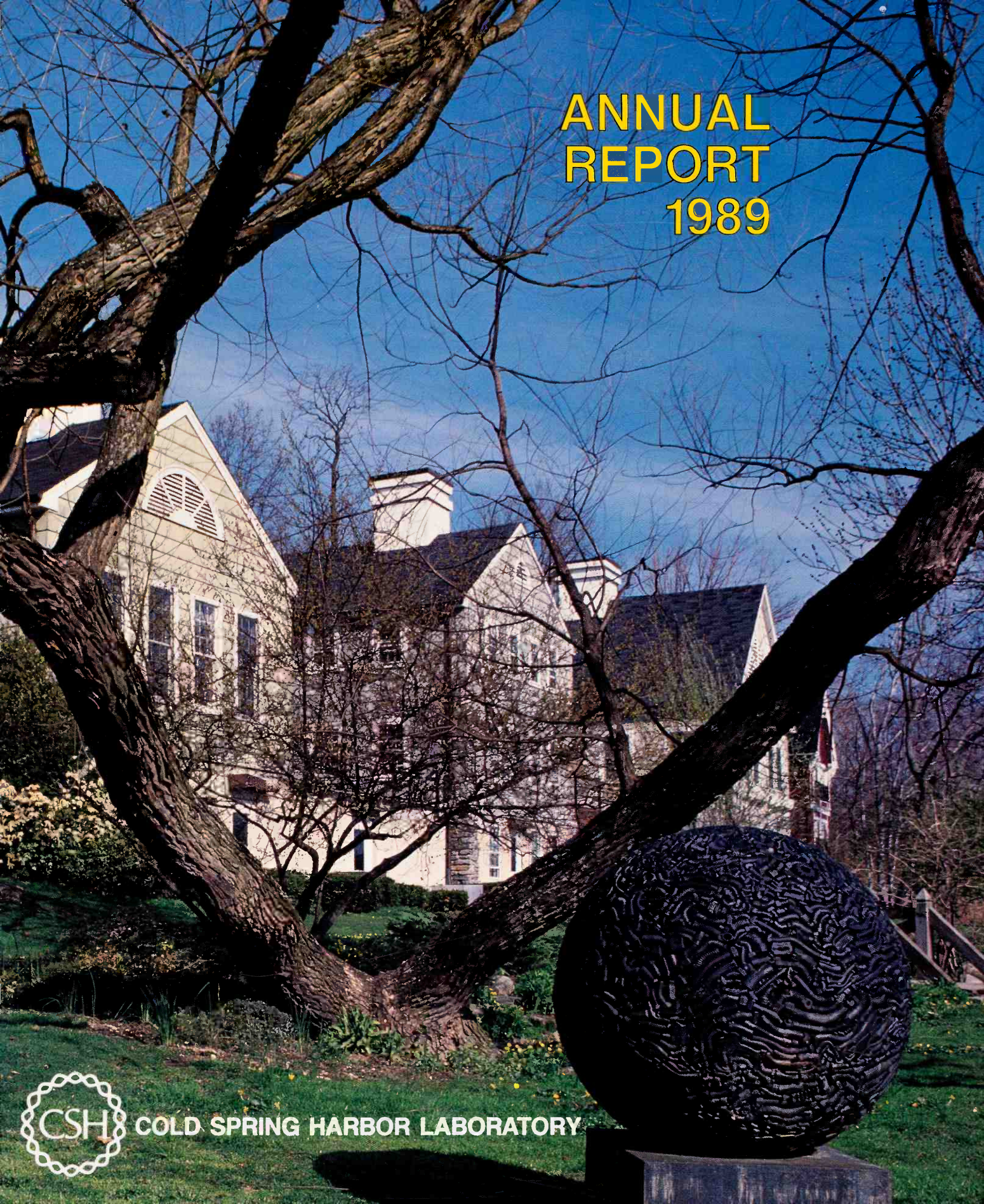
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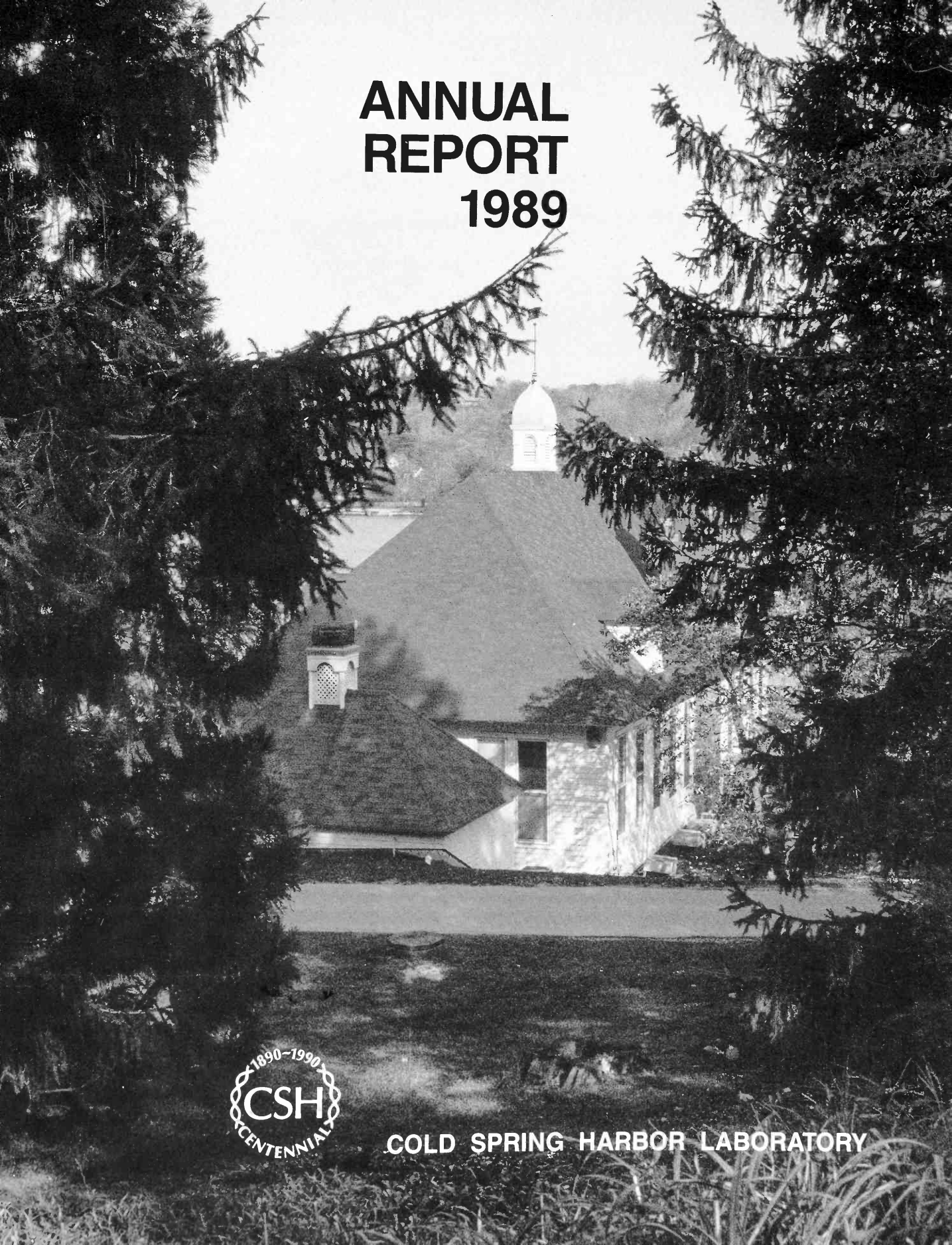

