez, A., B.A., Ph.D., Boston University, Massachusetts
Waldenstrom, A., M.Sc., Ph.D., Lund University, Sweden
Zhang, Z., B.S., M.S., Max-Planck Institute of Psychiatry, Munich, Germany

SEMINARS

Ball, G., Johns Hopkins University School of Medicine, Baltimore, Maryland: Ethological approaches to learning.
Bear, M., Brown University/HHMI, Providence, Rhode Island: Long-term depression.
Byrne, J., University of Texas–Houston Medical School: Nonassociative learning in *Aplysia*. Introduction to the cellular study of learning. Associative learning in *Aplysia*. Overview of membranes and synaptic transmission.
Eichenbaum, H., Boston University, Massachusetts: Role of LTP and LTP in learning. Memory systems in rodents.
Gilbert, C., The Rockefeller University, New York: Dynamics of adult visual cortex.
Greenough, W., University of Illinois, Urbana: Morphological correlates of learning and experience.
Holland, P., Duke University, Durham, North Carolina: Introduction to learning theory I. Introduction to learning theory II.
LeDoux, J., New York University, New York: Fear conditioning.
Malinow, R., Cold Spring Harbor Laboratory: Long-term potentiation I.
Martin, K., University of California, Los Angeles: Mechanisms of site-specific synaptic facilitation I. Mechanisms of site-specific synaptic facilitation 2.
Mayford, M., University of California, San Diego: Gene-knockout/transgenic approaches to study learning.
McGaugh, J., University of California, Irvine: Modulation of memory.
Miller, E., Massachusetts Institute of Technology, Cambridge: Role of prefrontal cortex in memory.
Raymond, J., Stanford University, California: Adaptive modifications of the VOR.
Schulman, H., Stanford University School of Medicine, California: Overview of second-messenger systems and their role in learning and memory I. Overview of second-messenger systems and their role in learning and memory II.
Squire, L., University of California, San Diego: Memory systems in nonhuman primates. Human memory and disorders of memory.
Thompson, R., University of Southern California, Los Angeles: Classical conditioning of discrete behavioral responses.
Tully, T., Cold Spring Harbor Laboratory: Genetic approaches to study learning in *Drosophila*.

Molecular Mechanisms of Human Neurological Diseases

July 23–29

INSTRUCTORS	**A. Aguzzi,** University Hospital of Zurich, Switzerland **S. Gandy,** New York University and Nathan S. Kline Institute of Psychiatric Research, Orangeburg, New York **J. Hardy,** Mayo Clinic, Jacksonville, Florida
ASSISTANT	**V. Lingappa,** University of California, San Francisco

How and why do neurons die in specific human neurological disorders? What are the molecular and biochemical manifestations of specific genetic lesions in neurodegenerative disorders? Do different pathological deaths share common mechanisms? What practical treatments are contemplated? This lecture course explored possible answers to these important questions. Recent advances in neurogenetics and in molecular and cell biology have begun to shed light on the mechanisms that underlie nervous system injury in disease states such as Alzheimer's disease, Parkinson's disease, frontotemporal dementia, stroke, tauopathies, synucleinopathies, prion diseases, and polyglutamine repeat disorders. Taking advantage of small class size and extensive discussion, invited faculty lecturers examined critical issues in their areas of expertise. Overviews were provided and course participants did not have to be familiar with neurological diseases. The course focused principally on the specific hypotheses and approaches driving current research. Emphasis was placed on the highly dynamic interface between basic and clinical investigation, including the interdependence of clinical research and disease model

development, and the value of disease research in understanding the function of the normal nervous system. This year's lecturers were Adriano Aguzzi, Richard Frackowiak, Paul Fraser, Sam Gandy, Tim Greenamyre, John Hardy, David Holtzman, Peter St. George-Hyslop, Virginia Lee, Vishu Lingappa, James Meschia, Diane Murphy, Bob Vassar, John Trojanowski, Mike Hutton, Marcy MacDonald, Parsa Kazemi-Esfarjani, and David Westaway.

PARTICIPANTS

Alonso, A., B.S., M.S., Ph.D., NYS Institute for Basic Research, Staten Island, New York
Bernacchia, A., B.S., Ph.D., University of Bologna, Italy
Ching, Y.P., B.Sc., Ph.D., Hong Kong University, Hong Kong
Chou, T., B.S., Ph.D., University of Pennsylvania, Philadelphia
Cole, N., B.S., Ph.D., National Institutes of Health, Bethesda, Maryland
Corvetti, L., M.D., International School for Advanced Studies, Trieste, Italy
Deitz, W., Yale University, New Canaan, Connecticut
Fraidakis, M., M.D., M.S., Karolinska Institute, Stockholm, Sweden
Frenkel, D., M.S., Tel Aviv University, Israel
Heppner, F., M.D., University Hospital Zurich, Switzerland
Kohler, R., Ph.D., Lion Bioscience Research, Inc., Cambridge, Massachusetts
Kroening, A.-K., Dipl., Institute of Clinical Neurobiology, Wuerzburg, Germany
Lynch-Ballard, E., M.D., University of Texas, Houston
Maggi, L., Ph.D., SISSA-ISAS, Trieste, Italy
Majumder, S., B.Sc., Ph.D., University of Texas, M.D. Anderson Cancer Center, Houston
Mattiazzi, M., B.S., Ph.D., University of Bari, Italy
Sigurdson, C., B.S., Colorado State University, Fort Collins
Ritz, B., M.D., Ph.D., University of California, Los Angeles
Roy, M., B.A., Ph.D., Stanford University, California
Sharmeen, L., M.Sc., Ph.D., Pfizer Global R&D, Ann Arbor, Michigan

SEMINARS

Aguzzi, A., University Hospital of Zurich, Switzerland: Molecular biology of prion diseases.
Frackowiak, R., Wellcome Trust, London, United Kingdom: Structural and function basis of neurodegenerative illness.
Fraser, P., University of Toronto, Canada: Biophysics of amyloidogenesis.
Gandy, S., New York University and Nathan S. Kline Institute of Psychiatric Research, Orangeburg, New York: Regulation of amyloidosis by gonadal hormones.
Greenamyre, T., Emory University, Atlanta, Georgia: Clinical features of Parkinson's. A model for Parkinson's based on an environmental toxin.
George-Hyslop St., P., University of Toronto, Canada: Genetics and molecular biology of Alzheimer's disease.
Hardy, J., Mayo Clinic Jacksonville, Florida: An overview of neurogenetics.
Holtzman, D., Washington School of Medicine, St. Louis, Missouri: Molecular biology of stroke. Genetics and transgenes of apolipoprotein E. Depletion of peripheral abeta as a strategy to lower brain amyloid load.
Hutton, M., Mayo Clinic Jacksonville, Florida: Genetics and transgenesis of tauopathies.
Kazemi-Esfarjani, P., Califorina Institute of Technology, Pasadena: Modeling triple repeat diseases in *Drosophila*.
Lee, V., University of Pennsylvania School of Medicine, Philadelphia: Biochemistry of tauopathies.
MacDonald, M., Massachusetts General Hospital, Charlestown: Trinucleotide repeat diseases.
Lingappa, V., University of California, San Francisco: Cell biology of prion proteins biogenesis.
Meschia, J., Mayo Clinic, Jacksonville, Florida: Clinical features of stroke syndromes.
Murphy, D., NINDS, National Institutes of Health, Rockville, Maryland: Introduction to NIH.
Trojanowski, J., University of Pennsylvania, Philadelphia: Synucleinopathies.
Vassar, B., Northwestern University Medical School, Chicago, Illinois: Molecular biology and transgenesis of BACE, a/k/a beta secretase.
Westaway, D., University of Toronto, Canada: Transgenic modeling of Alzheimer disease. Immunotherapy of Alzheimer's disease.

C. elegans

July 24–August 13

INSTRUCTORS
A. Chisholm, University of California, Santa Cruz
M. Hengartner, University of Zurich, Switzerland
E. Jorgensen, University of Utah, Salt Lake City
R. Korswagen, University Hospital Utrecht, The Netherlands

ASSISTANTS
T. Harris, Cold Spring Harbor Laboratory
M. Hammarlund, University of Utah, Salt Lake City

This course was designed to familiarize investigators with *C. elegans* as an experimental system, with an emphasis on both classical genetic analysis and reverse genetic approaches. A major goal was to teach students how to successfully exploit the information generated by the *C. elegans* genome project. The course was suited both for those who have current training in molecular biology and some knowledge of genetics, but have no experience with *C. elegans*, and for students with some prior worm experience who wished to expand their repertoire of expertise. The following topics were covered both in the laboratory and by lectures from experts in the field: worm pushing, *C. elegans* databases and worm bioinformatics, anatomy and development, forward genetics, chemical and transposon mutage-

nesis, generation of transgenic animals, expression pattern analysis, reverse genetics, construction and screening of deletion libraries, and RNA inactivation. The course was designed to impart sufficient training to the students in the most important attributes of the *C. elegans* system to enable them to embark on their own research projects after returning to their home institutions.

PARTICIPANTS

Aikawa, J.-I., B.A., Ph.D., RIKEN, Saitama, Japan
Bazzoni, G., B.A., M.D., Mario Negri Institute for Pharmacological Research, Milan, Italy
Carlow, C., B.S., Ph.D., New England Biolabs, Beverly, Massachusetts
Degtyareva, N., B.S., Ph.D., University of North Carolina, Chapel Hill
Hansen, M., B.S., M.S., Copenhagen University, Denmark
Holmberg, C., M.S., Ph.D., Turku Centre for Biotechnology, Finland
Kirk, K., B.S., Ph.D., New Mexico Technology, Socorro
Kiyokawa, E., M.D., Ph.D., RIKEN, Saitama, Japan
Larsen, M., M.D., Ph.D., University of Southern Denmark, Odense, Denmark
Lechleiter, J., B.A., Ph.D.. University of Texas Health Sciences Center, San Antonio
Pintard, L., Ph.D., Swiss Institute of Cancer Research, Lausanne, Switzerland
Porter, M., B.Sc., Ph.D., University College London, United Kingdom
Riedl J., B.S., Ph.D., University of Utrecht, The Netherlands
Seitz, S., M.S., Max-Planck Institute of Molecular Genetics, Berlin, Germany
Strauss, K., Max-Planck Society, Tüebingen, Germany
Tewari M., B.A., Ph.D., Harvard Medical School, Boston, Massachusetts

SEMINARS

Aroian, R., University of California, San Francisco: Pathogenesis, toxicity assays.
Bowerman, B., University of Oregon, Eugene: Early embryogenesis, ts screens, and movies.
Chisholm, A., University of California, Santa Cruz: Eph signaling; microscopy.
Garriga, G., University of California, Berkeley: Neuronal development, screening RNAi phenotypes.
Hengartner, M., University of Zurich, Switzerland: Cell death; suppressor genetics.
Jin, Y., University of California, Santa Cruz: Synapse development, transformation + a little GFP.
Jorgensen, E., University of Utah, Salt Lake City: Synaptic function, forward genetics.
Kenyon, C., University of California, San Francisco: Aging, aging assay, laser ablation.
Korswagen, R., University Hospital Utrecht, The Netherlands: Wnt signaling, suppressor screens.
Stein, L., Cold Spring Harbor Laboratory: *C. elegans* genome Wormbase.
Plasterk, R., Hubrecht Laboratory, Utrecht, The Netherlands: RNAi, mechanisms, RNAi.
Vidal, M., Dana-Farber Cancer Institute, Boston, Massachusetts: Vulval signaling, proteomics.
Villeneuve, A., Stanford University School of Medicine, California: Meiosis, recombination, and balancers.

Biology of Developmental Disabilities in Children

July 30–August 3

INSTRUCTORS **B.J. Casey,** Weill Medical College of Cornell University, New York
Y. Munakata, University of Denver, Colorado

This course explored basic developmental principles of the brain and behavior and their application to understanding the biology of developmental disabilities. Leading authorities in areas of higher cognitive functions, including language and cognitive control, presented models of typical and atypical brain development. Students were exposed to a wide spectrum of methods currently being used in developmental science, including neuroimaging, computational modeling, human genetics, and animal models. Emphasis was placed on a converging methods approach, and these methods were discussed in both lectures and workshops throughout the course in the context of models of developmental disabilities. This year's lecturers were Annette Karmiloff-Smith, Isabel Gautier, Michelle de Haan, Kathleen Thomas, Helen Neville, April Benasich, Bruce McCandliss, Adele Diamond, John Fossella, and William Greenough.

PARTICIPANTS

Berger, Sarah, B.A., Ph.D., New York University, New York
Brunssen, S., B.S., M.S., University of North Carolina, Chapel Hill
Choudhury, N., B.A., Ph.D., Rutgers University, Newark, New Jersey
Cleary, M., B.A., Ph.D., Indiana University, Bloomington
Eigsti, I.-M., B.A., Ph.D., The University of Rochester, New York
Fan, J., B.S., Ph.D., The Sackler Institute, Cornell University, New York
Hay, D., B.A., Ph.D., University of Saskatchewan, Canada
Herbert, M., Ph.D., M.D., Massachusetts General Hospital, Charlestown

Liu, D., B.S., Ph.D., University of Michigan, Ann Arbor
MacKenzie, M., B.Sc., M.Sc., University of Michigan, Ann Arbor
McDuffie, A., B.A., M.A., Vanderbilt University, Nashville, Tennessee
Morton, B., Ph.D., University of Denver, Colorado
Raz, A., M.S., Ph.D., Cornell University, New York
Raz, S., B.A., Ph.D., University of Memphis, Tennessee
Ruz, M., B.S., M.S., University of Granada, Spain
Sheridan, M., B.S., Washington University, St. Louis, Missouri
Thomas, Jennifer, BA, M.S., Rutgers University, Newark, New Jersey
Tottenham, N., B.A., Ph.D., University of Minnesota, Minneapolis
Wang, A., B.A., Ph.D., University of California, Los Angeles

SEMINARS

Benasich, A., Rutgers University, Newark, New Jersey: Typical and atypical development of language and speech.
Casey, B.J., Sackler Institute, New York; Munakata, Y., Denver University, Colorado: Overview of upcoming week.
Casey, B.J., Sackler Institute, New York: Impact of disruption of frontostriatal circuitry on development.
De Haan, M., University College London, United Kingdom: Developmental principles of facing processing.
Diamond, A., Eunice Kennedy Shriver Center, Waltham, Massachusetts: Development of prefrontal cortex and related disorders.
Fossella, J., The Rockefeller University, New York: Human genetics workshop.
Gauthier, I., Vanderbilt University, Nashville, Tennessee: Organization of face and object processing.
Greenough, W., University of Illinois, Urbana: Environmental and genetic factors in atypical and typical development.
Karmiloff-Smith, A., Institute of Child Health, London, United Kingdom: Different approaches to relating genes to developmental disorders.
Manakata, Y., Denver University, Colorado: Workshop on computational modeling of prefrontal function.
McCandliss, B., Sackler Institute, New York: Workshop on speech and language interventions.
Neville, H., University of Oregon, Eugene: Plasticity and organization of language development.
Posner, M., Sackler Institute, New York: Putting the course in perspective?
Thomas, K., Sackler Institute, New York: Workshop on pediatric neuroimaging: ERP, MRI, fMRI.

Eukaryotic Gene Expression

July 24–August 13

INSTRUCTORS
B. Dynlacht, Harvard University, Cambridge, Massachusetts
J. Goodrich, University of Colorado, Boulder
J. Lees, Massachusetts Institute of Technology, Cambridge
M. Timmers, University Medical Centre Utrecht, The Netherlands

ASSISTANTS
P. Danielian, Massachusetts Institute of Technology, Cambridge
M. Klejman, University Medical Centre Utrecht, The Netherlands
J. Rayman, Harvard University, Cambridge, Massachusetts

This course was designed for students, postdocs, and principal investigators who have recently ventured into the dynamic area of gene regulation. The course focused on state-of-the-art strategies and techniques employed in the field. Students made nuclear extracts, performed in vitro transcription, and measured RNA levels using primer extension. Emphasis was placed on biochemical studies of protein-DNA and protein-protein interactions. Detailed characterizations of the DNA-binding properties of site-specific transcription factors were carried out using electrophoretic mobility shift and DNase I footprinting assays. These assays were used to study protein-DNA interactions in crude extracts and using recombinant proteins. Coimmunoprecipitation and binding assays were employed to investigate protein-protein interactions with the general transcription machinery. During the past few years, the gene

regulation field has begun to emphasize the importance of in vivo approaches to studying protein-DNA and protein-protein interactions. Students were therefore exposed to the chromatin immunoprecipitation technique. Experience with basic recombinant DNA techniques was a prerequisite for admission to this course. Lectures by the instructors covered the current status of the gene expression field, theoretical aspects of the methodology, and broader issues regarding strategies for investigating the regulation of gene expression in eukaryotes. Guest lecturers discussed contemporary problems in eukaryotic gene regulation and technical approaches to their solution. The speakers this year included Laura Attardi, Marisa Bartolomei, Steve Buratowski, Ken Burtis, Mike Carey, Brian Dynlacht, Jim Goodrich, Shiv Grewal, Leemor Joshua-Tor, Bob Kingston, Jackie Lees, Lorenz Poellinger, Bob Roeder, Marc Timmers, Laszlo Tora, and Toshi Tsukiyama.

PARTICIPANTS

Agostinho, M., University of Libson, Portugal
Bahadur, U., B.Sc., M.S., Indian University of Science, Bangalore, India
Bubier, J., B.A., Marquette University, Milwaukee, Wisconsin
Corrigan, A., B.S., Aeomica Disease Profiling Nycomed, Amersham, Sunnyvale, California
Gartel, A., B.S., Ph.D., University of Illinois, Chicago
Giraldo, P., M.Sc., Centro Nacional de Biotechnologia, Madrid, Spain
Glisovic T., B.S., M.S., Pharmaceutical Biosciences, Uppsala, Sweden
Kossler, H., Dipl., Institut für Biochemie, Gottingen, Germany
Lieto, L., B.S., Ph.D., NIAID, National Institutes of Health, Rockville, Maryland
Melnick, A., M.D., Mt. Sinai Medical Center, New York
Nykanen, P., M.S., Turku Centre for Biotechnology, Finland
Richter, H., B.S., M.Sc., University of Copenhagen, Bagsvaerd, Denmark
Shureiqi, I., B.S., M.D., University of Texas, Houston
Skowronska-Krawczyk, D., M.S., Ph.D., University of Geneva, Switzerland
Stanhill, A., B.Sc., M.Sc., Hebrew University, Jerusalem, Israel
Upreti, M., M.S., Ph.D., Jawaharlal Nehru University, New Delhi, India

SEMINARS

Attardi, L., Stanford University, California: Identification of apoptosis-specific p53 target genes.
Bartolomei, M., University of Pennsylvania, Philadelphia: Mechanism of genomic imprinting.
Buratowski, S., Harvard Medical School, Boston, Massachusetts: Connecting transcription to chromatin and mRNA processing.
Burtis, K., University of California, Davis: Production and use of cDNA microarrays for analysis of gene expression in *Drosophila*.
Carey, M., University of California School of Medicine, Los Angeles: Enhanceosome assembly and function.
Dynlacht, B., Harvard University, Cambridge, Massachusetts: Transcriptional regulation during the mammalian cell cycle.
Goodrich, J., University of Colorado, Boulder: Mechanisms of human RNA ploymerase II transcription.
Grewal, S., Cold Spring Harbor Laboratory: Linking histone modifications to gene activity and more.
Joshua-Tor, L., Cold Spring Harbor Laboratory: Transcription factors in 3D in *Drosophila*.
Kingston, B., Harvard University, Boston, Massachusetts: A biochemical approach to epigenetic regulation: SWI/SNF and polycomb.
Lees, J., Massachusetts Institute of Technology, Cambridge: The in vivo roles of the E2F transcription factors.
Poellinger, L., Karolinska Institute, Stockholm, Sweden: Mechanism of action of stress-inducible transcription factors.
Roeder, B., The Rockefeller University, New York: Role of general and gene-specific coactivators in transcription.
Timmers, M., Utrecht University, The Netherlands: Analysis of basal transcription faction B-TFIID: Putting the heat on TBP.
Tora, L., IGBMC, Illkirch, France: The role of TAFs in regulation of RNA polymerase II transcription.
Tsukiyama, T., Fred Hutchinson Cancer Research Center, Seattle, Washington: Function of yeast ISWI chromatin remodeling complexes in transcription.

Imaging Structure and Function in the Nervous System

July 24–August 13

INSTRUCTORS
K. Delaney, Simon Fraser University, Burnaby, BC, Canada
V. Murthy, Harvard University, Cambridge, Massachusetts
K. Svoboda, Cold Spring Harbor Laboratory

ASSISTANTS
J. Burrone, Harvard University, Cambridge, Massachusetts
J. Bollmann, Max-Planck Institute for Medical Research, Heidelberg, Germany
I. Davison, Simon Fraser University, Burnaby, BC, Canada
S. Levi, Washington University, St. Louis, Missouri
S. Shi, University of California, San Francisco

Advances in light microscopy, digital image processing, and the development of a variety of powerful fluorescent probes present expanding opportunities for investigating the nervous system, from synaptic spines to networks in the brain. This intensive laboratory and lecture course provided participants with the theoretical and practical knowledge to utilize emerging imaging technologies. The primary emphasis of the course was vital light microscopy. Students learned the principles of light microscopy, as well as use of different types of electronic cameras, laser scanning systems, functional fluorophores, delivery techniques, and digital image-processing software. In addition to transmitted light microscopy for viewing cellular motility, the course examined a variety of molecular probes of cell function, including calcium-sensitive dyes, voltage-sensitive dyes, photo-activated ("caged") compounds, and exocytosis tracers. Issues arising in the combination of imaging with electrophysiological methods were covered. Particular weight was given to multiphoton laser scanning microscopy and to newly available biological fluorophores, especially green fluorescent protein (GFP) and its variants. A spectrum of neural

and cell biological systems, including living animals, brain slices, and cultured cells were used. Applicants had a strong background in the neurosciences and in cell biology. Lecturers in the 2001 course included Kerry Delaney, William Betz, Holly Cline, Ann Marie Craig, Richard Day, Winfried Denk, Joseph Fetcho, Paul Forscher, Laurence Katz, David Kleinfeld, Fred Lanni, Richard Lewis, Jeff Lichtman, Jerome Mertz, Venkatesh Murthy, Karel Svoboda, Roger Tsien, and Samuel Wang.

PARTICIPANTS

Brenowitz, S., B.A., Oregon Health Sciences University, Portland
Chang, S., B.S., Ph.D., Yale University-HHMI, New Haven, Connecticut
Franks, K., B.S., Ph.D., The Salk Institute, La Jolla, California
Guatimosim, C., B.S., Ph.D., Univeresidade Federal de Minas Gerais, Brazil
Hess, D., B.S., University of Pennsylvania School of Medicine, Philadelphia
Iwanaga, Y, , B.S., Ph.D., Max-Planck Institute for Brain Research, Frankfurt, Germany
Portera-Cailliau, C., B.A., Ph.D., Columbia University, New York
Stocca, G., M.S., Ph.D., Vanderbilt University, Nashville, Tennessee
Tashiro, Y., B.A., M.A., Osaka University, Japan
Tsao, D., B.S., Ph.D., Harvard Medical School, Boston, Massachusetts
Wyart, C., B.S., Ph.D., Laboratoire des Fluides Complex-es, Strasbourg, France
Zakharenko, S., M.D., Ph.D., HHMI, Columbia University, New York

SEMINARS

Betz, W., University of Colorado, Denver: Imaging secretion, FM-1-43.
Bollman, J., Max-Planck Institute for Medical Research, Heidelberg, Germany: Ca^{2+} uncaging.
Cline, H., Cold Spring Harbor Laboratory: In vivo imaging in tadpoles.
Craig, A.M., Washington University School of Medicine, St. Louis, Missouri: Immunofluorescence. Imaging synaptic proteins.
Day, R., University of Virginia, Charlottesville: GFP basics. Fret basic and applications.
Delaney, K., Simon Fraser University, Burnaby, Canada: Introduction to calcium imaging.
Denk, W., Max-Planck Institute for Medical Research, Heidelberg, Germany: 2-photon microscopy.
Fetcho, J., SUNY Stony Brook, New York: In vivo imaging in zebrafish.
Forscher, P., Yale University, New Haven, Connecticut: DIC microscopy.
Katz, L., Duke University Medical Center, Durham, North Carolina: Imaging, past and future.
Kleinfeld, D., University of California, La Jolla: In vivo imaging of blood flow.
Lanni, F., Carnegie-Mellon University, Pittsburgh, Pennsylvania: Fluorescence microscopy.
Lewis, R., Stanford University School of Medicine, California: Imaging, past and future.
Lichtman, J., Washington University, St. Louis, Missouri: Confocal microscopy.
Mertz, J., Ecole Superieure de Physique et de Chimie Industrielles, France: Nonlinear microscopies
Murthy, V., Harvard University, Cambridge, Massachusetts: Imaging secretion, FM-1-43. GFP probes to image secretion.
Svoboda, K., Cold Spring Harbor Laboratory: Basic optics. Applications of 2-photon.
Tsien, R., University of California, San Diego: GFP-based probes.
Wang, S., Princeton University, New Jersey: Uncaging.

Yeast Genetics

July 24–August 13

INSTRUCTORS	**D. Amberg,** SUNY Syracuse, New York **D. Burke,** University of Virginia, Charlottesville **O. Cohen-Fix,** NIDDK, National Institutes of Health, Bethesda, Maryland
ASSISTANTS	**Y. Green,** NIDDK, National Institutes of Health, Bethesda, Maryland **L. Grulich,** SUNY, Syracuse, New York **K. Ross,** NIDDK, National Institutes of Health, Bethesda, Maryland **L. Topper,** University of North Carolina, Chapel Hill

This course is a modern, state-of-the-art laboratory course designed to teach the students the full repertoire of genetic approaches needed to dissect complex problems in the yeast *Saccharomyces cerevisiae*. Combinations of classical genetic approaches are emphasized, including the isolation and characterization of mutants, tetrad analysis, complementation, and mitotic recombination. Molecular genetic techniques, including various types of yeast transformation, gene replacement with plasmids and PCR, construction and analysis of gene fusions, and generation of mutations in cloned genes, were also emphasized. Students used the classical and molecular approaches to gain experience in identifying and interpreting various kinds of genetic interactions including suppression and synthetic lethality. Students were immersed in yeast genomics and performed and interpret experiments with

DNA arrays. Students gained first-hand experience in modern cytological approaches such as epitope tagging and imaging yeast cells using indirect immunofluorescence, GFP-protein fusions, and a variety of fluorescent indicators for various subcellular organelles. Lectures on fundamental aspects of yeast genetics were presented along with seminars given by outside speakers on topics of current interest. This years speakers included Joaquin Arino, Kerry Bloom, Charlie Boone, Fred Cross, Dean Dawson, David Levin, Susan Michaelis, Jodi Nunnari, Mark Rose, Rodney Rothstein, Jeremy Thorner, and Mark Tyers.

PARTICIPANTS

Aulds, J., B.S., Ph.D., Upstate Medical University, Syracuse, New York
Baehr, A., M.S., Ph.D., University of Heidelberg, Germany
Bagnat, M., Ph.D., MPI-GBG, Dresden, Germany
Balcer, H., B.S., Ph.D., Brandeis University, Waltham, Massachusetts
Giota, F., M.Sc., Ph.D., Botanical Institute, Heverlee-Leuven, Belgium
Gottlieb, T., B.A., Ph.D., Fred Hutchison Cancer Research Center, Seattle, Washington
Hogan, S., B.S., M.S., Genome Therapeutics Corporation, Waltham, Massachusetts
Kumamoto, C., B.A., Ph.D.,Tufts University, Boston, Massachusetts
Lehtinen, S., M.Sc., Ph.D., University of Tampere, Finland
Li, L., B.S., M.S., St. John's University, Jamaica, New York
Santos, J., B.S., Ph.D., National Institutes of Environmental Health Sciences, Durham, North Carolina
Schneider, K., B.S., Ph.D., Massachusetts Institute of Technolgy, Cambridge
Schrift, G., B.S., University of Iowa, Iowa City
Van Berkum, N., B.S., M.S., University Medical Center Utrecht, The Netherlands
Wiedemann, N., Dipl., University of Freiburg, Germany
Witt, S., B.S., Ph.D., Louisiana State University Health Sciences Center, Shreveport

SEMINARS

Arino, J., Universidad Autonoma de Barcelona, Spain: Ppz phosphatases: Looking for a job.
Bloom, K., University of North Carolina, Chapel Hill: Chromosome and nuclear dynamics in yeast.
Boone, C., University of Toronto, Canada: SGA and functional genomics in yeast.
Tyers, M., Washington University, St. Louis, Missouri: Genome-wide synthetic lethal arrays for discovery of gene function and pathway mapping. More genome-wide synthetic lethal arrays for discovery of gene function and pathway mapping.
Cross, F., The Rockefeller University, New York: Strange arithmetic in genetic analysis of mitotic exit.
Dawson, D., Tufts University, Boston, Massachusetts: Finding your partner without loosing your sister; challenges for meiotic chromosomes.
Levin, D., Johns Hopkins University, Baltimore, Maryland: Cell wall integrity signaling.
Michaelis, S., Johns Hopkins Medical School, Baltimore, Maryland: Nonclassical biogenesis of yeast α-factor: CaaX processing and export via an ABC transporter.
Nunari, J., University of California, Davis: Regulation of mitochondrial behavior.
Rose, M., Princeton University, New Jersey: Nuclear (con)fusion during mating—Kar54 where you are?
Rothstein, R., Columbia University, New York: Genome gymnastics: Using recombination as a tool (and some biology, too!)
Stearns, T., Stanford University, California: The DNA damage checkpoint is nuclear limited in yeast.
Thorner, J., University of California, Berkeley: Septins, septin filament assembly, and a novel cell cycle checkpoint.

Cellular Biology of Addiction

August 7–13

INSTRUCTORS **R. Blakely,** Vanderbilt University School of Medicine, Nashville, Tennessee
B. Madras, Harvard Medical School, Southborough, Massachusetts
N. Volkow, Brookhaven National Laboratory, Upton, New York

ASSISTANT **G. Miller,** Harvard Medical School, Boston, Massachusetts

Drug addiction is the most costly neuropsychiatric disorder faced by our nation. The primary objectives of this workshop were to provide an intense dialogue of the fundamentals, state-of-the-art advances, and major gaps in the cell and molecular biology of drug addiction. Targeted to new or experienced investigators, the course combined formal presentations and informal discussions to convey the merits and excitement of cellular and molecular approaches to drug addiction research. With the advent of genomics and proteomics, an extraordinary opportunity now exists to develop comprehensive models of neuroadaptative processes fundamental to addiction, craving, and relapse to drug use and to brain function, in general. A range of disciplines and concepts were represented, including (1) current views on addiction; (2) the contribution of noninvasive brain imaging to identify drug targets and adaptive processes; (3) the association between neuroadaptative processes to drugs and behavioral manifestations; (4) linkage of genotype to drug response; (5) tolerance, sensitization, and adaptation at the cellular level; and (6) regulation of receptor, enzyme function, and associated neural activity. This workshop course provided an integrated view of current and novel research on neuroadaptive responses to addiction, fostered discussion on collaboration and integration, provided critical information needed to

construct a model of addiction as a disease, and molecular targets for biological treatments. Beyond the plane of scientific endeavor, the information from these approaches are vital for formulating public policy and for enlightening the public on the neurobiological consequences of drug use and addiction. This workshop course was designed to generate interest in cell and molecular biology, open conduits for collaborations and novel routes to investigating the neurobiology of addiction.

PARTICIPANTS

Benveniste, H., M.D., Ph.D., Brookhaven National Laboratory/ SUNY Upton, New York
Brown, J., B.S., Ph.D., New England Regional Primate Research Center, Southborough, Massachusetts
Caine, S.B., B.A., Ph.D., Harvard Medical School, Belmont, Massachusetts
Carey, R., B.A., Ph.D., VA Medical Center, Syracuse, New York
Corti, C., B.Sc., Ph.D., GlaxoSmithKline, Verona, Italy
Dawes, M., B.A., M.D., University of Texas Health Science Center, San Antonio
Falerio, L., Ph.D., Brown University, Providence, Rhode Island
Gulley, J., B.S., Ph.D., University of Colorado Health Sciences Center, Denver
Keefe, K., B.A., Ph.D., University of Utah, Salt Lake City
Lindsey, K., B.A., Ph.D., Emory University, Atlanta, Georgia
Lloyd, S., B.A., M.S., St. Jude Children's Research Hospital, Memphis, Tennessee
Maldonaldo-Vlaar, C., B.S., Ph.D., University of Puerto Rico, San Juan
Maraganore, D., B.S., M.D., Mayo Clinic, Rochester, Minnesota
Mayne, L., B.Sc., University of Sussex, Brighton, United Kingdom
Morrell, J., B.S., Ph.D., Rutgers University, Newark, New Jersey
Rahim, R., B.S., Ph.D., Temple University, Philadelphia, Pennsylvania
Rubakhin, S., M.Sc., Ph.D., University of Illinois, Urbana-Champaign
Schramm, N., B.S., Ph.D., Vanderbilt University, Nashville, Tennessee
Silveri, M., B.S., Ph.D., McLean Hospital, Belmont, Massachusetts
Taha, S., B.S., Ph.D., University of California, San Francisco
Thomas, M., B.A., Ph.D., University of Michigan, Ann Arbor
Whittington, R., B.A., M.D., Columbia University, New York
Xu, M., B.S., Ph.D., University of Cincinnati, Ohio
Yun, I., B.A., M.S., University of California, San Francisco

SEMINARS

Allen, R., Oregan Health Sciences University, Portland: Opioid regulation of cell responses.
Blakely, R., Vanderbilt University School of Medicine, Nashville Tennessee: The serotonin transporter: Structure, trafficking, and in vivo imaging.
Collins, A., University of Colorado, Boulder: A primer for using genetic strategies to study nicotine abuse.
Crabbe, J., Oregon Health Sciences University, Portland: Genes and their evironment.
Devi, L., New York University School of Medicine, New York: Receptor dimerization and functional consequences.
Edwards, R., University of California, San Francisco: Vesicular and membrane transporters: Modulators of transporters and synaptic activity.
Evans, C., Regents University of California, Los Angeles: Endogenous opiates in reward and adaptive responses to opiates.
Kreek, M.J., The Rockefeller University, New York: Peptide gene polymorphisms and potential implications for addiction to opiates.
Lester, H., California Institute of Technology, Pasadena: Ion channel receptors and transporters: Role in brain function.
Madras, B., Harvard Medical School, Southborough, Massachusetts: Brain imaging of psychostimulant targets: Relevance to cocaine addiction and medications development.
Pollack, J., National Institute of Drug Addiction, Baltimore, Maryland: NIDA support for genetic and molecular approaches to drug abuse.
Robinson, T., University of Michigan, Ann Arbor: Drug-induced neuroplasticity: Implications for motivational and emotional processes in addiction.
Rudnick, G., Yale University School of Medicine, New Haven, Connecticut: Transporter structure and function.
Self, D., University of Texas Southwestern Medical Center: Drug craving and relapse in drug addiction.
Tsien, R., University of California, San Diego: Monitoring and manipulating cell signals and proteins.
Volkow, N., Life Sciences, Brookhaven National Laboratory, Upton, New York: Imaging the effects of chronic drug use in human brain.
Von Zastrow, M., University of California, San Francisco: Differences in cellular response to opiates in vitro: A model for cellular tolerance?
Vrana, K., Wake Forest University Medical School, Winston-Salem, North Carolina: Functional genomics of substance abuse.
Wong, D., Johns Hopkins University, Baltimore, Maryland: Neurotoxic effects of drugs of abuse: Imaging, mechanisms, and correlation with neuropsychological tests.
Worley, P., Johns Hopkins University School of Medicine, Baltimore, Maryland: Immediate early genes and synaptic plasticity.