COLD SPRING HARBOR LABORATORY

ANNUAL REPORT 1989

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Front cover: Delbruck / Page Laboratories, as seen with "Nuts and Bolts" sculpture by Michael Malpass.
Back cover: Jones Laboratory, oldest laboratory at Cold Spring Harbor, was built in 1893.
(Photos by Margot Bennett)

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(*Back row*) T.M. Jessell, J.R. Reese, T. Whipple, D.B. Pall, J.R. Warner, J.E. Darnell, Jr., T. Maniatis (*Middle row*) D.L. Luke III, O.R. Grace, W. Everdell, F.M. Richards, Mrs. G.G. Montgomery, Jr., G.W. Cutting, Jr., S. Strickland, J. Klingenstein (*Front row*) Mrs. J.H. Hazen, Mrs. S. Hatch, B.D. Clarkson, J.D. Watson, L.J. Landeau, W.S. Robertson. Not shown: D. Botstein, W.M. Cowan, Mrs. C. Dolan, T.J. Knight, W.R. Miller, D.D. Sabatini, T.J. Silhavy

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DIRECTOR'S REPORT

The understanding of cancer as an aberration of the normal processes of cell growth and division has long stood out as a prime, if not ultimate, goal of the world's biomedical research community. When Everest was conquered, we saw the challenge of cancer in terms of that long-sought-after "Himalayan" goal. Now, with time, we see that we are assaulting a more-K2-like peak, where the ice falls ahead pose complexities that even the resolute and strong know may be close to the limits of human endurance.

When I was a boy growing up in Chicago, cancer was only talked about in whispers, a scourge that struck at random and against which we had no medical means of fighting back, particularly if it had spread beyond its point of origin. The only hope had to be the new facts that science would one day discover. After I became a student at the University of Chicago in 1943, I became aware of the research monies given to its Medical School by Albert and Mary Lasker. They wanted the world to have the scientific knowledge that would reveal the real faces of the enemy. Blindly thrashing against a foe that we knew only by name and not by form or substance could only perpetuate our fears. So, the Laskers put real life into the body known as the American Society for Cancer Control, then an assembly of doctors, many of whom were habituated to keeping the truth from their patients. Their efforts transformed this ineffective organization into the American Cancer Society, a national body founded in 1944 that would work for the public good by using the scientific mind, as opposed to the surgeon's scalpel, as the means ultimately to banish cancer from the human vocabulary.

It was in this same year that Avery, MacLeod, and McCarty published their historic paper showing that DNA molecules, not proteins, were the hereditary molecules of bacteria. At that time, very few scientists worked on or were excited by DNA or with RNA, its equally mysterious companion nucleic acid. So the Avery result did not immediately galvanize a then-unfocused biological community into a DNA-dominated mentality. I heard first of Avery's experiments through Sewell Wright's course on physiological genetics that I attended in the spring of 1947, my senior year in college. DNA's potential significance, however, only hit me with a vengeance when I moved on in the fall to Indiana University as a graduate student. There, my first-term courses brought me into the center of the gene replication dilemma: How could genetic molecules with their very great specificities be exactly copied?

Salvador Luria in his virus course excited me about these still very mysterious disease-causing agents, while Hermann J. Muller in his advanced genetics course recounted his lifelong odyssey in search of the secrets of the gene. The simplicity

of Luria's bacterial viruses (phages) immediately fascinated me, and in the spring of 1949, I started my Ph.D. thesis research in his lab. A year earlier, Luria had become one of the first recipients of the American Cancer Society's (ACS) new research grants. These were very important monies for the fledgling scientific area that later was to be known as molecular biology. The National Science Foundation (NSF) had not yet come into existence and the National Institutes of Health (NIH), much less its National Cancer Institute (NCI) component, had only a minuscule budget for scientific investigators outside its own Bethesda walls. At that time, the ACS did not have its own staff for evaluating grant proposals, a task then given to the Committee on Growth of the National Research Council.

Support of Luria's research by the ACS was not at all surprising. Several types of viruses were known to cause cancer in a variety of animals, and it was natural to think that some human cancers might also have viral etiologies. But in those early post-war years, no one really knew what a virus was, and directly attacking how the cancer-inducing viruses acted was not a realistic objective. Many viruses, including Luria's little phages, contained DNA, and we often speculated that DNA was their genetic component. But there were other viruses that totally lacked DNA and instead had RNA components. Conceivably, both forms of nucleic acid carried genetic information, but there still existed many scientists who suspected that maybe neither DNA nor RNA was a truly genetic molecule. Perhaps there was a fatal flaw to the Avery experiment that no one had yet caught.

General acceptance of the primary genetic role of DNA only came when Francis Crick and I found the double helix. The fact that it had a structure that was so perfect for its self-replication could not be a matter of chance. When I first publicly presented the double helix at the 1953 Cold Spring Harbor Symposium, there was virtually immediate and universal acceptance of its implications. At long last, we had the reference molecule on which to base our thinking about how living cells operate at the molecular level. And the DNA viruses immediately could be viewed in a more approachable fashion. The DNA molecules within them were clearly their chromosomes and, within a year, Seymour Benzer produced a detailed genetic map of the *r*II gene of phage T4 in which the mutant sites along a gene were correctly postulated to be the successive base pairs along the double helix.

Less obvious was whether RNA also could be a genetic molecule. By then, we believed that RNA functioned as an informational intermediate in the transfer of genetic information from the base pair sequences of DNA to the amino acid sequences in proteins (DNA→RNA→protein). As such, RNA did not need to be capable of self-replication, it only needed to be made on DNA templates. Vigorous proof that RNA also could be genetic molecules came from Alfred Gierer's 1956 demonstration in Tübingen that RNA purified from tobacco mosaic virus was infectious.

Viruses by then had become perceived as tiny pieces of genetic material surrounded by protective coats made up of protein (and sometimes lipid) molecules that ensured their successful passage from one cell to another. At first, we believed that the proteins used to construct their outer coats were the only proteins coded by the viral chromosomes. The enzymes used to replicate their DNA were believed initially to be those of their host cells. This assumption, however, created the dilemma of how the RNA of RNA viruses was replicated if the RNA within their host cells was made entirely on DNA templates. One way out of the dilemma was to postulate that some types of DNA-made RNA were later selectively amplified by RNA-templated RNA synthesis catalyzed by host cell enzymes. By 1959, however,

Seymour Cohen's and Arthur Kornberg's labs began to show that phage chromosomes coded for many of the enzymes needed to replicate their DNA. This opened up the possibility that animal viruses also coded for the enzymes that duplicate their DNA. If true, I thought we might have the first real clue as to how viruses cause cancer.

Dissolving the deep enigma surrounding viral carcinogenesis first became a goal of mine when I learned of tumor viruses in Luria's 1947 virus course. A young uncle of mine was then dying of cancer, and it was that fall when I first acutely sensed the need for science to fight back. This desire was rekindled in the spring of 1958 when I was visiting Luria's lab, by then in Urbana. There I heard Van Potter from the University of Wisconsin, Madison, give a lecture on the biochemistry of cancer. From him I first realized that the cells of higher organisms, unlike those of bacteria, needed specific signals to divide. In fact, the majority of cells in our bodies are not dividing but are in an apparent resting state where DNA synthesis is not occurring. A DNA virus infecting such cells would be unable to multiply unless it coded for one or more enzymes that specifically functioned to move their quiescent host cells into the "S" phase of DNA synthesis. Conceivably, insertion of animal viral genomes into the chromosomes of resting host cells would convert them into dividing cells with the signal for DNA synthesis always turned on.

This idea came to me when I was preparing a lecture on cancer to beginning Harvard students whom I was trying to excite with the new triumphs of molecular biology. It was they who first learned of my hypothesis. That viral carcinogenesis might have such a simple answer dominated my thoughts all that spring of 1959. In May, I presented it as my prize lecture for that year's Warren Award of the Massachusetts General Hospital. Francis Crick and I shared the award and my presentation came after he told an overflow audience how transfer RNA was the "adaptor" molecule he had earlier postulated for reading the messages of RNA templates. Thanks to Mahlon Hoagland and Paul Zamecnik's new experiments, there were no doubts as to whether Francis' ideas were on track. His talk had the virtue of being not only elegant, but also right. On the other hand, my talk had to seem more hot air than future truth. I left the Museum of Science Lecture Hall depressed at the thought that I had appeared at least an order of magnitude less intellectually powerful than Francis. Clearly, I might have given a more convincing talk if I had a plausible hypothesis as to why RNA viruses also sometimes induce cancer. For, as opposed to the situation with DNA, resting animal cells are constantly making RNA. Conceivably, there were two very different mechanisms through which the DNA and RNA viruses caused cancer. The other possibility was that my idea, although pretty, was just wrong.

During that spring, I started some experiments with John Littlefield, who had been purifying the Shope papilloma virus from rabbit warts. At that time, it was the smallest known DNA tumor virus and we expected its DNA to have a molecular weight of about four million. Surprisingly, using sedimentation analysis, we measured an apparent molecular weight of some seven million, with some molecules of seemingly twice that size that we thought might be end-to-end dimers. Unfortunately, we never looked at them in the electron microscope. If we had, we would have discovered that the Shope papilloma DNA is circular and that the faster-sedimenting molecules were not dimers, but a supercoiled form of the uncoiled simple circle that has a molecular weight of five million. In retrospect, I felt stupid, since earlier I had spent much time arranging for Harvard's Biological Laboratories to get an electron microscope. But, circles were not yet in the air and I never expected to see anything interesting.

Then the only, and not always dependable, source of the Shope papilloma virus was the Kansas trapper Earl Johnson. So, the discovery of a more accessible and even smaller mouse virus was beginning to revolutionize tumor virology. In 1958, Sarah Stewart and Bernice Eddy, working at the National Cancer Institute, opened up the DNA tumor virus field to modern virological methods through being able to propagate Ludwig Gross' "paratoid" virus in mouse cells growing in cell culture. They renamed this virus "polyoma," after its unexpected property of inducing a broad spectrum of tumors following inoculation into immunologically immature newborn mice. Quickly, several molecularly oriented virologists, including Renato Dulbecco at Caltech, Leo Sachs in Israel, and Michael Stoker in Glasgow, took up the polyoma virus system, moving on several years later to the newly discovered, similarly sized monkey tumor virus SV40. Easily workable cell culture systems to study the multiplication of these viruses as well as their cancer-inducing (transforming) properties were in place by the early 1960s. The time had thus come to ask whether these DNA viruses contained one or more specific cancer-inducing genes. So, there was an aura of real excitement permeating our 1962 Symposium on "Animal Viruses." I came down from Harvard and listened to Dulbecco give the closing summary at which the then-young Howard Temin and David Baltimore were much in evidence.

I did not, however, then join in the cancer gene quest. My earlier experiments on papilloma DNA were diversions from a ribosome-dominated lab that unfortunately was still in the dark as to where to go next. But when we found the first firm evidence for messenger RNA in March of 1960, the course of my Harvard lab for the next decade was firmly set. We wanted to understand how messenger RNA was made and then functioned to order the amino acids on ribosomes during protein synthesis. But I continued to emphasize tumor viruses in my Harvard lectures, which eventually formed the basis for my first book *The Molecular Biology of the Gene* (1965). Its last chapter, "A Geneticist's View of Cancer," discussed the DNA and RNA tumor viruses, concluding with the statement that through study of the simple DNA and RNA tumor viruses, we have our best chance of understanding cancer.

My taking on the Directorship here early in 1968 at last gave me the opportunity to get into the DNA tumor virus field. The great era of phage and bacterial genetics research at Cold Spring Harbor was coming to an end and we needed a new intellectual focus as a reason for our existence. Fortunately, that summer, our animal virus course brought to us several individuals who were just starting research with tumor viruses. The lecturer to excite me most was Joe Sambrook, then a postdoc in Dulbecco's Salk Institute lab where he worked with Henry Westphal on the integration of SV40 DNA into the chromosomal DNA of cells made cancerous by SV40. Soon after meeting Joe, I asked him to move here and start a DNA tumor virus lab. He quickly accepted, wrote a successful grant application, and arrived here the following June.

Within several years, Joe was leading a very high powered group in James lab consisting of Henry Westphal, Carel Mulder, Phil Sharp, Walter Keller, and Mike Botchan whose main purpose was to identify the SV40 gene(s?) that leads to cancer. By then, George Todaro and Robert Huebner, members of the NIH Special Cancer Virus Program, proposed using the name "oncogene" for a cancer-causing gene and this designation rapidly caught on. Through work here and at several other key sites, including the Salk Institute, NIH, and the Weizmann Institute, the SV40 oncogene(s?) was shown to be identical to the so-called "early gene(s)" that functions at the start of the SV40 replication cycle. This was a most gratifying result, compatible with my brainstorm of a decade earlier that the DNA viral on-

cogenes function to convert host cells into states capable of supporting DNA synthesis.

Key tools in everyone's analysis were the newly discovered restriction enzymes that cut DNA molecules at precise nucleotide sequences. A given restriction enzyme was used to cut up a viral genome into discrete pieces that could then be isolated from each other by a powerful new ethidium bromide agarose gel procedure developed here by Phil Sharp, Bill Sugden, and Joe Sambrook. Restriction enzymes came to the laboratory when Carel Mulder brought Herb Boyer's *Eco*RI into the James lab and later went on to isolate the *Sma*I enzyme. Soon afterward, Joe Sambrook and Phil Sharp began to use *Hpa*I and *Hpa*II enzymes, whose use was pioneered by Ham Smith and Dan Nathans at Johns Hopkins. More and more restriction enzymes came on line through the efforts of Rich Roberts, who arrived here in late 1972. About 50% of the world's commonly used restriction enzymes were discovered over the next decade in the Roberts laboratory.