Macromolecular Crystallography

October 15

INSTRUCTORS **W. Furey,** VA Medical Center, Pittsburgh, Pennsylvania
G. Gilliland, National Institute of Standards and Technology, Gaithersburg, Maryland
A. McPherson, University of California, Irvine
J. Pflugrath, Rigaku/MSC, Inc., The Woodlands, Texas

Crystallography and X-ray diffraction yield a wealth of structural information unobtainable through other methods. This intensive laboratory/computational course focused on the major techniques used to determine the three-dimensional structures of macromolecules. It was designed for scientists with a working knowledge of protein structure and function, but who are new to macromolecular crystallography. Topics covered included crystallization (proteins, nucleic acids, and complexes), crystal characterization, X-ray sources and optics, synchrotrons, crystal freezing, data collection, data reduction, multiple isomorphous replacement, multiwavelength anomalous diffraction phase determination, solvent flattening, molecular replacement and averaging, electron density interpretation, structure refinement, molecular graphics, noncrystallographic summary, simulated annealing, and coordinate desposition. Participants learned through extensive hands-on experiments. They crystallized and determined a protein structure, along with lectures on the theory and informal discussions behind the techniques. Applicants were familiar with the creation and editing of simple text files on UNIX workstations, using a screen-based editor such as vi, emacs, or jot.

PARTICIPANTS

Adams, E., B.S., Ph.D., Stanford University School of Medicine, California
Bertero, M., Ph.D., University of British Columbia, Canada
Bohle, S., B.A., Ph.D., University of Wyoming, Laramie
Bredt, D., M.D., Ph.D., University of California, San Francisco
Celie, P., Ph.D., Netherlands Cancer Institute, Amsterdam
Edayathumangalam, R., B.Sc., M.Sc., Colorado State University, Fort Collins
Gibson, T., B.A., Ph.D., University of Wisconsin, Madison
Hubbard, J., B.Sc., Ph.D., GlaxoSmithKline, Stevenage, United Kingdom
Kosinski-Collins, M., B.S., Ph.D., Massachusetts Institute of Technology, Cambridge
Nielsen, R., B.A., University of Aarhus, Denmark
Ohren, J., B.A., M.S., Pfizer Global Research and Development, Ann Arbor, Michigan
Petros, A., B.S., Ph.D., Abbott Laboratories, Abbott Park, Illinois
Ramirez, U., B.S., Ph.D., Northwestern University, Chicago, Illinois
Shuman, S., B.A., Ph.D., Memorial Sloan-Kettering Cancer Institute, New York
Sironi, L., B.S., Ph.D., European Institute of Oncology, Milano, Italy
Vannini, A., B.S., M.S., Instituto di Richerche di Biologia Molecolare, Angeletti, Rome, Italy

SEMINARS

Adams, P., Lawrence Berkeley Laboratory, Berkeley, California: Simulated annealing refinement. Introduction to CNS.
Brodersen, D., MRC Laboratory of Molecular Biology, United Kingdom: Crystallographic methods for macromolecular assemblies. Insights from the atomic resolution structure of the 30S ribosomal subunit.
Dodson, E., University of York, United Kingdom: CCP4: System and tutorial.
Furey, W., VA Medical Center, Pittsburgh, Pennsylvania: Isomorphous replacement and anomalous scattering I. Isomorphous replacement and anamalous scattering II. Symmetry operators. Phase improvement by solvent flattening/negative density transaction. Crystallographic symmetry operations and their application. Noncrystallographic symmetry averaging. Structure determination and analysis of the pyruvate dehydrogenase. Multienzyme E1 complex.
Hendrickson, W., Columbia University, New York: MAD phasing: Theory and practice.
Hung, L.-W., Brookhaven National Laboratory, Upton, New York: Practical aspects of MAD methods. Solve tutorial.
Joshua-Tor, L., Cold Spring Harbor Laboratory: Crystal structures of two intermediates in the assembly of the papillomavirus replication and initiation complex. Structure presentation.
Kjeldgaard, M., Aarhus University, Denmark: Electron density fitting from A to O.
McPherson, A., University of California, Irvine: Crystallization of macromolecules I. Crystallization of macromolecules II. Vectors, waves, symmetry, and planes. Preliminary crystal characterization, crystallographic symmetry, and unit cells. Fundamental diffraction relationships I. Fundamental diffraction relationships II. Fourier transforms and the electron density equations. Patterson techniques. Heavy atoms and anomalous scatterers.
Navaza, J., CNRS-GIF, Gif-sur-Yvette, Switzerland: Molecular replacement.
Perrakis, A., NKI, Netherlands Cancer Institute, Amsterdam: ARP/wARP: The underlying concepts and algorithms for automated procedures for phase improvement, extension, and model building ARP/wARP: Implementation and examples of applications.
Pflugrath, J., Rigaku/MSC, Inc., The Woodlands, Texas: Data collection: Design and setup. Cryocrystallography. Data collection: Integration and post processing. Application of anomalous scattering from sulfur atoms.
Richardson, J., Duke University Medical Center, Durham, North Carolina; Richardson, D., Duke University Medical Center, Durham, North Carolina: Contact dots.
Sweet, R., Brookhaven National Laboratory, Upton, New York: Introduction to X-ray crystallography. X-ray sources and optics.
Tronrud, D., University of Oregon, Eugene: Macromolecular refinement I. Macromolecular refinement II. TNT tutorial.
Westbrook, J., Rutgers University, Piscataway, New Jersey: The protein data bank.
Xu, R.-M., Cold Spring Harbor Laboratory: Structural basis of NAD^+-dependent protein deacetylation.

Bioinformatics: Writing Software for Genome Research

October 17

INSTRUCTORS	**S. Rozen,** Whitehead Institute for Biomedical Research, Cambridge, Massachusetts **L. Stein,** Cold Spring Harbor Laboratory
ASSISTANTS	**A. Day,** University of California, Los Angeles **E. Birney,** EMBL-EBI, Hinxton, United Kingdom **J. Barnett,** Massachusetts Institute of Technology, Cambridge **R. Halgren,** Michigan State University, East Lansing **J. Kissinger,** University of Pennsylvania, Philadelphia **V. Mootha,** Whitehead Institute, Cambridge, Massachusetts **C. Mungal,** University of California, Berkeley **L. Palmer,** Cold Spring Harbor Laboratory

The desktop computer is rapidly becoming an indispensable tool in the biologist's toolchest. The success of the human genome project has created an explosion of information: billions of bits of biological information stashed electronically in databases around the globe just waiting for the right key to unlock them. New technologies such as DNA microarrays and high-throughput genotyping create an information overload that the traditional laboratory notebook cannot handle. To exploit the information revolution in biology, biologists must move beyond canned Web interfaces and Excel spreadsheets. They can take charge of the data by creating their own software to fetch, manage, and integrate it.

The goal of this course was to provide biologists with the tools needed to deal with this changing landscape. Designed for students and researchers with little prior knowledge of programming, this 2-week course taught the fundamentals of the Unix operating system, Perl scripting, dynamic Web page development with the CGI protocol, and database design. The course was combined with formal lectures with hands-on experience in which students work to solve a series of problem sets drawn from common scenarios in biological data acquisition, integration, and laboratory workflow management. For their final projects, students posed problems using their own data and worked with each other and the faculty to solve them.

PARTICIPANTS

Alcazar, R., B.S., Ph.D., Johns Hopkins University, Baltimore, Maryland
Brown, C., B.S., Ph.D., Washington State University, Pullman
Butland, S., B.Sc., M.Sc., University of British Columbia, Vancouver, Canada
Caudy, M., B.S., Ph.D., Burke Medical Research Institute of Cornell Medical College, White Plains, New York
Chang, J., B.S., Ph.D., Whitehead Institute/Massachusetts Institute of Technology, Cambridge
Futcher, B., B.Sc., SUNY Stony Brook, New York
Hong, N., B.S., Ph.D., University of California, San Francisco
Huang, S., B.S., Ph.D., Memorial Sloan-Kettering Cancer Center, New York
Johnston, D., B.Sc., Ph.D., The Natural History of Museum, London, United Kingdom
Jordan, B., B.A., Massachusetts Institute of Technology, Center for Cancer Research, Cambridge
Lamb, J., B.Sc., Ph.D., Dana-Farber Cancer Institute, Boston, Massachusetts
Miska, E., B.A., Ph.D., Massachusetts Institute of Technology, Cambridge
Moreno, C., B.S., Ph.D., Emory University, Atlanta, Georgia
Murphy, C., B.S., Ph.D., University of California, San Francisco
Nash, J., B.Sc., Ph.D., National Research Council of Canada, Ontario
Nelson, R., B.S., Ph.D., St. Louis University, Missouri
Palomino Mondragon, M., B.S., University of California, Irvine
Rathore, D., B.S., Ph.D., National Institutes of Health, Bethesda, Maryland
Reiter, L., B.S., Ph.D., University of California, San Diego
Schoepfer, R., M.D., University College London, United Kingdom
Singer, T., M.S., Ph.D., Cold Spring Harbor Laboratory
Tanurdzic, M., B.Sc., Ph.D., Purdue University, West Lafayette, Indiana
Taylor, K., B.S., Ph.D., Cedars Sinai Medical Center, Los Angeles, California
Wei, Y., B.S., Ph.D., Blackstone Technology Group, West Caldwell, New Jersey
Zhao, X., B.S., Ph.D., Cold Spring Harbor Laboratory
Zito, K., B.S., Ph.D., Cold Spring Harbor Laboratory

SEMINARS

Daly, M., Whitehead Institute/Massachusetts Institute of Technology, Cambridge: LD.
Gaasterland, T., The Rockefeller University, New York: Genomic analysis.
Hughey, R., University of California, Santa Cruz: HMMS.
Jamison, C., George Mason University, Manassas, Virginia: Object-oriented Perl.
Lewis, S., Lawrence Berkeley Laboratory, Berkeley, California: Gene ontologies.
Marth, G., National Institutes of Health/National Center for Biotechnolgy Information, Bethesda, Maryland: Sequence pipeline.
Pearson, W., University of Virginia, Charlottesville: Sequence alignment.
Pietzsch, R., Pfizer Global Research and Development, Groton, Connecticut: Practical and flexible database.
Slonim, D., Genetics Institute, Wyeth-Ayerst Research, Cambridge, Massachusetts: Expression topic.

Immunocytochemistry, In Situ Hybridization, and Live Cell Imaging

October 20–November 2

INSTRUCTORS
G. Matera, Case Western Reserve University, Cleveland, Ohio
J. Murray, University of Pennsylvania School of Medicine, Philadelphia
J. Swedlow, University of Dundee, United Kingdom

ASSISTANTS
A. Binnie, Oxford University, United Kingdom
M. Difilippantonio, National Cancer Institute/NIH, Bethesda, Maryland
T. Howard, University of New Mexico, Albuquerque
K. Hu, University of Pennsylvania, Philadelphia
M. Platani, University of Dundee, United Kingdom

This course focused on specialized techniques in microscopy, in situ hybridization, immunocytochemistry, and live cell imaging related to localizing DNA, RNA, and proteins in fixed cells as well as protein and RNA dynamics in living cells. The course emphasized the use of the latest equipment and techniques in fluorescence microscopy, including confocal laser scanning microscopy, deconvolution methods, digital image processing, and time-lapse imaging of living specimens. The course was designed to present students with state-of-the-art technology and scientific expertise in the use of light microscopy to address basic questions in 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 were the preparation of tagged nucleic acid probes, fixation methods, detection of multiple DNA sequences in single nuclei or chromosome spreads, comparative genomic hybridization, cellular localization of RNA, localization of nucleic acids and proteins in the same cells, use of a variety of reporter molecules and nonantibody fluorescent tags, indirect antibody labeling, detection of multiple proteins in a single cell, and the use of GFP variants to study protein expression, localization, and

dynamics. In each method, several experimental protocols were presented allowing the students to assess the relative merits of each and to relate them to their own research. Students were encouraged to bring their own nucleic acid, protein, or antibody probes to the course, which were used in addition to those provided by the instructors. The laboratory exercises were supplemented with lectures given by invited distinguished scientists, who presented up-to-the-minute reports on current methods and research using the techniques being presented.

PARTICIPANTS

Ben T., H., B.S., M.S., Biozentrum, Basel, Switzerland
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Groth, A., B.S., M.S., Danish Cancer Society, Copenhagen, Denmark
Henel, G., B.S., Mayo Graduate School, Rochester, Minnesota
Houston, A., B.S., Ph.D., University College Cork, Ireland
Jahnel, R., B.S., M.S., Freie Universtat Berlin, Germany
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Wei, C., B.S., Ph.D., University of Texas, M.D. Anderson Cancer Center, Houston

SEMINARS

Dernburg, A., Lawrence Berkeley National Laboratory, Berkeley, California: Chromosome FISH.
Fraser, S., California Institute of Technology, Pasadena: Imaging the cell movements and lineages of vertebrate embryos with light and MRI microscopy.
Matera, G., Case Western Reserve University, Cleveland, Ohio: RNA FISH.
Murray, J., University of Pennsylvania School of Medicine, Philadelphia: Basic Introduction to light and fluorescence microscopy. Immunocytochemistry.
Murray, J., University of Pennsylvania School of Medicine, Philadelphia; Swedlow, J., National Cancer Institute/NIH, Dundee, United Kingdom: Principles of confocal microscopy and deconvolution techniques.
Pederson, T., University of Massachusetts Medical School, Worcester: Tracking RNA molecules in the nucleus.
Ried, T., National Cancer Institute/NIH, Bethesda, Maryland: Mechanisms and consequences of chromosomal aberrations in cancer cells.
Singer, R., Albert Einstein Collge of Medicine, Bronx, New York: Cytoplasmic organization of mRNA.
Spring, K., National Heart, Lung, and Blood Institute/NIH, Bethesda, Maryland: Cameras and light sources.
Straight, A., Harvard Medical School, Boston, Massachusetts: Applications of green fluorescent protein in microscopy.
Swedlow, J., University of Dundee, United Kingdom: Live-cell imaging.

Computational Genomics

October 31–November 5

INSTRUCTORS	**W. Pearson,** University of Virginia, Charlottesville **R. Smith,** GlaxoSmithKline, King of Prussia, Pennsylvania
ASSISTANT	**A.J. Mackey,** University of Virginia, Charlottesville

Beyond BLAST and FASTA: This course presented a comprehensive overview of the theory and practice of computational methods for gene identification and characterization from DNA sequence data. The course focused on approaches for extracting the maximum amount of information from protein and DNA sequence similarity through sequence database searches, statistical analysis, and multiple sequence alignment. Additional topics included gene recognition (exon/intron prediction), identifying signals in unaligned sequences, and integration of genetic and sequence information in biological databases. The course combined lectures with hands-on exercises; students were encouraged to pose challenging sequence analysis problems using their own data. The course maked extensive use of local WWW pages to present problem sets and the computing tools to solve them. Students used Windows and Mac workstations attached to a Unix server; participants had to be comfortable using the Unix operating system and a Unix text editor. The course was designed for biologists seeking advanced

training in biological sequence analysis, computational biology core resource directors and staff, and scientists in other disciplines, such as computer science, who wish to survey current research problems in biological sequence analysis.

PARTICIPANTS

Brigido, M., B.A., Ph.D., Universidade de Brasilia, Asa norte, Brasilia
Chen, H.-C., B.S., M.S., National Center for Biotechnology Information, Bethesda, Maryland
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Guo, D., B.S., Ph.D., Virginia Bioinformatics Institute/Virginia Technology, Blacksburg
Hoffmann, H., B.S., Ph.D., AlphaGene, Lunenburg, Massachusetts
Huang, S., B.S., Ph.D., Memorial Sloan-Kettering Cancer Center, New York
Lovato, M., B.A., The Scripps Research Institute, La Jolla, California
Matise, T., B.S., Ph.D., Rutgers University, Piscataway, New Jersey
Miller III, H., B.S., Ph.D., E. Tennessee State University, Johnson City
Moloshok, T., B.S., Ph.D., Fox Chase Cancer Center, Philadelphia, Pennsylvania
Olofsson, P., M.S., Ph.D., Inflammation Research, Lund, Sweden
Park, Y., B.S., Ph.D., NCBI, National Institutes of Health, Bethesda, Maryland
Pear, W., B.A., Ph.D., University of Pennsylvania, Philadelphia
Strand, M., B.A., Ph.D., ARO/NIEHS, Research Triangle Park, North Carolina
Vincent, B., B.S., Louisana State University, New Orleans
Welsh, J., B.Sc., M.S., National Research Council, Halifax, Canada
Westerman, R., B.S., Purdue University, West Lafayette, Indiana
Xie, Q., B.S., Ph.D., GlaxoSmithKline, King of Prussia, Pennsylvania
Zhao, C., B.S., Ph.D., Marshfield Medical Research Foundation, Marshfield, Wisconsin
Zhao, Y., B.S., M.D, University of Southern California, Los Angeles

SEMINARS

Altschul, S., National Library of Medicine, Bethesda, Maryland: Statistics of sequence similarity scores. Iterated protein database searches with PSI-BLAST.
Ashburner, M., EMBL-EBI, Cambridge, United Kingdom: Gene ontologies—From structure to function. The *Drosophila* Genome Project—What have we learned.
Bateman, A., The Sanger Centre, Hinxton, United Kingdom: Introduction to protein family databases. Multiple sequence alignment with hidden Markov models.
Cooper, P., National Center for Biotechnology Information, Bethesda, Maryland: NCBI Databases. NCBI tools for genome analysis.
Pearson, W., University of Virginia, Charlottesville: Protein evolution—biology. Algorithms for pairwise sequence comparison. Motifs in protein and DNA sequences.
Smith, R., GlaxoSmithKline, King of Prussia, Pennsylvania: Introduction to multiple sequence alignment.
Yandell, M., Celera Genomics, Rockville, Maryland: Eukaryotic gene identification. Bioinformatics on a genome scale.

Phage Display of Combinatorial Antibody Libraries

November 6–19

INSTRUCTORS **C. Barbas,** The Scripps Research Institute, La Jolla, California
D. Siegel, University of Pennsylvania School of Medicine, Philadelphia
G. Silverman, University of California, San Diego

ASSISTANTS **R. Fuller,** Scripps Research Institute, La Jolla, California
C. Goodyear, University of California, San Diego
K. Noren, New England Biolabs, Beverly, Massachusetts
C. Tuckey, New England Biolabs, Beverly, Massachusetts

Recent advances in the generation and selection of antibodies from combinatorial libraries allow for the rapid production of antibodies from immune and nonimmune sources. This intensive laboratory/lecture course focused on the construction of combinatorial antibody libraries expressed on the surface of phage and selection of desired antibodies from the library. Students learned the theoretical and practical aspects of constructing combinatorial libraries from immune and nonimmune sources as well as the construction of synthetic antibody libraries. Antibodies were selected from the library by panning. Production, purification, and characterization of Fab fragments expressed in *E. coli* were also covered. Studies resulting in specific peptide-bearing phage were also performed.

The lecture series, presented by a number of invited speakers, emphasized the PCR of immunoglobulin genes, the biology of filamentous phage and the utility of surface expression libraries, expression of antibodies in *E. coli* and mammalian cells, antibody structure and function, catalytic antibodies, directed protein evolution, retroviral and cell display libraries, the immunobiology of antibody activity, and recent results on the use of antibodies in therapy. The theory and practical implications for selection from phage displayed libraries of random peptides, cDNA products, and semisynthetic proteins were also explored.

PARTICIPANTS

An, Z., B.S., Ph.D., Merck Reseach Laboratories, Rahway, New Jersey
Banerjee, C., B.Sc., Ph.D., Dyax Corp, Cambridge, Massachusetts
Boffey, J., B.Sc., Ph.D., Glasgow University, Scotland
Brown, E., B.Sc., Glasgow Caledonian University, Scotland
Eckert, D., B.S., Ph.D., Merck Research Labs, Rahway, New Jersey
Gerena, R., B.S., Ph.D., Immunicon Corporation, Huntingdon Valley, Pennsylvania
Handali, S., M.D., Tulane University, New Orleans, Louisiana
Huebner, J., M.D., Brigham & Women's Hospital, Boston, Massachusetts
Jeong, Y., Ph.D., Weill Medical College of Cornell University, New York
Knapp, B., M.S., Ph.D., Max-Planck Institute, Freiburg, Germany
Krah, R., B.A., Ph.D., IDEXX Laboratories Inc., Westbrook, Maine
Lovett, M., B.A., Howard Hughes Medical Institute and University of California, San Francisco
Mori, T., B.S., Ph.D., National Cancer Institute, Frederick, Maryland
Schmechel, D., Ph.D., National Institute for Occupational Safety and Health, Morgantown, West Virginia
Schmucker, D., B.S., Ph.D., Harvard Medical School and Dana-Farber Cancer Institute, Boston, Massachusetts
Wayner, E., B.S., Ph.D., Fred Hutchinson Cancer Research Center, Seattle, Washington

SEMINARS

Barbos, C., The Scripps Research Institute, La Jolla, California: Software and hardware for genomes: Polydactyl zinc finger proteins and the control of endogenous genes.
McHeyzer-William, M., Duke University Medical Center, Durham, North Carolina: Regulation and development of antibody responses in vitro.
Model, P., The Rockefeller University, New York: Biology of the filamentous phage.
Noren, C., New England Biolabs, Beverly, Massachusetts: Phage peptide libraries: The Ph.D. for peptides.
Pasqualini, R., University of Texas M.D. Anderson Cancer Center, Houston: In vivo panning.
Sidhu, S., Genetech, Inc., S. San Francisco, California: Mapping protein and cellular function with phage display.
Siegel, D., University of Pennsylvania School of Medicine, Philadelphia: Cell surface selection of combinatorial Fab libraries.
Silverman, G., University of California, San Diego: Repertoire cloning of SLE autoantibodies.
Wilson, I., The Scripps Research Institiute, La Jolla, California: Structural biology of the immune system.

The Laboratory would like to acknowledge the generosity of the following companies who loaned equipment and reagents to the various courses:

ALA Scientific Instruments, Inc.
A-M Systems Inc.
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Roche Diagnostics
SD Instruments Inc.
Santa Cruz Biotechnology Inc.
Savant Instruments
Schleicher & Schuell Inc.
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Technical Manufacturing Corp.
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Universal Imaging Corporation
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Waters Corp.
Western Technology Marketing, Inc.

SEMINARS

Invited Speaker Program

Each year, Cold Spring Harbor Laboratory invites speakers from outside the institution to present their latest findings on a weekly basis. These seminars keep the CSHL staff current on the latest developments and broaden their perspectives. Graduate students and postdoctoral fellows meet with the seminar speakers for lunch immediately after the seminar, allowing an opportunity for the exchange of ideas in an informal setting.

January

Dr. Michael Dustin, Washington University School of Medicine. The immunological synapse. (Host: Leemor Joshua-Tor)
Dr. Nat Heintz, The Rockefeller University. Molecular mechanisms of cerebellar development. (Host: Josh Huang)
Dr. William Newsome, HHMI/Stanford University School of Medicine. Making decisions: The brain's link between perception and action. (Host: Tony Zador)

February

Dr. Steven Henikoff, Fred Hutchinson Cancer Research Center. Targeted screening for induced mutations. (Host: Rob Martienssen)
Dr. Yi Xian Zheng, Carnegie Institution of Washington. The nucleation and organization of the microtubule cytoskeleton. (Host: Greg Hannon)
Dr. Joseph Nevins, Duke University Medical Center. Synergy in pathways that regulate G1 cell cycle progression. (Host: Michael Zhang)
Dr. David Allis, University of Virginia. Deciphering the histone code: A growing "tale" about four "tails." (Host: David Spector)

March

Dr. Timothy Nilsen, Case Western Reserve University. *Trans*-splicing: Novel SL RNP-specific factors and the splice site recognition problem. (Host: Greg Hannon)
Dr. Derek Van Der Kooy, University of Toronto. How to make a mammalian brain. (Host: Ed Stern)
Dr. Robert Benezra, Memorial Sloan-Kettering Cancer Center. Id proteins are required for tumor angiogenesis. (Host: Rob Lucito)
Dr. Nick Dyson, Massachusetts General Hospital Cancer Center. Control of cell proliferation by the E2F transcription factor. (Host: Scott Lowe)

April

Dr. Donald Ingber, Children's Hospital, Harvard Medical School. The mechanics of cell and tissue regulation. (Host: David Helfman)
Dr. Kevan Shokat, University of California, San Francisco. Unnatural ligands for engineered receptors: New tools for chemical genetics. (Host: Tatsuya Hirano)
Dr. Doug Hanahan, University of California, San Francisco. Acquired cells and capabilities mediating tumorigenesis in mice. (Host: Winship Herr)

October

Dr. Robert Desimone, NIMH, National Institutes of Health. How the brain pays attention. (Host: Tony Zador)
Dr. Sangram Sisodia, University of Chicago. Molecular neurobiology of Alzheimer's disease. (Host: Yi Zhong)

November

Dr. Lorraine Pillus, University of California, San Diego. Modifying chromatin to modulate chromosomes and gene expression. (Host: Shiv Grewal)

December

Dr. Gary Felsenfeld, NIDDK, National Institutes of Health. Establishment and maintenance of chromatin boundaries. (Host: Nouria Hernandez)

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. The seminars also afford a necessary opportunity for the graduate students and postgraduate staff to develop their skills in organizing, presenting, and defending their research.

January

Wun Chey Sin (Cline Lab): Rho in light-induced development.
Eric Drier (Yin Lab): Memory enhancement by a mammalian atypical PKC isoform, PKM-Zeta in *Drosophila melanogaster*.
Rui-Ming Xu: Structural basis of NAD-dependent protein deaceylation.
Marja Timmermans: Do plants get cancer?: Repression of stem cell fate during leaf development.

February

Maria Soengas (Lowe Lab): Struggling between life and death: Oncogenes, tumor suppressors and apoptosis.
Joanna Wysocka (Herr Lab): HCF-1 is targeted to chromatin by its VP16 interaction domain to promote dell proliferation.
Bill Tansey: Transcriptional regulation and the ubiquitin-proteasome pathway—or—do transcription factors have to die? Ub the Judge.

March

Bruce Stillman: Inheritance of the genome.
Scott Powers, Tularik Genomics/CSHL: Discovery of amplicons and candidate oncogenes.
Andrew Neuwald: Identifying determinants of protein structure and function through sequence CHAIN analysis.

April

Dmitri Chklovskii: Maps in your brain: What can we learn from them?

David Jackson: Outside control: Communication and proliferation in plant stem cells.
Arne Stenlund: Initiation of papillomavirus DNA replication proceeds through an ordered assembly pathway.

October

Shiv Grewal: Epigenetic control of higher-order chromatin assembly.
Michael Zhang: Computational molecular biology of gene expression and regulation.
T.C. Meng (Tonks Lab): Redox-dependent signal transduction: Signaling through reversible oxidation and inactivation of protein tyrosine phosphatases.

November

Jose Esteban (Malinow Lab): Receptor trafficking in the developing brain: A story of forced retention and conditional release.
Geraldine Pawlak (Helfman Lab): Interactions between cell signaling and the cytoskeleton during oncogenic transformation.

December

Mary Byrne (Martienssen Lab): Stem cell fate and organogenesis in *Arabidopsis*.
Eli Hatchwell (Spector Lab): Histone tail modifications during the initiation of X-chromosome inactivation.

BANBURY CENTER

BANBURY CENTER DIRECTOR'S REPORT

The Banbury Center continues to be used almost year-round. There were 26 meetings in the Banbury meetings program, and Laboratory staff came to the Center for seven internal meetings. The graduate students of The Watson School of Biological Sciences held in March a week-long course on evolution, and local community groups used our facilities on five occasions. Of the more than 650 participants in the meetings in 2001, 104 (14%) came from abroad. American participants came from 40 states, with 3 states—New York, California, and Maryland—providing one third of the visitors. The program of meetings was, as usual, catholic in its range of topics.

Science Policy

Napster is a good example of how the Internet is making, and provoking, extraordinary changes in the dissemination of anything that can be digitized. The world of scientific publishing is not immune from these changes and *Electronic Access to Scientific Literature* (John Inglis and Jan Witkowski, April 16–18) was held to examine ideas and initiatives intended to lower the barriers to electronic access to the scientific literature. Stimulated by the PubMed Central Initiative, the participants examined this and its implications for commercial publishers.

Great hope is being held for the applications of genomics to health care. This depends on having not only the sequence of the human genome, but also appropriate technologies for using that information and, just as importantly but often neglected, the appropriate health care and regulatory policies. The purpose of *Integrating Genomics Technologies in Health Care: Practice and Policy Challenges* (Finley Austin and Thame Kreiner, February 25–28) was to assess where the technology is heading; to educate participants in what is happening in fields other than their own; and to consider broader issues such as social impact, safety, and oversight.

Another health care area where practical applications are heavily dependent on policy is in vaccine development. *Making Vaccines for the Developing World: Access to and Deployment of New Technologies* (Melinda Moree and Philip K. Russell, October 9–11) examined the ways in which early access to enabling technologies is important for efficient and cost-effective vaccine development. There may be difficulty in gaining such access except in cases where the price of the vaccine can be set to pay for royalties on enabling technologies. There was discussion of what can be done to promote partnerships between public sector vaccine developers and the technology companies.

Genomics

The availability of complete genome sequences has spurred a tremendous increase in sequence analysis, but this is being done largely on an ad hoc basis, with Web Sites using different sets of nomenclature rules, query systems, and user interfaces. The information becomes fragmented, and it is difficult for biologists to know what is available. The *Genomic Annotation Workshop: DNA Replication* (Lincoln Stein and Ewan Birney, March 17–22) was the first in a series of workshops that will take a well-circumscribed topic—in this case DNA replication—and bring biologists expert in the topic together with informaticists. Together, they will create curated annotations of the genome that summarize the current state of knowledge in the field. The workshop was very successful and the results were presented at the *DNA Replication* meeting.

Cancer

Three meetings on cancer were held at Banbury in 2001, all dealing with important issues in cancer treatment. Two—*Interferons: Biological Mechanisms and Disease Treatments I* and *II*—dealt with the

Meier House provides accommodations for meeting participants at Banbury Center.

clinical uses of interferon. Organized by Josh Fidler, and by Ernest Borden and Judah Folkman, respectively, the meetings were particularly interesting in that they were not restricted to interferon and cancer, but reviewed the actions of interferon in virus infections as well as in multiple sclerosis. The meetings also discussed the clinical trials of interferon and whether extending the findings to multi-institutional Phase II trials will yield insights into the biology, pathology, and mechanisms of antitumor actions of interferons.

New Concepts for Clinical Cancer Trials (Douglas Hanahan, Jan Witkowski, Judah Folkman, Robert Kerbel, and Jim Pluda, November 4–7) discussed new findings indicating that the manner in which cancer treatments are given is important. It seems that different dosing schedules combined with lower doses could increase the efficacy and reduce the toxicity and side effects of traditional cytotoxic drugs, and perhaps also of radiotherapy and some investigational drugs. However, whereas a maximum tolerated dose is relatively easy to establish, it is not as clear how to determine optimal dosing at the lowest possible levels while maintaining maximal efficacy in these kinds of schedules. The possible benefits for patients are so great that these problems must be overcome.

The J.P. Morgan H&Q Executives Conference

David Deming and J.P. Morgan H&Q continue to support what is one of the highlights of the Banbury Center year. This year's meeting—*Controlling Cancer*—tackled the controversial idea that an all-out war on a patient's cancer by chemotherapy, radiation, and surgery—all of which cause considerable suffering to the patient—may not be the best strategy. Instead, it may be possible to contain cancer, treating it as a chronic disease. We had a marvelous set of speakers including Doug Hanahan, Scott Lowe, Bob Kerbel, Kari Alitalo, Raghu Kalluri, Brian Sawyers, and Joseph Schlessinger.

Neuroscience

The two Banbury Center meetings on neuroscience dealt with contrasting aspects of brain function. One—*Cortical Maps* (Dmitri Chklovskii and Alexei Koulakov, October 13–16)—was primarily experimental. The goal of the meeting was to stimulate discussion between neuroscientists working on different aspects of cortical maps: theoretical and experimental, physiological and anatomical, and developmental and functional.

In contrast, *Can a Machine Be Conscious?* (David Chalmers, Rod Goodman, Owen Holland, Christof Koch, and Jerome Swartz, May 13–16) dealt with a topic with strong psychological and philosophical underpinnings. A wealth of new experimental information about the brain has been gathered by neuroscientists, and the organizers felt that the time was ripe for a meeting to discuss how these data inform classic thinking on consciousness. The topic was focused by considering the design and construction of a conscious machine. Could a machine ever be said to be conscious? Or is consciousness something only biologically evolved animals and humans can ever possess?

Biology

Meetings on basic research in biology are the foundation of the Banbury Center program, and there were some especially interesting meetings in this category in 2001.

Discovered 10 years ago, various families of seven-transmembrane receptor genes have been identified that encode chemosensory receptors, for example, nematode and fruit fly odorant receptors, vomeronasal receptors, and taste receptors. With recent advances in genome sequencing projects, genome-wide studies of these families have become feasible. However, no meetings have been dedicated to the molecular biology of chemosensory receptor genes, which form the largest known gene families in animal genomes. *Molecular Biology of Chemosensory Receptors: The First Decade* (Peter Mombaerts and Stuart Firestein, March 11–14) was devoted to a review of all that is known of these molecules. In conjunction with this meeting, *Chemosensory Receptor Classification* (Peter Mombaerts, Stuart Firestein, and Randall Reed, March 15) was a workshop on possible approaches for classification of receptors and their families.

A meeting on *Mammalian Cloning: Biology and Practice* held at the Center in 2000 discussed technical aspects of the high failure rate of cloning. *Stability and Reversal of the Differentiated State* (Robin Lovell-Badge, David Stocum, and Jan Witkowski, October 28–31) concentrated on the biology that might underlie these failures, examining the fundamental biology of differentiation and its reversal. Participants worked on imprinting, X-inactivation, methylation, stem cells, germ cells, regeneration, and cloning in organisms as diverse as amphibia, mammals, and plants.

Epithelial and endothelial tubes are a fundamental structural unit of organ design, but very little is known about how epithelial and endothelial tubes form, how their sizes and shapes are regulated, and how tubular structures are maintained. *Epithelial and Endothelial Tube Morphogenesis* (Mark Krasnow and W. James Nelson, November 11–14) reviewed what is known from basic cell biological studies and genetic analysis of tube formation and maintenance in organisms as diverse as *C. elegans*, *Drosophila*, zebrafish, and mice, and the identification of genes involved in human tubulogenesis disorders.

The History and Mechanisms of Animal and Plant Evolution (John Doebley, Charles Marshall, and Nipam Patel, October 21–24) took on a big topic—how it is that plants and animals have come to be the way they are today. Participants included paleontologists, developmental biologists, geneticists, and morphologists. All were expected to contribute their own special knowledge to discussions of the relationship between micro- and macro-evolution, the early diversification of phyla, the genetic basis of evolutionary changes in morphology and development, and the adaptive forces underlying evolutionary change.