By then, James lab was also working with a second DNA tumor virus, a human adenovirus that Ulf Pettersson had brought from Uppsala in 1971. Its life cycle also was divided into an early phase and a late phase, with the genes carrying its oncogenic potential also being "early" genes. A clear next objective was to find and identify the messenger RNAs for proteins encoded by the early and late adenovirus genes. Ray Gesteland's and Rich Roberts' groups in Demerec lab took on this task, which soon began to generate mystifying results. All of the late mRNAs seemed to possess a common terminal segment even though they were encoded by widely separated sequences of DNA. Resolution of the paradox occurred in late March of 1977, when Rich Roberts orchestrated a team consisting of Tom Broker, Louise Chow, Rich Gelinas, and Dan Klessig to their monumental discovery of RNA splicing. Independently, Phil Sharp and Susan Berget, then at MIT, made the same great discovery after initially observing with the electron microscope that the 5′ end of a late adenovirus messenger RNA did not behave as expected.

The discovery of RNA splicing was a once-in-a-lifetime event that completely transformed all of eukaryotic biology, and our 1977 Symposium, where the discovery was first publicly announced, was an occasion of intense intellectual ferment. Afterwards, new implications arose virtually weekly. Among the first was understanding that the T(umor)-antigen-coding SV40 early gene specified two different cancer-causing proteins. They are derived by two different ways of splicing the early SV40 messenger RNA. The once thought single SV40 T(umor) antigen in fact consists of large (T) and small (t) components, with the main cancer-causing activity due to the large T antigen. Splicing also occurs with the early adenovirus mRNAs, with two of the resulting protein products of the E1A and E1B genes playing essential roles in early viral replication as well as having oncogenic activity.

Now we realize that the subsequent working out of how these tumor virus oncogenes actually induce cancer would have been virtually impossible if the procedures of recombinant DNA had not been discovered in 1973. They have allowed us to study the action of individual oncogenes as well as to prepare the large amounts of highly purified oncogenic proteins needed to study their molecular functioning. We had, however, to wait six long years after Herb Boyer and Stanley Cohen gave us the first generally applicable recombinant DNA procedures until the stringent NIH prohibitions against using recombinant DNA to clone viral oncogenes were dropped. Only in early 1979 could the recombinant DNA era of tumor virology take off.

Not only were the DNA tumor viruses ripe for analysis, but how to think about the RNA tumor viruses was also known. Through work in the 1960s by Harry Rubin, Peter Vogt, and Howard Temin, the defective nature of most RNA tumor

viruses had been firmly established. Their replication requires the simultaneous presence of a normal helper virus. In acquiring their cancerous potentials, the RNA tumor viruses had somehow lost part of their own genomes. The way such RNA genomes, be they normal or defective, are replicated was first correctly hypothesized by Howard Temin. In 1964, he suggested that the infecting RNA molecules served as templates to make DNA genomes, which, in turn, integrated as proviruses into host cell chromosomes. Proof came in 1970 when Howard Temin working with Satoshi Mizutani, and independently David Baltimore, discovered within mature RNA tumor virus particles the enzyme reverse transcriptase. Soon the name "retroviruses" became used to encompass all those RNA viruses that replicate their RNA through a DNA intermediate.

Studies on the Rous sarcoma virus (RSV) provided the first deep insights on how the RNA tumor viruses cause cancer. RSV mutants that were unable to transform cells were found by Peter Duesberg and his collaborators frequently to lose part of a specific region of the genome that they called *sarc*. Its true nature became known from Mike Bishop's and Harold Varmus' 1976 seminal experiments showing that the *sarc* sequences within RSV are highly homologous to those of a normal cellular *sarc* equivalent. This finding immediately suggested that the cancer-causing signals of retroviral genomes have nothing to do with the replication processes of retroviruses. Instead, they originated from illegitimate recombinant events that replaced normal retroviral base pairs with DNA segments bearing cellular genes. A year later, the protein product of *sarc* itself was isolated and found independently by Ray Erickson and Art Levinson to be a protein kinase, an enzyme capable of adding phosphate groups to preexisting proteins.

Over the next decade, more than 30 additional oncogenes were isolated from RNA tumor viruses. In each case, they closely resemble a normal cellular gene. The proteins these oncogenes encode have seemingly very diverse roles; some are growth factors, others are receptors, many are kinases, and still others code for proteins that bind to DNA and control transcription. Unifying the roles of all these oncogenes, as well as of their normal cellular equivalents, is their involvement in the signal transduction processes that control whether a cell divides, remains quiescent, or becomes terminally differentiated. In normal cells, the functioning of these signal transduction genes is tightly regulated, so that they function only when cell division is needed. In contrast, the functioning of their oncogene derivatives is unregulated and leads to overexpression of their respective protein products. Proto-oncogene is the term now used to designate normal genes that can be converted into oncogenes by mutations or abnormal recombinational events.

We thus see that the oncogenes of DNA and RNA tumor viruses work in fundamentally different fashions. Those of DNA tumor viruses play essential roles in the replication of the viral genomes, with their cancer-causing attributes related to the tricks by which they turn their normal quiescent host cells into factories primed for DNA synthesis. In contrast, the oncogenes of retroviruses play no role in their replication, having arisen by genetic accidents that dissociate growth-promoting genes from their normal regulatory signals.

When they were first found, the question had to be faced whether the oncogenes of retroviruses were essentially laboratory artifacts and not related to human cancer. One way to settle the matter was to devise procedures that directly looked for human oncogenes by asking whether DNA isolated from human cancer cells could convert a normal cell into a cancerous cell. At MIT, Bob Weinberg first convincingly showed that this could be done using the DNA infection (transfection) procedures that Mike Wigler helped to develop while he was a graduate

student at Columbia University. By that time, Mike had joined our staff and was focusing on ways to clone the genes that his transfection procedures had functionally introduced into cells. In 1981, Wigler and Weinberg, working with the same cancer cell line, used different cloning procedures to isolate the first known human oncogene. More importantly, this bladder cell oncogene turned out to be virtually identical to the viral *RAS* oncogene isolated several years before by Ed Scolnick when he was at NCI. *RAS* was just the first of several retroviral oncogenes shown to be a cause of human cancer. Now no one doubts that the study of retroviral oncogenes bears directly on the understanding of human cancer.

The NIH-3T3 cultured mouse cells that Wigler and Weinberg made cancerous by the addition of the *RAS* oncogenes were later found to be more predisposed to cancer than cells obtained from the organs of living animals. Now we realize that Weinberg's and Wigler's classic experiments would have failed if they had used cells whose growth regulation was more normal. Two years later in 1983, Earl Ruley, here at James lab, showed that the *RAS* oncogene only transforms normal rat kidney cells to a cancerous state when a second oncogene is simultaneously added. In his experiments, either the adenovirus E1A oncogene or the retroviral *myc* oncogene could complement the activity of *RAS*. At the same time, Helmut Land and Luis Parada working at MIT in Weinberg's lab independently came to the same conclusion: Normal cells do not become fully cancerous through acquiring a single oncogene, but instead they become cancerous progressively, as other oncogenes successively come into action.

When the rush to use recombinant DNA procedures to study oncogenes began, we had to worry whether the research expertise and facilities at Cold Spring Harbor were up to the task. So we brought to James lab in 1980 John Fiddes from the University of California at San Francisco, where he was one of the first serious gene cloners. At the same time, we persuaded Tom Maniatis, who, before moving on to Caltech, had helped develop cDNA cloning as a member of our staff, to introduce a summer molecular cloning course. Started in 1980, this course gave rise in 1982 to the extraordinarily successful cloning techniques book *Molecular Cloning: A Laboratory Manual* by Maniatis, Fritsch, and Sambrook, which sold over 60,000 copies in its first edition. We also planned and constructed in 1983 a major south addition to Demerec Laboratory aimed at strengthening our facilities for protein chemistry and mutagenesis analysis. Equally important, we realized that we could not successfully exploit the monoclonal antibody procedures discovered in Cambridge, England, by George Kohler and Cesar Milstein in 1975 until we built a further addition to James lab. We had already constructed, in 1970, a south addition for offices and a seminar room and, in 1972, a west addition for virus culture. We needed to use monoclonal antibodies first to purify and then to better define the surfaces of oncogenic proteins. Planning for the north (Sambrook) addition allowed us to get Ed Harlow straight from his Ph.D. at the Imperial Cancer Research Fund (ICRF) Laboratories in London. There he had made monoclonal antibodies against both the SV40 T antigen and the still very mysterious p53 protein, a cellular constituent that David Lane and Lionel Crawford at ICRF had found to bind to the SV40 T antigen.

Upon arriving here, Ed focused on the key adenovirus oncogenic protein E1A to see which proteins it bound. He hoped that by identifying the cellular components with which it interacted, clues would emerge as to how E1A caused cancer. To spot these cellular proteins, he used a monoclonal antibody against E1A to precipitate it from extracts of adenovirus-infected cells. Then, he displayed the resulting precipitate on a gel to see whether proteins in addition to E1A could be detected. In this way, several unknown cellular proteins were found to bind to E1A.

What any of them did remained a mystery until *Nature* in the fall of 1987 published a paper by Wen-Hwa Lee on the properties of Rb, a newly identified DNA binding protein that helps to prevent cancer. When Rb is absent in a human due to mutations in both of his two *Rb* genes, retinoblastoma (cancer of the retina) develops. Those individuals who inherit a bad *Rb* gene from one of their parents are at risk for this cancer, frequently developing retinoblastoma at an early age when a cancer-causing mutation occurs in the remaining good *Rb* gene. Harlow's lab noticed that the Rb protein had been assigned a size very similar to one of the proteins (105K) that binds tightly to the E1A protein. Hoping that they might be the same, Harlow's lab began a collaboration with Bob Weinberg's lab, one of the three groups that had just cloned the *Rb* gene. Happily, the two proteins (Rb and 105K) proved to be identical, suggesting that E1A's oncogene potential lies partially in its ability to neutralize the anti-cancer activity of the Rb protein. I say partially because besides binding to Rb, E1A binds also to several other, still to be functionally identified, molecules, each of which also may be a cancer-preventing (anti-oncogene) protein.

The implication of Harlow's discovery widened with the subsequent finding that the T antigens of SV40 and polyoma also tightly bind Rb, as does an oncogenic protein coded by a papilloma virus that causes warts. Moreover, the once mysterious p53 protein that binds to the SV40 (polyoma) T antigen was shown last year by Arnold Levine and Bert Vogelstein also to be an anti-oncogenic protein. Although p53 does not bind to E1A, it does bind to E1B, a second "early" adenovirus protein that potentiates the oncogenic transformation potential of the E1A protein.

The study of tumor viruses has thus advanced fundamental cancer research more than anyone could have predicted when serious research on them began some three decades ago. The RNA tumor viruses have revealed almost all of the oncogenes known today. Without them, we would largely be in the dark as to the molecular players in the signaling processes that lead to cell division. Equally important have been the insights gained from learning how the oncogenes of the DNA tumor viruses work. The knowledge that they prevent anti-oncogenes from functioning gives us a new way to identify anti-oncogenes and will materially advance our understanding of hereditary predispositions to cancer.

We must remember, however, that the small hyperplastic tumors that result from gain of oncogenes that promote cell growth and division are generally benign. These usually tiny tumors generally only grow to life-threatening size when they become infiltrated (vascularized) by newly growing blood vessels that bring to them the oxygen and nutrients needed for their growth. Douglas Hanahan elegantly showed this through experiments done in collaboration with Judah Folkman. In our Harris laboratory, Doug introduced oncogenes into the germ cells of mice to produce transgenic mice in which these cell-division-signaling genes are expressed in early development as well as throughout adult life. His targeting of the SV40 T antigen to function in the pancreas resulted in large numbers of benign hyperplasmic growths in the insulin-producing islets. Only a small percentage of these benign growths, however, turned into rapidly growing tumors with infiltrating blood vessels. They did so by acquiring the ability to send out angiogenic (blood-vessel-forming) signals that induced neighboring endothelial cells to form new blood vessels. Finding out which molecules carry these signals will soon become a major objective for the coming decade of cancer research. If we could find inhibitors of these angiogenic growth factors, we might have in hand a powerful new way to stop cancers from growing. Most importantly, the endothelial cells

that line our blood vessels effectively do not divide in adult life. So, as Judah Folkman has long dreamed, inhibitors of tumor angiogenesis would not necessarily affect the healthy functioning of our preexisting blood vessels.

The truly cancerous cells of solid tumors (as opposed to those of the circulating cells of the blood) also show failure of their normal cellular affinities and spread (metastasize) to many unwanted tissue sites. Our first molecular clue as to the oncogenic changes that create cells capable of metastasis came this past January from the cloning of an oncogene on human chromosome 18 that leads to a final step in the progression of normal colon cells into their highly malignant equivalents. Through a very difficult feat of gene cloning, Bert Vogelstein found that this oncogene codes for a cell membrane protein involved in cell-cell recognition. Over the next few years, the losses of many additional cell-recognition proteins are likely to be implicated in the unwanted spread of tumor cells from their original sites of origin.

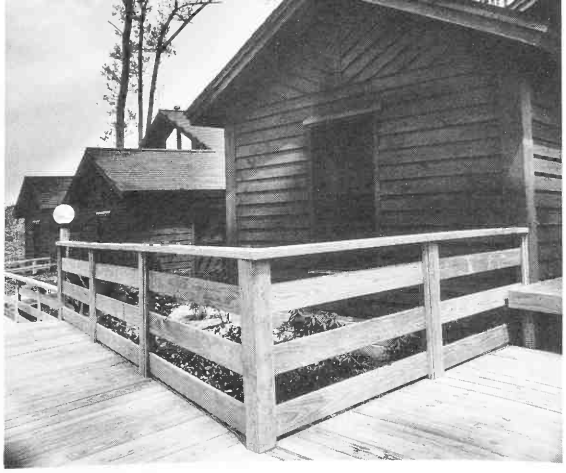
At long last, we may thus have the proper intellectual framework to understand most of the more common life-threatening cancers. At their essence are three types of genetic changes: (1) those that make cells divide when they should not, (2) those that lead to the formation of blood vessels that infiltrate into and so nourish the growing tumors, and (3) those that modify cell-recognizing molecules in ways leading to losses of their respective cell's ability to recognize their normal cellular partners. We have indeed come a very long way, but there remain myriad further details to unravel about both currently known oncogenes and the many more oncogenes yet to be discovered. Many of these new observations will initially unsettle us and momentarily make us despair of ever being able to have a fair fight against an enemy that so constantly changes the face it presents. But now is most certainly not the moment to lose faith in our ability to triumph over the inherent complexity that underlies the existence of the living state.