The theme of including animal and plant studies was continued in *Common and Contrasting Mechanisms of Pathogen Virulence and Host Resistance in Plant and Animal Diseases* (Jorge Galan and Brian Staskawicz, April 8–11). This discussion meeting brought together researchers working on micro-

bial pathogenesis and host resistance mechanisms in both plant and animal systems. Researchers working on animal cells have advantages in cell biology and biochemistry, whereas the plant community has led in the genetic dissection of host response to pathogen infection. Key questions discussed included whether plant and animal pathogens share common mechanisms of pathogenesis and whether the signaling mechanisms involved are conserved between plant and animal hosts.

Neurological Disorders

The Fragile X syndrome is one of the best-characterized developmental genetic disorders, and rapid advances are being made in understanding the biological processes involved. *Understanding the Neural Basis of Fragile X* (William Greenough, David Nelson, and Don Bailey, March 4–7) reviewed the data coming from the wide range of studies on various aspects of basic and clinical research relating to the neural basis of Fragile X, including the nervous system phenotype in Fragile X syndrome and animal models, and FMRP synthesis, regulation, and the mRNAs to which it binds.

The other two meetings in this category discussed neurological disorders that are poorly characterized. Autism is a particularly controversial subject, especially in relation to its possible environmental causes. *Microbiology, Immunology, and Toxicology of Autism and Other Neurodevelopmental Disorders* (Ian Lipkin, February 11–14) tackled a broad range of topics relating to autism, including the genetics and neurobiology of autism, the general issue of the toxicology of neurodevelopmental disorders, and the possible role of vaccines as a cause of autism.

Another major neurological disorder that remains intractable is depression, and one area that has been especially neglected is childhood depression. Banbury took a small step to rectify this by holding a meeting on *Childhood Depression: A Critical Review* (Boris Birmaher and Ian Goodyer, February 20–23). First-episode depression occurs in the 8- to 18-year age group, and therefore this is an impor-

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tant group to study from an etiological as well as clinical perspective. The meeting examined features such as why it is that depressions are uncommon in prepubertal children compared to postpubertal adolescents, and why childhood depressives do not respond to antidepressants, whereas adolescent depressives do.

Workshop for Judges

Banbury Center has always had a strong interest in the public understanding of science, having had workshops for science journalists and congressional staff for many years. These have continued in the *Basic Issues of Science* workshops, held in conjunction with the Federal Judicial Center in Washington. The workshops do not deal with the role of science in the courtroom but rather introduce the concepts and styles of science to the judges. This was, as always, a delightful meeting, and one highlight was the presentation and discussion by Peter Neufeld, who established the Innocence Project with Barry Scheck. (Peter Neufeld first came to Banbury for the 1989 meeting on *DNA and Forensic Science*, a historic meeting that had an important role in the early debates about DNA fingerprinting.)

The American Eugenics Image Archive Project

This project, a collaborative effort with the Dolan DNA Learning Center, is proving to be a great success. In 2001, Dave Micklos and I implemented a further step in the project with a meeting *American Eugenics and the New Biology: Perspectives and Parallels* (David Micklos and Jan Witkowski, May 6–8). The meeting provided an opportunity for opinion leaders and policymakers from government, science, health care, education, and the mass media to learn about America's past involvement in eugenics from leading experts. As we had hoped, the eclectic mix of high-level participants and presenters created lively exchanges.

We also held a meeting of the Project's Eugenics Advisory Board (David Micklos and Jan Witkowski, September 30–October 1). This was a most important meeting in that the Board recommended to the National Institutes of Health that the site be made public in its entirety.

The Watson School of Biological Sciences—Topics in Biology

Banbury Center again held the week-long *Topics in Biology* course for students of The Watson School of Biological Sciences. This year the topic was evolution, and the lead instructor was Nipam Patel. The breadth of the course was great, running from reviewing the immense variety of life to the details of molecular evolution. A highlight of the week was a visit to the American Museum of Natural History.

Other Meetings

Several Laboratory groups came to Banbury for review and planning meetings, as did the Dolan DNA Learning Center and the Cold Spring Harbor Laboratory Press. As usual, we made the Center available to a small number of local community groups, notably Lloyd Harbor Village, The Cold Spring Harbor School District, and Holiday House.

Meier House

The Meier House was purchased by the Laboratory in 1999 to provide additional accommodation for Banbury Center meetings and to serve as a place where writers could come for periods of time to work in peace on their books. It has been an invaluable addition to the Banbury estate, and there is no doubt that participants in our meetings have enjoyed using it.

Funding

The sources of support for Banbury Center meetings are given for each meeting in the following list of programs. The Center and the scientists who participated in our meetings are very grateful for the generosity of all those institutions that provided this support.

Acknowledgments

Participants in Banbury Center meetings invariably make two comments. The first is on the beauty of the estate, and the second, on how well the meetings are organized. The praise belongs to the staff of the Center and the Laboratory. Bea Toliver, Ellie Sidorenko, and Katya Davey make sure that the meetings run properly, and Chris McEvoy, Andy Sauer, and Joe Ellis keep the estate looking beautiful. The staff of the Audiovisual Department helped the meetings run smoothly by dealing with the complexities of matching computers and PowerPoint. The Meetings Office works with us on the increasingly difficult task of interleaving the Grace Auditorium and Banbury Center meetings, and Housekeeping has looked after all the visitors for us. Finally, the Center could not work at all without the enthusiasm of its organizers and participants.

Jan Witkowski

Entrance to Banbury Center

Microbiology, Immunology, and Toxicology of Autism and Other Neurodevelopmental Disorders

February 11–14

FUNDED BY **Cure Autism Now Foundation, McNeil Consumer Healthcare, National Alliance for Autism Research (NAAR), and the University of California, Davis M.I.N.D. Institute**

ARRANGED BY **W.I. Lipkin,** University of California, Irvine

Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
W.I. Lipkin, University of California, Irvine

SESSION 1: Scope of the Problem: Epidemiology of Autism and Neurodevelopmental Disorders
Co-Chairpersons: G. Dawson, University of Washington, Seattle and **J. Cordero,** Centers for Disease Control and Prevention, Atlanta, Georgia

E. Susser, HIV Center for Clinical and Behavioral Studies, New York, New York: Epidemiological approaches to neurodevelopmental disorders.
E. Fombonne, Institute of Psychiatry, King's College London, United Kingdom: Epidemiology of autism.
B. Taylor, Royal Free & University College Medical School, London,United Kingdom: MMR in the UK and prevalence of autism spectrum disorders.
M. Yeargin-Allsopp, Centers for Disease Control and Prevention, Atlanta, Georgia: Past and future perspectives in autism epidemiology.
J. Cordero, Centers for Disease Control and Prevention, Atlanta, Georgia: Review of epidemiology presentations.

SESSION 2: Host Factors: Genetics of Autism
Chairperson: E. London, National Alliance for Autism Research, Princeton, New Jersey

C. Gilliam, Columbia Genome Center, New York, New York: Genetics of autism (AGRE Project).
M.A. Pericak-Vance, Duke University Medical Center, Durham, North Carolina: Genetics.
K.G. Becker, National Institute on Aging, Baltimore, Maryland: Comparative genomics of autism, Tourette's Syndrome, dyslexia, and immune disorders.

E. Fombonne, E. Susser

SESSION 3: Anatomy and Neurobiology of Autism
Chairperson: D.G. Amaral, University of California, Davis, Sacramento

T.L. Kemper, Boston University School of Medicine, Massachusetts: Neuropathology of autism.
N. Minshew, University of Pittsburgh Medical Center, Pennsylvania: Cortical dysfunction in autism.
D.C. Chugani, Children's Hospital of Michigan, Detroit: Neurochemical pathways in autism.
G. Dawson, University of Washington, Seattle: Early indices of brain dysfunction in autism.
E. Courchesne, University of California, San Diego, La Jolla: Functional and structural imaging studies in autism.

SESSION 4: Immunology of Neurodevelopmental Disorders
Chairperson: W.I. Lipkin, University of California, Irvine

S.E. Swedo, National Institute of Mental Health, Bethesda, Maryland: PANDAS in OCD spectrum disorders.
W.I. Lipkin, University of California, Irvine: A murine PANDAS model.
E. Hollander, Mount Sinai School of Medicine, New York, New York: D8/17 and autism.
S. Jacobson, National Institute of Neurological Disorders & Stroke, Bethesda, Maryland: Viral paradigms for chronic CNS diseases.
K.B. Nelson, National Institute of Neurological Disorders & Stroke, Bethesda, Maryland: Neonatal predictors of neurodevelopmental disorders.

SESSION 5: Animal Models
Chairperson: S. Jacobson, National Institute of Neurological Disorders & Stroke, Bethesda, Maryland

M. Hornig, University of California, Irvine: Infectious and immune factors in neurodevelopmental damage.
K.M. Carbone, Food and Drug Administration, Bethesda, Maryland: Viral immune models of autism.
D.G. Amaral, University of California, Davis, Sacramento: The role of the amygdala in social behavior: Implications for autism.
L.R. Young, Emory University, Atlanta, Georgia: Social interactions in prairie voles.

SESSION 6: Toxicology of Neurodevelopmental Disorders
Chairperson: N. Minshew, University of Pittsburgh Medical Center, Pennsylvania

M. Aschner, Wake Forest University School of Medicine, Winston-Salem, North Carolina: Neuropathogenesis of mercury intoxication.
S. Barone, Environmental Protection Agency, Research Triangle Park, North Carolina: Effects of gestational mercury exposure on neurotrophic factor signaling and altered development of the nervous system.
S. Bernard, ARC Research, Cranford, New Jersey: Mercury and autism pathogenesis.

SESSION 7
Co-Chairpersons: W.I. Lipkin, University of California, Irvine and **E. Susser,** HIV Center for Clinical and Behavioral Studies, New York, New York

A.J. Wakefield, Royal Free & University College Medical School, London, United Kingdom: MMR, the gut, and autism.
J. O'Leary, Coombe Women's Hospital, Dublin, Ireland: Molecular detection of measles virus sequences in white cells and gastrointestinal tissues of children with neurodevelopmental disorders.
M.A. Afzal, National Institute for Biological Standards & Control, Herts, United Kingdom: Vaccines, Crohns disease, and autism.
F. DeStefano, Centers for Disease Control & Prevention, Atlanta, Georgia: Review of vaccines and autism epidemiology.
W.I. Lipkin, University of California, Irvine: Closing summary.

E. Hollander, S. Swedo, B. Taylor

Childhood Depression: A Critical Review

February 20–23

FUNDED BY **Cold Spring Harbor Laboratory**

ARRANGED BY **B. Birmaher,** University of Pittsburgh Medical Center, Pennsylvania
I.M. Goodyer, University of Cambridge, United Kingdom

Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Conceptual Aspects
Chairperson: I.M. Goodyer, University of Cambridge, United Kingdom

S.J. Suomi, National Institute of Child Health and Human Development, Bethesda, Maryland: Childhood depression: A critical review.

L. Wolpert, University College London, United Kingdom: The evolutionary psychology of depression.

R. Hornsby, Sir Robert Mond Memorial Trust, London, United Kingdom: A personal perspective (on adolescent despair).

SESSION 2: Clinical Aspects
Co-Chairpersons: I.M. Goodyer, University of Cambridge, United Kingdom and **L. Wolpert,** University College London, United Kingdom

J. Kaufman, Yale University, New Haven, Connecticut: Are child-, adolescent-, and adult-onset depression one and the same disorder?

P.J. Ambrosini, MCP-Hahnemann University, Philadelphia, Pennsylvania: Irritability in adolescent affective disorders.

E. Fombonne, King's College London, United Kingdom: Long-term outcomes of child and adolescent depression: The Maudsley study.

I. Goodyer, L. Wolpert, J. Costello

SESSION 3: Biological Studies
Chairperson: L. Wolpert, University College London, United Kingdom

A.C. Angold, Duke University Medical Center, Durham, North Carolina: The roles of various types of stressor, parental depression, testosterone, and estradiol in relation to changing rates of depression.
J.A. Graber, Columbia University, New York, New York: Psychosocial predictors of depression in adolescents.
B. Birmaher, University of Pittsburgh Medical Center, Pennsylvania: Biological studies in never depressed children at high risk to develop MDD.

SESSION 4: Social Environment Influences
Chairperson: B. Birmaher, University of Pittsburgh Medical Center, Pennsylvania

J. Costello, Duke University, Durham, North Carolina: Child and adolescent depression: Prodromal signs and symptoms.
J. Garber, Vanderbilt University, Nashville, Tennessee: Psychosocial predictors of depression in adolescents.
M.A. Rueter, University of Minnesota, St. Paul: Familial influences on early (prepubertal) versus later (postpubertal) first onset of MDE.
D. Williamson, University of Pittsburgh Medical Center, Pennsylvania: Environmental risk factors for depression in children and adolescents.

SESSION 5: Affective-Cognitive Processes
Co-Chairpersons: B. Birmaher, University of Pittsburgh Medical Center, Pennsylvania and **E. Fombonne,** King's College London, United Kingdom

L. Murray, University of Reading, United Kingdom: Identifying cognitive vulnerability to depression in five-year-olds.
I. M. Goodyer, University of Cambridge, United Kingdom: Are sensitive minds bad for the brain? Mood-linked cognitions as psychoendocrine insults.
L. Mufson, New York State Psychiatric Institute, New York: IPT-A: Translating efficacy into effectiveness research.

SESSION 6: Pharmacological Treatment
Chairperson: E. Fombonne, King's College London, United Kingdom

N. Ryan, University of Pittsburgh Medical Center, Pennsylvania: SSRIs treat child and adolescent major depression while TCAs are ineffective: Is this a real difference in noradrenergic versus serotonergic therapeutic strategies or is it merely an artifact of available studies?
R.A. Kowatch, University of Cincinnati Medical Center, Ohio: Pharmacological trials in pediatric bipolars: Is placebo necessary?

SESSION 7: Family/Genetic Approaches
Chairperson: S.J. Suomi, National Institute of Child Health and Human Development, Bethesda, Maryland

R.D. Todd, Washington University School of Medicine, St. Louis, Missouri: Gene-environment interactions in the development of early-onset depression: Results from an epidemiologically based twin study of brain morphometry.
M.M. Weissman, Columbia University, New York, New York: Children and depression: Results from top-down and bottom-up studies.

SESSION 8: Neuroimaging
Chairperson: S.J. Suomi, National Institute of Child Health and Human Development, Bethesda, Maryland

H. Blumberg, Yale University, New Haven, Connecticut: Structural and functional MRI studies of bipolar disorder in adolescents and adults.
K.N. Botteron, Washington University School of Medicine, St. Louis, Missouri: Structural brain differences in early-onset depression: Neurodevelopmental or neurodegenerative mechanisms.

Integrating Genomics Technologies in Health Care: Practice and Policy Challenges

February 25–28

FUNDED BY **Burroughs Wellcome Fund, Hoffmann-La Roche Inc., and Affymetrix, Inc.**

ARRANGED BY **M.J.F. Austin,** Hoffmann-La Roche Inc., Nutley, New Jersey
T. Kreiner, Affymetrix, Inc., Santa Clara, California

Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
M.J.F. Austin, Hoffmann-La Roche Inc., Nutley, New Jersey

SESSION 1: Technologies and Analysis
Chairperson: L. Babiss, Hoffmann-La Roche Inc., Nutley, New Jersey

J. Warrington, Affymetrix, Inc., Santa Clara, California: Microarray applications in diagnostics.
W.H. Koch, Roche Molecular Systems, Alameda, California: Microarray-based genotyping of polymorphic drug metabolism: Pharmacogenetics lessons galore.
E. Lai, Glaxo Wellcome Inc., Research Triangle Park, North Carolina: Application of SNP technologies in health care: What have we learned and what are the challenges?
E.S. Winn-Deen, Celera Genomics, Rockville, Maryland: Diagnostics in the postgenomic era.
J. Hoh, Rockefeller University, New York, New York: Problems in the analysis of microarray data from first principles.
J.D. Terwilliger, Columbia University, New York, New York: Why the HGP will likely have minimal if any effect on public health and medicine in the foreseeable future.

SESSION 2: Applications I
Chairperson: U. Francke, HHMI, Stanford University Medical Center, California

N.C. Dracopoli, Bristol-Myers Squibb, Princeton, New Jersey: Application of pharmacogenomics in clinical drug development.
C. Dykes, Variagenics, Inc., Cambridge, Massachusetts: Candidate gene studies within clinical development.
N.A. Holtzman, The Johns Hopkins Medical Institutions, Baltimore, Maryland: Separating genotype from genohype: Will we be able to predict and prevent common complex diseases?
B.R. Korf, Partners Center for Human Genetics and Harvard Medical School, Boston, Massachusetts: Integration of genetics into medical practice.
D.K. Burns, Glaxo Wellcome Inc., Research Triangle Park, North Carolina: Moving genetics from research to impacting clinical practice: The GlaxoSmithKline perspective.

M. Austin, D. Burns, E. Winn-Deen

SESSION 3: Applications II
Chairperson: U. Francke, HHMI, Stanford University Medical Center, California

A. Guttmacher, National Human Genome Research Institute, Bethesda, Maryland: From research laboratory to neighborhood health center: The NHGRI's efforts to translate DNA into health care.

C.C. Morton, Brigham & Women's Hospital, Boston, Massachusetts: Genomic resources applied in the clinical cytogenetics laboratory.

W. Uhlmann, University of Michigan, Ann Arbor: Genetic counseling in our current health care system.

G. Wiesner, Case Western Reserve University, Cleveland, Ohio: The GREAT system: Linking genomics to primary care.

R.L. White, DNA Sciences, Inc., Fremont, California: DNA Sciences' Gene Trust Project.

SESSION 4: Regulatory and Policy Issues
Chairperson: L. Knowles, The Hastings Center, Garrison, New York

G. Koski, National Institutes of Health, Rockville, Maryland: Realism and regulation of research in the genomics era.

P.F. Terry, Genetic Alliance, Washington, D.C.: Policy issues: A consumer perspective.

B. Koenig, Stanford University, California: Update on the Secretary's Advisory Committee on Genetic Testing (SACGT); Identifying "Race": Looking through a genomics prism.

J. Doll, U.S. Patent and Trademark Office, Arlington, Virginia: The ground rules for genomic patenting.

H.T. Greely, Stanford University Law School, California: Regulatory and health financing constraints on clinical genomics.

SESSION 5: Closing Discussion: Managing the Future—A Group Discussion to Identify Key Needs and Next Steps
Interlocutors: T. Kreiner, Affymetrix, Inc., Santa Clara, California and **M.J.F. Austin,** Hoffmann-La Roche Inc.

L. Babiss, Hoffmann-La Roche Inc., Nutley, New Jersey: Technology and analysis key trends.

U. Francke, HHMI, Stanford University Medical Center, California: Applications and clinical applications key trends.

L. Knowles, The Hastings Center, Garrison, New York: Regulatory and policy issues.

Meeting in session.

Understanding the Neural Basis of Fragile X

March 4–7

FUNDED BY **National Institute of Mental Health (NIMH), with additional support from National Institute of Child Health and Human Development (NICHD) (through a grant to the University of Illinois, Urbana)**

ARRANGED BY **W. Greenough,** University of Illinois, Urbana
D.L. Nelson, Baylor College of Medicine, Houston, Texas
D. Bailey, University of North Carolina at Chapel Hill

Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
K. Clapp, FRAXA Research Foundation, Newburyport, Massachusetts

SESSION 1: Human Phenotype
Chairperson: L. Crnic, University of Colorado Health Sciences Center, Denver

R.J. Hagerman, MIND Institute, University of California, Davis Medical Center, Sacramento: Spectrum of involvement in Fragile X syndrome and in permutation area.

D. Bailey, University of North Carolina, Chapel Hill: Opportunities for integrative biobehavioral research in Fragile X syndrome.

E. Berry-Kravis, Rush Children's Hospital, Chicago, Illinois: Pharmacological approaches to seizures, behavior, and cognition in Fragile X syndrome: Present and future.

SESSION 2: Animal Phenotype
Chairperson: D. Bailey, University of North Carolina, Chapel Hill

W.T. Greenough, University of Illinois, Urbana: FMRP expression and neural morphology.

W.T. Brown, New York State Institute for Basic Research, Staten Island: Toward an improved mouse model of Fragile X.

B. Oostra, Erasmus Universiteit Rotterdam, The Netherlands: Mouse models for Fragile X syndrome.

L. Crnic, University of Colorado Health Sciences Center, Denver: Abnormalities in the auditory startle response in FMR1-targeted mutants on several genetic backgrounds.

R.E. Paylor, Baylor College of Medicine, Houston, Texas: More behavioral phenotyping of mouse models of Fragile X.

H. Siomi, University of Tokushima, Japan: Cell culture and whole animal approaches to understanding the function of the FMR1 protein in *Drosophila*.

J.R. Larson, University of Illinois, Chicago: Olfactory learning and memory in mice.

K. Clapp, W. Greenough

SESSION 3: FMR1 mRNA Transport, Regulation of FMRP Synthesis, and Interacting Proteins
Chairperson: W.T. Greenough, University of Illinois, Urbana

D.L. Nelson, Baylor College of Medicine, Houston, Texas: Consequences of mutation of the *FMR1/FXR* genes in mouse and fly.

G.J. Bassell, Albert Einstein University, Bronx, New York: FMRP trafficking in cultured hippocampal neurons.

G. Neri, Universita Cattolica, Rome, Italy: Pharmacological reactivation of the fully mutated *FMR1* gene.

P. Chiurazzi, Universita Cattolica, Rome, Italy: Methylation analysis of the promoter of the inactive and reactivated fully mutated *FMR1* gene.

P.J. Hagerman, University of California, Davis: Expression of the Fragile X gene.

K.M. Huber, Brown University, Providence, Rhode Island: Role for dendritic protein synthesis in hippocampal long-term depression.

K.J. Mack, University of Wisconsin, Madison: Sensory experience increases, and seizures reduce, expression of the Fragile X mental retardation protein.

R.P. Bauchwitz, St. Luke's-Roosevelt Institute of Health Sciences, New York: Further analysis of Fmr1 transgenic and knockout mice.

R.F. Kooy, University of Antwerp, Belgium: Differentially expressed sequences in the Fragile X knockout mouse.

S.T. Warren, Emory University School of Medicine, Atlanta, Georgia: DNA chip analysis of FMRP function and the consequence of its absence.

SESSION 4: Cellular Functions of FMRP
Chairperson: D.L. Nelson, Baylor College of Medicine, Houston,Texas

I.J. Weiler, University of Illinois, Urbana-Champaign: Some translational deficits in Fmr-1 knockout.

M.C. Rattazzi, New York State Institute for Basic Research, Staten Island: Toward gene therapy of Fragile X syndrome.

SESSION 5: Interacting RNAs and Proteins
Chairperson: P.J. Hagerman, University of California, Davis

B. Bardoni, CNRS, Illkirch, France: Search for FMRP function through the characterization of four novel FMRP-interacting proteins.

H. Moine, CNRS, Strasbourg, France: Characterization of a specific binding target for FMRP on its own mRNA.

J. Darnell, Rockefeller University, New York, New York: Identification of sequence-specific RNA targets for KH2 and newly characterized high-affinity RNA-binding domains of FMRP.

A.T. Hoogeveen, Erasmus University Medical School, Rotterdam, The Netherlands: FMRP and its related proteins.

E.W. Khandjian, Hop. St. Francois d'Assise, Quebec, Canada: The expanding Fragile X protein family: RNA-binding proteins and more?

Coffee break on back porch of Banbury Conference Center.

Molecular Biology of Chemosensory Receptors: The First Decade

March 11–14

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program, with additional support from Senomyx, Inc.**

ARRANGED BY **P. Mombaerts,** The Rockefeller University, New York, New York
S. Firestein, Columbia University, New York, New York

Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Opening Lecture:
L.B. Buck, Harvard Medical School, Boston, Massachusetts

SESSION 1: Mammalian Odorant Receptors: Genomics
Chairperson: P. Mombaerts, The Rockefeller University, New York, New York

D. Lancet, Weizmann Institute of Science, Rehovot, Israel: Mining the genome: The complete human olfactory receptor repertoire.
S. Zozulya, Senomyx, Inc., La Jolla, California: The repertoire of human functional odorant receptor candidates.
B.J. Trask, Fred Hutchinson Cancer Research Center, Seattle, Washington: Comparative OR genomics: Large-scale change and polymorphism.
J. Strotmann, University of Hohenheim, Stuttgart, Germany: Olfactory receptors with atypical features.
S. Firestein, Columbia University, New York, New York: Molecular pharmacology of odor receptors.

SESSION 2: Other Chemosensory Receptors
Chairperson: R. Axel, HHMI, Columbia University, New York, New York

R. Tirindelli, Universita di Parma, Italy: The coexpression of putative pheromone receptors suggests another mode of information processing in the olfactory systems.
L. Vosshall, The Rockefeller University, New York, New York: Determinants of spatial convergence in the *Drosophila* olfactory system.
H. Amrein, Duke University Medical Center, Durham, North Carolina: Identification and expression of a large family of *Drosophila* taste receptor genes.
C.I. Bargmann, University of California, San Francisco: Odor discrimination and olfactory receptor expression in *C. elegans*.
H. Robertson, University of Illinois at Urbana-Champaign: Molecular evolution of the nematode and insect chemoreceptor superfamilies.

L. Buck, R. Axel, N. Ryba

SESSION 3: Taste Receptors
Chairperson: M. Zoller, Senomyx, Inc., La Jolla, California

J.F. Battey, National Institute on Deafness and Other Communication Disorders, Bethesda, Maryland: Discovery of genes selectively expressed in taste receptor cells.
N. Ryba, National Institute of Dental and Craniofacial Research, Bethesda, Maryland: Bitter taste receptors.
N. Chaudhari, University of Miami School of Medicine, Florida: Comparing *umami* taste with the properties of a cloned taste receptor.

SESSION 4: Odorant Receptors: Molecular Biology
Chairperson: R.F. Margolskee, HHMI, Mount Sinai School of Medicine, New York, New York

A. Chess, Whitehead Institute, Cambridge, Massachusetts: Genome-wide coordination of olfactory receptor genes.
T. McClintock, University of Kentucky, Lexington: Intracellular trafficking of olfactory receptors.
B. Davis, National Institute on Deafness and Other Communication Disorders, Rockville, Maryland: The growth and the taste and smell programs at the NIDCD, 1990–2000.

SESSION 5: Odorant Receptors: Development
Chairperson: L.B. Buck, Harvard Medical School, Boston, Massachusetts

B. Key, University of Queensland, Brisbane, Australia: Changing the cell surface glycocode on olfactory axons via transgenesis: The combined role of odorant receptors and cell surface.
C.A. Greer, Yale University School of Medicine, New Haven, Connecticut: Axonal targeting and organization of the olfactory bulb glomerulus.
R.R. Reed, HHMI, The Johns Hopkins University, Bethesda, Maryland: Engineering altered activity in the mammalian olfactory system.
H. Sakano, University of Tokyo, Japan: Mutually exclusive expression of odorant receptor transgenes.

SESSION 6: Odorant Receptors: Function
Chairperson: S. Firestein, Columbia University, New York, New York

G.M. Shepherd, Yale University Medical School, New Haven, Connecticut: Odor determinants, receptor-binding pockets, and odor images: Convergence of experiment and theory.
C. Wetzel, Ruhr-University-Bochum, Germany: Functional expression and characterization of olfactory receptors of vertebrates and invertebrates.
D. Giorgi, Institut de Genetique Humaine, Montpellier, France: Identification of specific ligands of mouse OR912-93.
S. Korsching, University of Cologne, Germany: Concentration and structure-dependent odor space in the mouse olfactory bulb.
K. Touhara, University of Tokyo, Japan: Reconstitution of mouse olfactory receptors that recognize an overlapping set of odorants.

Closing Lecture:
R. Axel, HHMI, Columbia University, New York, New York

Genomic Annotation Workshop: DNA Replication

March 17–22

FUNDED BY **National Institutes of Health**

ARRANGED BY **L.D. Stein,** Cold Spring Harbor Laboratory
E. Birney, European Bioinformatics Institute, Cambridge,United Kingdom

WORKSHOP MEMBERS:

L.I. Aravind, National Center for Biotechnology Information, National Library of Medicine (NLM), Bethesda, Maryland
J. Ashurst, Sanger Centre, Cambridge, United Kingdom
A.G. Bateman, Sanger Centre, Cambridge, United Kingdom
E. Birney, European Bioinformatics Institute, Cambridge, United Kingdom
M. Clamp, Sanger Centre, Cambridge, United Kingdom
R. Copley, EMBL Heidelberg, Germany
E.E. Eichler, Case Western Reserve University, Cleveland, Ohio
J. Gilbert, Sanger Centre, Cambridge, United Kingdom
A. Kanapin, European Bioinformatics Institute, Cambridge, United Kingdom
A. Neuwald, Cold Spring Harbor Laboratory
K. Pruitt, National Library of Medicine (NLM), Bethesda, Maryland
J. Stalker, Sanger Centre, Cambridge, United Kingdom
L.D. Stein, Cold Spring Harbor Laboratory
B. Stillman, Cold Spring Harbor Laboratory
E. Stupka, European Bioinformatics Institute, Cambridge, United Kingdom

Bioinformaticists at work.

The Basic Issues of Science

April 1–4

FUNDED BY **The Federal Judicial Center**

ARRANGED BY **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory

SESSION 1

J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory and F.M. Smith, Federal Judicial Center, Washington, D.C.: Welcoming remarks.

G.E. Allen, Washington University, St. Louis, Missouri: Eugenics: Past, present, and future.

SESSION 2

J. Maienschein, Arizona State University, Tempe: From Darwin to Dolly: Developments in the biological sciences in the 20th century.

J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory: Fundamentals of genetics.

SESSION 3

J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory: The Human Genome Project: Origins and future.

D. Blacker, Massachusetts General Hospital, Boston, Massachusetts: Using molecular human genetics: Alzheimer's disease.

SESSION 4

P. Reilly, Interleukin Genetics, Inc., Waltham, Massachusetts: Social implications of genetic research.

SESSION 5

P. Neufeld, Innocence Project, New York, New York: Using DNA forensics.

SESSION 6

I. Pepperberg, Massachusetts Institute of Technology, Cambridge, Massachusetts: What Alex the Grey Parrot tells us about human cognition and language.

R. Park, University of Maryland, College Park, Maryland: Science and pseudoscience and the courtroom.

Federal Judicial Conference attendees.

Common and Contrasting Mechanisms of Pathogen Virulence and Host Resistance in Plant and Animal Diseases

April 8–11

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **J. Galan,** Yale University School of Medicine, New Haven, Connecticut
B.J. Staskawicz, University of California, Berkeley

Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Innate Immunity and Host Defense Responses
Chairperson: B.J. Staskawicz, University of California, Berkeley

K. Anderson, Memorial Sloan-Kettering Institute, New York, New York: Genetic analysis of the response to infection in *Drosophila*.

G. Nunez, University of Michigan Medical School, Ann Arbor: Regulation of apoptosis and NF-κB activation by Nod proteins.

S. Miller, University of Washington, Seattle: Lipid A modifications and the resistance of gram-negative bacteria to innate immunity.

B.J. Staskawicz, University of California, Berkeley: NDR1, a putative GPI-anchored protein specifying bacterial disease resistance in *Arabidopsis thaliana*.

J. Parker, The Sainsbury Laboratory, Norwich, United Kingdom: Connecting local and systemic plant resistance pathways.

X. Dong, Duke University, Durham, North Carolina: Signal transduction in systemic acquired resistance.

SESSION 2: Bacterial Modulators of Host Responses
Chairperson: J. Galan, Yale University School of Medicine, New Haven, Connecticut

J. Galan, Yale University School of Medicine, New Haven, Connecticut: Mimicry of cellular proteins as a strategy for the modulation of cellular responses by bacterial pathogens.

H. Wolf-Watz, University of Umea, Sweden: Modulation of expression and translocation of *Yersinia* virulence effectors after target cell contact.

D. Philpott, Institut Pasteur, Paris, France: How *Shigella* plants the seeds of inflammation into intestinal cells.

K. Orth, University of Michigan Medical Center, Ann Arbor: Black death, Black spot: *Yersinia* effector is a ubiquitin-like protein protease.

A. Collmer, Cornell University, Ithaca, New York: The Hrp (type III secretion system) effector proteins of *Pseudomonas syringae* pv. tomato DC3000.

U. Bonas, Martin-Luther University, Halle, Germany: Type III effector proteins and their role in the *Xanthomonas*-plant interaction: Recognition or disease?

U. Bonas, J. Dangl, B. Staskawicz, P. Schulze-Lefert

SESSION 3: Mechanisms of Microbial/Host Interactions I
Chairperson: D. Portnoy, University of California, Berkeley

G.B. Martin, Cornell University, Ithaca, New York: *Pseudomonas* effector proteins that interact with the tomato Pto kinase.

J. Dangl, University of North Carolina, Chapel Hill: Type III effectors in the *P. syringae* interaction with *Arabidopsis*.

R. Innes, Indiana University, Bloomington: Recognition of the *Pseudomonas* virulence protein AvrPphB by *Arabidopsis*.

D. Portnoy, University of California, Berkeley: A PEST-like sequence in listeriolysin O essential for *Listeria monocytogenes* pathogenicity.

J.F. Miller, University of California, Los Angeles: Parasite adaptation to a dynamic host: *Bordetella*-phage interactions.

S. Hultgren, Washington University, St. Louis, Missouri: Pathogenic fiber formation in bacteria: Structure, function, and role in diseases of the urinary tract.

SESSION 4: Mechanisms of Microbial/Host Interactions II
Chairperson: J.D.G. Jones, The Sainsbury Laboratory, Norwich, United Kingdom

J.D.G. Jones, The Sainsbury Laboratory, Norwich, United Kingdom: Mode of action of tomato Cf- genes that confer resistance to *Cladosporium fulvum*.

J. Ellis, CSIRO, Canberra, Australia: Specificity, repression, and activation of rust resistance proteins.

S. Hulbert, Kansas State University, Manhattan: NBS genes in cereals.

K. Shirasu, The Sainsbury Laboratory, Norwich, United Kingdom: RAR1: A link from R genes to ubiquitination machinery.

C. Roy, Yale School of Medicine, New Haven, Connecticut: Implications for pathogen evolution: Hijacking of a eukaryotic host gene by *Legionella pneumophila*.

E. Groisman, Washington University School of Medicine, St. Louis, Missouri: The SPI-2 pathogenicity island of *Salmonella* enteric.

SESSION 5: Plant Defense Responses
Chairperson: F.M. Ausubel, Massachusetts General Hospital, Boston

P. Schulze-Lefert, Max-Planck-Institut für Zuchtungsforschtung, Koln, Germany: Activation of and rescue from cell death in plant disease resistance.

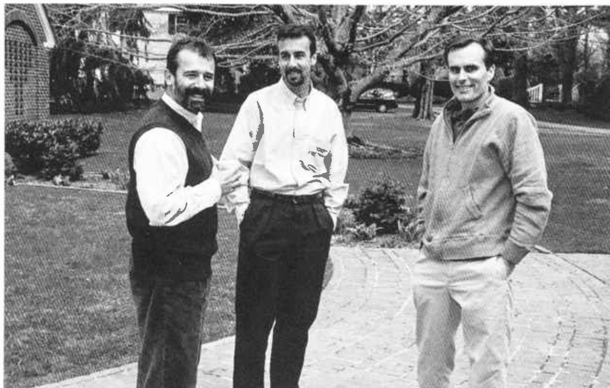
B. Baker, University of California, Berkeley: Molecular-genetic characterization of the N-mediated TMV disease resistance pathway.