Given enough time, and the financial and moral resources that will let those born optimistic stay that way, the odds for eventual success in beating down cancer are on our side.

HIGHLIGHTS OF THE YEAR

Our Complex of Super Cabins Is Dedicated

For many scientists around the world, Cold Spring Harbor Laboratory is a temporary home away from home during the various meetings and courses, which brought more than 5400 scientists here last year. For many of these visitors, the unheated Page Motel and its four austere companion cabins were all a seemingly inadequate feature of the hill above James lab. We were thus relieved to complete the six heated guest cabins on the upper campus last spring. Each has four bedrooms and very comfortably houses eight visitors. On August 26, we dedicated them to six old friends of the Laboratory. The ceremony took place during the annual phage meeting, paying tribute to the earlier days of the Laboratory when several of the honorees spent summers here as part of the "Phage Group." The six cabins are now named for Herbert W. Boyer, Bentley Glass, Harry Eagle, Salvador E. Luria, Franklin W. Stahl, and Günther S. Stent. In his remarks, Günther

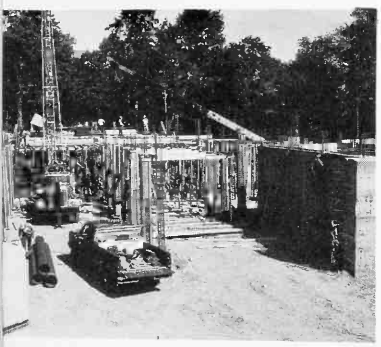


B. Glass, H. Eagle, M. Meselson, G. Stent, F. Stahl, and J.D. Watson at Cabin Dedication Ceremony

echoed sentiments that I believe many of the "first-generation" molecular biologists share about those early days: "From the professional point of view, I discovered the pleasures of research . . . I was reborn as a biologist and launched into the as-yet-unnamed molecular biology." I was disappointed that Salva could not attend the ceremony because of his health and that Herb could not fit the cross-country trip into his schedule. Matt Meselson gave a cleverly amusing speech on the pros and cons of sexual reproduction, graciously replacing Herb with little notice.

I note with much pleasure the pleasing appearance of the cabins, which were most tastefully decorated by our trustee Wendy Hatch, along with my wife Liz and Laura Hyman. Outside, the cabins are most skillfully interconnected with wooden walkways that next year will directly connect to the Neuroscience Center.

Neuroscience Center Progresses



Neuroscience Center progress

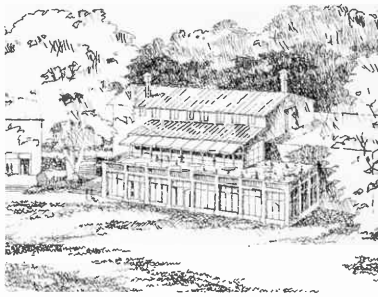
The landscape of our upper campus is changing rapidly as construction on the Neuroscience Center has been moving ahead on schedule. Since the parking garage was completed and the cornerstones were dedicated in late spring, the contractors have already completed the outer shell of the Beckman Neuroscience Laboratory. Work is in progress on Hazen Tower and the shell of Dolan Hall has already reached the second floor. Seeing these structures rise makes the day seem just around the corner when our research staff will reach 225, up from today's 150. This size staff will allow just the right mix of researchers from several fields and will not need major expansion in the foreseeable future. Walking by the construction when I get the chance, I can already see that the buildings fit comfortably into the hillside. The upcoming 55th Symposium on "The Brain" should help give the Neuroscience Center the visibility it needs to attract the high-caliber researchers we expect.

Major Gifts Received

Thankfully, there are several major gifts to report this year. A recent grant from the Samuel S. Freeman Charitable Trust of $1 million will support our cancer research. After Lita Annenberg Hazen's generous gift last year for construction of the Hazen Tower, I was pleasantly surprised to receive another similar gift late in the year for the Unrestricted Fund. This gift will allow me to say "yes" to more of the immediate and ever-changing needs of the Laboratory. The Kresge Foundation gave us a generous challenge grant of $500,000 for Dolan Hall, provided we raise the almost

$2 million remaining for full funding. The Fannie E. Rippel Foundation also gave a challenge grant of $250,000 for equipping the Neuroscience Center, after we raise an additional $500,000. These two endorsements will be powerful tools for our development office. Challenge grants also emphasize the importance of every dollar contributed, since we must raise the additional funds before we receive anything from the foundations. The Neuroscience Center received boosts of $300,000 and $100,000 from the Pew Charitable Trusts and the Booth Ferris Foundation, respectively. Within the Neuroscience Center, three rooms have already been named (benefactors are in parentheses): The Oakes Ames Seminar Room (Mrs. Pauline A. Plimpton and Mr. Amyas Ames), the Gardner Neuroscience Library (Mr. and Mrs. Robert B. Gardner, Jr.), and the Lucy and Edward Pulling Seminar Room (Mr. and Mrs. George W. Cutting, Jr.). Our AIDS research program has received $100,000 from the Mellam Family Foundation.

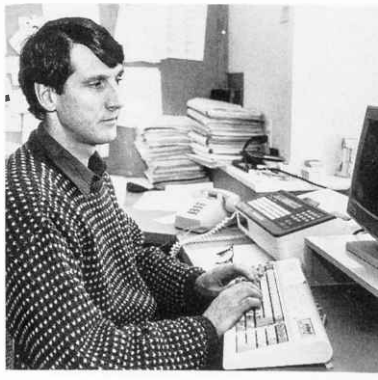
State of the Second Century Campaign



Through the diligent efforts of a corps of staff members and volunteers over the last year, the campaign has, to date, exceeded everyone's expectations. Since the public phase of the campaign started in April, we have raised almost $9.3 million. The tireless efforts of David L. Luke III as chairman of the campaign and chairman of the Corporate Committee of the campaign have certainly been the major factor in the campaign's success and the Laboratory is deeply indebted to him. To reach our goal, we must raise at least another $7 million, and hopefully more. These monies are required for some of the most important items for our continued financial health, such as the need for endowed chairs to offer our best scientists steady support, $2 million to renovate McClintock lab, and $2 million of the $3 million total needed to completely overhaul and expand the sorely inadequate kitchen and dining facilities in Blackford Hall.

The Blackford renovations and the planning for six more super cabins are part of a broader program to upgrade many of our infrastructure facilities. This is essential in view of the expanding staff and growing meetings and course attendance. The Corporate Committee of the campaign has undertaken to raise the needed funds from pharmaceutical and biotechnology-oriented companies in the U.S. and abroad that have sent their scientists here for meetings and courses. Headed by David L. Luke III, the committee already has commitments from Bristol-Myers Squibb Co., Burroughs Wellcome Co., Merck & Co., Pall Corp., and Westvaco Corp. Our goals for this Infrastructure Fund also include enlarged waste-treatment facilities, better fire protection, energy management, new data processing and office equipment, upgraded maintenance facilities, road construction/repair, and expanded research support facilities.

David Beach, a Hughes Investigator, Co-organizes New Yeast Course



David Beach

Late in the year, we received the good news that David Beach had been named a Howard Hughes Medical Institute Investigator. David thus becomes the first Hughes Investigator in the Laboratory's history and joins a very select group of about 175 top researchers throughout the country. With this high distinction comes research support for seven years plus funds to expand and renovate McClintock laboratory for new lab space for David and his team. HHMI recognized David's work on cell cycle control and the *cdc2* gene that has helped bring the fission yeast *S. pombe* to the forefront of cancer research. Recent observations of a pos-

sible connection between *cdc2* and the *src* proto-oncogene have stirred interest throughout the scientific community. His evidence shows that either the *src* protein or a tyrosine kinase with similar activity regulates *cdc2*. *cdc2* has already been shown to be a component of the maturation promoting factor (MPF).

Continuing our expansion to a year-round teaching institution, the fourth and last of our new set of spring and fall advanced courses was taught in October on "The Molecular Genetics of Fission Yeast." This course grew out of the prominence fission yeast has had in recent years, again partly due to co-organizer David Beach's research. As with the other three new courses, this yeast course was more than successful and has been scheduled again for fall 1990.

Ed Harlow

Harlow Wins Milken Award

The Laboratory and the scientific community quickly realized the importance of Ed Harlow's discovery concerning the retinoblastoma anti-oncogene–E1A protein interaction. More public bodies are now recognizing this great breakthrough, and the Milken Family Medical Foundation honored Ed in December with a cancer research award for his research. The award from the foundation, located in Sherman Oaks, California, includes $50,000. This is only the second year that the Milken Foundation has given this cancer award, and Ed was one of just three recipients in basic science this year, sharing the honor with Bert Vogelstein of Johns Hopkins Medical School and Charles Scher of St. Jude's Medical Center in Memphis.

Molecular Cloning II Arrives

The long-awaited second edition of *Molecular Cloning: A Laboratory Manual* arrived in November, bringing to an end the four years of hard work by Joe Sambrook, Ed Fritsch, Tom Maniatis, Nina Irwin, Nancy Ford and her staff, and the numerous scientific advisers. Their long hours of testing and retesting methods, going over manuscripts, and even adding the latest techniques in the middle of editing have created a new standard, filling a void left by the outdated first edition. The manual now spans three volumes and over 1600 pages. With 25,000 advance orders and another 10,000 within the first few months after publication, I am confident that this book will become the leader of a now-crowded field of similar texts.

Also to be noted is the enormous success of "Antibodies," the splendid lab manual by Ed Harlow and David Lane published in late 1988. By now over 16,000 copies have been sold, and already it is the second most popular book we have ever published.


J. Salk, J.D. Watson

Jonas Salk Visits Laboratory

After his early 1950s discovery eradicated polio in this and other countries and made it possible for parents to relax in the summertime, Jonas Salk has turned his attentions to another publicly terrifying disease—AIDS. On December 7, Jonas discussed his strategy for controlling HIV infection and the disease using a vaccine. He presented his work at a Banbury conference on AIDS and afterward spoke to a more general audience at Grace Auditorium. He reported that his "whole-virus" vaccine method, similar to the one he used in the 1950s against polio, has showed evidence of halting or slowing the progression of the infection. Having celebrated his 75th birthday recently, it is heartening to see that Jonas is still working at full force.

Undergraduate Research Program

The Undergraduate Research Program returned to full financial health in 1989 with the return of funding from the National Science Foundation, which unexpectedly had been lost in 1988. From a pool of 106 well-qualified applicants (43 male, 63 female), program director Winship Herr put together a fine group of 16 undergraduates. The group was split equally male/female and students came from as far as Great Britain (3) and Yugoslavia (1). In addition to the NSF funds, contributions to cover the costs of the program were received from the Burroughs Wellcome Fund, Baring Brothers Ltd., the Robert P. Olney Fund, and Miles, Inc./Bayer AG. Attesting to the intensity and interest the program sparks, one student, Steven Palmer, temporarily interrupted his studies to spend a year continuing his research at Cold Spring Harbor.

Robertson Research Fund

Thanks to the careful administration of the Robertson Research Fund, our largest endowment, the unstable market of the last two years has had a negligible impact on the income from the fund. This has allowed us to continue support for younger scientists who have not yet established themselves and secured their own grant support. Last year, the Outstanding Junior Fellow Awards went to Carol Greider, who came to us from the University of California, Berkeley, and Eric Richards, who moved here after finishing his Ph.D. at Massachusetts General Hospital of Harvard Medical School. Carol, who works on the biochemistry of the enzyme telomerase, has also made the observation that there may be a link between age and telomere length. Eric is studying the regulation of telomere length in the small-genome plant *Arabidopsis* as well as trying to clone its centromere. Our still growing plant program and the labs of Robert Franza and John Anderson received interim support in anticipation of grant funds. Other Robertson funds went for most needed salaries and stipends for postdoctoral fellows and visiting scientists.

54th Symposium Focuses on Immunological Recognition

After a 13-year hiatus, immunology returned to the Symposium with 446 attendees hearing 94 speakers over 14 sessions. Remembering the 41st Symposium, when DNA manipulation was in its infancy, crystallography lacked the powerful imaging computers, and transgenics were not yet reality, this meeting showed how far our understanding of the development and functioning of the immune system has come. As in past years, the meeting was fully registered months in advance, thanks largely to co-organizer John Inglis. With his extensive background in immunology, he and I managed to bring together the many very high caliber speakers, including Lee Hood, Susumu Tonegawa, Philippa Marrack, Hugh McDevitt, Irv Weissman, and Walter Bodmer. A traditional break from the grueling lecture schedule, the Dorcas Cummings Memorial lecture was given by Gustav Nossal, Australia's best-known scientist. His overview of the use of colony-stimulating factors in medicine and the autoimmune nature of diabetes was one of our best lectures ever for a public audience. From what I saw and heard about the 19 dinner parties that followed the lecture (a 35-year-old tradition), "a good time was had by all."

S. Klimasauskas, V. Shick, E. and M. Ustav, G. and N. Yenikolpov, and K. Galactionov

Iron Curtain around Science Drops, Too

The events of the last year in Eastern Europe have reshaped not only the political and economic climates of the planet, but also the scientific atmosphere. I have been fortunate to be involved in forging new ties with the scientific community in the Soviet Union, since I was recently elected to the Soviet Academy of Sciences. Due to the removal of what visiting scientist Grigory Yenikolopov called "a hostage mentality," Soviets have been able to leave the country with their families to pursue research in other countries temporarily. As a result, after having had only one Soviet researcher here in our first 99 years, we now have five visiting scientists from the Soviet Union and one from Poland. They are Konstantin Galactionov from Leningrad, Grigori Yenikolopov and Valentin Shick from Moscow, Saulius Klimasauskas from Lithuania, and Mart Ustav from Estonia.

J. Greenberg, G. Cutting

LIBA and Associates Membership Up

Last year, under the guidance of Chairman George W. Cutting, Jr., and with considerable help from Jane Greenberg, LIBA membership soared to 781 members, an increase of 47% over 1988. Annual giving also increased, allowing LIBA to support fellowships for Ashok Dubey, Anindya Dutta, Jeffrey Kazazz, and Erich Grotewold. The New Investigator Start-Up Fund awarded grants to Carol Greider and Arne Stenlund.

The Associates Program, only three years old, also had a very good year in 1989. Over $205,000 was donated by 135 associates, a membership increase of 22%. At the annual meeting in January, John Reese, a LIBA Director and new Laboratory trustee, was put in charge of the newly formed Associates Committee to continue expansion of the program. This annual giving, added to LIBA funds, is an important source of dependable unrestricted funds that enables the Laboratory to react quickly to promising research projects.