SESSION 6: Model Systems and Genomics
Chairperson: F.M. Ausubel, Massachusetts General Hospital, Boston

F.M. Ausubel, Massachusetts General Hospital, Boston: Modeling human pathogenesis in nonvertebrate model hosts.

P.F. Predki, DOE Genome Institute, Walnut Creek, California: Rapid characterization of pathogen genomes via high-throughput draft sequencing.

S. Lory, Harvard Medical School, Boston, Massachusetts: Eavesdropping on communications between pathogens and their host using DNA microarrays.



J. Galan, E. Groisman, C. Roy

Electronic Access to Scientific Literature

April 16–18

FUNDED BY **Cold Spring Harbor Laboratory**

ARRANGED BY **J. Inglis,** Cold Spring Harbor Laboratory Press
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
J. Inglis, Cold Spring Harbor Laboratory Press

SESSION 1: Users' Wants and Needs
Chairperson: B. Alberts, National Academy of Sciences, Washington, D.C.
B. Stillman, Cold Spring Harbor Laboratory: What do scientists want? (followed by additional comments, participating scientists)
A. Okerson, Yale University, New Haven, Connecticut: What do libraries want?
Additional comments, participating librarians.

SESSION 2: Cross-Publisher Initiatives
Co-Chairpersons: B. Alberts, National Academy of Sciences, Washington, D.C. and **N. Cozzarelli,** PNAS-University of California, Berkeley
D. Lipman, National Center for Biotechnology Information, NIH, Bethesda, Maryland: PubMed Central
Discussion of PubMed Central
K. Hunter, Elsevier Science, New York, New York: CrossRef

SESSION 3: What Are Individual Publishers Doing?
Chairperson: P. Campbell, Nature Publishing Group, London, United Kingdom
Brief presentations and discussions by publishers.

SESSION 4: What Next?
Co-Chairpersons: J. Inglis, Cold Spring Harbor Laboratory Press and **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory

B. Alberts, J. Sack, J. Fox, N. Cozzarelli

M. Eisen

R. Bovenshulte, K. Hunter

Interferons: Biological Mechanisms and Disease Treatments I

April 29–May 1

FUNDED BY **Herbert J. Siegel Fund for Cancer Pharmacogenomics**

ARRANGED BY **I.J. Fidler,** M.D. Anderson Cancer Center, Houston, Texas

Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1

Chairperson: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

I.J. Fidler, M.D. Anderson Cancer Center, Houston, Texas: Introduction and antiangiogenic properties of interferon α/β.

J.E. Darnell, The Rockefeller University, New York, New York: The stats in cancer.

B.B. Aggarwal, M.D. Anderson Cancer Center, Houston, Texas: Molecular basis for the antiproliferative and anti-inflammatory effects of interferon.

J.M. Folkman, Children's Hospital, Boston, Massachusetts: Antiangiogenic therapy with low-dose interferon α.

SESSION 2

Chairperson: E.C. Borden, Cleveland Clinic Foundation, Taussig Cancer Center, Ohio

C.P.N. Dinney, M.D. Anderson Cancer Center, Houston, Texas: Interferon gene therapy for superficial bladder cancer.

J.M. Kirkwood, University of Pittsburgh Cancer Institute, Pennsylvania: Adjuvant therapy.

A.M.M. Eggermont, University Hospital Rotterdam, The Netherlands: Adjuvant therapy with intermediate doses of IFN and the rationale of the Peg-Intron Eortc 18991 trial.

B. Clarkson, Memorial Sloan-Kettering Cancer Center, New York, New York: New inhibitors of *abl* tyrosine kinase and other targets for specific therapies of CML.

S. Rich, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois: The role of growth factors and inhibitors in the pathobiology of primary pulmonary hypertension.

SESSION 3

Chairperson: I.J. Fidler, M.D. Anderson Cancer Center, Houston, Texas

E.C. Borden, Cleveland Clinic Foundation, Taussig Cancer Center, Ohio: Genes, apoptosis, and angiogenesis.

P.B. Chapman, Memorial Sloan-Kettering Cancer Center, New York, New York: Utility of high-dose IFNα in melanoma.

F. Randazzo, Chiron Corporation, Emeryville, California: Analyzing pathways in cancer by combining functional analysis with gene expression profiling of small tumor biopsy samples.

S. Lowe, Cold Spring Harbor Laboratory: Cellular senescence induced by oncogenic *ras*.

S.E. Krown, Memorial Sloan-Kettering Cancer Center, New York: Interferon therapy for Kaposi's sarcoma: Evidence for activity of low-dose daily interferon.

P. Chapman, E. Bergsland

I.J. Fidler, J. Folkman

American Eugenics and the New Biology: Perspectives and Parallels

May 6–8

FUNDED BY **National Human Genome Research Institute, National Institutes of Health**

ARRANGED BY **D. Micklos,** DNA Learning Center, Cold Spring Harbor Laboratory
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: History

G.E. Allen, Washington University, St. Louis, Missouri: Progressive origins of eugenics and the Eugenics Record Office.

E.A. Carlson, State University of New York, Stony Brook: Bad seed, corrupted germ plasm, prized pedigrees, and eugenic worth.

SESSION 2: Impacts

S. Selden, University of Maryland, College Park: Fitter families for future firesides: State fairs and the construction of merit and race in America, 1913–1930.

P.A. Lombardo, University of Virginia, Charlottesville: Immigration and sterilization in the United States.

N.A. Holtzman, The John Hopkins Medical Institutions, Baltimore, Maryland: Is eugenics dead and buried?

SESSION 3: Resources

D. Micklos, DNA Learning Center, Cold Spring Harbor and J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory: Introduction to the image archive on the American eugenics movement.

SESSION 4: Lessons

D. Paul, Brandeis University, Waltham, Massachusetts: Comparative eugenics.

D. Hamer, National Cancer Institute, Bethesda, Maryland: Genetics of human behavior.

Session 5: General Discussion

J. Apple, P. Seawell

G. Allen, N. Holtzman, P. Woolf

Can a Machine Be Conscious?

May 13–16

FUNDED BY **The Swartz Foundation**

ARRANGED BY **C. Koch,** California Institute of Technology, Pasadena
D. Chalmers, University of Arizona
R. Goodman, California Institute of Technology, Pasadena
O. Holland, Clinton House, Stroud, United Kingdom
J. Swartz, The Swartz Foundation, East Setauket, New York

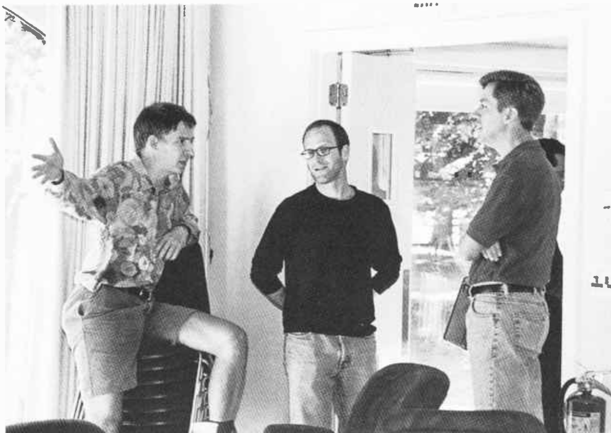
Introduction and Welcome:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
J. Swartz, The Swartz Foundation, East Setauket, New York

SESSION 1
Chairperson: C. Koch, California Institute of Technology, Pasadena

D.J. Chalmers, University of Arizona, Tucson: Machine consciousness: Problems and prospects.
C. Koch, California Institute of Technology, Pasadena: From biological to machine consciousness.
G. Tononi, University of Wisconsin, Madison: Recipes for consciousness.
E. Rolls, University of Oxford, United Kingdom: Consciousness and dual routes to action in neural network machines.

SESSION 2
Chairperson: R.M. Goodman, California Institute of Technology, Pasadena

M.A. Goodale, University of Western Ontario, Canada: Why we need two cortical visual systems: A teleassistance model.
C.D. Frith, University College London, United Kingdom: The importance of other minds.
L. Steels, Free University of Brussels, Belgium: The role of language in the emergence of consciousness.
J.C. Hawkins, Handspring Inc., Mountain View, California: What neuroanatomy and the physiology of our senses tell us about consciousness and intelligence.
S. Blackmore, University of the West of England, Bristol, United Kingdom: Consciousness in meme machines.



C. Koch, A. Zador, J. Hawkins

SESSION 3

Chairperson: D.J. Chalmers, University of Arizona, Tucson

N. Block, New York University Faculty of Arts & Sciences, New York: What are experiments about consciousness really about?

E. Dietrich, Virginia Institute of Technology, Blacksburg: Can a machine be conscious? Sure, but it won't help.

I. Aleksander, Imperial College, London, United Kingdom: Robot usable models of visual consciousness.

R.M. Goodman, California Institute of Technology, Pasadena and O. Holland, Clinton House, Stroud, United Kingdom: Autonomous robots + dynamic environment + intelligent control = consciousness?

SESSION 4

Chairperson: O. Holland, Clinton House, Stroud, United Kingdom

B. Baars, The Neurosciences Institute, San Diego, California: Self systems in the brain constrain conscious contents: A global workspace perspective.

S. Franklin, University of Memphis, Tennessee: Conscious software agents. We've got one running. How conscious is it? I don't know.

S. Dehaene, Service Hospitalier Frederic Joliot, Orsay, France: Cerebral processing of conscious and unconscious stimuli: A neural workspace hypothesis.

A.J. Clark, University of Sussex, Brighton, United Kingdom: Consciousness and perceptual knowledge.

A. Cleeremans, Free University of Brussels, Belgium: Being virtual: Consciousness and self as graded, adaptive phenomena.

SESSION 5

Chairperson: C. Koch, California Institute of Technology, Pasadena

D. Psaltis, California Institute of Technology, Pasadena: Awareness-based computing.

I. Aleksander, J. Witkowski

Interferons: Biological Mechanisms and Disease Treatments II

September 9–11

FUNDED BY **Herbert J. Siegel Fund for Cancer Pharmacogenomics**

ARRANGED BY **E.C. Borden,** Cleveland Clinic Foundation, Ohio
J. Folkman, Children's Hospital, Boston, Massachusetts

Introductions:
J.D. Watson, Cold Spring Harbor Laboratory
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: OFMs: Mechanisms and Antiangiogenic Effects
Chairperson: I.J. Fidler, M.D. Anderson Cancer Center, Houston, Texas

J. Folkman, Children's Hospital, Boston, Massachusetts: Introduction: IFNs overview of antiangiogenesis results.
E.C. Borden, Cleveland Clinic Foundation, Ohio: Objective and the interferon system.
I.J. Fidler, M.D. Anderson Cancer Center, Houston, Texas: INFNs: Preclinical antiangiogenesis mechanisms.
J.E. Darnell, The Rockefeller University, New York, New York: IFN-induced signaling.
A. Ezekowitz, Massachusetts General Hospital, Boston: Life-threatening hemangiomas.
A.W. Yasko, M.D. Anderson Cancer Center, Houston, Texas: Giant cell tumors of bone.

SESSION 2: Considerations in Clinical Study Designs
Chairperson: E.C. Borden, Cleveland Clinic Foundation, Ohio

S. Weiss, Emory University Hospital, Atlanta, Georgia: Pathology of angiogenic neoplasms.
D. Lindner, Cleveland Clinic Foundation, Ohio: IFNs in preclinical tumor models.
S.E. Krown, Memorial Sloan-Kettering Cancer Center, New York, New York: Effective dose and schedule for Kaposi's sarcoma.
A.M.M. Eggermont, University Hospital Rotterdam, The Netherlands: Doses and schedules in melanoma—European experience.
B.R.G. Williams, Cleveland Clinic Foundation, Ohio: Assessment of mechanisms by gene array.
R. Kalluri, Beth Israel Deaconess Medical Center, Boston, Massachusetts: Tumstatin and INFN-α gene expression.
J.M. Pluda, CTEP National Cancer Institute, Rockville, Maryland: Potential for combination with other antiangiogenic agents.

A. Eggermont, J. Folkman

SESSION 3: Discussion and Presentation of Preclinical and Phase II/III Cooperative Group Clinical Study Designs

Discussion Groups:
A. Ezekowitz, Massachusetts General Hospital, Boston: Hemangiomas.
A.W. Bleyer, University of Texas, M.D. Anderson Cancer Center, Houston: Giant cell tumors of bone.
G. Thomas Budd, Cleveland Clinic Foundation, Ohio: Angiosarcomas.
J.E. Darnell, The Rockefeller University, New York, New York: Critical preclinical questions.

Recommendations for Future Studies:
Co-Chairpersons: E.C. Borden, Cleveland Clinic Foundation, Ohio and **J. Folkman,** Children's Hospital, Boston, Massachusetts: Next steps and summary.

J. Darnell, B. Williams

Meeting of the Editorial Advisory Panel: Digital Image Archive on the American Eugenics Movement

September 30–October 1

FUNDED BY **National Human Genome Research Institute, National Institutes of Health**

ARRANGED BY **D. Micklos,** Dolan DNA Learning Center, Cold Spring Harbor Laboratory
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Welcome and Review

Overview of Image Archive
Full-text Searching
Bulletin Board
New Collections: Ellis Island, CSHL, Estabrook Archives
Banbury Center Meeting

SESSION 2: Discussion of NIH Restrictions and Conditions for Lifting "People under Scrutiny," Potential for Abuse, and Concern about Re-exploration of Disabled People

Conditions fulfilled:
Additional EAP member
Posting of editorial policy
Use agreement

Conditions remaining:
EAP discussion of separating site into educational and archival components
Report of previous Working Group discussion
Site use and relevant site feedback
Resolution

SESSION 3: Tour of Dolan DNA Learning Center

SESSION 4: Independent Exploration of Eugenics Site, Bioinformatics Lab
Assignment of new images to topic areas

SESSION 5: Discussion of Future Plans
Collection trip to Eugenics Society and University College Archives, London
Future collection trips

S. Selden, P. Lombardo, and P. Ryan at the Dolan DNA Learning Center.

Making Vaccines for the Developing World: Access to and Deployment of New Technologies

October 9–11

FUNDED BY **Albert B. Sabin Vaccine Institute, Inc.**

ARRANGED BY **M. Moree,** PATH/Malaria Vaccine Initiative, Seattle, Washington
P.K. Russell, Albert B. Sabin Vaccine Institute, Potomac, Maryland

Co-Chairpersons:
L.K. Gordon, VaxGen, Inc., Brisbane, California
R. Rabinovich, PATH/Malaria Vaccine Initiative, Rockville, Maryland

Welcome and Introductions:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
D.L. Douglas, Albert B. Sabin Vaccine Institute, New Canaan, Connecticut
P.K. Russell, Albert B. Sabin Vaccine Institute, Potomac, Maryland
R. Rabinovich, PATH/Malaria Vaccine Initiative, Rockville, Maryland and **Lance K. Gordon,** VaxGen, Inc., Brisbane, California: Charge to the conference.
P.K. Russell, Albert B. Sabin Vaccine Institute, Potomac, Maryland: Recommendations from previous meetings.
R.T. Mahoney, International Vaccine Institute, Sedona, Arizona: Management of intellectual property.
H. Kettler, Institute for Global Health, San Francisco, California: Development of intellectual property: The Biotechnology Foundation.

SESSION 1: Case Studies: Access to Technologies for the Developing World
Chairperson: M. Moree, PATH/Malaria Vaccine Initiative, Seattle, Washington

R. Insel, University of Rochester Medical Center, New York: Pneumococcal vaccines.
M.A. Liu, Bill & Melinda Gates Foundation, Seattle, Washington: DNA vaccine technology.

SESSION 2: Perspectives
Chairperson: P.K. Russell, Albert B. Sabin Vaccine Institute, Potomac, Maryland

T. A. Young, Texas A&M University System, College Station: Academic technology transfer office—Rewards, motivations, accountability.
L. Gordon, VaxGen Inc., Brisbane, California: Biotech—Strategies for the development and partnering of platform technologies.
R. Rabinovich, PATH/Malaria Vaccine Initiative, Rockville, Maryland: NGOs—Access to platform technologies.

SESSION 3: Small Group Sessions: Identification of Problems

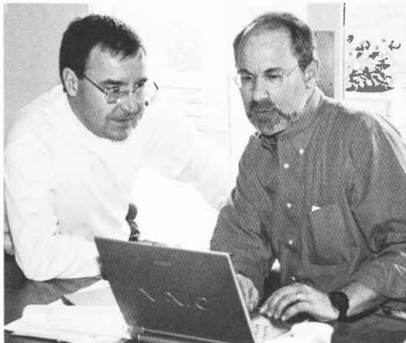
How to access and compile scientific data for the evaluation of platform technologies for products for the developing world?
Can the initial licensing step be used to gain access to platform technologies for the developing world?
How can licensing practices for companies be modified to further access?
What different access to technology strategies may be necessary for products that have dual markets vs. products that only have a developing world market?

SESSION 4

Reporting from Small Group Discussions: **Chairperson: R. Rabinovich,** PATH/Malaria Vaccine Initiative, Rockville, Maryland

Recommendations for Action: **Chairperson: L.K. Gordon,** VaxGen, Inc., Brisbane, California

Future Agenda: **Chairperson: P.K. Russell,** Albert B. Sabin Vaccine Institute, Potomac, Maryland



C. Schirvel, R. Insel

Cortical Maps

October 13–16

FUNDED BY **Marie H. Robertson Memorial Fund for Neurobiology**

ARRANGED BY **D. Chklovskii,** Cold Spring Harbor Laboratory
A. Koulakov, University of Utah, Salt Lake City

Welcome and Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
D. Chklovskii, Cold Spring Harbor Laboratory

SESSION 1
Chairperson: A. Koulakov, University of Utah, Salt Lake City

N. Swindale, University of British Columbia, Vancouver: Visual cortex polymaps.
G.G. Blasdel, Harvard University, Boston, Massachusetts: Optical imaging in primate visual cortex.
A. Grinvald, The Weizmann Institute of Science, Rehovot, Israel: Visualization of cortical architecture and dynamics.
A. Koulakov, University of Utah, Salt Lake City: Wiring economy and polymaps.
D. Chklovskii, Cold Spring Harbor Laboratory: Orientation preference maps: A wiring optimization approach.
D.C. Van Essen, Washington University School of Medicine, St. Louis, Missouri: Cortical maps and mappings between monkeys and humans.

SESSION 2
Chairperson: D. Chklovskii, Cold Spring Harbor Laboratory

Z.F. Kisvarday, Ruhr-University Bochum, Germany: Area- and age-related changes of pinwheel center positions in relation to ocular dominance map.
E. Kaplan, Mount Sinai School of Medicine, New York, New York: Color, size, and all the rest: How do they all fit inside the visual cortex?
M. Sur, Massachusetts Institute of Technology, Cambridge: Orientation maps and local processing in visual cortex.
J.C. Horton, University of California, San Francisco: Lessons for column formation in primate striate cortex from a precise topographic map generated from the shadows of retinal blood vessels.
K.D. Miller, University of California, San Francisco: Which properties should be organized into maps?—A developmental model.

G. Blasdel, S. Löwell

SESSION 3

Chairperson: A. Grinvald, The Weizmann Institute of Science, Rehovot, Israel

S. Löwell, Leibniz Institute for Neurobiology, Magdeburg, Germany: Is there a genetic influence on the layout of visual cortical maps?

U.T. Eysel, Ruhr-University of Bochum, Germany: Optical imaging of cortical maps and the effects of visual cortex lesions: A demonstration of general robustness and subtle modifiability of cortical maps.

D. Fitzpatrick, Duke University, Durham, North Carolina: Relating patterns of connectivity to functional maps in the visual cortex.

E.M. Callaway, The Salk Institute for Biological Studies, La Jolla, California: Relationships between afferent input, local circuits,and functional organization of primary visual cortex.

G.J. Goodhill, Georgetown University Medical Center, Washington, D.C.: The effect of variable elastic topologies on the structure of ocular dominance and orientation maps.

L. Krubitzer, University of California, Davis: Cortical maps: Genetic and epigenetic contributions to the phenotype.

SESSION 4

Chairperson: N. Swindale, University of British Columbia, Vancouver, Canada

J.S. Lund, University of Utah, Salt Lake City: Anatomical substrates for local and global integration of visual information: Intrinsic and feedback connections of macaque V1 cortex.

A. Shmuel, University of Minnesota, Minneapolis: Functional organization of the feedback connection from V2 to V1 of the primate.

A.W. Roe, Yale University School of Medicine, New Haven, Connecticut: The ANDs, and NOTs of feature-specific integration in V1 and V2 of the primate.

K. Obermayer, Berlin, Germany: Role of nonlinear intracortical interactions in cortical map formation.

F. Wolf, Max-Planck Institute, Gottingen, Germany: Are orientation preference maps optimized for visual function?

M.V. Tsodyks, The Weizmann Institute of Science, Rehovot, Israel: Visual maps and population activity in the visual cortex.

SESSION 5

Chairperson: G.G. Blasdel, Harvard University, Boston, Massachusetts

D.-S. Kim, University of Minnesota Medical School, Minneapolis: Functional MRI of cortical maps—Scopes, future, and limitations.

V. Dragoi, Massachusetts Institute of Technology, Cambridge: Inhomogeneities in the structure of orientation maps and their consequences for cortical function.

A. Das, The Rockefeller University, New York, New York: Cortical architecture and its role in early vision.

M. Rosa, Monash University, Victoria, Australia: Small brains, great maps: The visual cortex in a dwarf primate, the marmoset monkey.

P. Ak, Mount Sinai School of Medicine, New York, New York: Representation of spatial frequency selectivity in the mammalian primary visual cortex.

Banbury Conference Center

J.P. Morgan H&Q/Cold Spring Harbor Laboratory Executive Conference on Controlling Cancer

October 18–20

ARRANGED BY **J.D. Watson,** Cold Spring Harbor Laboratory
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1

J.D. Watson, Cold Spring Harbor Laboratory: Welcoming remarks.

D. Hanahan, Hormone Research Institute, University of California, San Francisco: Cancer as an organ.

SESSION 2

S. Lowe, Cold Spring Harbor Laboratory: Genes, cell death, and therapies.

C. Sawyers, Jonsson Comprehensive Cancer Center, University of California, Los Angeles: Gleevec and other kinase inhibitors in cancer therapies.

R. Kerbel, Sunnybrook and Women's Health Sciences Centre, Toronto, Canada: Anti-angiogenic drugs as a new therapeutic approach for cancer.

L. Hudson, J. Watson

SESSION 3

V. Mittal, Cold Spring Harbor Laboratory: DNA array technology and cancer.

R. Lucito, Cold Spring Harbor Laboratory: Using array technology to detect genomic mutations in cancer.

SESSION 4

K. Alitalo, Finnish Academy of Sciences, University of Helsinki, Finland: Lymphangiogenesis: A new target for cancer treatment.

J. Schlessinger, Signaling pathways: Targets for drug discovery.

R. Kalluri, Beth Israel Deaconess Medical Center, Boston, Massachusetts: Regulation of angiogenesis and tumor growth by vascular basement membranes.

Discussion: Cancer therapies—What next?

Closing Remarks:
J.D. Watson, Cold Spring Harbor Laboratory

G. Milne, C. Seiden

The History and Mechanisms of Animal and Plant Evolution

October 21–24

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **J. Doebley,** University of Wisconsin, Madison
C. Marshall, Harvard University, Cambridge, Massachusetts
N.H. Patel, HHMI, University of Chicago, Illinois

SESSION 1: Investigating Microevolutionary Change
Chairperson: N.H. Patel, HHMI, University of Chicago, Illinois

F. Nijhout, Duke University, Durham, North Carolina: The microevolution of developmental mechanisms.
A. Long, University of California, Irvine: Using linkage disequilibria to dissect complex traits.
J. Doebley, University of Wisconsin, Madison: The evolution of plant form: Examples from maize and its relatives.
D. Stern, Princeton University, New Jersey: A developmental genetic approach to understanding microevolutionary morphological variation.
G.C. Gibson, North Carolina State University, Raleigh: Will we ever have the power to dissect quantitative traits down to the nucleotide level?

SESSION 2: Variation, Adaptation, and Speciation
Chairperson: J. Doebley, University of Wisconsin, Madison

P.M. Brakefield, University of Leiden, The Netherlands: Does development constrain butterfly eyespot evolution?
D.W. Schemske, Michigan State University, East Lansing: Ecological genetics of adaptation and speciation.
H.A. Orr, The University of Rochester, New York: Are mathematical theories of adaptation possible?

SESSION 3: Evolution of Genetic Pathways
Chairperson: C. Marshall, Harvard University, Cambridge, Massachusetts

C. Queitsch, University of Chicago, Illinois: Hsp90 buffers genetic variation, environmental responses and developmental stability in *Arabidopsis thaliana*.
A.S. Wilkins, BioEssays, Cambridge, United Kingdom: How a complex genetic pathway (might have) evolved: The case of the *Drosophila* sex determination pathway.
G. O'Dell, University of Washington, Seattle: The robustness of evolved genetic networks would be astonishing were it not essential.

A. Wilkins, J. Gerhart, M. Akam

SESSION 4: Plant Evolution

Chairperson: M. Akam, University of Cambridge, United Kingdom

M.J. Donoghue, Yale University, New Haven, Connecticut: Homoplasy, heterotopy, and constraints on evolution in plants.

J. Maloof, The Salk Institute for Biological Studies, San Diego, California: Natural variation in *Arabidopsis* light signaling.

SESSION 5: Arthropod Evolution

Chairperson: M.Q. Martindale, University of Hawaii, Honolulu

D.E.G. Briggs, University of Bristol, United Kingdom: Morphological data from Cambrian fossils: Evidence for pattern and process?

W. Arthur, University of Sunderland, United Kingdom: How do evolutionary differences in segment number arise? Developmental and population studies of a centipede model system.

M. Akam, University of Cambridge, United Kingdom: Arthropod relationships: New trees and their implications for the evolution of development.

N.H. Patel, HHMI, University of Chicago, Illinois: Evolution of arthropod segmentation and body patterning.

SESSION 6: Chordate and "Basal" Metazoan Evolution

Chairperson: P.W.H. Holland, University of Reading, United Kingdom

J.C. Gerhart, University of California, Berkeley: Hemichordates and the origin of chordates.

B.J. Swalla, University of Washington, Seattle: The role of the innate immune system in the evolution of the chordates.

P. Janvier, Museum National d'Histoire Naturelle, Paris, France: From jawless to jawed vertebrates: A "blackbox" of vertebrate evolution.

M.Q. Martindale, University of Hawaii, Honolulu: Axial patterning in "radially" symmetrical metazoans.

SESSION 7: Evolutionary Changes in Gene Regulation

Chairperson: G.C. Gibson, North Carolina State University, Raleigh

M. Ludwig, University of Chicago, Illinois: Evolution of *cis*-regulatory elements.

G. Wray, Duke University, Durham, North Carolina: Evolution of a gene regulatory system: The Endo16 promoter of camarodont sea urchins.

SESSION 8: The Origins of Animal Multicellularity

Chairperson: N. Shubin, University of Chicago, Illinois

P.W.H. Holland, University of Reading, United Kingdom: Origins of animal multicellularity.

A. Adoutte, Centre de Genetique Moleculaire du CNRS, France: The new animal phylogeny: Implications for understanding the evolution of development.

SESSION 9: The Geological Record

Chairperson: J. Gerhart, University of California, Berkeley

C. Marshall, Harvard University, Cambridge, Massachusetts: Evolution as the filtering of development by geology.

D. Erwin, National Museum of Natural History: Evolutionary innovation: Construction of ecospace.

SESSION 10: Discussion

Co-Moderators: J. Doebley, University of Wisconsin, Madison; **C. Marshall,** Harvard University, Cambridge, Massachusetts; and **N.H. Patel,** HHMI, University of Chicago, Illinois

Wine and Cheese Party

Stability and Reversal of the Differentiated State

October 28–31

FUNDED BY **Yamanouchi USA Foundation**

ARRANGED BY **R. Lovell-Badge,** National Institute for Medical Research, London, United Kingdom
D. Stocum, Indiana University-Purdue University, Indianapolis
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Introduction and Welcome:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Pluripotency, ES Cells, and Germ Cells
Chairperson: R. Lovell-Badge, National Institute for Medical Research, London, United Kingdom

A. Smith, University of Edinburgh, United Kingdom: Pluripotency and lineage restriction in embryonic stem cells.

M.A. Surani, University of Cambridge, United Kingdom: The mammalian germ line: Origin, pluripotency, and epigenetic reprogramming.

P.J. Donovan, Thomas Jefferson University, Philadelphia, Pennsylvania: Transformation of germ cells into pluripotent stem cells.

R.G. Martinho, New York University Medical School, New York: Transcriptional regulation in *Drosophila* primordial germ cells.

SESSION 2: Nuclear Reprogramming
Chairperson: M.C. Raff, University College, London, United Kingdom

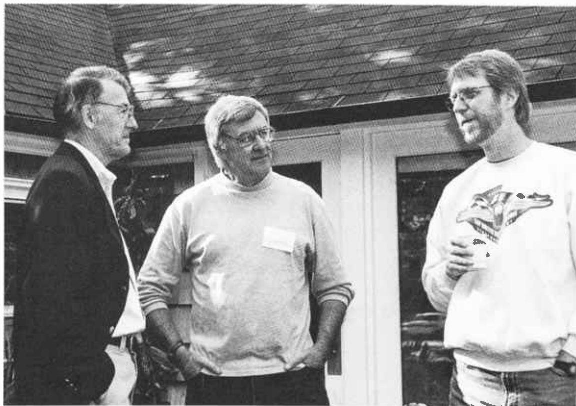
R. Jaenisch, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: Nuclear cloning and the reprogramming of the genome.

P. Mombaerts, The Rockefeller University, New York, New York: Derivation of ES cell lines via nuclear transfer in mouse.

B. Knowles, The Jackson Laboratory, Bar Harbor, Maine: Molecular control of the oocyte to embryo transition.

K.E. Latham, Temple University School of Medicine, Philadelphia, Pennsylvania: Incomplete or progressive reprogramming of somatic cell nuclei during cloning in mice.

K.J. McLaughlin, University of Pennsylvania, Kennett Square: Visualizing reprogramming of Oct4 in mouse clones.



B. Carlson, D. Stocum, J. Watson

SESSION 3: Current Status of Stem Cell Regulations
Chairperson: R. Jaenisch, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts

L. Skirboll, Office of Science Policy, NIH, Bethesda, Maryland: The development of U.S. stem cell policy.
A. Colman, PPL Therapeutics plc, Edinburgh, United Kingdom: Patent and regulatory issues concerning research and therapeutic applications of human ES cells.
J. Burley, Queen's College, Oxford, United Kingdom: Ethical and policy issues.

SESSION 4: Regeneration and Reversal of the Differentiated State
Chairperson: P. Mombaerts, The Rockefeller University, New York, New York

D. Stocum, Indiana University-Purdue University, Indianapolis: Regenerative biology and medicine.
J. Brockes, University College London, United Kingdom: Plasticity of the differentiated state during urodele amphibian regeneration.
W.-S. Kim, Sogang University, Seoul, Korea: Dedifferentiation in the regenerating salamander limbs.
B.M. Carlson, University of Michigan, Ann Arbor: Stability and instability of differentiation in long-term denervated and aging mammalian skeletal muscle.
M.C. Raff, University College London, United Kingdom: Reversing direction in the oligodendrocyte lineage.

SESSION 5: Cell Fate Decisions and Potential
Chairperson: D. Stocum, Indiana University-Purdue University, Indianapolis

S. Huang, Children's Hospital/Harvard Medical School, Boston, Massachusetts: Switching between cell fates: Attractors in cell regulatory networks.
D. Jackson, Cold Spring Harbor Laboratory: Control of communication and proliferation in plant stem cells.
J.C. Watson, Indiana University-Purdue University, Indianapolis: Photoregulated protein kinases in plants.
M. Timmermans, Cold Spring Harbor Laboratory: Suppression of stem cell fate during lateral organ development in maize.
B. Petersen, University of Florida, Gainesville: In vitro trans-differentiation of adult hepatic stem cells and their potential plasticity.
R. Lovell-Badge, National Institute for Medical Research, London, United Kingdom: Stem cell genes and cell fate decisions.

SESSION 6: Adult Stem Cells and Their Potential
Chairperson: A. Smith, University of Edinburgh, United Kingdom

Diana Clarke, Curis Inc., Cambridge, Massachusetts: Understanding stem cells derived from the adult brain and pancreas.
F.M. Watt, Imperial Cancer Research Fund, London, United Kingdom: Stem cell renewal and lineage commitment in mammalian epidermis.
F.D. Miller, McGill University, Montreal, Canada: Isolation and characterization of multipotent adult stem cells from mammalian dermis.
D. Meletis, Karolinska Institute, Stockholm, Sweden: Stem cells and regulation of differentiation.

SESSION 7: General Discussion
Co-Chairpersons: R. Lovell-Badge, National Institute for Medical Research, London, United Kingdom and **D. Stocum,** Indiana University-Purdue University, Indianapolis

B. Knowles

New Concepts for Clinical Cancer Trials

November 4–7

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **D. Hanahan,** University of California, San Francisco
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Steering Committee:
J. Folkman, Children's Hospital, Boston, Massachusetts
R.S. Kerbel, Sunnybrook & Women's Health Sciences Centre, Toronto, Canada
J.M. Pluda, CTEP National Cancer Institute, Rockville, Maryland

Introduction and Welcome:
D. Hanahan, University of California, San Francisco
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Metronomic Dosing of Chemotherapy
Chairperson: J. Folkman, Children's Hospital, Boston, Massachusetts

J. Folkman, Children's Hospital, Boston, Massachusetts: The concept of metronomic dosing and its realization in traditional mouse models.

J. Folkman, Children's Hospital, Boston, Massachusetts: Metronomic chemotherapy: The impact of suppressing p53 in tumor endothelium.

Experience(s) in the Clinic with Metronomic Chemotherapy

E. Bergsland, University of California, San Francisco: Challenges related to putting metronomic chemotherapy into clinical practice.

Y. Takahashi, Kanazawa University, Japan: Survival without tumor shrinkage: Tumor dormancy therapy.

B.A. Kamen, Cancer Institute of New Jersey, New Brunswick: Pediatric experience: Empiric validation of "metronomics."

L. Hlatky, Dana-Farber Cancer Institute, Boston, Massachusetts: Optimal dosing entails balanced metronomic suppression of the vascular and tumor cell compartments.

General Discussion: Issues, problems, possible actions to expedite clinical evaluation of metronomic strategies, assessing schedules and doses, biomarkers, etc.

SESSION 2: Combining Metronomic Chemotherapy with Investigational Drugs
Chairperson: R.S. Kerbel, Sunnybrook & Women's Health Sciences Centre, Toronto, Ontario, Canada

R.S. Kerbel, Sunnybrook & Women's Health Sciences Centre, Toronto, Ontario, Canada: Demonstrating the principle of combining metronomic chemotherapy with investigational anti-angiogenic drugs.

Other Preclinical Results

R. Giavazzi, Mario Negri Institute for Pharmacological Research, Bergamo, Italy: Combination treatments with experimental drugs and chemotherapies in preclinical models.

G. Bergers, University of California, San Francisco: Assessment of metronomic chemotherapy in mouse models of multistage carcinogenesis.

H.M. Pinedo, Free University Hospital, Amsterdam, The Netherlands: Phenomena to be considered in clinical trials aiming to achieve angiogenesis inhibition.

S.G. Eckhardt, University of Colorado Cancer Center, Denver: Ex vivo analyses of biological activity.

J.M. Pluda, CTEP National Cancer Institute, Rockville, Maryland: NCI experiences with new clinical trial designs and agents.

General Discussion: Issues, problems, possibilities

E. Bergsland, R. Kerbel

SESSION 3: Combining Metronomic Dosing of Chemotherapies with Approved Agents Being Used Offline (e.g., Celebrex and Thalidomide)
Chairperson: R.S. Kerbel, Sunnybrook & Women's Health Sciences Centre, Toronto, Ontario, Canada

R.S. Kerbel, Sunnybrook & Women's Health Sciences Centre, Toronto, Canada: Preclinical and clinical studies on metronomic antiangiogenic therapy.

Results in Animal Models or Clinical Trials/Experiences

S. Baruchel, Hospital for Sick Children, Toronto, Canada: Low-dose chemotherapy and Celebrex as an anti-angiogenic approach: Results of a pediatric clinical trial.
R. Buckstein, Sunnybrook Regional Cancer Centre, Toronto, Canada: High-dose Celebrex and low-dose cyclophosphamide in relapsed/refractory Hodgkin's lymphoma.
M.W. Kieran, Dana-Farber Cancer Institute, Boston, Massachusetts: Combination therapies.

General Discussion of Topics 2 and 3: Issues, problems, opportunities. Possible actions to validate metronomic dosing strategies, and to assess when metronomic vs. MTD strategies are warranted in combinatoric trials.

SESSION 4: The Case of Interferon-α
Chairperson: J. Folkman, Children's Hospital, Boston, Massachusetts

J. Folkman, Children's Hospital, Boston, Massachusetts: The childhood hemangioma experience.
R. Kalluri, Beth Israel Deaconess Medical Center, Boston, Massachusetts: Endogenous interferon suppresses angiogenesis in response to the collagen IV fragment tumstatin, an angiogenesis inhibitor.

General Discussion: Issues, problems, possible actions to address the use of metronomic, low doses of IFNα, both alone and in combination with other agents.

SESSION 5: Targeting Different Stages in Cancer Progression
Chairperson: D. Hanahan, University of California, San Francisco

D. Hanahan, University of California, San Francisco: The lessons from mouse models of multistage carcinogenesis: Stage-specific efficacy of MMP inhibitors, and tyrosine kinase receptor inhibitors.

Relevant Experiences from Clinical Trials and Mouse Models (e.g., the problems and promise of MMP-1 in Phase III Trials)

G. Bergers, University of California, San Francisco: Targeting peri-endothelial support cells.
J.S. Humphrey, Bristol-Myers Squibb PRI, Wallingford, Connecticut: Clinical trials of MMP1, then and now.
R.S. Herbst, M.D. Anderson Cancer Center, Houston, Texas: Clinical trials with RTK-1.
L. Murray, SUGEN, Inc., South San Francisco, California: Clinical trials with RTK-1.
J. Folkman, Children's Hospital, Boston, Massachusetts: How do you equate mouse stages to human stages, when considering the implications of stage-specific efficacy?

General Discussion: Issues and problems: The path forward to address the concept of stage-specific efficacy in clinical trials.

SESSION 6: Finale: The Path Forward
Chairperson: J.M. Pluda, CTEP National Cancer Institute, Rockville, Maryland

J.M. Pluda, CTEP National Cancer Institute, Rockville, Maryland: The NCI perspective.
I.M. Chico, U.S. Food and Drug Administration, Rockville, Maryland: The FDA perspective.
A. Barge, AstraZeneca Pharmaceuticals, Cheshire, United Kingdom: Big pharma perspective.
L. Norton, Memorial Sloan-Kettering Cancer Center, New York, New York: Oncologist perspective.

Wrap-up Discussion: Action items, initiatives, cooperative opportunities (Possible topics: How can mouse models continue to help move forward the evaluation of these new concepts, of stage-specific efficacy, metronomic and low dosing, etc? Can/should stage-specific, metronomic, and low-dose trials be expedited for radiation and chemotherapy as single agents, so as to potentially allow broader combinatroic testing with approved and experimental drugs? If yes, how best to do so?)

A Final Question to the Participants: Should we seek to convey some of the perspectives forthcoming from this workshop to the wider community?

Epithelial and Endothelial Tube Morphogenesis

November 11–14

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **M.A. Krasnow,** Stanford University School of Medicine, California
W.J. Nelson, Stanford University School of Medicine, California

Welcome and Goals of Meeting:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Mechanisms of Tube Formation—In Vitro Studies
Chairperson: A.L. Hubbard, Johns Hopkins University School of Medicine, Baltimore, Maryland

K.E. Mostov, University of California, San Francisco: Epithelial tubulogenesis, polarity, and membrane traffic.

W.J. Nelson, Stanford University School of Medicine, California: Cell dynamics in epithelial morphogenesis.

G.E. Davis, Texas A&M University System Health Science Center, College Station, Texas: Cdc42 and Rac1 control endothelial cell vacuole and lumen formation in three-dimensional extracellular matrices.

A.L. Hubbard, Johns Hopkins University School of Medicine, Baltimore, Maryland: Vesicle traffic in polarized epithelial cells.

M.E. Gerritsen, Genentech, Inc., South San Francisco, California: Gene expression profiling during angiogenesis in vitro.

SESSION 2: Mechanisms of Tube Formation—In Vivo Studies
Chairperson: H. Skaer, University of Cambridge, United Kingdom

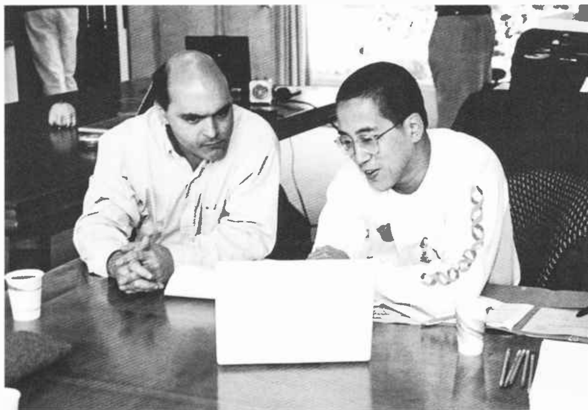
B.M. Weinstein, National Institute of Child Health and Human Development, Bethesda, Maryland: Studying blood vessel formation in the developing zebrafish.

C.H. Damsky, University of California, San Francisco: Pseudovasculogenesis: Formation of a hybrid fetal/maternal vascular system during development of the human placenta.

G.R. Dressler, University of Michigan, Ann Arbor: Cell migration and adhesion in the developing kidney.

H. Skaer, University of Cambridge, United Kingdom: Cell recruitment in tubulogenesis: Parallels between *Drosophila* and mammalian kidney development.

D.J. Andrew, Johns Hopkins University School of Medicine, Baltimore, Maryland: Genetic control of tube morphology in the *Drosophila* salivary gland.



B. Weinstein, D. Li

SESSION 3: Mechanisms of Tube Formation—Signaling and Regulation
Chairperson: A.P. McMahon, Harvard University, Cambridge, Massachusetts

A.P. McMahon, Harvard University, Cambridge, Massachusetts: Tubule branching in the lung.

E. Keshet, The Hebrew University Hadassah Medical School, Jerusalem, Israel: Organ-specific induction of vascular networks in the adult.

W. Birchmeier, Max-Delbruck-Centrum, Berlin-Buch, Germany: c-met signaling in tubulogenesis.

C. Samakovlis, Stockholm University, Sweden: Bnl (FGF) signaling regulates apical cell dynamics and epithelial tube length in the *Drosophila* trachea.

SESSION 4: Tubulogenesis Mutants and Diseases I
Chairperson: G.J. Beitel, Northwestern University, Evanston, Illinois

G.J. Beitel, Northwestern University, Evanston, Illinois: Mechanisms of length and diameter control in the epithelial tubes of the *Drosophila* tracheal system.

M. Buechner, University of Kansas, Lawrence: Regulation of the diameter of the excretory canals, a single-celled tubular epithelium in *C. elegans*.

F. Karim, Exelixis, Inc., South San Francisco, California: Genetic dissection of pathways controlling branching morphogenesis and vascular remodeling.

I. Drummond, Massachusetts General Hospital, Charlestown: Epithelial patterning and function in the zebrafish pronephric kidney.

G. Germino, Johns Hopkins University School of Medicine, Baltimore, Maryland: Role of PKD protein in establishing and maintaining tubular structure.

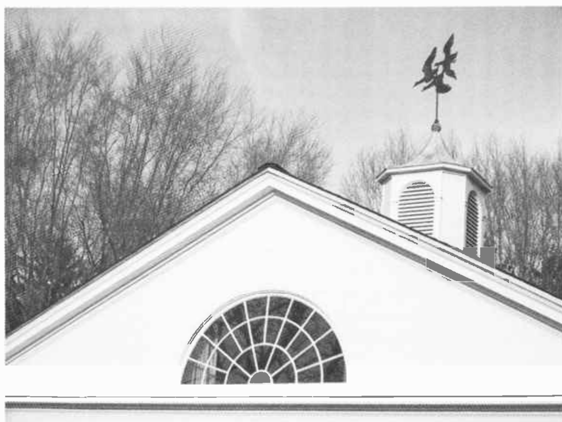
SESSION 5: Tubulogenesis Mutants and Diseases II/Engineering and Evolution of Blood Vessels
Chairperson: D. Marchuk, Duke University Medical Center, Durham, North Carolina

D. Marchuk, Duke University Medical Center, Durham, North Carolina: Vascular morphogenesis: What we have learned from inherited vascular dysplasias.

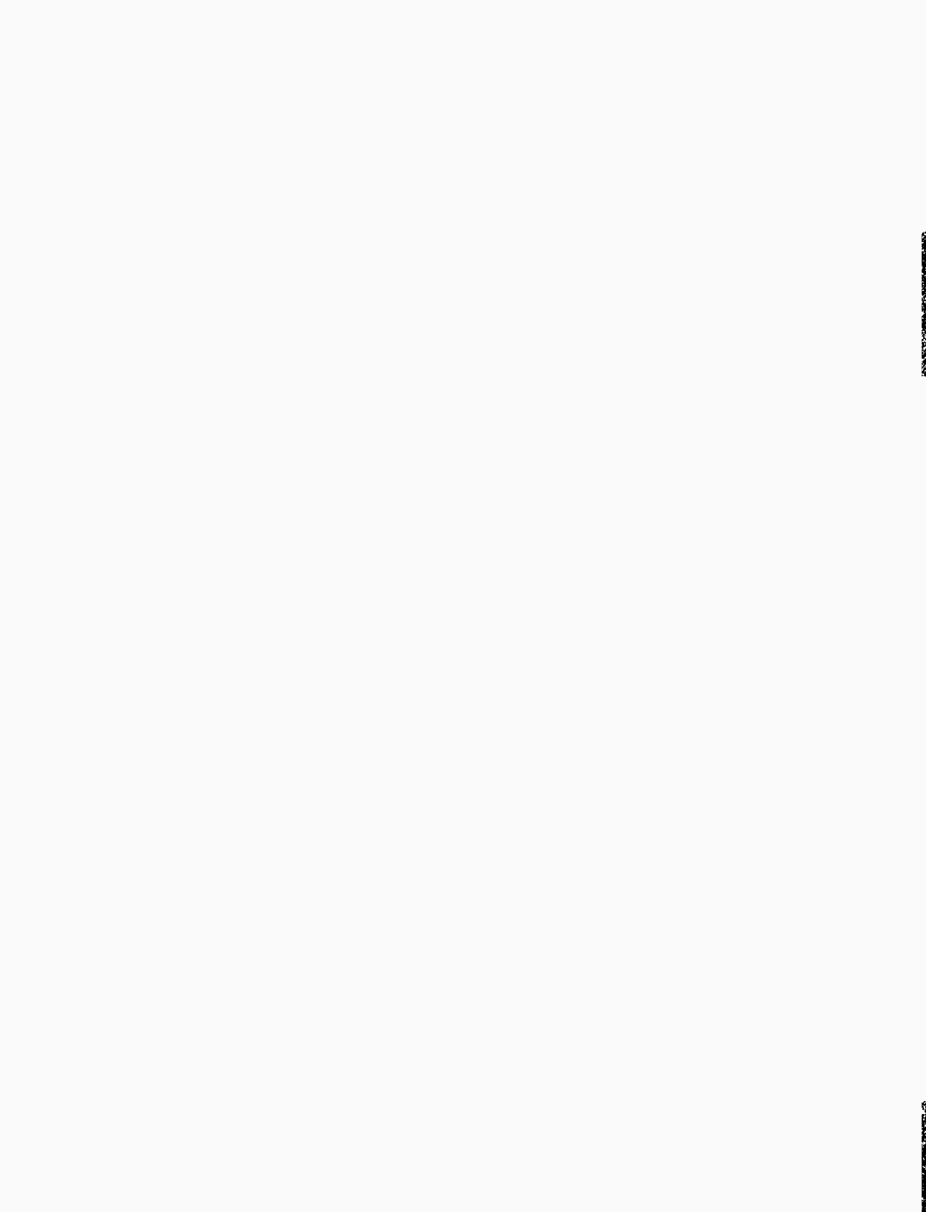
D.Y. Li, University of Utah, Salt Lake City: Mouse angiogenesis mutants: Role of matrix in morphogenesis.

D. Radisky, Lawrence Berkeley Laboratory, California: Mechanisms leading to luminal morphogenesis in the mammary gland.

M.A. Krasnow, Stanford University School of Medicine, California: Developmental control of blood cell migration by the *Drosophila* VEGF pathway: Implications for evolution of the vascular system.



DOLAN DNA LEARNING CENTER



DOLAN DNA LEARNING CENTER

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Erin Wahlgren

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Hong Zhou

BIOMEDIA

Adrian Arva
Shirley Chan
Uwe Hilgert
Eun-Sook Jeong
Susan Lauter
Gisella Walter
Margaret Woodbury
Wen-Bin Wu
Chun-hua Yang

Searching for Meaning in the Human Genome

Albert Einstein spent a great portion of his life pondering the forces that govern the universe. His quantum theory sketched out the grand rules by which elementary particles move and interact to construct all matter. In response to the perception that quantum mechanics had reduced life to a set of mathematical probabilities, Einstein famously said, "I cannot believe that God would choose to play dice with the universe." In a similar way, the publication of the human genome sequence, in February 2001, gives us cause to ponder the forces that have shaped our own genetic inheritance and wonder if anyone is playing dice with our genes.

As the race to sequence the human genome heated up, the Cold Spring Harbor Laboratory Meetings Office came up with its own game of chance to divert the attention of visiting gene experts. Called Genesweep, it is a competition to guess the actual number of human genes. Up to within several weeks of the initial publications of the genome sequence, most bets hovered around 100,000 genes, with some going as high as 150,000.

The announcement that two independent sequencing efforts had arrived at a number of approximately 30,000 genes therefore came as a surprise even to experts. This raised an obvious question: How can humans get along with only about twice as many genes as a fruitfly (with 13,000 genes) or a microscopic roundworm (with 19,099 genes)? At least part of the answer lies in the range of informed bets in the Genesweep contest. Bets on larger numbers were in part based on experiments which sample genes that are expressed (active) in different kinds of cells. These methods, and the higher estimates they produced, were favored by the so-called "gene mining" companies—who obviously would like more genes to sell to their clients.

There is good reason for this seeming overestimate. Prior to protein synthesis, the DNA code is transcribed into a complementary RNA code. Through a process called RNA splicing, discovered independently at Cold Spring Harbor Laboratory and the Massachusetts Institute of Technology, the RNA code is cut and spliced to produce different kinds of directions. Thus, although most genes are represented only once in the human genome, a single gene can be alternatively spliced to produce several different proteins. In this way, the genomes of higher organisms can get a lot of proteins out of relatively few genes.

One would have thought that the two independent sequencing projects would have produced enough information to identify every single human gene. However, several recent assessments suggest that both genome projects may have missed as many genes as they found. In fact, it is very difficult to compare or move between the data sets produced by the two projects. Each used different experimental methods and different computer software to search out genes among the raw data. Many genes are missing from gaps in sequence, where adjacent chromosome pieces have yet to be linked

together. Regions containing large amounts of repetitive DNA, especially around the centromeres, will likely never be completely sequenced and assembled—although few genes are likely to be missed there. Thus, a year after what seemed to the general public the definitive effort to sequence the human genome, we still can only guess at the number of genes that make us human. The Genesweep prize has yet to be awarded.

Rightly, the general public and most scientists focus on the genes revealed by the Human Genome Project. Of course, naming and knowing all of our genes will be a great step forward in understanding how our cells work—and exactly what goes wrong in various diseases. Making human life healthier and, presumably, happier are the practical outcomes of the Human Genome Project. However, just as meaning sometimes lies between the lines of prose or verse, some of the meaning of our genome lies between the genes. Indeed, most of the human genome is not the stuff of genes.

The road to sequencing the human genome began in the 1950s and 1960s. During this period, the discovery of the structure of DNA and the cracking of the genetic code showed the rules that allow living matter to be faithfully reassembled in each generation. Although reducing inheritance to a set of chemical probabilities may have unveiled the mystery of procreation, it did not rob life of meaning. For James Watson, Cold Spring Harbor President and co-discoverer of the DNA structure, it was enough that DNA is "a beautiful molecule." Unfortunately, it takes a fair appreciation of chemistry to understand what Jim Watson means by this. Whereas the DNA structure is more than most people can fathom, the genetic code—the directions for making proteins—can be followed by any fifth grader. Therefore, the cracking of the genetic code was comforting, because, at the very least, it makes good sense.

At the same time, other scientists were turning up evidence that protein-coding regions make up only a fraction of the genomes of higher organisms, including humans. Much of this non-gene DNA is found around the centromere, a knob-like swelling in each chromosome. Here, short DNA sequences are repeated tens to thousands of times, creating a vast DNA desert virtually devoid of genes. It is thought that these short DNA repeats arise when the enzyme involved in copying DNA "stutters," losing its place among a string of repeats and adding an extra unit from time to time. Thus, the repeated regions increase in size over the long course of evolution.

Longer DNA repeats are found outside the centromeres. Ranging from several hundred to several thousand DNA units in length, these repeats are not due to duplication errors. Rather, they are transposable elements—so-called jumping genes—that have copied themselves and moved from chromosome to chromosome. Although transposable elements were first discovered in corn, by Cold Spring Harbor Laboratory scientist Barbara McClintock, they have since been identified in every organism studied—including humans.

In the 1970s, a third type of non-gene DNA, termed an intron, was discovered. Up until that time, most of what was known about gene structure had come from studying bacteria in which each gene generally is a continuous stretch of DNA code, beginning with a start codon and ending with a stop codon. The genes of higher organisms (eukaryotes) turned out to be more complex. In these genes, the genetic code is interrupted by numerous introns, giving rise to "split genes." The average human gene has tens of introns, and the intron sequences are almost always longer than the coding sequences. Prior to protein synthesis, the phenomenon of RNA splicing removes the introns to produce a continuous genetic code.

By the start of the Human Genome Project, the going estimate was that these three types of noncoding DNA weighed in at 95% of the human genetic endowment! In comparison, the entire genetic code of all the genes needed for human life was thought to constitute only about 5% of our total complement of DNA. The draft sequence showed that this estimate was overgenerous by a factor of three. It now appears that as little as 1.5% of the human genome actually carries genetic code for making proteins.

Why so little gene information and why so much "junk?" What kind of way is this to run a genome? At least some fraction of noncoding DNA is far from junk. Noncoding regions between genes contain specific sequences—called promoters and enhancers—that regulate when, where, and how much of a protein is produced. This coordinated activity of genes is especially important during embryonic growth and development. Noncoding regions within genes—introns—contain information that directs RNA splicing, which allows a diversity of proteins to be produced from a single gene. Indeed, gene reg-

ulation and splicing are considered the means through which an essentially identical set of mammalian genes is elaborated into a human, a mouse, or a monkey. Thus, it is not the genes that set humans apart, it is what we do with them.

The noncoding DNA that surrounds promoters, enhancers, and splice signals can be explained as the necessary evolutionary "grist" from which these gene regulators emerged. Short repeated units can be dismissed as mere mistakes in the complex machinery needed for replicating DNA. From a metaphysical standpoint, the transposable elements are more difficult to explain. Two transposable elements, called L1 and *Alu*, are the most frequent gene-sized sequences in the human genome. About 1 million copies of *Alu* and 500,000 copies of L1 compose about 27% of human DNA by weight. Each of these hundreds of thousands of copies arose from an individual "jump" at some point in human evolution. L1 transposons came into the genome about 150 million years ago, so we share these sequences with many vertebrates, including fish and mice. *Alu* is only about 65 million years old—young enough that its jumping is confined to primates, the "monkey" branch of the tree of life. Several thousand *Alu* elements are found only in humans, so they have made the jump in the past 6 million years—after humans diverged from a common ancestor with chimps. L1 carries a gene for the enzyme reverse transcriptase (RT), which converts L1 RNA into a mobile DNA copy. This same enzyme enables retroviruses, such as human immunodeficiency virus (HIV), to insert into positions on the human chromosome from which they cause infection. Current thinking holds that the retroviruses borrowed the RT gene from transposons in the host genome.

Interestingly, the L1 RT enzyme has an additional function analogous to restriction enzymes, the "scissors" that allow biologists to precisely manipulate DNA. L1 makes staggered nicks on each side of the DNA molecule, thus providing a site into which a reverse-transcribed DNA copy can integrate into a new chromosome position. L1 appears to search through DNA and make its first cut in a region containing roughly the same string of DNA letters, termed a consensus sequence. But the second nick is unpredictable. Thus, the jumping about that is mediated by L1 RT lies somewhere between predictability and randomness.

Regardless, with no functional genes at all, *Alu* is thought to rely on RT enzyme produced by L1. This makes *Alu* a parasite of L1 in a molecular symbiosis that puts a point on the axiom:

Great fleas have little fleas
Upon their back to bite 'em
And little fleas have lesser fleas
And so ad infinitum.

Biologists struggle to find the meaning of transposons that take so much space in the human genome. Some believe transposons have been successful because they have enabled the genomes they inhabited to compete more successfully on the battleground of evolution. *Alu* jumping peaked 40 million years ago, reaching a rate of perhaps one new jump in each newborn primate. This period roughly coincided with the evolutionary "radiation" that led to the development of forerunners of modern branches of the primate family. *Alu* accumulates in gene-rich regions, and its jumping may be activated under stressful conditions. These facts have led some to speculate that *Alu* transposition has had a positive role in primate evolution, creating gene variations that provided a selective advantage for evolving primates as they adapted to changes in the earth's environment. Other biologists believe that the success of *Alu* and other transposons rests entirely in their own reproductive ability. *Alu*, which makes no protein, may exist solely for its own replication. This fits Richard Dawkins' moniker of "selfish DNA." If one continued on this line of thought, one might craft a definition of life from the viewpoint of DNA:

Life is the perpetuation and amplification of a DNA sequence through time.

According to this view, *Alu* is a supremely successful life form, with a million copies of itself perpetuated in each of the billions of humans and primates alive today.

Of course, most people would not like to entertain the possibility that our genomes are merely vessels for the reproduction of some selfish and fidgety DNA. Those so inclined might find some cheer

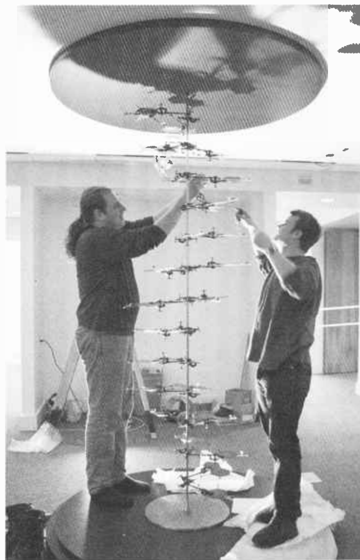
knowing that (HOORAY!) some transposons have gone extinct like the dinosaurs. The *Alu*/L1 symbiosis replaced an earlier and virtually identical symbiosis between a sequence called Mer and L2. Although they have not jumped for at least 100 million years, Mer and L2 "fossils" still litter about 5% of the human genome.

All told, various types of transposons make up more than half of our genetic endowment. If possession is nine-tenths of the law, one might ask "Who's in charge of this genome, anyway?" The truth is, we will probably never know for certain whether transposons are part of the plan or just little fleas playing dice with our DNA.

The Genes We Share

By year's end, we were well into production of our own tribute to the human genome project—a new exhibit entitled *The Genes We Share*. The exhibit takes a global look at the incredible genetic similarity of all human beings, as well as the differences that make each person unique. The 2000-square-foot exhibit will include an array of historical items, audiovisual installations, and interactive exhibits. To make way for the new work, we dismantled the long-standing exhibition, *Story of a Gene*, which focused on the biology, medical applications, and social issues of human growth hormone. It nearly broke our hearts to paint over the *Cellarium*, a room-size depiction of the interior of a human cell.

The Genes We Share celebrates the 50th anniversary of the discovery of the DNA structure, which set the stage for the recent efforts to determine the entire DNA sequence of the human genome. As visitors explore the exhibition, they will be encouraged to view the human genome as a record of our shared ancestry, an instruction manual for our bodies, and a source of information that can foreshadow a person's future health.



Designers from the London Science Museum install the floor-to-ceiling replica of the original DNA model built by Watson and Crick.

Visitors will be greeted in the front hall with photomurals of crowded "peoplescapes," illustrating the similarities and differences between the people of the world. The main hall (the former *Cellarium*) shifts focus to the individual. An interactive area will allow visitors to observe and make records of their own physical features and to compare these with the features of other individuals. Shifting focus even closer, microscopic footage and high-resolution animations will highlight body structures and biological processes that we all share. The endpoint of this area will explore DNA variations in our genomes that make each of us unique. DNA and personality profiles of identical twins, Matt and Danny, will explore the relationship between nature (DNA) and nurture (environment).

Other installations in the central hall will shift focus back to the level of human populations and how genetic differences have evolved between them. An interactive map of the world will depict the migration paths of our earliest ancestors and illustrate some of the environmental factors that influenced human evolution. An interactive table, in the shape of a cellular component called a mitochondrion, will allow

visitors to explore the use of DNA to study human evolution and trace our ancestry. Mini-displays highlight unusual DNA "memorabilia"—including the hair of Anna Anderson-Manahan (who claimed to be the Russian Duchess, Anastasia) and the mitochondrial DNA sequence of the Nobel laureate, James D. Watson.

The smallest exhibition space, located beneath the projection room of the Multitorium, will explore the anatomical, genetic, and behavioral changes that evolved to set humans apart from other primates. Skeletons of a chimpanzee, a modern human, and Neanderthal (the first adult reconstruction ever displayed) will be set within the recreation of a cave environment. Recreations of prehistoric paintings, as well as Neanderthal and Cro-Magnon burials, will encourage visitors to think about the earliest evidence of human self-awareness. By comparing mitochondrial DNA sequences, visitors will see evidence of genetic changes that accumulated through evolutionary time, since humans shared common ancestors with chimps and Neanderthals.

The interpretation of the human DNA sequence will be introduced by an interactive exhibit, "Stories in Our Genes," in which Matt Ridley presents a guided tour through human chromosomes, based on his popular book, *Genome*. This leads into elements dealing directly with DNA structure and sequencing. An eight-foot-tall reconstruction of the original metal DNA model made by Watson and Crick in 1953 represents the beginning of the quest to understand ourselves. A working DNA sequencer, operating daily to sequence DNA submitted by student classes from around the United States, will illustrate the ubiquity of DNA sequencing technology. Finally, exhibits on DNA "chips" and gene therapy will encourage the viewer to ponder how DNA will affect their future lives and health.

We Become the Dolan DNA Learning Center

On June 8, 2001, we rededicated a facility doubled in size by completion of the *Biomedia* Addition. The ceremony honored the generosity of Charles and Helen Dolan, from whom our institution now takes its name. The Dolan Family Foundation's lead gift toward the $5 million expansion was the culmination of years of support, stretching back to when Helen was a member of the original trustee committee that guided the founding of the DNALC. Charles is a quiet pioneer of the cable television industry, and his company, Cablevision, was the major donor to an earlier 1993 renovation of the DNALC. This venture created our 104-seat auditorium as a venue for *Long Island Discovery*, a multimedia presentation that has been seen by more than 76,000 people visiting the DNALC.

The keynote address was given by Dr. Peter Bruns, Vice President of Grants and Special Programs of the Howard Hughes Medical Institute, which has supported our educational programs for the past seven years. The event also marked the unveiling of a large-scale portrait of Charles and Helen in the formal entrance to the *Biomedia* Addition. The impressionist painting was created by Lewis Miller, an Australian artist whose works at CSHL include a monumental portrait of President James Watson and character sketches of noted scientific visitors. Mr. Miller won the 1998 Archibald Prize, Australia's most prestigious award for portraiture.

The dedication also marked the end of a difficult renovation period, during which the multimedia group sought temporary offices on the main lab campus and the instructional group was crammed into makeshift offices in a former exhibit gallery. United again in our new facility, our educators and multimedia designers can once again collaborate in the manner that has enabled us to create novel instructional materials.

Our new facility, designed by the noted architectural firm Centerbrook, is a joy to inhabit. The entire upper level is given over to multimedia production. Enclosed offices along the length of the building provide quiet spaces for writing, and two open bays and a conference area provide open spaces for collaborative work. The area is home to an eclectic staff with varied backgrounds in research biology, science communication, computer programming, exhibition development, and graphic design.

New and redeveloped spaces on the lower level accommodate our programs for student enrichment and teacher training. Three teaching laboratories are organized around "island" benches of our own design, which encourage cooperative learning. The laboratories incorporate the latest multimedia

With all major renovations complete, the DNALC was rededicated on June 11, 2001. Clockwise, from top left: The new west entry to the *BioMedia* Addition; upgraded teaching laboratory; garden border in front of the DNALC depicting the DNA helix; terrace and shade garden adjacent to the new lunchroom; and the Lewis Miller portrait of Charles and Helen Dolan, hanging inside the west entrance.

projection technology and stereo sound, allowing instructors to integrate digitized lab results with an array of education resources—including live WWW content, molecular animations, videotapes, and DVDs. An adjacent prep lab, complete with DNA sequencer and several types of centrifuges, supports the most advanced experiments by staff and student interns.

The striking, octagonal computer laboratory is the symbolic heart of the building, emphasizing the increasing use of computers to "mine" the genetic information encoded in DNA molecules. Like today's genome scientists, DNALC students now have the opportunity to move effortlessly between in vitro ("in glass," or test tube) manipulations of DNA biochemistry to *in silico* (computer) manipulations of the genetic information stored in DNA. For example, in the biochemistry lab, students can isolate their own DNA, amplify a variable region by PCR, and analyze the results by gel electrophoresis. Then, in the computer laboratory, student DNA types are entered into a database and the class "population" is compared to world population using statistical measures.

Instructional staff share a communal office, and a reception/business office sets a professional tone to our interaction with visiting teachers and administrators. In addition, updated restrooms have replaced the antiquated (and tiny) facilities installed in 1925 for elementary children who were the building's first clientele. The original girls' lavatory found new use as a corridor to provide common access to the three teaching laboratories. A new lunchroom allows students to have a relaxed meal looking out on our shade garden, rather than huddling in the hallway or on a school bus.

Instructional Programs

Despite the mayhem of operating a building under construction—including a three-month abandonment of two teaching laboratories during renovation of the original building—a total of 20,868 teachers and students, representing 102 school systems, took part in DNALC laboratory activities during 2001. Amazingly, this number very nearly matches the level of calendar year 2000, when we hosted 21,750 workshop and lab participants. With three functional laboratories up and running by late spring, we increased summer programs by 22%; 557 students participated in workshops at the DNALC as well as at John F. Kennedy High School (Bronx), Brooklyn Technical High School (Brooklyn), and Central Islip High School (Central Islip).

Our *DNA Sequencing Service* doubled in size, processing over 2,000 samples submitted by 64 high schools, 38 universities, and 5 community colleges. Developed several years ago with funding from the National Science Foundation, this service offers students the unique opportunity to use their own DNA to learn elements of modern genomic biology. The exponential growth of this program has been made possible by the gift of a 377 DNA sequencer and ongoing donations of sequencing reagents and instrument service by Applied Biosystems, which allows the DNALC to increase the size of our program while continuing to provide the service for free. Technical support for the *Sequencing Service* is provided by several research staff at the main CSHL campus: Joan Alexander, Spencer Teplin, Ray Preston, and Patrick Smith.

Using a kit developed by the DNALC and distributed by Carolina Biological Supply Company, students isolate DNA from their own hair roots or cheek cells. Their DNA is mixed with freeze-dried PCR reagents to amplify (clone) a highly variable region of the mitochondrial genome. The amplified samples are then mailed to the DNALC, where high school interns perform the final DNA sequencing reactions. The student DNA sequences are then databased in an educational workspace at our WWW site, from which students can launch a number of analyses, including BLAST searches and CLUSTAL sequence alignments. A companion WWW site, *Genetic Origins*, contains everything needed to reproduce the experiment and use the results to explore unanswered questions in human evolution.

For the past several years, middle-school field trips had combined lab work with interpretive use of the *Story of a Gene* exhibit. Thus, dismantling the exhibit required us to rethink how we could continue to provide a rich experience for middle-school students while we develop the new exhibit, *The Genes We Share*. We turned this quandary into an opportunity to quickly integrate the computer laboratory into the life of the DNALC.

Working directly with multimedia designer Chun-hua Yang, the middle-school staff developed a guided multimedia module for middle-school visitors. The result was an interactive mystery based on the true story of Anastasia Romanov. During a typical session, a DNALC instructor uses video clips from the NOVA video, *Anastasia: Dead or Alive*, to introduce the Romanov family and circumstances of their murder in 1918.

Middle-school students participate in the Anastasia computer experience led by educator Maureen Cowan.

Students then work collaboratively at their computer stations, using forensic methods to determine the ages and sexes of skeletal remains from a common grave in Siberia. Comparing DNA from the skeletons to living descendants of the Russian royal family confirms the identity of the five Romanovs, but leaves open the fate of the youngest daughter, Anastasia. Following this story, students compare the lives and physical features of Anna Anderson, a woman who many people, including some surviving Russian royalty, believed was Anastasia. A comparison of Anna Anderson's DNA with that from the bones in the Siberian grave recreates scientific experiments conducted in the 1990s and provides the final word on the mystery of Anastasia. Teacher and student reaction to the *Anastasia* module has been almost overwhelming. We believe the module is one of the very best examples of effective use of multimedia in science education. By year's end we had begun development of a WWW version to make this stunning work available worldwide.

In the spring, we continued our honors seminar series for local students, *Great Moments in DNA Science*. The program attracted 476 secondary science students to a range of topics in modern biology:

- Dr. Gregory Hannon, Cold Spring Harbor Laboratory: "The Role of Cell Proliferation Control in Cancer"
- Robert Baumann, Suffolk County Crime Laboratory: "The Use of DNA in Criminal Investigations"
- Dr. Howard Rosenbaum, American Museum of Natural History: "Working With Whales: DNA as a Useful Tool for Conserving the World's Largest Creatures"

Expanding the DNALC Model to the East and West

Director Dave Micklos meets with students in Singapore.

The year provided us with new opportunities to disseminate our unique way of working. Following up on a visit in October 2000 from the Minister of Education, RAdm Teo Chee Hean, of the Republic of Singapore, we worked with ministry officials to explore the possibility of setting up a sister institution in the island republic noted for student science achievement. During a week-long visit in February, DNALC director David Micklos was given an extensive tour of all levels of the science education and research system, including elementary and secondary schools, junior and technical colleges, and the national university and research institutes. In May, Dave and Scott Bronson returned to Singapore to instruct a *DNA Science* Workshop for high school and junior college faculty, and four Singaporean educators visited the DNALC in July to attend the Pfizer *Leadership Institute*.

In the following months, continuing discussions led to the proposal to establish two institutions in Singapore based closely on the DNALC model. One, at the Singapore Science Centre, will focus on student enrichment and public outreach. The other, at the National Institute of Education, will focus on teacher training. We anticipate a high-level visit from the Ministry in 2002 and a subsequent multi-year agreement for the substantial transfer of intellectual property to the Singapore centers—including teacher training, technical assistance, WWW site mirroring, and joint curriculum development. With central control of a school system about the size of Chicago, we regard this as a unique opportunity to experiment with the rapid and large-scale deployment of instructional methods we have developed over the past 15 years.

Closer to home, a different opportunity presented itself when CSHL Trustee Arthur Spiro proposed to develop a "DNALC West" in collaboration with the Research Institute of North Shore-Long Island Jewish (NS-LIJ) Health System. In this case, it was agreed that the DNALC will operate a teaching lab hosted at NS-LIJ, mirroring lab field trips and summer workshops conducted at the DNALC. By year's end, plans were in hand for a teaching laboratory and student lunchroom to be located in space adjacent to the system's diagnostic laboratories, just off the Northern State Parkway in Lake Success. The NS-LIJ facility will allow us to better serve school districts in western Nassau County, Brooklyn, Queens, and Manhattan.

Howard Hughes *VectorNet* Programs

With funding from the Howard Hughes Medical Institute (HHMI), we continued our longstanding effort to introduce students and faculty to the use of modern networked computing in genomic biology. Central to this effort is *VectorNet*, a stand-alone, portable computer laboratory, consisting of 12 user laptops and a laptop server. The program is based on our earlier work with *Vector* mobile DNA laboratories—specially designed Ford vans and reagent kits we introduced in the mid-1980s to deliver teacher training in molecular genetics to sites around the country. Vans and DNA footlockers are now standard methods for resource sharing between schools in many regions of the country. In a similar way, *VectorNet* was designed to prove the feasibility of "backpacking" a bioinformatics computer laboratory essentially anywhere.

The student component, *New York City Genes* is a collaborative project with the *Gateway to Higher Education Program*, a major science education initiative by Mt. Sinai Medical School and NYC public schools. In 2001, we trained 55 high school teachers from New York City to use the *VectorNet* Laboratory to access the DNALC's rich Internet content, data analysis tools, and on-line bioinformatics facilities. In the spring, the *VectorNet* system was rotated to Brooklyn Technical High School (Brooklyn), the High School for the Humanities (Manhattan), and Stevenson High School (Bronx) where it was used by some 250 primarily minority students in grades 9–11. The availability of a set of networked computers in the biology classroom allowed students to move between lab experiments and computer analysis of their own DNA polymorphisms.

Bioinformatics is a relatively young discipline that attempts to analyze the information content of DNA. Since it merges biology and information technology, the field offers an interesting means to involve computer-literate students in science. Most bioinformatics tools and data sets are freely available on the Internet. Students and teachers have the unprecedented opportunity to use the same tools that biologists do to explore the human genome.

The *Vector Bioinformatics* Workshop aims to enable teaching faculty to take advantage of the treasure trove of DNA data flowing from the Human Genome Project, while improving basic computer skills. During its first summer, 78 high school and college faculty participated in workshops held at four independent research institutions. We relished the chance to visit these wonderful institutions and renew friendships with local organizers who were essential to our summer success:

Susan Cooper, Trudeau Institute, Saranac Lake, New York
Nancy Hutchison, Fred Hutchinson Cancer Research Center, Seattle, Washington
Ellen Potter, Salk Institute, La Jolla, California
Philip Silverman, Oklahoma Medical Research Foundation, Oklahoma City

The week-long workshop guides participants in logical steps from DNA isolation through sequence basics to *in silico* gene discovery. The workshops begin with basic methods for analyzing patterns in DNA sequences and progress to on-line algorithms that identify gene features, including open reading frames and intron/exon boundaries. Participants then use genome browsers to find genes in on-line databases, identify their chromosome locations, identify homologs in other organisms, and explore their involvement in normal and disease processes. Conceptually, the week culminates in the investigation of pharmacogenetics and the research efforts to identify single nucleotide polymorphisms (SNPs) that predict disease susceptibility and drug response.

The computer work is punctuated by biochemistry labs that use the teacher's own DNA as a starting point for on-line analysis, using the DNALC's custom *BioServers*. Simple DNA types at the PV92 locus on chromosome 16—developed *via* PCR and gel electrophoresis—are the basis for studies of human population genetics and tests of competing theories of human origins. Making use of the DNALC's *Sequencing Service* (described above), sequencing the control region reveals SNPs in each participant's own DNA and provides a foundation for sequence comparisons and database searches.

Pfizer *Leadership Institute*

Originally supported by the National Science Foundation during summers 1993 through 1995, the *Leadership Institute in Human and Molecular Genetics* returned in 2001 with Pfizer Foundation funding. Affectionately known as "DNA Boot Camp," this *Institute* is to high school biology teachers what the CSHL postgraduate research courses are to scientists—an intense immersion into high-level experimentation and thinking. During their three-week stay in July, participants are housed on the main campus of CSHL, where they can interact with CSHL staff and visiting scientists at meals and seminars.

The 20 faculty participants in 2001 represented 14 different states, and a rigorous selection process ensured that they were among the top 5% of high school biology teachers nationwide. As evidence of their professionalism, 85% of the participants had advanced degrees, and they averaged 18 years' teaching experience. The schools they represented ranged from well-financed private institutions to large urban high schools with limited budgets; 55% of their schools have a minority population of 19–52%, and 25% of them provide lunch assistance for their student population. The intent of the *Institute* is to further develop their content and leadership skills so they can function as regional experts in advanced biology instruction.

The *Institute* incorporates high-level theoretical, laboratory, and computer work—including human and plant DNA polymorphisms, DNA sequencing, bioinformatics, and computer multimedia. Seminars presented by CSHL research scientists Richard McCombie, Rob Martienssen, Vivek Mittal, and David Helfman extended coursework to real-life research problems. A field trip to the American Museum of National History, in New York City, provided an opportunity to tour "The Genomic Revolution" exhibit with curator Rob DeSalle, to attend a symposium on "Teaching in the Genome Age," and to interact with Woodrow Wilson Fellows taking part in a similar institute at Princeton.

The *Institute* culminated with independent time during which participants were free to follow up on laboratory or computer work of particular interest to them, and to prepare curriculum materials for use in their own classrooms. Projects included identification of genes/transgenes in plants, bioinformatics, and Web page construction. One teacher brought bone material from a 1000-year-old South American mummy to the workshop. During the independent work period, she and two colleagues extracted and analyzed the mummy's mitochondrial DNA. Other teachers learned how to do electronic PCR or explored parallels between modern genetics and the ill-fated eugenics movement.

Pfizer Leadership participants and DNALC staff on the final day of the three-week-long workshop.

DNA Interactive

In the spring, CSHL was abuzz with news of Jim Watson's plan for a five-part PBS television series, *Genetic Journey*, to celebrate the 50th anniversary of his discovery of the double helix. This will surely be the most notable public science event of 2003. Thus, we were thrilled when, in the fall, the DNALC was brought into the project to develop *DNA Interactive (DNAi)*, an interactive WWW "portal" through

which teachers and students can enter a rich world of on-line resources about the DNA revolution. Development of the site, as well as supporting vidoegraphy and animations, is being funded by a $2 million grant from the Howard Hughes Medical Institute.

DNAi will be organized around five mini-courses that loosely follow the content of the TV series. Each mini-course will draw a variety of media elements into a common window, or "player"—including video interviews from *Genetic Journey*, schematic animations, high-resolution graphic animations, interactive problems, downloadable lesson plans and student workbooks, and connections to science education standards. *DNAi*'s unique content will be complemented by a revolutionary site structure. Working from the premise that teachers gain "ownership" of their curriculum by organizing knowledge drawn from a number of sources, *DNAi* will allow teachers to customize information and multimedia tools to best suit their unique instructional needs and preferences.

DNAi will employ a sophisticated content management system, which will be used by the DNALC staff to create the content of the site and allow teachers to customize the content according to their own needs. A user profile is stored on the *DNAi* server, and upon logging on, a teacher's customized content is assembled "on the fly" from content stored in the *DNAi* multimedia database. The site will be customizable on three levels. On the first level, teachers can create a custom class page, incorporating interactive page design of the sort offered on the start pages of Yahoo, MSNBC, and other on-line companies. The class page includes a message board to alert students to current work and assignments, a news feed of genetics articles from around the WWW, and a message board that at once anchors class discussions within wider discussions at the *DNAi* site.

On a second level, teachers will be able to edit modules within the mini-courses, shortening them or adding customized narrative. On a third level, teachers will be able to search, by keyword, all of the multimedia content at the site, then edit the returned items for display in a custom player. Each selected item can then be annotated by the teacher for class presentation or homework assignment. This will give biology teachers the multimedia equivalent of Powerpoint—a simple presentation builder linked to a database of thousands of searchable video clips, animations, molecular graphics, photographs, and narrative elements.

DNAi represents a tremendous opportunity for the DNALC to make good use of the sophisticated Internet publishing capabilities it has built over the past several years. We believe that *DNAi* will introduce a new way for biology teachers and students to interact on the WWW, and we expect it to draw between 500,000 and 1 million visitors per month. Upon publication of *DNAi*, in spring 2003, we will be positioned for additional large-scale projects in biological multimedia.

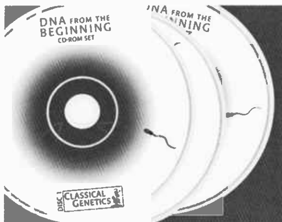
Our WWW Domain Expands

The *Gene Almanac* WWW site is a portal to a number of content sites developed and managed by the DNALC. In 2001, the sites received a combined visitation of 1.7 million, an increase of 54% over the previous year. *DNA from the Beginning* is still the most popular site, but within months of its launch, the new site *Your Genes, Your Health* was poised to overtake it in popularity.

In anticipation of future growth, we improved our site architecture and updated our server hardware. We also implemented common sense-domain names, which will aid visitors in finding our sites:

Gene Almanac: http://www.genealmanac.org
DNA from the Beginning: http://www.dnaftb.org
Your Genes, Your Health: http://www.yourgenesyourhealth.org
Archive of the American Eugenics Movement: http://www.eugenicsarchive.org
Genetic Origins: http://www.geneticorigins.org
Bioservers: http://www.bioservers.org

In December 2000, we posted the final chapters of our Internet-accessible tutorial *DNA from the Beginning* (*DNAFTB*). To make this work available to those without fast Internet connections, in November we released a CD-ROM version for stand-alone play. Animation, video, and photographic



Above: Postcard to promote the *DNA from the Beginning* CD set. At right: The *Your Genes, Your Health* Web Site has become the most popular component of the DNALC Web Site.

resources of the 41-chapter work are so extensive that they fill three disks. The set is available at our WWW site for $49.99, using PayPal to expedite credit card purchases.

With completion of *DNAFTB*, the *Biomedia* staff began developing a companion site entitled *Your Genes, Your Health* (*YGYH*). A multimedia guide to genetic disorders, *YGYH* is targeted at patients and families who are urgently looking for understandable information about specific genetic disorders. The site deals with relatively common disorders, and focuses on how ongoing molecular genetics research is improving diagnostics and the types of treatment that are currently available.

YGYH was launched in April with Fragile X syndrome, followed by Marfan syndrome, cystic fibrosis, hemophilia, and Duchenne/Becker muscular dystrophy. The hemophilia module was a Yahoo Web Site pick of the week. Modules on phenylketonuria, Huntington disease, neurofibromatosis, sickle cell, hemochromatosis, thalassemia, Tay-Sachs, Alzheimer, Down syndrome, and either asthma or diabetes will follow in 2002. Each disorder starts with short facts for quick browsing. More in-depth information is provided by animations on the disorder's cause, inheritance, and diagnosis. Video interviews with patients, health-care providers, and researchers provide insiders' perspectives on having and treating genetic disorders. Based on feedback received from this Web Site, these insiders' perspectives are extremely helpful for the newly diagnosed. Each disorder also has links to support groups and foundations, which advise us during development.

In February 2001, the *Biomedia* group obtained a 3-year, $850,000 grant from the National Institutes of Health to build a multimedia Internet site called *Inside Cancer*. The Web Site is geared toward the general public and will be especially useful to teachers and students. It will be a resource for people who want authoritative information on the workings of a cancer cell. Animations and video interviews with cancer researchers and other experts will help people understand the complex science and issues of cancer. The *Inside Cancer* site will have five modules: (1) *What is Cancer* shows how cancers develop from a single cell. (2) *Causes & Prevention* identifies behaviors and environmental factors that increase cancer incidence. (3) *Diagnosis & Treatment* explains traditional and experimental strategies for identifying and fighting cancer. (4) *Cancer in the Laboratory* explains key experiments that have advanced our understanding of cancer at the cellular and molecular levels. (5) *Pathways to Cancer* is a three-dimensional tour showing how growth signals are relayed from the cell exterior to the nucleus and how they are perturbed in cancer cells.

Eugenics Image Archive

The *Image Archive on the American Eugenics Movement* Web Site continues to be a popular resource. In 2001, the *Archive* received 84,000 visitors who requested 428,000 documents. We increased the

holdings of the *Archive* by nearly 50% when we photographed 700 additional objects from the CSHL Archives and two other sites. At the International Center of Photography (ICP) in Manhattan, we were able to gather an eclectic set of images from the exhibition *Perfecting Mankind: Eugenics and Photography*. We were very excited to receive permission to access collections at the University College, London, where we spent a frenetic two days in October in the collections of Francis Galton, Karl Pearson, and Lionel Penrose. It was thrilling to see Galton's notebooks for *Hereditary Genius*, the work that started the eugenics movement; data from his work on fingerprint analysis and composite portraiture; and personal correspondence with his cousins Charles and Leonard Darwin.

To allow people to have the sense of working with original documents that represent the objectives and methods of the eugenics movement, about half of the *Archive* consists of photographs of rare documents and correspondence. However, the content of these *photographs* cannot be searched. Thus, in 2001 we began a major project to transcribe each written document in the collection. Under this system, each image of a document has a corresponding text file, which can be searched along with titles. Thus, a search returns both the archival photograph and a corresponding text file, which is easier to read and from which quotations can be easily extracted.

On May 6–8, we held the first of three Banbury meetings to be sponsored under our grant from the National Human Genome Research Institute. *American Eugenics and the New Biology: Perspectives and Parallels* aims to familiarize "opinion leaders" about this dark saga in American science. The meeting drew 31 participants from diverse fields, including family genetics, education, ethics, journalism, government, industry, and philanthropy. Sessions on the history and lessons learned from the eugenics movement included presentations by four members of our Advisory Panel: Gar Allen, Elof Carlson, Paul Lombardo, and Steve Selden.

Garland Allen, Washington University, St. Louis, Missouri
Elof Carlson, SUNY, Stony Brook, New York
Katie Clapp, FRAXA Research Foundation, Newburyport, Massachusetts
Pat Colbert-Cormier, Lafayette High School, Louisiana
Nancy Fisher, Regence Blue Cross, Seattle, Washington
Henry Friedlander, City University of New York, New York
Daniel Kevles, California Institute of Technology, Pasadena
Philip Kitcher, University of California, San Diego
Martin Levitt, American Philosophical Society, Philadelphia, Pennsylvania
Paul Lombardo, University of Virginia, Charlottesville
Nancy Press, Oregon Health Sciences University, Portland
Philip Reilly, Shriver Center for Mental Retardation, Waltham, Massachusetts
Pat Ryan, Carolina Biological Supply Company, Burlington, North Carolina
Marsha Saxton, World Institute on Disability, San Francisco, California
Steven Selden, University of Maryland, College Park
Terry Sharrer, National Museum of American History, Washington, D.C.

Dean Hamer of the National Cancer Institute discusses the genetics of human behavior with participants at the first *American Eugenics and the New Biology* meeting in May.

Staff and Interns

We were sorry to bid farewell to several staff members in 2001. Vin Torti, the Chief Development Officer for the DNALC, left in July for a position at Long Island Institute of Technology. He is currently working as the development officer at Winthrop Hospital. Our scientific coordinator, Veronique Bourdeau, went on maternity leave in September and had a baby boy. The whole family then moved to Montreal, where Vero's husband took a position at the local university. In the future, we hope to work with Vero to bring some of the DNALC's programs to the Great White North. After contributing key writing to the *DNAFTB* and *YGYH* WWW sites, Susan Conova left in December for a position in the external relations department of Columbia Health Science, where she is a science writer and communicator.

With the increase in physical space at the DNALC, we were able to plan for a new exhibit. Bronwyn Terrill joined us in February to develop the new DNALC exhibit *The Genes We Share*, which will be open to the public in 2002. Bronwyn, our Australian import, has a background in science communication and museum exhibit development and has worked at several major science museums and organizations in Australia.

The *Biomedia* group expanded in response to the DNALC's growing web presence. Wen-Bin (Bin) Wu started in April as a new multimedia designer. He has a background in print design and a masters degree in computer design from Rochester Institute of Technology. Bin's eye for simplicity and elegance and his attention to detail have brought a new look to our Web Sites. Adrian Arva was an eagerly awaited addition to the *Biomedia* staff. Adrian responded to our on-line advertisement for a computer programmer in September of 2000. His interest in Web Site development and bioinformatics, and his medical/science background, made him an ideal choice for our web programmer. It took a little longer for the U.S. and Romanian governments to agree with our choice, but Adrian finally began working at the DNALC in May.

The middle-school instructional group also saw changes. In September, we were thrilled to welcome Tricia Maskiell back to the middle-school staff. Tricia had left her position at the DNALC in 2000 when her husband's job required a move to New Hampshire. One of the first things Tricia did when they moved back to New York in 2001 was to contact the DNALC and take on a part-time position. Danielle Sixsmith, another middle-school teacher, went on maternity leave this summer, and returned in November with a new addition to her family—a baby girl. Danielle is working part-time, and will head up DNALC West, the satellite facility we are currently developing with the North Shore–Long Island Jewish Health System.

To make full use of our enlarged teaching facilities, in the fall we recruited Jennie Aizenman and Hong Zhou to the instructional group. In addition to teaching high school classes, Jennie will develop new labs for the students. She received her Ph.D. in molecular microbiology and immunology from the Johns Hopkins University School of Public Health and did postdoctoral work at Rockefeller University. Hong helps manage our high school interns and our *DNA Sequencing Service*. The wife of CSHL scientist Jerry Yang, Hong has a masters degree in molecular biology and has worked in science labs in China and in the United States.

Veteran interns Yan Liang Huang (Harborfields High School) and Janice Lee (Oyster Bay High School) assisted us throughout the year training new interns and working in our new laboratory classrooms and prep labs. Janice is researching RNAi in fission yeast with CSHL's Tom Volpe (Delbruck). She also completed her research of PCR analysis of the CF1 mutation in *Arabidopsis* and, over the summer, helped train the younger interns.

Other veteran interns, whom we miss tremendously, left mid-year to pursue their scientific interests. Among them, Caroline Lau (Syosset High School) was chosen for the prestigious CSHL "Partners for the Future" program. She has been teamed with David Jackson (Delbruck), and is studying cell-to-cell communication in *Arabidopsis thaliana*. Rebecca Shoer (Syosset High School) is researching new protocols for microarray techniques with Vivek Mittal at the Woodbury Genome Center. Jordan Komisaro (Long Beach High School) is researching *Arabidopsis* genetics with Rob Martienssen (Delbruck). Daniel Goldberg (Half Hollow Hills East) has joined a researcher at NS-LIJ. Rebecca (Becca) Yee, a former high school intern at the DNALC, used her summer break from Wellesley College to help instruct and coor-

dinate new interns. Her expertise and skill were greatly appreciated during the hectic workshop season. Visiting summer interns were Daniel DeRoulet, Daniel Davison, Brendan O'Kane, and Dennis O'Kane.

Processing and sequencing mitochondrial DNA sent from around the country has become an important responsibility of the intern force. Sirish Kondabolu (Half Hollow Hills High School) and Jonathan Mogen (Half Hollow Hills High School), along with senior intern Andrew Diller, have kept pace beautifully as we continue to meet the increasing demand on the DNALC's on-site sequencing program.

Joining the intern crew in the spring were Jared Winoker (Syosset High School), Marie Mizuno (Cold Spring Harbor High School), Benjamin Blond (Syosset High School), and Kunal Kadakia (Syosset High School). Jared and Kunal have been studying the allelic frequency of the *Alu* insertion on chromosome 16. Benjamin has been working with Jennie Aizenman on mutagenesis of enhanced GFP and BFP proteins. Marie is researching apoptosis with Yuri Lazebnik (Hershey).

Fall 2001 newcomers included Eric Paniagua (Long Island School for the Gifted), Saroja Bangaru (Long Island School for the Gifted), Michelle Louie (Kings Park High School), Hala Mostafa (Kings Park High School), Gerad Ryan (Kings Park High School), Jarrett Linder (Half Hollow Hills High School), Alex Witkowski (Cold Spring Harbor High School) and Wayne Chiang (Cold Spring Harbor High School).

The *Biomedia* group also had talented and enthusiastic interns to help with the day-to-day operations of Web Site production. Tracy Mak (Syosset High School) and Felix Hu (Northport High School) as returning high school interns were indispensable in taking care of all the details. Felix was extremely helpful over the summer with software and computer installations. Summer college interns Eun-Sook Jeong (C.W. Post), Kun-Feng Chen (C.W. Post), and Matthijs Muller (Free University of Amsterdam, Netherlands) helped with initial production of the *Your Genes, Your Health* Web Site. We also welcomed Watson School graduate student Elizabeth Thomas to assist us with WWW development.

2001 Workshops, Meetings, and Collaborations

January 16–18	National Institutes of Health, *A Decade of ELSI Research* Conference, Bethesda, Maryland
January 22–23	*Your Genes, Your Health* interview, Katherine Clapp, FRAXA Research Foundation, Newburyport, Massachusetts
January 24	*Your Genes, Your Health* interview, Debbie Stevenson, FRAXA Research Foundation, National Fragile X Foundation, New York, New York
January 25	Site visit by Rebecca Burkhadt, Long Island Business News
January 29	*Your Genes, Your Health* interview, Dr. Ted Brown, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, New York
January 30	Site visit to American Museum of Natural History and the International Center of Photography, New York, New York
February 13	Site visit by Axel Jahns, Brinkmann Instruments
February 15–20	American Association for the Advancement of Science Annual Meeting, San Francisco, California
February 16–25	Site visit to the National Institute of Education, Singapore
March 14	Site visit by Dr. Rod Wing, Dr. Jerry Trapnell, and Dr. Harold Cheatham, Clemson University, South Carolina
March 18	Presentation for students and parents, Parrish Art Museum, Southampton, New York
March 20	Site visit by Dr. James Bonacum, American Museum of Natural History, New York, New York
March 22	Howard Hughes Medical Institute, *New York City Genes* teacher training, York College, Queens, New York
March 22–25	National Science Teachers Association Annual Meeting, St. Louis, Missouri
March 29	Workshop presentation for National Institute of Science Meeting, Atlanta, Georgia
March 30	National Human Genome Research Institute ELSI Project, *Eugenics Image Archive*, collection visit to International Center of Photography, New York, New York
	Your Genes, Your Health interview, Dr. Richard Devereux, New York Presbyterian Hospital, New York, New York
April 4	Site visit by Judy Winkler, Invention Factory Science Center, Trenton, New Jersey
	National Institute of Social Sciences Board Meeting, New York, New York
	Presentation for *Career Day* at Sayville High School, Sayville, New York
April 6	*Your Genes, Your Health* interview, Julie Kurnitz, New York, New York
April 13	Laboratory for Pine Creek and George Washington High Schools, Colorado Springs, Colorado
April 20	Site visit to American Museum of Natural History, New York, New York
April 25	National Institute of Social Sciences Meeting, Harvard Club, New York, New York
April 26	Site visit by Wendy Law and David Masterman, Fred Hutchinson Cancer Research Center, Seattle, Washington
April 28	Presentation for students and parents, Planting Fields Arboretum State Historic Park, Oyster Bay, New York
May 1	*Great Moments in DNA Science* Honors Students Seminar, CSHL
May 4	*Your Genes, Your Health* interview, Dr. Allan Rubenstein, Mount Sinai School of Medicine, New York, New York
May 6–8	National Institutes of Health ELSI conference, *American Eugenics and the New Biology: Perspective and Parallels*, Banbury Center, CSHL
May 7	*Great Moments in DNA Science* Honors Students Seminar, CSHL
May 10	Site visit by Dr. Peter Bruns, Vice President for Grants and Special Programs, Howard Hughes Medical Institute
May 11	Site visit by Jesse Raiford, Maximum Science Studio
May 14	Site visit by Christine Herbes-Sommers and Sandy Haller, *Human Race* project, California Newsreel
May 15	*Great Moments in DNA Science* Honors Students Seminar, CSHL
	Your Genes, Your Health interview, Cara Kaek and daughter Noel, Long Island, New York
May 16	Site visit by Robert M. Frehse, Jr., Vice President and Executive Director, The Hearst Foundation, New York City
May 18–20	American Society for Microbiology Education Conference, Orlando, Florida
May 22	Site visit by Dean Madden and John Schollar, University of Reading, United Kingdom
May 28–June 1	*DNA Science* Workshop, National Institute of Education, Singapore
May 29	*Your Genes, Your Health* interview, Paul Brayshaw, Washington D.C.
May 30	*Your Genes, Your Health* interview, Price family, Washington D.C.
	Your Genes, Your Health interview, Dr. Katherine High, Children's Hospital of Philadelphia, Pennsylvania

June 8	Dolan DNA Learning Center Building Dedication
June 11–13	Teacher training workshop for Singapore Ministry of Education
June 12	*Your Genes, Your Health* interview, Dr. Catherine Manno, Children's Hospital of Philadelphia, Pennsylvania
June 15	Site visit and interview by Scott Feldman, News 12 Long Island
June 18–22	Howard Hughes Medical Institute, *Vector Bioinformatics* Workshop, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma
June 25–29	*Fun With DNA* Workshop, DNALC
	World of Enzymes Workshop, DNALC
	DNA Science Workshop, DNALC
July 2–20	Pfizer *Leadership Institute in Human and Molecular Genetics*, DNALC
July 6	Site visit by Karen Arenson, *The New York Times*
July 7	Howard Hughes Medical Institute, *New York City Genes* teacher training, American Museum of Natural History, New York, New York
July 9–13	*Green Genes* Workshop, DNALC
	Genomic Biology & PCR Minority Workshop, Central Islip High School, New York
July 16–20	*Fun With DNA* Workshop, DNALC
	World of Enzymes Workshop, DNALC
	DNA Science Minority Workshop, Brooklyn Technical High School, New York
July 18	*Your Genes, Your Health* interview, Dr. Clement Ren, Stony Brook University Medical Center, New York
July 23–27	*Fun With DNA* Workshop, DNALC
	Green Genes Workshop, DNALC
	DNA Science Minority Workshop, John F. Kennedy High School, Bronx, New York
	Genomic Biology & PCR Workshop, DNALC
	Howard Hughes Medical Institute, *Vector Bioinformatics* Workshop, Salk Institute for Biological Studies, La Jolla, California
July 25	*Your Genes, Your Health* interview, Dr. Jane Halperin, genetic counselor, New York, New York
July 26	*DNA from the Beginning* interview, Dr. Nathaniel Comfort, Baltimore, Maryland
July 30–August 3	*Fun With DNA* Workshop, DNALC
	World of Enzymes Workshop, DNALC
	DNA Science Workshop, DNALC
	DNA Science Workshop, Brooklyn Technical High School, New York
	Howard Hughes Medical Institute, *Vector Bioinformatics* Workshop, Fred Hutchinson Cancer Research Center, Seattle, Washington
July 31	*Your Genes, Your Health* interview, Suzanne Burger, Westchester County, New York
August 6–10	*Green Genes* Workshop, DNALC
	DNA Science Workshop, DNALC
August 9	*Your Genes, Your Health* interview, Dr. Alfred Spiro, Albert Einstein College of Medicine, Bronx, New York
	DNA from the Beginning interview, Dr. Scott Gilbert, Swarthmore College, Pennsylvania
August 13–17	*Fun With DNA* Workshop, DNALC
	Genetic Horizons Workshop, DNALC
	Genomic Biology & PCR Workshop, DNALC
	Howard Hughes Medical Institute, *Vector Bioinformatics* Workshop, Trudeau Institute, Saranac Lake, New York
August 20–24	*Fun With DNA* Workshop, DNALC
	World of Enzymes Workshop, DNALC
	DNA Science Workshop, DNALC
August 22	Site visit by Michael Dowling, Roy Zuckerberg, and Dan DeRoulet, North Shore-Long Island Jewish Health System
August 23	*Your Genes, Your Health* interview, Dr. Selma Snyderman, Mount Sinai School of Medicine, New York, New York
August 27	Site visit by Michael Gilman and Gregory Peterson, Biogen, Cambridge, Massachusetts
	Your Genes, Your Health interview, Erin Buckley, Huntington, New York
August 31	Site visit by Clare Matterson, Wellcome Trust, London, U.K., and D. Burke, Pricewater-house Coopers
August 27–31	*Fun With DNA* Workshop, DNALC
	DNA Science Workshop, DNALC
September 7–9	Workshop presentation for students taking part in the *Human Race* project, Allston, Massachusetts

September 18	Site visit by David Bowtell and Joe Sambrook, Peter MacCallum Cancer Institute, East Melbourne, Australia
September 20	Gateway Institute for Higher Education Coordinator Meeting, City College, New York, New York
September 21	*Your Genes, Your Health* interview, Dr. Chris Ross, Johns Hopkins University School of Medicine, New York, New York
	Your Genes, Your Health interview, Suzanne Doggett, Huntington's Disease Society of America, New York, New York
	Your Genes, Your Health interview, Nancy and Barry Goldring, Huntington's Disease Society of America, New York, New York
September 30–October 1	National Human Genome Research Institute ELSI Project, *Eugenics Image Archive*, Editorial Advisory Panel Meeting, Banbury Center, CSHL
	Your Genes, Your Health interview, Pat Ryan, Carolina Biological Supply Company, Burlington, North Carolina
October 2	*Your Genes, Your Health* interview, Dr. Kusum Viswanathan, Brookdale University Hospital and Medical Center, New York
	Your Genes, Your Health interview, Dr. Kenneth Rivlin, Brookdale University Hospital and Medical Center, New York
October 3	Inter School Exchange Faculty Dinner, DNALC
October 4	Site visit by Dr. Michael Schroeder, Eppendorf AG, Hamburg, Germany
October 5	Site visit by Piero Benedetti, Padova University, Italy
October 15	Site visit by Linda Winston, American Museum of Natural History, New York, New York
October 17	Presentation for *Long Island School to Career Partnership*, Huntington Hilton, New York
October 18	*Inside Cancer* interview, Dr. Scott Lowe, CSHL
October 18–19	Exhibit interview, Dr. Matt Ridley, International Center for Life, Newcastle, United Kingdom
	Inside Cancer interview, Dr. Judah Folkman, Harvard Medical School, Massachusetts
October 20	*Inside Cancer* interview, Dr. Doug Hanahan, University of California-San Francisco
October 22–26	National Human Genome Research Institute ELSI Project, *Eugenics Image Archive*, collection visit to University College, London, United Kingdom
October 25	*Your Genes, Your Health* interview, Maya Priest, New York, New York
October 29–31	Howard Hughes Medical Institute Precollege Directors Meeting, Chevy Chase, Maryland
November 6	*Inside Cancer* interview, Dr. Mike Wigler, CSHL
November 7–10	National Association of Biology Teachers Annual Meeting, Montreal, Quebec, Canada
November 14	Site visit to Biogen, Cambridge, Massachusetts
November 15	Site visit by Barbara Speziale, Clemson University, South Carolina
November 16	Site visit by Kip Powers and David Tesseo, Science Center of Southeastern Connecticut, New London
November 20	Site visit by Marcia Welsch, Elaine Sands and Gayle Insler, Adelphi University, Garden City, New York
November 28	Site visit by Katie Barbour and Doug Crain, Flying Colors Media
December 3	Site visit by Ann McDermott, Oncogene Pharmaceuticals, Melville, New York
	Site visit to New York Institute of Technology, Old Westbury, New York
December 4–5	National Human Genome Research Institute ELSI Review Panel, Bethesda, Maryland
December 7	Site visit by Dr. Sheldon Karnilow and Mike DeStio, Half Hollow Hills School District, Dix Hills, New York
December 12	Museum exhibit interview, Syd Mandelbaum, Cedarhurst, New York
December 18	Site visit to Biogen, Cambridge, Massachusetts

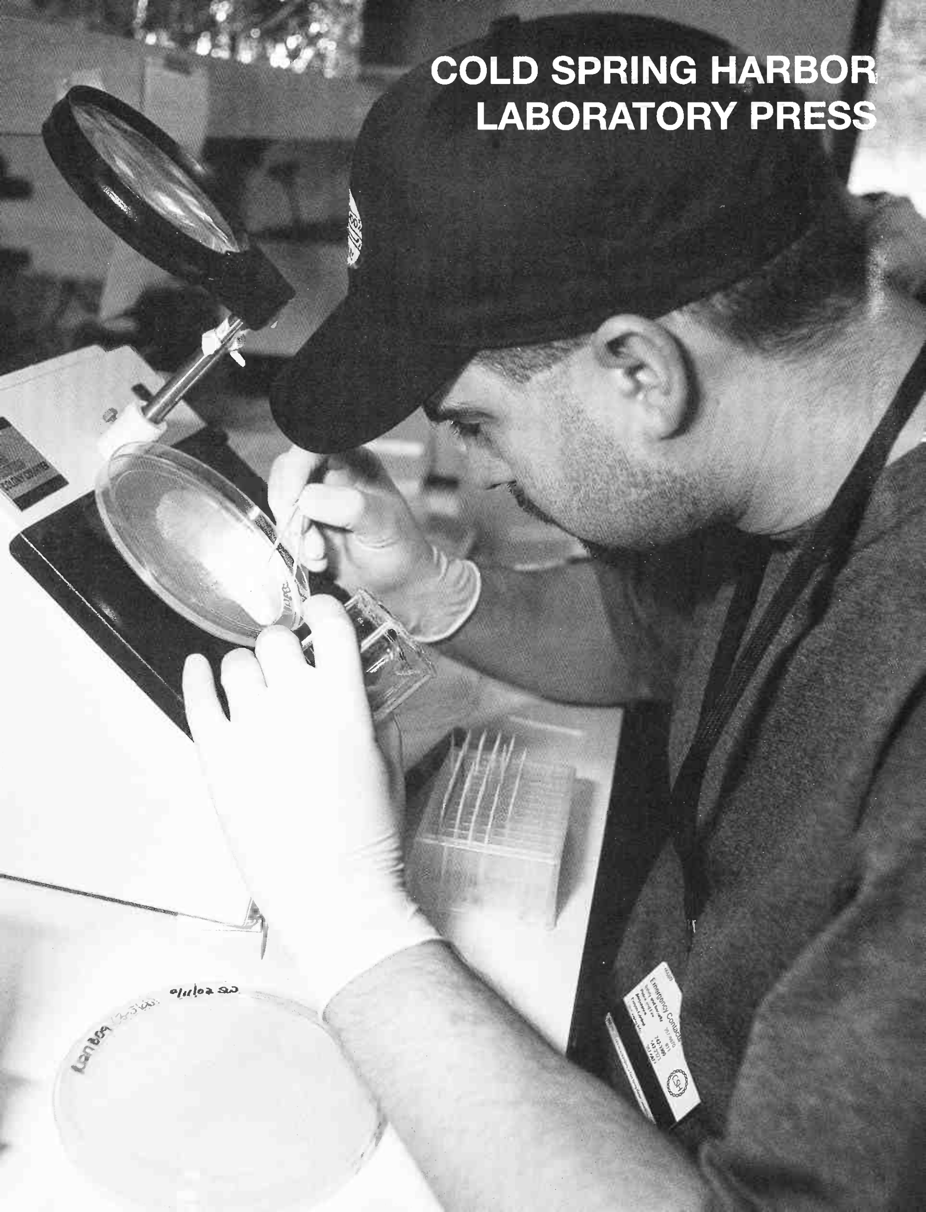
Sites of Major Faculty Workshops 1985–2001

Key: High School **College** *Middle School*

ALABAMA	University of Alabama, Tuscaloosa	1987–1990
ALASKA	University of Alaska, Fairbanks	1996
ARIZONA	Tuba City High School	1988
ARKANSAS	Henderson State University, Arkadelphia	1992
CALIFORNIA	**Foothill College, Los Altos Hills**	**1997**
	University of California, Davis	1986
	San Francisco State University	**1991**
	University of California, Northridge	**1993**
	Canada College, Redwood City	1997
	Pierce College, Los Angeles	**1998**
	California Lutheran University, Thousand Oaks	1999
	Laney College, Oakland	1999
	California State University, Fullerton	**2000**
	Salk Institute for Biological Studies, La Jolla	2001
COLORADO	Colorado College, Colorado Springs	1994
	United States Air Force Academy, Colorado Springs	**1995**
	University of Colorado, Denver	1998
CONNECTICUT	Choate Rosemary Hall, Wallingford	1987
DISTRICT OF COLUMBIA	**Howard University**	**1992,1996**
FLORIDA	North Miami Beach Senior High School	1991
	University of Western Florida, Pensacola	1991
	Armwood Senior High School, Tampa	1991
	University of Miami School of Medicine	2000
GEORGIA	Fernbank Science Center, Atlanta	1989
	Morehouse College, Atlanta	**1991,1996**
	Morehouse College, Atlanta	1997
HAWAII	Kamehameha Secondary School, Honolulu	1990
ILLINOIS	Argonne National Laboratory	1986,1987
	University of Chicago	**1992,1997**
INDIANA	Butler University, Indianapolis	1987
IDAHO	University of Idaho, Moscow	1994
IOWA	Drake University, Des Moines	1987
KANSAS	University of Kansas, Lawrence	1995
KENTUCKY	Murray State University	1988
	University of Kentucky, Lexington	1992
	Western Kentucky University, Bowling Green	1992
LOUISIANA	Jefferson Parish Public Schools, Harvey	1990
	John McDonogh High School, New Orleans	1993
MAINE	Bates College, Lewiston	1995
MARYLAND	Annapolis Senior High School	1989
	Frederick Cancer Research Center, Frederick	1995
	McDonogh School, Baltimore	1988
	Montgomery County Public Schools	1990–1992
	St. John's College, Annapolis	*1991*
	University of Maryland, School of Medicine, Baltimore	**1999**
MASSACHUSETTS	Beverly High School	1986
	CityLab, Boston University School of Medicine	1997
	Dover-Sherborn High School, Dover	1989
	Randolph High School	1988
	Winsor School, Boston	1987
	Boston University	**1994,1996**
MICHIGAN	Athens High School, Troy	1989
MISSISSIPPI	Mississippi School for Math & Science, Columbus	1990,1991
MISSOURI	Washington University, St. Louis	1989
	Washington University, St. Louis	**1997**
NEW HAMPSHIRE	St. Paul's School, Concord	1986,1987
	New Hampshire Community Technical College, Portsmouth	**1999**
NEVADA	University of Nevada, Reno	1992
NEW YORK	Albany High School	1987
	Bronx High School of Science	1987
	Columbia University, New York	1993

	Cold Spring Harbor High School	1985,1987
	DeWitt Middle School, Ithaca	1991,1993
	DNA Learning Center	1988–1995, 2001
	DNA Learning Center	**1990,1992, 1995,2000**
	DNA Learning Center	*1990–1992*
	Fostertown School, Newburgh	*1991*
	Huntington High School	1986
	Irvington High School	1986
	Junior High School 263, Brooklyn	*1991*
	Lindenhurst Junior High School	*1991*
	Mt. Sinai School of Medicine, New York	1997
	Orchard Park Junior High School	*1991*
	Plainview-Old Bethpage Middle School	*1991*
	State University of New York, Purchase	1989
	State University of New York, Stony Brook	1987–1990
	Titusville Middle School, Poughkeepsie	*1991,1993*
	Wheatley School, Old Westbury	1985
	U.S. Military Academy, West Point	**1996**
	Stuyvesant High School, New York	1998–1999
	Trudeau Institute, Lake Saranac	2001
NORTH CAROLINA	North Carolina School of Science, Durham	1987
OHIO	Case Western Reserve University, Cleveland	1990
	Cleveland Clinic	1987
	North Westerville High School	1990
OKLAHOMA	School of Science and Mathematics, Oklahoma City	1994
	Oklahoma City Community College	**2000**
	Oklahoma Medical Research Foundation, Oklahoma City	2001
PENNSYLVANIA	Duquesne University, Pittsburgh	1988
	Germantown Academy	1988
SOUTH CAROLINA	Medical University of South Carolina, Charleston	1988
	University of South Carolina, Columbia	1988
TEXAS	J.J. Pearce High School, Richardson	1990
	Langham Creek High School, Houston	1991
	Taft High School, San Antonio	1991
	Trinity University, San Antonio	**1994**
	University of Texas, Austin	**1999**
	Austin Community College-Rio Grande Campus	2000
UTAH	University of Utah, Salt Lake City	1993
	University of Utah, Salt Lake City	**1998**
	University of Utah, Salt Lake City	**2000**
VERMONT	University of Vermont, Burlington	1989
VIRGINIA	Eastern Mennonite University, Harrisonburg	1996
	Jefferson School of Science, Alexandria	1987
	Mathematics and Science Center, Richmond	1990
	Mills Godwin Specialty Center, Richmond	1998
WASHINGTON	**University of Washington, Seattle**	**1993,1998**
	Fred Hutchinson Cancer Research Center, Seattle	1999, 2001
WEST VIRGINIA	Bethany College	1989
WISCONSIN	Marquette University, Milwaukee	1986,1987
	University of Wisconsin, Madison	1988,1989
	Madison Area Technical College	**1999**
WYOMING	University of Wyoming, Laramie	1991

AUSTRALIA	Walter and Eliza Hall Institute and University of Melbourne	1996
CANADA	Red River Community College, Winnipeg, Manitoba	1989
ITALY	Porto Conte Research and Training Laboratories, Alghero	1993
	International Institute of Genetics and Biophysics, Naples	1996
PANAMA	**University of Panama, Panama City**	**1994**
PUERTO RICO	University of Puerto Rico, Mayaguez	1992
	University of Puerto Rico, Mayaguez	**1992**
	University of Puerto Rico, Rio Piedras	**1993**
	University of Puerto Rico, Rio Piedras	1994
RUSSIA	Shemyakin Institute of Bioorganic Chemistry, Moscow	1991
SINGAPORE	National Institute of Education	2001
SWEDEN	Kristineberg Marine Research Station, Fiskebackskil	1995


COLD SPRING HARBOR
LABORATORY PRESS

2001 PUBLICATIONS

LABORATORY MANUALS

Arabidopsis: A Laboratory Manual
Detlef Weigel and Jane Glazebrook

Protein-Protein Interactions: A Molecular Cloning Manual
Erica Golemis (ed.)

CSHL MONOGRAPH SERIES

Stem Cell Biology
Daniel R. Marshak, Richard L. Gardner, and David Gottlieb (eds.)

SYMPOSIUM

Biological Responses to DNA Damage
Cold Spring Harbor Symposia on Quantitative Biology
Volume LXV

TEXTBOOKS

Bioinformatics: Sequence and Genome Analysis
David W. Mount

Genes & Signals
Mark Ptashne and Alexander Gann

OTHER TITLES

A History of Genetics (reprint edition)
A.H. Sturtevant

An A to Z of DNA Science: What Scientists Mean When They Talk about Genes and Genomes
Jeffre L. Witherly, Galen P. Perry, and Darryl L. Leja

A Passion for DNA: Genes, Genomes, and Society (paperback edition)
James D. Watson

At the Helm: A Laboratory Navigator
Kathy Barker

"Discovering the Double Helix" (Video)
James D. Watson

Lateral DNA Transfer: Mechanisms and Consequences
Frederic Bushman

Safety Sense: A Laboratory Guide

The Unfit: A History of a Bad Idea
Elof Axel Carlson

CHILDREN'S BOOKS

Enjoy Your Cells
Fran Balkwill and Mic Rolph

Germ Zappers
Fran Balkwill and Mic Rolph

JOURNALS

Genes & Development (Volume 15, 24 issues)
T. Grodzicker and D. Solter (eds.)

Genome Research (Volume 11, 12 issues)
A. Chakravarti, R. Gibbs, E. Green, R. Myers, and L. Goodman (eds.)

Learning & Memory (Volume 8, 6 issues)
J.H. Byrne (ed.)

Protein Science (Volume 10, 12 issues)
M. Hermodson (ed.)

OTHER

CSHL Annual Report 2000

Banbury Center Annual Report 2000

Administration and Financial Annual Report 2000

COLD SPRING HARBOR LABORATORY PRESS

For this publishing house, 2001 was a year of new beginnings and satisfactory outcomes. Revenue rose by 14% to $9,940,000, and our contribution to the economy of the Laboratory, combining overhead allocations and operating margin, exceeded $1 million. Financial highlights of the year included the strong first-year sales of the new edition of the manual *Molecular Cloning*, significant advances in subscriptions to *Genome Research* and *Protein Science*, and robust journal advertising sales that withstood the sharpest industry downturn in a generation.

Books for Scientists

The book program produced a front list of unusual interest and diversity. Of the 15 new titles published, 8 were for the professional use of research investigators.

Stem Cell Biology, edited by Daniel Marshak, Richard Gardner, and David Gottlieb, acknowledged the tidal wave of scientific and popular interest in stem cells and their clinical applications but succeeded in the editors' goal of providing a longer and more authoritative view of a field in which, despite many advances, much remains to be learned before new treatments can be established.

Lateral DNA Transfer: Mechanisms and Consequences, by Frederic Bushman, also addressed a topic of emerging biological interest—the fascinating consequences of the exchange of genetic information between species. The immense scope of the topic touches on all levels of biological organization, and the author's ability to assemble and synthesize such information was highly praised.

We were also well satisfied by the warm reception that greeted *At the Helm: A Laboratory Navigator* by Kathy Barker. Newly appointed principal research investigators must recruit, motivate, and lead a research team, manage personnel and institutional responsibilities, and compete for funding while maintaining the outstanding scientific record that got them their position in the first place. Small wonder, then, that many of these young scientists feel ill-prepared, especially since their training contains no formal education in leadership roles and responsibilities. *At The Helm* addresses that need and there is no other book like it. After investing several years in research and interviews, Kathy Barker has produced a remarkable source of advice and wisdom that new and aspiring principal investigators have greeted with enthusiasm and relief.

The year's strongest sales were achieved by the newly released third edition of the classic laboratory manual *Molecular Cloning*, by Joe Sambrook and David Russell. This extraordinary book has been a best seller since its first publication in 1982, and in its new edition has once again become a must-have information source for all laboratories using molecular approaches to the study of genes, cells, and proteins. Yet, even its 2300 pages cannot encompass all aspects of current technology in adequate depth, and we have begun the development of a series of advanced manuals that carry the authoritative stamp of *Molecular Cloning* to new levels of detail. The first of these, *Protein–Protein Interactions*, edited by Erica Golemis, was published at the end of the year and is already proving successful. Our collection of manuals was further expanded with the valuable addition of *Arabidopsis* by Detlef Weigel and Jane Glazebrook, the first techniques book in plant biology that Cold Spring Harbor has published in several years. And the books for scientists were rounded out by *Symposium 65: Biological Responses to DNA Damage*, the latest volume in the annual series of symposia at the Laboratory that for more than 60 years has provided analysis and interpretation of the most interesting problems in biology.

The backlist of professional titles showed strong sales, led by *At the Bench*, *Using Antibodies*, and *Transcriptional Regulation in Eukaryotes*. With the support of an educational grant from the Office of AIDS Research at the National Institutes of Health, we were able to bring back into print the classic 1997 volume *Retroviruses*, edited by John Coffin, Stephen Hughes, and Harold Varmus, and also to produce a CD version that can be sent free of charge to research and clinical facilities overseas where literature resources are limited.

Books for the Public

Our commitment to the publication of a limited number of quality titles with an audience beyond scientists continued this year with the release of two books for general readers, a videotape, and the first two of a planned series of four children's books.

The Unfit: A History of a Bad Idea, by Elof Axel Carlson, is an account of the history of social essentialism or the human propensity for identifying individuals as genetically inferior on the basis, for example, of race, social class, or behavior. This thought-provoking work was widely reviewed and uniformly praised as a careful, balanced, and much needed contribution to the literature of eugenics. The use and potential abuses of genetic knowledge are becoming a matter of increasing public interest and concern, yet for citizens untrained in science, the technical terms used by experts in the field often make the arguments hard to follow. *An A to Z of DNA Science*, by Jeffre Witherly, Galen Perry, and Darryl Leja, is designed to help nonspecialists understand what scientists mean when they talk about genes and genomes; it is a small pocket-sized book in which terms are defined and helpfully illustrated.

A decade ago, we began distributing a unique series of four children's books by Fran Balkwill and Mic Rolph that presented information about genes, cells, and proteins in an entertaining, straightforward way, with lively colorful illustrations. The series was immensely popular among scientists, the only market we had the rights to address with these books. Ten years is a long time in biological research and much has changed. Now, Balkwill and Rolph have created a new series of books, entitled Enjoy Your Cells, in which the science is entirely undated, the pictures entirely new, and the scope expanded. The first books in this enjoyable series, *Enjoy Your Cells* and *Germ Zappers*, were released in the fall, and we now have the opportunity to bring them to the attention of the broader educational market worldwide.

Books for Students

Our textbook publishing initiative made a strong start with the appearance of *Bioinformatics: Genome and Sequence Analysis*, by David Mount. Published in March, it quickly became the book of choice for courses in this emerging discipline and, by year's end, had been adopted in more than 40 schools, including such major research universities as Harvard, Princeton, and the University of California at Berkeley.

The advanced textbook *Genes & Signals*, by CSHL Trustee Mark Ptashne and staff member Alex Gann, was published in December, to glowing advance praise from leading scientists. Inspection copies for seniors' courses are already in review at more than 80 schools and sales in its first few months are very strong.

Development began on a number of other textbook titles, including a second edition of *DNA Science*, which is based on courses taught at the Laboratory's Dolan DNA Learning Center, and *Discovering Genomics, Proteomics, and Bioinformatics* by Malcolm Campbell, which will be co-published with the leading textbook publisher Benjamin Cummings. Also in development with Benjamin Cummings is a fifth edition of Jim Watson's classic text *Molecular Biology of the Gene*, which is being revised by Richard Losick (Harvard), Tania Baker and Steve Bell (Massachusetts Institute of Technology), Mike Levine (University of California at Berkeley), and Alex Gann (Cold Spring Harbor Laboratory).

In the development of all of these projects, the writing center at the Meier House has proved to be an extraordinary asset, and more than a dozen authors spent productive and pleasant periods in residence during the year.

On-line Publishing

In 2001, the Press Web Site was redesigned to permit easier use and more effective marketing activities, e-commerce-enabled, and relaunched for separate American and European access, with prices

displayed in appropriate currencies. On-line purchasing grew substantially in 2001 to represent greater than 12% of total book sales, and a steadily increasing number of scientists have registered at the Site to receive advance information and special offers.

Three Web Sites are specifically devoted to individual books: MolecularCloning.com, BioinformaticsOnline.org, and GenesandSignals.org. These sites require registration, and the first two require book purchase for access. The sites offer services such as the opportunity to download and print book content, links to other on-line resources, and downloads of graphic images for teaching purposes. More than 7000 scientists have registered as users of these sites and the number continues to grow.

Journal Publishing

Circulation of *Genes & Development* in 2001 reflected continued strength in institutional subscriptions, with a decline in individual subscriptions as an ever larger number of institutions networked the electronic edition of the journal to their entire staff. Manuscript submission continued to be strong, and the journal's impact factor rose to 19.67, maintaining its position among the top ten journals in all of experimental biology. Davor Solter stepped down as European Editor in December after 4 years in the position that required attention to a steadily increasing number of manuscripts. His wise and careful judgment was a great asset to the journal, and we are most grateful for his work on the its behalf. Davor was succeeded by Rudi Grosschedl (University of Munich), whom we are glad to welcome on board.

The circulation of *Genome Research* rose by a remarkable 14% in 2001, with significant growth in both individual and institutional subscriptions. Manuscript submission increased by 10% and seems likely to increase still more, assisted by the implementation at year's end of an entirely electronic manuscript submission and review system. The journal's impact factor rose to 7.61.

Learning & Memory increased in circulation by 22%. The manuscript submission rate was maintained and the journal's impact factor rose sharply to 4.01, an achievement in neuroscience, a field that now offers scientists the opportunity to publish in well over 100 journals.

The first year of our contract with The Protein Society to publish its journal *Protein Science* had a satisfying outcome. The journal was redesigned in print and re-launched in an electronic form that made it readily available to subscribers for the first time. Targeted marketing resulted in a 40% increase in institutional subscriptions and advertising income rose more than fourfold.

In a difficult year for the advertising industry, sales in our journals rose by 13.5%, an excellent result.

Encouraged by the early success of our partnership with The Protein Society, we entered into discussions with The RNA Society about its journal *RNA*. After responding to the Society's competitive request for a proposal, we were delighted to reach agreement to publish the journal on the Society's behalf beginning in January 2003.

Sales, Marketing, and Distribution

Our sales and marketing programs now reflect the more complex and international business environments in which we operate. Promotional activities in 2001 included direct mail of redesigned newsletters, catalogs, and brochures, as well as meeting exhibits, advertising in print publications and on-line, and print and broadcast media publicity. We attended 11 professional meetings, displaying new and established books in such numbers that a double exhibit booth has now become a standard necessity. Effective use was made of the conferences at the Laboratory to draw scientists' attention to our new publications.

Our frontlist received a gratifying number of favorable reviews in widely read publications such as *Nature*, *Science*, *Cell*, and the *Trends* journals. Several book signings at bookstores and our exhibit stands brought out crowds eager to meet the authors.

A first foray into the complex and competitive task of gaining textbook adoptions was highly successful and propelled David Mount's *Bioinformatics* to the position of market leader in this emerging

field of study by year's end. A children's book marketing consultant was hired to advise us on reaching the trade and educational markets. As part of this exercise, a children's book flyer was mailed to 95,000 teachers, librarians, and booksellers in September to announce the arrival of the first two books in the Enjoy Your Cells series.

There was consolidation of the recently established European Sales office, and its sales target was exceeded by 20%. A fruitful relationship was established with the on-line retailer Amazon.com, which rapidly became one of our top three customers. New sales representation was established in Latin America and the United Kingdom and Ireland, and new distributors were appointed in Turkey, Egypt, Singapore, Thailand, and Brazil. Three of our titles, on prions, stem cells, and bioinformatics, were picked as Book of the Month Club Selections, and *The Unfit* was named Book of the Year in Scholarly Publishing by Amazon.com.

Staff

The staff members of the Press (as of December 2001) are listed elsewhere in this volume. It is a pleasure to acknowledge the dedication and high standards they bring to their duties. A year of new beginnings and satisfactory outcomes is only made possible by their talent and willingness to shoulder extra burdens.

In the spring, the Press moved to handsome new offices on the Woodbury campus that house 41 of our 47 staff members (we are a distributed organization, with staff at locations in San Diego, New Mexico, and Connecticut; a warehouse in Hauppauge; and a sales and distribution center in Oxford, UK). The transition from three separate sites to one center of administrative operations has been a happy one, with significant gains in efficiency and communication. The complexities of the move were admirably coordinated and executed by our colleagues in the Facilities Department.

In 2001, we welcomed several new members to the staff: Bill Keen as Finance Director, David Crotty as Commissioning Editor, Bibiane Garite, and Michele Schoudel. Nora Rice became a full time member of the Development staff.

Finally, I would like to say how grateful I am to the senior members of the Press staff: Jan Argentine, Editorial Development Manager; Ingrid Benirschke, Marketing Manager; Kathryn Fitzpatrick, Marketing Manager; Marcie Siconolfi, Advertising Sales Manager; Nancy Hodson, Operations Manager; Bill Keen, Finance Director; Guy Keyes, Sales and Distribution Manager; Denise Weiss, Production Manager; the editors of our journals, Terri Grodzicker at *Genes & Development* and Laurie Goodman at *Genome Research*; and my versatile assistant Elizabeth Powers. Publishing is a team-based enterprise, in which all functions are interdependent. The Press and the Laboratory are fortunate to have in key positions individuals who carry out their duties with responsibility, intelligence, and grace.

John R. Inglis

FINANCE

FINANCIAL STATEMENTS

CONSOLIDATED BALANCE SHEETS
December 31, 2001 and 2000

Assets:	*2001*	*2000*
Cash and cash equivalents	$ 25,917,330	27,456,990
Accounts receivable:		
Publications	2,070,714	2,187,811
Other	1,396,569	937,537
Grants receivable	5,194,624	3,652,363
Contributions receivable	6,681,094	8,130,610
Publications inventory	2,027,130	2,327,313
Prepaid expenses and other assets	2,436,437	1,691,542
Investments	185,947,777	208,166,091
Investment in employee residences	4,495,774	3,047,429
Restricted use asset	1,400,000	–
Land, buildings and equipment, net	108,104,940	99,323,744
Total assets	$ 345,672,389	356,921,430
Liabilities and Net Assets:		
Liabilities:		
Accounts payable and accrued expenses	$ 5,928,294	7,928,869
Notes payable	202,154	227,932
Bonds payable	45,200,000	45,200,000
Deferred revenue	2,615,372	2,695,061
Total liabilities	53,945,820	56,051,862
Net assets:		
Unrestricted	170,584,179	177,939,557
Temporarily restricted	13,179,303	9,796,302
Permanently restricted	107,963,087	113,133,709
Total net assets	291,726,569	300,869,568
Total liabilities and net assets	$ 345,672,389	356,921,430

CONSOLIDATED STATEMENT OF ACTIVITIES
Year ended December 31, 2001
With comparative totals for the year ended December 31, 2000

	Unrestricted	*Temporarily Restricted*	*Permanently Restricted*	*2001 Total*	*2000 Total*
Revenue and other support:					
Public support (contributions and non-government grant awards)	$ 7,758,771	13,070,798	1,421,839	22,251,408	24,803,360
Government grant awards	20,728,880	–	–	20,728,880	20,097,385
Indirect cost allowances	14,274,887	–	–	14,274,887	12,885,076
Program fees	2,677,232	–	–	2,677,232	2,551,373
Publications sales	9,940,567	–	–	9,940,567	8,683,809
Dining services	2,628,965	–	–	2,628,965	2,616,113
Rooms and apartments	1,890,226	–	–	1,890,226	1,985,658
Royalty and licensing fees	2,234,554	–	–	2,234,554	1,093,573
Investment income (interest and dividends)	8,285,120	–	–	8,285,120	8,132,622
Miscellaneous	541,080	–	–	541,080	547,831
Total revenue	70,960,282	13,070,798	1,421,839	85,452,919	83,396,800
Net assets released from restrictions	9,687,797	(9,687,797)	–	–	–
Total revenue and other support	80,648,079	3,383,001	1,421,839	85,452,919	83,396,800
Expenses:					
Research	39,464,156	–	–	39,464,156	35,923,808
Educational programs	11,490,571	–	–	11,490,571	9,103,007
Publications	9,800,811	–	–	9,800,811	8,281,363
Banbury Center conferences	1,026,037	–	–	1,026,037	1,165,989
Dolan DNA Learning Center programs	1,468,244	–	–	1,468,244	1,105,658
Watson School of Biological Sciences programs	1,104,729	–	–	1,104,729	698,779
General and administrative	9,739,917	–	–	9,739,917	9,252,253
Dining services	3,551,730	–	–	3,551,730	3,635,324
Total expenses	77,646,195	–	–	77,646,195	69,166,181
Excess of revenue and other support over expenses	3,001,884	3,383,001	1,421,839	7,806,724	14,230,619
Other changes in net assets:					
Net (depreciation) appreciation in fair value of investments	(10,357,262)	–	(6,592,461)	(16,949,723)	4,798,458
(Decrease) increase in net assets	(7,355,378)	3,383,001	(5,170,622)	(9,142,999)	19,029,077
Net assets at beginning of year	177,939,557	9,796,302	113,133,709	300,869,568	281,840,491
Net assets at end of year	$ 170,584,179	13,179,303	107,963,087	291,726,569	300,869,568

CONSOLIDATED STATEMENTS OF CASH FLOWS
Years ended December 31, 2001 and 2000

	2001	*2000*
Cash flows from operating activities:		
(Decrease) increase in net assets	$ (9,142,999)	19,029,077
Adjustments to reconcile (decrease) increase in net assets to net cash provided by operating activities:		
Depreciation and amortization	4,620,198	3,974,311
Net depreciation (appreciation) in fair value of investments	16,949,723	(4,798,458)
Contributions restricted for long-term investment	(7,504,820)	(9,350,612)
Restricted use asset	(1,400,000)	–
Changes in assets and liabilities:		
Increase in accounts receivable	(341,935)	(1,954,474)
Increase in grants receivable	(1,542,261)	(893,014)
Decrease (increase) in contributions receivable	1,425,568	(3,600,936)
Decrease (increase) in publications inventory	300,183	(188,439)
Increase in prepaid expenses and other assets	(744,895)	(370,229)
(Decrease) increase in accounts payable and accrued expenses	(2,000,575)	3,088,218
Decrease in deferred revenue	(79,689)	(183,304)
Net cash provided by operating activities	538,498	4,752,140
Cash flows from investing activities:		
Capital expenditures	(13,401,394)	(21,991,646)
Proceeds from sales and maturities of investments	58,387,861	71,313,900
Purchases of investments	(53,119,270)	(70,827,688)
Net change in investments in employee residences	(1,448,345)	(295,849)
Net cash used in investing activities	(9,581,148)	(21,801,283)
Cash flows from financing activities:		
Permanently restricted contributions	1,421,839	5,625,670
Contributions restricted for investment in land, buildings and equipment	6,082,981	3,724,942
Decrease in contributions receivable	23,948	349,221
Repayment of notes payable	(25,778)	(23,986)
Net cash provided by financing activities	7,502,990	9,675,847
Net decrease in cash and cash equivalents	(1,539,660)	(7,373,296)
Cash and cash equivalents at beginning of year	27,456,990	34,830,286
Cash and cash equivalents at end of year	$ 25,917,330	27,456,990
Supplemental disclosures:		
Interest paid	$ 1,671,874	2,063,031
Noncash investing and financing activities:		
Contributed property	$ 1,400,000	–

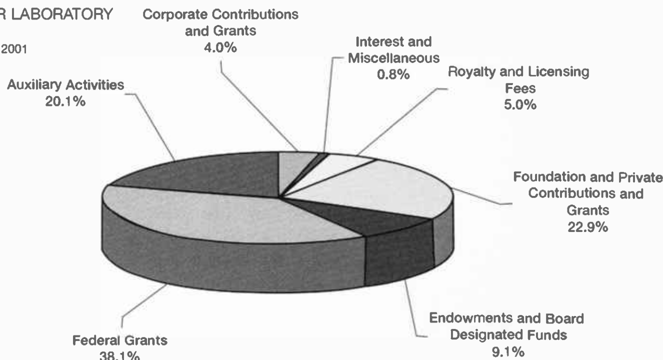
COMPARATIVE OPERATING HISTORY 1997–2001
(Dollars in Thousands)

	1997	1998	1999	2000	2001
Revenue:					
Main Lab:					
Grants and contracts	$ 22,743	24,025	27,179	30,345	34,716
Indirect cost reimbursement	9,910	11,054	11,207	12,718	14,134
Other	8,472	9,441	9,426	10,618	12,528
CSHL Press	5,238	6,341	6,400	8,684	9,941
Banbury Center	1,495	1,444	1,848	1,856	1,666
Dolan DNA Learning Center	875	1,334	1,392	1,471	1,878
Watson School of Biological Sciences	–	–	218	682	927
Total revenue	48,733	53,639	57,670	66,374	75,790
Expenses:					
Main Lab:					
Research and training	22,743	24,025	27,179	30,345	34,716
Operation and maintenance of plant	5,274	5,549	5,765	6,589	7,027
General and administrative	3,625	3,378	3,844	6,162	6,492
Other	5,759	7,328	7,863	7,075	9,505
CSHL Press	5,320	6,141	6,077	8,186	9,515
Banbury Center	1,437	1,321	1,614	1,702	1,536
Dolan DNA Learning Center	887	1,228	1,280	1,362	1,801
Watson School of Biological Sciences	–	–	218	682	927
Total expenses, excluding depreciation and amortization	45,045	48,970	53,840	62,103	71,519
Excess before depreciation, amortization, and designation of funds	3,688	4,669	3,830	4,271	4,271
Depreciation and amortization	(3,371)	(3,443)	(3,526)	(3,974)	(4,620)
(Designation) release of funds (2)	–	(750)	–	(297)	349
Net operating excess	$ 317	476	304	–	–

(1) The above amounts are presented on a combined basis for all funds for which Cold Spring Harbor Laboratory prepares operating budgets.

(2) Funds designated to underwrite future direct and indirect expenses of the research programs.

COLD SPRING HARBOR LABORATORY
SOURCES OF REVENUE
YEAR ENDED DECEMBER 31, 2001



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 2001.

GRANTS January 1, 2001–December 31, 2001

COLD SPRING HARBOR LABORATORY

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*2001 Funding*[+]	
FEDERAL GRANTS				
NATIONAL INSTITUTES OF HEALTH				
Program Projects	Dr. Herr	1/92–12/01	$ 4,320,915	
	Dr. Stillman	8/90–7/05	3,580,211	
Research Support	Dr. Cline	12/99–11/04	467,478	
	Dr. Cline	3/98–2/01	444,611	
	Dr. Enikolopov	8/99–5/04	334,926	
	Dr. Enikolopov	9/94–11/03	352,630	
	Dr. Grewal	3/00–2/05	268,800	
	Dr. Hannon	9/00–8/05	282,484	
	Dr. Hannon	3/01–2/03	166,416	*
	Dr. Helfman	9/99–8/04	386,119	
	Dr. Hengartner/Dr. Herr	5/00–4/04	231,395	
	Dr. Hernandez	7/00–6/04	199,500	
	Dr. Hirano	5/96–4/02	341,685	
	Dr. Hirano	7/01–6/05	292,355	*
	Dr. Huang	8/01–6/06	415,521	*
	Dr. Joshua-Tor	12/01–11/04	245,534	
	Dr. Joshua-Tor	5/01–3/06	378,522	*
	Dr. Krainer	7/89–6/02	451,541	
	Dr. Krainer	6/01–5/06	409,100	*
	Dr. Lowe	7/99–6/04	422,942	
	Dr. Malinow	5/00–4/05	518,529	
	Dr. Malinow	4/95–3/03	390,463	
	Dr. McCombie	1/99–12/03	445,131	
	Dr. McCombie	9/99–9/02	2,101,669	
	Dr. Neuwald	10/98–8/06	221,922	
	Dr. Skowronski	4/98–3/03	487,735	
	Dr. Spector	4/90–3/03	486,349	
	Dr. Stillman	6/00–5/04	521,562	
	Dr. Svoboda	12/98–11/03	395,180	
	Dr. Tonks	8/91–3/03	608,814	
	Dr. Tonks	5/99–6/05	349,125	
	Dr. Tully	4/94–3/02	363,034	
	Dr. Tully	10/00–9/03	266,917	
	Dr. Wigler	7/98–4/02	934,450	
	Dr. Wigler	9/00–7/03	752,766	
	Dr. Xu	1/98–12/02	302,854	
	Dr. Xu	8/01–7/06	290,636	*

*New Grants Awarded in 2001.
[+]Includes Direct & Indirect Cost.

Grantor	Program/Principal Investigator	Duration of Grant	2001 Funding+
	Dr. Yin	9/99–4/02	366,630
	Dr. Zhang	9/00–8/03	465,416
	Dr. Zhang	8/00–7/03	299,250
	Dr. Zhong	6/00–5/04	290,938
Fellowships	Dr. Bubulya	2/01–1/02	37,516 *
	Dr. Sacco-Bubulya	7/00–1/02	20,098
	E. Govek	5/01–4/03	22,860 *
	Dr. Hofmann	12/00–12/02	44,212
	Dr. Ronemus	12/01–11/04	41,996 *
	Dr. Ruthazer	3/99–2/02	43,772
Training Support	Training in Cancer Cell Biology and Tumor Virology	7/94–12/03	329,702
Course Support	Cancer Research Center Workshops	4/92–3/05	287,005
	Neurobiology Short-term Training	5/82–4/01	160,527
	Immunocytochemistry, In Situ Hybridization and Live Cell Imaging	7/98–6/03	71,882
	Advanced Genome Sequence Analysis	4/95–3/04	75,186
	Cell and Developmental Biology of *Xenopus*	4/96–3/04	18,480
	C. elegans	8/98–6/06	62,405
	Macromolecular Crystallography	9/00–8/05	49,521
	Cellular Biology of Addiction	4/01–3/02	40,000 *
	Making and Using DNA Microarrays	9/99–6/04	49,609
	Bioinformatics: Writing Software for Genome Research	7/00–6/05	47,086
Meeting Support	Genome Sequencing and Biology	4/90–3/02	37,026
	The Cell Cycle	4/00–3/03	9,000
	Telomeres and Telomerase	5/01–4/02	20,700 *
	Learning and Memory	4/01–3/02	13,000 *
	Proteolysis and Biological Control	4/01–3/06	11,000 *
	Tyrosine Phosphorylation and Cell Signaling	4/01–3/06	7,000 *
	66th CSHL Symposium on Quantitative Biology: The Ribosome	5/01–4/06	5,000 *
	Microbial Pathogenesis and Host Defense	7/01–6/02	12,000 *
	Neurobiology of *Drosophila*	8/01–7/06	21,775 *
	Eukaryotic DNA Replication	8/01–7/06	10,000 *
	Mechanisms of Eukaryotic Transcription	8/01–7/06	8,000 *
	Programmed Cell Death	8/01–7/02	12,000 *
NATIONAL SCIENCE FOUNDATION			
Cooperative Agreement	Dr. McCombie	2/99–1/02	271,526
Research Support	Dr. Cline	5/99–8/03	115,000
	Dr. Helfman	2/99–1/03	111,847
	Dr. Martienssen	9/01–8/05	476,040 *
Training Support	Undergraduate Research Program	6/00–5/03	58,000
Course Support	Advanced Bacterial Genetics	5/99–4/02	64,929
	Arabidopsis Molecular Genetics	4/00–3/03	67,062
	Cell and Developmental Biology of *Xenopus*	9/98–8/02	21,396
	Computational Neuroscience: Vision	8/00–7/02	18,679

*New Grants Awarded in 2001.
+Includes Direct & Indirect Cost.

Grantor	Program/Principal Investigator	Duration of Grant	2001 Funding+
Meeting Support	Learning and Memory	3/01–2/02	10,588 *
	Tyrosine Phosphorylation and Cell Signaling	4/01–3/02	6,000 *
	Biology of Proteolysis	7/01–6/02	5,000 *
	Neurobiology of *Drosophila*	7/01–6/02	15,745 *
	Mechanisms of Eukaryotic Transcription	7/01–6/02	3,000 *
	Eukaryotic DNA Replication	7/01–6/02	4,000 *
	Eukaryotic mRNA Processing	7/01–6/02	3,000 *
UNITED STATES DEPARMENT OF AGRICULTURE			
Research Support	Dr. Martienssen	12/00–11/03	85,000
	Dr. McCombie/Dr. Martienssen	9/01–9/03	500,000 *
	Dr. Jackson	9/01–8/03	195,000 *
	Dr. Stein	9/00–8/04	1,582,903
	Dr. Stein	9/00–8/03	946,112
	Dr. Timmermans	9/01–8/03	130,000 *
UNITED STATES DEPARTMENT OF THE ARMY			
Research Support	Dr. Hannon	6/00–5/03	126,000
	Dr. Helfman	7/99–6/02	117,600
	Dr. Lazebnik	6/01–5/04	215,812 *
	Dr. Van Aelst	7/99–6/02	117,600
	Dr. Van Aelst	9/01–8/03	167,000 *
	Dr. Zhong	9/99–8/02	247,892
Fellowship Support	Dr. Du	11/01–10/03	49,000 *
	Dr. Hamaguchi	4/00–3/04	59,000
	Dr. Hannon	6/00–5/01	59,000
	Dr. Jin	5/99–5/02	43,080
	Dr. Kannanganattu	1/01–2/04	48,191 *
	Dr. Mendez	7/99–6/02	42,000
	Dr. Narita	7/01–6/04	48,004 *
	Y. Seger	7/01–7/04	22,000 *
Meeting Support	Microbial Pathogenesis and Host Defense	9/01–2/02	12,379 *
MISCELLANEOUS GRANTS			
Research Support			
AKC Canine Health Foundation	Dr. McCombie/Dr. Hannon	7/01–6/02	34,000 *
Rita Allen Foundation	Dr. Lowe	9/99–8/02	50,000
	Dr. Hannon	10/00–9/03	50,000
Alzheimer's Association	Dr. Malinow	8/99–7/02	60,000
American Cancer Society	Dr. Wigler, Professorship	1986–2012	70,000
	Dr. Wigler, Supply Allowance	2001	10,000
Andrew's Buddies Corporation	Dr. Krainer	1/00–12/01	40,000
Michael Scott Barish Human Cancer Grant sponsored by the 1 in 9: The Long Island Breast Cancer Action Coalition	Dr. Wigler	2001	85,000 *
Chris-Craft Industries, Inc.	Siegel Funds for Cancer Pharmacogenomics	6/01–5/03	175,000 *
Cleiman Memorial Foundation	Dr. Tully	1/01–12/01	1,000 *
D.A.R.P.A./New York University Consortium Agreement	Dr. Wigler	9/01–9/03	79,367 *

*New Grants Awarded in 2001.
+Includes Direct & Indirect Cost.

Grantor	Program/Principal Investigator	Duration of Grant	2001 Funding*
William Theodore Denslow Foundation	Dr. Tonks	2001	8,000 *
Helen and Charles Dolan	Institutional Advancement	6/01–11/02	750,000 *
Donaldson Charitable Trust	Dr. Enikolopov	12/01–11/02	250,000 *
Genetica	Dr. Hannon	11/00–10/01	68,217
Joseph G. Goldring Foundation	Dr. Stillman	7/99–6/02	60,000
Gillespie Family Fund	DNA Microarray Technology Support	8/01–12/01	351,376 *
Thomas S.T. Gimbel & Lesley B. Gimbel Gift	Utrophin Medical Research Project	1/01–12/01	2,500 *
Bernard F. and Alva B. Gimbel Foundation	Utrophin Medical Research Project	1/01–12/01	6,000 *
The Lorraine & Lloyd Glidden Foundation–Neurofibromatosis Inc.	Dr. Van Aelst	4/01–3/02	25,000 *
Irving A. Hansen Memorial Foundation	Dr. Tansey	8/01–7/02	30,000 *
Helicon Therapeutics, Inc.	Dr. Tully	7/01–6/02	168,000 *
Helen Hoffritz Charitable Trust	Dr. Cline	2001	30,000 *
Human Frontier Science Program Organization (H.F.S.P.)	Dr. Tully	8/99–8/02	46,645
Long Islanders Against Breast Cancer	Dr. Wigler	2001	93,652 *
Louis Morin Charitable Trust	Dr. Joshua-Tor	12/01–11/02	125,000 *
Elizabeth McFarland Breast Cancer Research Grant	Dr. Wigler	2001	26,010 *
March of Dimes	Dr. Hengartner	6/01–5/02	54,402
G. Harold & Leila Y. Mathers Charitable Foundation	Dr. Svoboda	3/99–6/02	290,140
The Maxfield Foundation Consortium Program	Dr. Lazabnik	12/00–11/01	10,000
Neurofibromatosis Foundation, Arizona	NF Target Discovery Group Support	6/01–5/02	5,000 *
Neurofibromatosis Foundation, Massachusetts Bay Area	Dr. Van Aelst	4/01–3/02	13,000 *
NIH/Baylor College of Medicine Consortium Agreement	Dr. Mills	9/01–8/06	175,400 *
NIH/Columbia University Consortium Agreement	Dr. Lowe	9/00–7/05	498,416
NIH/Cal Tech Consortium Agreement	Dr. Stein	9/00–6/03	271,345
NIH/Nanoprobes, Inc., Consortium Agreement	Dr. Spector	4/99–3/02	31,939
NIH/Northwestern University Consortium Agreement	Dr. Spector	7/01–12/02	33,300 *
NIH/Downstate Medical Center Consortium Agreement	Dr. Yin	9/01–6/06	90,000 *
NIH/Sloan Kettering Consortium Agreement	Dr. Van Aelst	8/01–4/02	48,143 *
NIH/University of California, San Diego Consortium Agreement	Dr. Zhang	9/01–8/04	122,722 *
NIH/Washington University Consortium Agreement	Dr. Stein	3/99–2/03	46,413
	Dr. Stein	9/99–11/01	138,141
	Dr. Stein	9/99–9/02	15,541 *
Mr. and Mrs. Edmond Nouri	Dr. Tully	4/00–3/03	42,791
Novartis–Plant Consortium Program	Dr. Martienssen	1/98–12/02	135,000
NSF/Clemson University Consortium Agreement	Dr. Mc Combie	10/99–9/02	280,500
NSF/University of California, Berkeley Consortium Agreement	Dr. Jackson/Dr. Martienssen	10/01–9/06	218,320 *
NSF/Rutgers University Consortium Agreement	Drs. Spector/Martienssen/McCombie	10/00–9/05	336,819
NSF/University of Wisconsin Consortium Agreement	Dr. Martienssen	9/00–8/05	216,321

*New Grants Awarded in 2001.
*Includes Direct & Indirect Cost.

Grantor	Program/Principal Investigator	Duration of Grant	2001 Funding*
Promega Collaboration	Dr. Krainer	2/01–12/01	6,000 *
Rankowitz/Heffron	Dr. Zhong	4/98–3/02	25,000
Seraph Foundation	Dr. Enikolopov	11/00–4/02	28,000
Ann and Herb Siegel	Siegel Funds for Cancer Pharmacogenomics	6/01–5/03	500,000 *
SNP Consortium	Dr. Stein	4/99–9/02	288,019
SunGene–Plant Consortium	Dr. Martienssen	7/00–6/05	135,000
Tularik, Inc.	Dr. Wigler	10/97–10/03	660,000
Tober Foundation	Dr. Watson	11/01–10/02	10,000 *
U.S.D.A./Clemson University Consortium Agreement	Dr. McCombie	10/99–9/02	280,500
U.S.D.A./Yale University Consortium Agreement	Dr. McCombie/Dr. Martienssen	9/01–9/03	24,690 *
Westvaco–Plant Consortium	Dr. Martienssen	1/99–12/02	135,000
Zeneca Ltd.–Plant Consortium	Dr. Martienssen	7/99–7/03	135,000

Fellowships

Grantor	Program/Principal Investigator	Duration of Grant	2001 Funding*
Alzheimer's Association	Dr. Barria	8/99–7/01	40,000
	Dr. Zhu	9/01–11/01	7,699 *
American Cancer Society	Dr. Drier	7/99–6/02	34,000
	Dr. Hastings	1/00–12/02	32,000
Burroughs Wellcome Fund	Dr. Mainen	9/97–8/02	121,000
	Dr. Sabatini	9/01–8/02	59,000 *
Canadian Institutes of Health Research (C.I.H.R.)	Dr. Querido	8/99–7/02	25,520
Cancer Research Fund–Damon Runyon Walter Winchell	Dr. Z. Zhang	6/99–5/02	46,000
CSHL Association	Fellowship and Start Up Fund Support	2001	203,761
Danish Academy of Technical Sciences	Dr. Tonks/Dr. Andersen	2001	4,259 *
Deutsche Forschungsgeimeinschaft	Dr. Egger	2001	30,240 *
Deutsche Forschungsgeimeinschaft	Dr. Ehrlich	2001	25,678 *
Dorothy Rodbell Cohen Foundation	Dr. Mittal	4/01–3/02	20,000 *
The Ellison Medical Foundation	Dr. Grewal	9/99–9/03	50,000
	Dr. Tong	1/01–12/01	10,000 *
EMBO Award	Dr. Di Cristo	2001	27,655 *
Epilepsy Foundation	Dr. Aizenman	7/01–6/02	40,000 *
Fraxa Research Foundation	Dr. Margulies	3/01–2/02	38,000 *
	Dr. Zhu	8/01–11/01	10,830 *
Johns Hopkins/Ellison Foundation	Dr. Kass-Eisler	11/99–12/02	75,552
Helen Hay Whitney Foundation	Dr. Sheu	4/01–3/03	36,500 *
	Dr. Zito	7/01–6/02	41,000
	Dr. Sabatini	4/99–3/02	39,000
James R. Hudson, Jr.	Symposia Archives	5/01–5/02	60,000 *
Howard Hughes Medical Institute	Graduate Student Support	9/00–8/02	67,650
Human Frontier Science Program Organization (H.F.S.P.O.)	Dr. Cuvier	10/01–6/02	35,000 *
	Dr. Davenne	5/00–4/02	35,000
	Dr. de Stanchina	1/00–12/02	36,000
Japan Society for the Promotion of Science (J.S.P.S.)	Dr. Fukada	4/00–3/02	36,232
	Dr. N. Uchida	2001	41,000 *
Charles Henry Leach II Foundation	Dr. Enikolopov	1/97–12/01	26,000
Lehrman Institute	Dr. Svoboda	4/00–3/03	49,909
	Dr. Watson Archives	4/00–3/03	95,250
The Leukemia & Lymphoma Society Special Fellow Award	Dr. Duelli	12/00–12/03	35,000
	Dr. Julien	7/99–6/02	33,250
	Dr. Losada	7/01–6/04	50,000 *
	Dr. Schmitt	7/00–12/01	6,225
	Dr. Soengas	7/99–6/02	39,700

*New Grants Awarded in 2001.
*Includes Direct & Indirect Cost.

Grantor	Program/Principal Investigator	Duration of Grant	2001 Funding*
	Dr. Speck	7/01–6/03	40,000 *
	Dr. Tansey	7/01–6/06	100,000 *
Life Science Research Foundation	Dr. Vollbrecht	6/99–5/02	38,000
Long Beach Breast Cancer Coalition	Dr. Hamaguchi	11/01–10/02	4,000 *
NARSAD	Dr. Zhu	7/00–11/01	6,709
N.C.I.D.A.	Harbor Lecture Series	12/01–11/02	15,000 *
The David & Lucile Packard Foundation	Drs. Chklovskii/Mainen/Zador	7/01–6/04	316,086 *
Pew Charitable Trust	Dr. Svoboda	7/98–6/02	60,000
Quiagen	Mangone/Medline Workbench	9/01-8/02	11,980 *
Searle Scholars Program	Dr. Mainen	7/01–6/04	80,000 *
Alfred P. Sloan Foundation	Dr. Zador	9/00–9/02	20,000
Lauri Strauss Leukemia Foundation	Dr. Fridman/Dr. Tonks	4/01–3/02	15,000 *
St. Giles Foundation	Dr. Hatchwell	8/98–7/02	182,837 *
Swartz Foundation	Swartz Initiative for Computational Neuroscience Program	2001	32,314 *
Tularik, Inc.	Fellowship Support	1/98–12/03	150,000
Uehara Foundation	Dr. M. Uchida	2001	25,233 *
The V Foundation	Dr. Hamaguchi	4/00–3/02	50,000
The Wellcome Trust	Dr. Akerman	10/01–9/03	42,700 *
	Dr. Newey	10/01–9/03	65,302 *
Whitehall Foundation, Inc.	Dr. Huang	8/01–8/04	75,000 *
Course Support			
Howard Hughes Medical Institute	Advanced Neurobiology Courses	2001	330,000
John Merck Fund	Developmental Disabilities in Children	2001	23,000 *
Meeting Support			
Abbott Laboratories	Gene Expression and Signaling in the Immune System	2001	5,000 *
Amersham Pharmacia Biotech Inc.	Vector Targeting	2001	500 *
Amgen	Gene Expression and Signaling in the Immune System	2001	2,500 *
Berlex Biosciences	Vector Targeting	2001	2,000 *
Boehringer Ingelheim Pharmaceuticals	Gene Expression and Signaling in the Immune System	2001	3,000 *
Bruker Axs, Inc.	Structural Biology	2001	500 *
Canji, Inc.	Vector Targeting	2001	1,000 *
Gilson, Inc.	Structural Biology	2001	1,000 *
Digital Gene Technologies, Inc.	Corporate Contribution Program	2001	6,000
Hampton Research	Structural Biology	2001	1,000 *
Hoffmann-LaRoche, Inc.	Structural Biology	2001	1,000 *
Immunex, Inc.	Gene Expression and Signaling in the Immune System	2001	1,000 *
Klingenstein Foundation	Genetic Basis of Neurological and Behavioral Disorders	2001	22,000 *
Lehrman Institute	Pathways to Alzheimer's Disease	2001	43,989 *
Merck Research Laboratories	Vector Targeting	2001	1,000 *
MSC Molecular Structure Corp.	Structural Biology	2001	750 *
Osiris Therapeutics, Inc.	Stem Cell	2001	5,000 *
Pfizer	Gene Expression and Signaling in the Immune System	2001	1,000 *
Ruth & Milton Steinbach Foundation, Inc.	Genetic Basis of Neurological and Behavioral Disorders	2001	5,100 *
Vectorlogies, Inc.	Vector Targeting	2001	500 *
Wyeth Ayerst Pharmaceuticals	Gene Expression and Signaling in the Immune System	2001	3,000 *

*New Grants Awarded in 2001.
*Includes Direct & Indirect Cost.

Grantor	Program/Principal Investigator	Duration of Grant	2001 Funding*
FEDERAL SUPPORT			
The Federal Judicial Center	The Basic Issues of Science	2001	$ 16,195 *
NIH	Genomic Annotation Workshop: DNA Replication	2001	17,025
NIH–National Institute on Deafness and Other Communication Diseases	Chemosensory Receptor Classification Workshop	2001	3,990 *
NIH–National Human Genome Research Institute	American Eugenics and the New Biology: Perspectives and Parallels	2001	15,000
NIH–National Human Genome Research Institute	Meeting of the Editorial Advisory Panel: Digital Image Archive on the American Eugenics Movement	2001	4,770
NIH–National Institute of Mental Health and National Institute of Child Health and Human Development (through a grant to the University of Illinois, Urbana)	Understanding the Neural Basis of Fragile X	2001	33,842 *
NONFEDERAL SUPPORT			
Meeting Support			
Affymetrix, Inc.	Integrating Genomics Technologies in Healthcare: Practice and Policy Challenges	2001	7,500 *
Burroughs Wellcome Fund	Integrating Genomics Technologies in Healthcare: Practice and Policy Challenges	2001	10,000 *
Cure Autism Now Foundation	Microbiology, Immunology, and Toxicology of Autism and Other Neurodevelopmental Disorders	2001	5,000 *
Hoffmann-La Roche Inc.	Integrating Genomics Technologies in Healthcare: Practice and Policy Challenges	2001	12,263 *
McNeil Consumer Healthcare	Microbiology, Immunology, and Toxicology of Autism and Other Neurodevelopmental Disorders	2001	5,000 *
National Alliance for Autism Research (N.A.A.R.)	Microbiology, Immunology, and Toxicology of Autism and Other Neurodevelopmental Disorders	2001	5,000 *
Marie H. Robertson Memorial Fund for Neurobiology	Cortical Maps	2001	20,000
Albert B. Sabin Vaccine Institute, Inc.	Making Vaccines for the Developing World: Access to and Deployment of New Technologies	2001	24,598 *
Senomyx, Inc.	Molecular Biology of Chemosensory Receptors: The First Decade	2001	7,500 *
Herbert J. Siegel Fund for Cancer Pharmacogenomics	Interferons: Biological Mechanisms and Disease Treatments I	2001	22,233
Herbert J. Siegel Fund for Cancer Pharmacogenomics	Interferons: Biological Mechanisms and Disease Treatments II	2001	27,067
The Swartz Foundation	Can a Machine Be Conscious?	2001	30,603 *
UC Davis M.I.N.D. Institute	Microbiology, Immunology, and Toxicology of Autism and Other Neurodevelopmental Disorders	2001	5,000 *
Yamanouchi USA Foundation	Stability and Reversal of the Differentiated State	2001	22,065

*New Grants Awarded in 2001.
*Includes Direct & Indirect Cost.

DOLAN DNA LEARNING CENTER

Grantor	Program/Principal Investigator	Duration of Grant	2001 Funding*
FEDERAL GRANTS			
NATIONAL INSTITUTES OF HEALTH ELSI Research Program	Creation of a *Digital Image Archive on the American Eugenics Movement*	3/98–3/01	$ 227,994
NATIONAL INSTITUTES OF HEALTH	Creation of *Inside Cancer*	1/01–12/03	240,100
NATIONAL SCIENCE FOUNDATION	*A Partnership to Develop Advanced Technology Units on Genomic Biology*	8/97–7/01	47,644
DEPARTMENT OF ENERGY	*The Science and Issues of Human DNA Polymorphisms: An ELSI Training Program for High School Biology Teachers*	1/97–9/01	2,203
NONFEDERAL GRANTS			
Howard Hughes Medical Institute	Precollege Science Education Initiative for Biomedical Research Institutions	9/99–8/03	154,486
Josiah Macy, Jr. Foundation	*DNA from the Beginning/Your Genes, Your Health*	10/97–9/02	268,400
Pfizer Foundation	*Leadership Institute in Human and Molecular Genetics*	1/01–12/01	50,733

The following schools each awarded a grant for the *Genetics as a Model for Whole Learning Program:*

School	Amount
Bellmore-Merrick Central High School District	$ 5,305
Bethpage Union Free School District	1,425
Commack Union Free School District	250
Community School District #29	33,375
East Meadow Union Free School District	5,900
East Williston Union Free School District	1,400
Elwood Union Free School District	3,115
Farmingdale Union Free School District	980
Friends Academy	6,940
Garden City Union Free School District	5,545
Great Neck Union Free School District	6,515
Green Vale School	625
Harborfields Central School District	9,595
Half Hollow Hills Central School District	4,000
Hebrew Academy	1,325
Jericho Union Free School District	6,075
Lawrence Union Free School District	6,230
Locust Valley Central School District	16,000
Merrick Union Free School District	1,125
New York City School District #17	250
Northport–East Northport Union Free School District	6,160
Old Westbury School of the Holy Child	1,650
Port Washington Union Free School District	12,710
Rockville Center Union Free School District	4,525
St. Dominic Elementary School	2,450
St. Edwards	2,025
South Huntington Union Free School District	5,040
Syosset Central School District	20,250

The following schools each awarded a grant for *Curriculum Study:*

School	Amount
Bethpage Union Free School District	$ 1,100
Commack Union Free School District	1,100
East Meadow Union Free School District	2,200
Friends Academy	1,100
Garden City Union Free School District	1,100
Great Neck Union Free School District	2,200
Green Vale School	1,100
Half Hollow Hills Central School District	1,100
Herricks Union Free School District	1,100
Island Trees Union Free School District	1,100
Jericho Union Free School District	1,100
Lawrence Union Free School District	1,100
Levittown Union Free School District	1,100
Locust Valley Central School District	1,100
North Shore Central School District	1,100
Oceanside Union Free School District	1,100
Plainedge Union Free School District	2,200
Plainview–Old Bethpage Central School District	1,100
Portledge School	1,100
Port Washington Union Free School District	1,100
Ramaz School	1,100
Roslyn Union Free School District	1,100
Sachem Central School District	2,200
South Huntington Union Free School District	2,200
Syosset Central School District	1,100
West Hempstead Union Free School District	1,100

The generous commitments reported here make a real difference to advance the biomedical discovery at Cold Spring Harbor Laboratory. Gifts from individuals, corporations, and foundations are especially important to fund the innovation that will control cancer and improve our understanding of the brain. Investment in the people and facilities at the Laboratory is rapidly changing how we think about disease, helping us to better life sooner, rather than later.

Rod Miller, Vice President for Institutional Advancement

The individuals, foundations, and corporations listed on the following pages are responsible for the majority of research funding at Cold Spring Harbor Laboratory. Their commitment to our scientists is testimony to their confidence in the dedication of the very best minds in science to cure disease and improve the human condition. On behalf of our trustees and scientists, we sincerely thank these generous benefactors.

Diane Fagiola, Director of Development

Cold Spring Harbor Laboratory is a nonprofit research and educational institution chartered by the University of the State of New York. Less than half of the Laboratory's annual revenues are derived from Federal grants and contracts, and thus, we rely heavily on support from the private sector: foundations, corporations, and individuals. Contributions from the private sector are tax exempt under the provisions of Section 501(c)(3) of the Internal Revenue Code. In addition, the Laboratory is designated a "public charity" and, therefore, is enabled to receive funds resulting from the termination of "private foundations."

METHODS OF CONTRIBUTING TO COLD SPRING HARBOR LABORATORY

Gifts of Money can be made directly to Cold Spring Harbor Laboratory.

Securities: Stock certificates may be reassigned directly or transferred through your broker. Appreciated securities should be given outright, which will avoid capital gains taxes on the appreciated value. Securities that have decreased in value should be sold, and the proceeds donated. In this way, a donor will receive a deduction for both the loss and the charitable contribution.

Life Insurance: You may designate the Laboratory as the beneficiary of an existing or new policy, or irrevocably assign ownership of the policy. There are estate tax benefits in either case. If ownership is assigned, there is an immediate tax deduction.

Pooled Income Funds: Combine gifts from a number of donors in a pool for attractive investment and tax purposes.

Appreciated Real Estate or Personal Property: Sizable tax benefits can result from such donations; the Laboratory can use some in its program and can sell others.

Charitable Remainder Trusts can be structured to suit the donor's specific desires as to extent, timing, and tax needs.

Bequests: Most wills probably need to be updated. Designating Cold Spring Harbor Laboratory as beneficiary ensures that a bequest will be utilized as specified.

Conversion of Private Foundation to "Public" Status on Termination: This may be done by creating a separate fund within Cold Spring Harbor Laboratory whereby the assets of the private foundation are accounted for as a separate fund and used for the purposes specified by the donor. Alternatively, the private foundation can be established as a supporting organization of Cold Spring Harbor Laboratory.

Matching Gifts: Many employers will match gifts to Cold Spring Harbor Laboratory and/or the Watson School of Biological Sciences. Please check with your employer to augment your gift.

For additional information, please contact the Director of Development, Cold Spring Harbor Laboratory, One Bungtown Road, Post Office Box 100, Cold Spring Harbor, NY 11724, 516-367-8840.

CAPITAL AND PROGRAM CONTRIBUTIONS

January 1, 2001–December 31, 2001

Contributions of $5,000 and above, exclusive of Annual Fund

In 2001, Cold Spring Harbor Laboratory received significant support in the form of capital, program, and gifts-in-kind contributions from individuals, foundations, and corporations.

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Total $11,627,919

* New Pledges Awarded in 2001.

WATSON SCHOOL OF BIOLOGICAL SCIENCES CAPITAL CAMPAIGN

January 1, 2001–December 31, 2001

Contributions and pledges, exclusive of Annual Fund

The Watson School of Biological Sciences at Cold Spring Harbor Laboratory was established for the purpose of granting the Ph.D. degree. In its third year, the Watson School matriculated 6 additional students bringing the total number to 20. In 2001, the Laboratory received significant support from individuals, foundations, trusts, and corporations.

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Total	$3,397,228

*New Pledges Awarded in 2001.

ANNUAL CONTRIBUTIONS

Corporate Sponsor Program

For the first time in many years, we introduced a significant change in the structure of the Corporate Sponsor Program by introducing a new category—Corporate Benefactor. Mergers in the pharmaceutical and biotechnology industries are continuing to deplete the pool of companies that we can approach, even as the remaining companies increase greatly in size. In the extreme case, five members of the Program have, over the years, been combined to form a single company! The Corporate Benefactor membership is set at $50,000 with a proportionate increase in benefits. We are delighted that Pfizer Inc. became our first Benefactor, setting an example, we hope, to other companies.

The Corporate Sponsor Program continues to be critical to the vigorous meetings program held at Cold Spring Harbor Laboratory, whether at Grace Auditorium on the main Laboratory campus or at the Banbury Center. Without the strong foundation provided by the Program, we could not plan with confidence for the year's meetings, nor introduce new and unusual topics. We are, then, very grateful to the companies that joined us in 2001, and they are listed below.

The members of the Program receive special privileges in acknowledgment of their contributions. We waive all on-site fees for eight representatives of each company at our meetings. The number of meetings continues to increase so that in 2001, no fewer than 19 meetings took place in Grace Auditorium. Three scientists from each company may attend meetings at Banbury Center, where attendance is otherwise only by invitation of the organizers. Corporate Sponsors receive gratis copies of Cold Spring Harbor Laboratory Press publications including the journals *Genes & Development, Learning & Memory, Protein Science*, and *Genome Research*. We acknowledge our Sponsors in all relevant publications, including the books of abstracts given to each of the 7000 participants who come to the meetings each year. The names of the sponsoring companies are listed on the poster describing the meetings; this is mailed to approximately 17,000 scientists throughout the world. In addition, the companies are listed on the Cold Spring Harbor Laboratory Web Site and on the Meetings Office and Banbury Center Web pages.

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Albert B. Sabin Vaccine Institute, Inc.

Total	**$891,000**

Dolan DNALC Corporate Advisory Board Annual Fund

The Corporate Advisory Board, which was established in 1992, serves the Dolan DNA Learning Center as a liaison to the corporate community and assists in securing unrestricted support. As a means of raising support and awareness, the Corporate Advisory Board conducts the annual Golf Tournament and the Annual Fund. The proceeds benefit the Dolan DNA Learning Center and the Partners for the Future program. The Partners program teaches principles and methods of basic scientific research to eight Long Island high school seniors, who work with lab scientists for 10 hours a week after school, from October through March.

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Total	**$187,355**

Total Dolan DNALC Annual Fund **$309,709**

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Anne R. Meier

President's Report

2001 was another active year for our Association. We expanded our special events program, enhanced our community outreach effort, and continued our fund-raising in support of young scientists here at the Laboratory. Support for this wonderful institution continues despite the turmoil in the world around us.

The support of generous donors enables the Laboratory to staff its various research laboratories with top scientific research talent. A significant portion of the Annual Fund goes directly to support promising postdoctoral fellows who are vital to the Laboratory's continuing success. This year, the Cold Spring Harbor Laboratory Association raised $799,912 in our Annual Fund.

Leadership is key to the Association. We welcome David Banker, Lori Garofalo, Michael O'Brien, and Richard Torrenzano as new Directors. We thank Nick Bartilucci, Robin Hadley, and Eileen Pulling, our retiring Directors, who gave their talent and devoted time and effort to the Association.

Our February 4 Annual Meeting was followed by dinner and featured Dr. Shirley M. Tilghman, then the Howard A. Prior Professor of the Life Sciences at Princeton University and now the new President of Princeton. Dr. Tilghman's speech entitled "Genomic Imprinting—A Genetic Arms Race" was fascinating and enjoyed by all. Many thanks to Nick Bartilucci and Susan Sheeline who chaired this wonderful event.

On April 21, the Laboratory was transformed into a "Jazz Club" for the evening, featuring great music by the Harold Betters Quartet, Kenny Blake, and Eric Johnson, with a special guest appearance by Sheena Berkley. What made this evening truly special was the live recording of the concert preserved on CD for a lasting memory. The concert was followed by a buffet dinner and a late-set performance. A very special thanks is in order to the Committee co-chairs Mary Beth and Joe Donohue and Lisa and Gil Ott, and the Underwriting co-chairs Susan and Mark Hollo, Ginny and David Knott, and Cathy and Doug Soref. We are especially grateful to our Jazz Underwriters listed below. With their support and generosity, we were able to triple our proceeds from last year's event.

CSHLA President David H. Deming and guest speaker Dr. Walter C. Willett taken at the CSHL Association Annual Meeting in February.

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The Dorcas Cummings Lecture was held on June 3, 2001. Our guest speaker was Dr. Venki Ramakrishnan of MRC Laboratory of Molecular Biology, Cambridge, England. The title of his engaging lecture was "Protein Factories and Antibiotics." Our sincere thanks go to Cynthia Stebbins, Ann Seifert, and Susan Sheeline for their hard work in organizing the dinner parties that followed the lecture. A very

special thank you to the friends in our community who generously hosted these wonderful parties and entertained our own Laboratory scientists, friends, and visiting scientists from around the country and the world.

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Two other past events reappeared this year to their great success and our delight. The CSHL Association Partnership Picnic was held on September 23 at the home of Sandra and Stephen Lessing of Cold Spring Harbor. Mr. and Mrs. Francis W. Murray III of Cold Spring Harbor were very generous to host our Major Donor party on October 14. Many thanks to Mike and Judy as well as Frances Elder for her assistance in helping to organize this event.

The Third Annual Biotechnology Luncheon hosted by the Laboratory and sponsored by U.S. Trust Company was held on October 18. The guest speakers were Dr. Robert S. Kerbel, Head of Molecular & Cellular Biology Research at Sunnybrook & Women's College Health Science Centre in Toronto and the John & Elizabeth Tory Professor of Experimental Oncology at the University of Toronto; and Dr. George D. Yancopoulos, President & Chief Scientific Officer of Regeneron Laboratories, Tarrytown, New York. This year's topic was "Controlling Cancer: Anti-Angiogenic Therapy." Our guest speakers were introduced by Bruce Stillman, who also moderated the event. Much appreciation to Larry Remmel and Eileen Pulling for their hard work in making this event a sellout.

Many thanks to George W. Cutting, Jr., for hosting the first Partnership Program's "Oyster Fest" at the Laboratory held on December 7. George hosted the program's community members and Laboratory scientists for an informal social evening held in the Laboratory's Racker Room. George arranged to fly in oysters and smoked salmon from Alaska for this event, along with oysters served from the local area for a taste-test. A great time was had by all.

We thank each of you for your interest, time, and support. Have a great 2002.

David H. Deming

James A. Eisenman
1922–2001

Jim Eisenman, a firm but gentle man, was long a supporter of Cold Spring Harbor Laboratory. Together with his wife, Jan, and their three daughters, Jim had a vast impact on the Laboratory and always had its best interests at heart.

A Pennsylvania native, Jim received his B.S. in Mechanical Engineering from Brown University in 1943, one year ahead of schedule. After graduating, he worked as an engineer with Sperry Gyroscope on Long Island, and left to serve in the United States Merchant Marines during 1944 and 1945. He returned from World War II and graduated from St. John's Law School in Brooklyn in 1948; he married Janice Brackett in 1949.

Jim lived in Laurel Hollow for 47 years and was an active member of the local community. The land he purchased in Laurel Hollow was adjacent to Cold Spring Harbor Laboratory. For several decades, Jim lived next door to Al and Jill Hershey, and quickly developed an affection both for the Hershey family and for Cold Spring Harbor Laboratory. But it was the Laboratory's then-director, John Cairns, who brought Jim "into the fold" of Cold Spring Harbor Laboratory. In fact, the Cairns and Eisenmans were the best of friends and that friendship ultimately benefited Cold Spring Harbor Laboratory, for it was that camaraderie that cemented Jim's strong support of the Laboratory's mission and goals.

Jim practiced law, first with the firm of Campbell, Brumbaugh, Free and Graves, and then at his own patent law firm in Woodbury—Eisenman, Allsopp and Strack. In recent years, he was of counsel to Payne, Wood and Littlejohn, where he focused on corporate law and intellectual property. Jim also served as the Village District Judge in Laurel Hollow for more than two decades, and was on the board of directors of the St. Johnland Nursing Home in nearby Smithtown.

Maria Hedges, Jim Watson, Ahmad Bukhari, Jim Eisenman

Jim was a vital part of the Long Island Biological Association for more than 30 years. LIBA—the fund-raising and former governing body of Cold Spring Harbor Laboratory—had supported research at the Lab since its founding in 1924. Jim served as Director of LIBA from 1963 to 1989, and as its Treasurer from 1966 to 1989. In these roles, he helped Cold Spring Harbor Laboratory immeasurably—first to survive and then to aim for greatness.

On Friday, November 16, 2001, Jim succumbed to esophageal cancer at the age of 79. Survived by his wife, Jan, his daughters, four grandchildren, and a brother, Alvin, Jim was the heart of his family and of our community. His last wish even included the Laboratory, as he stipulated that donations be made in lieu of flowers to support our cancer research. To the end, Jim Eisenman remained a steadfast friend of Cold Spring Harbor Laboratory, of the community he lived in, and of the many who were lucky enough to know him. He will be sorely missed by us all.

James D. Watson

Rich Roberts, Jim Eisenman, Jan Eisenman

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