Baring Brothers & Co., Inc., Hosts Second Executive Conference

For top executives in the biotechnology industry, trying to combine the daily operation of their organizations with the need to keep up with rapidly changing technology is often an impossible task. For the second year straight, we were fortunate to have the leading investment banking firm Baring Brothers & Co., Inc., cosponsor a two-day conference for executives on an important and practical field of biology. The conference, organized by Douglas E. Rogers (a director at Baring Brothers), gave chairmen, presidents, CEOs, and other top executives an intensive

Baring Brothers executives at the DNA Learning Center lab

lesson on this year's topic: "Growth Factors in Development—Clues for New Therapies." Eight top scientists attended, including Stanley Cohen of Vanderbilt University, who won a Nobel Prize for the first purification of a growth factor. They discussed the use of growth factors to study and possibly treat a variety of diseases, including bone disorders and diseases of aging. A highlight of the conference was a laboratory experiment in the *Bio2000* teaching laboratory at the DNA Learning Center. There, the executives assumed the role of geneticist as they diagnosed a case of sickle cell anemia using the latest recombinant DNA techniques, including the polymerase chain reaction. Increasing their relationship with the Laboratory, Baring Brothers also gave us a grant that last summer sponsored two students from England in our Undergraduate Research Program.

Banbury Meetings Target Human Diseases

Continuing a shift in focus from an initial exclusive emphasis on risk assessment at our Banbury Center conferences, Director Jan Witkowski held several meetings pertaining directly to human diseases. In the fall, there were two meetings on AIDS—one on HIV gene expression, the other on the pathogenesis of HIV infection. An early fall meeting on the genetics of alcoholism, sponsored by the Christopher D. Smithers Foundation, forged important links among geneticists, physiologists, and psychiatrists as they try to coordinate their differing viewpoints. Various aspects of addiction in general were also addressed at a workshop for congressional aides in January 1990. Topics ranged from the biology of addiction to the potential impact on society of legalizing drugs. Jan's ambitious schedule of pertinent events at the Banbury meeting house would greatly please its donor Charles S. Robertson were he still alive to see the leading role of the Banbury Center in focusing on the public aspects of today's biological revolutions.

Changes in Scientific Staff

Early this year, we said farewell to Senior Staff Scientist Doug Hanahan whose affiliation with us began in 1978. Doug worked in Matt Meselson's lab at Harvard as a graduate student before earning his doctorate in 1983. Dividing his time between Harvard and Cold Spring Harbor, he became one of Harvard's prestigious Junior Fellows before joining our staff full time in 1985 as Staff Investigator. He settled into James lab, at first doing recombinant DNA experiments on the collagen gene, and quickly advanced to Senior Scientist in recognition of his acclaimed studies using transgenic mice. Our loss is the West Coast's gain; Doug now continues his work in San Francisco as an associate professor at the Hormone Research Institute, Department of Biochemistry, University of California.

Senior Staff Investigator Steven Briggs has returned to Pioneer Hi-Bred International, Inc., in Johnston, Iowa. Steve, who completed his Ph.D. at Michigan State in 1982, shared a joint appointment with Pioneer and the Laboratory and first came here in 1986 as a visiting scientist. Assuming a pivotal role in the rebuilding of a major plant genetics program at Cold Spring Harbor, he was promoted to Senior Staff Investigator in 1987. During his stay here, his efforts were focused on the genetic and molecular analyses of disease resistance in plants, isolating genes that confer resistance to corn pathogens, for example, rust.

Early in the year, Carmen Birchmeier accepted a position at the Max Delbrück Laboratory, Max Planck Institute, Cologne, West Germany. Prior to her arrival at the Laboratory, she worked on her doctorate with M.L. Birnsteil at the University of Zurich, followed by a postdoc period at the Max Planck Institute in Tübingen, Germany. Before being promoted to Staff Investigator, she trained in Mike Wigler's lab as a postdoc exploring new assays for the detection of human oncogenes.

Staff Associate Maureen McLeod, who worked in David Beach's lab, departed after having worked with us for five years. She received her Ph.D. from SUNY, Stony Brook in 1984 and worked on cell cycle regulation during her time here. She is now assistant professor at the SUNY Health Science Center, Department of Microbiology and Immunology, in Brooklyn.

New Staff Members

Joining the Laboratory as Senior Staff Investigator is Thomas Marr. Tom comes to us from the Los Alamos National Laboratory where he was a staff member and principal investigator in the theoretical biology and biophysics group. He collaborates in Hershey lab with Rich Roberts, pursuing his interest in computational molecular biology and the algorithms for the physical mapping of DNA.

Robert Martienssen has accepted a position as Staff Investigator and presently is working in Delbrück lab. He received his Ph.D. in plant molecular genetics from Cambridge University in 1986 and completed his postdoc at the University of California, Berkeley, where he worked with Michael Freeling. Rob is researching nuclear genes that regulate plastid development in maize.

New Visiting Scientists

In addition to our visitors from the Soviet Union, this year four visiting scientists representing a diverse number of countries are continuing their respective areas of research at Cold Spring Harbor. From Scotland we have Robin Allshire, who, after completion of his doctoral dissertation in Edinburgh in 1985, focused his efforts on chromosome structure and function at the Medical Research Council Cytogenics Unit. Now he works here in Delbrück lab. Doing experiments in Demerec lab is Clement Echetebu, a native of Nigeria, who is on leave from the University of Nigeria in Nsukka. Clement did his postgrad work in molecular biology at Moscow State and completed his doctorate at Odense University in Denmark.

Janos Posfai, who also is with Rich Roberts, is here for a second visit to continue the exploration of biological sequence analysis utilizing mathematical methods. Janos, on leave from the Institute of Biophysics Biological Research in Szeged, Hungary, first worked at the Laboratory in 1987. Yan Wang is here from Beijing, China, working in Demerec. He received his M.D. in 1968 and continued his postdoctoral work in the Department of Biochemistry at SUNY, Stony Brook, before coming to the Laboratory.

Staff Promotions

Loren Field and Dafna Bar-Sagi were both promoted to the position of Senior Staff Investigator from Staff Investigator. Loren, whose field of study is the molecular biology of the cardiovascular system, completed his postdoc with K.W. Gross at Roswell Memorial Park Institute in Buffalo. Arriving at the Laboratory in 1986 as a visiting scientist, he has utilized the powerful methods of germ-line DNA transformation to create transgenic mice that model diseases affecting the coronary system.

Ever since her arrival in 1984, Dafna's research has centered on the molecular mechanisms of growth control and oncogenic transformation, particularly the role of *RAS* proteins. She completed her early education in Israel and received her Ph.D. from SUNY, Stony Brook, in 1984 before joining the cell biology group in McClintock lab in September 1984 as a postdoc.

In 1987, Adrian Krainer was one of the first two young scientists appointed to the position of Cold Spring Harbor Fellow. This program encourages independent research by outstanding young scientists who, during their graduate studies, display exceptional promise of becoming leading scientists in the future. Adrian, who joined the Laboratory after working with Tom Maniatis at Harvard, continues to research RNA splicing in mammalian cells and now has accepted a position as Staff Investigator.

Visiting scientist Jacek Skowronski was also promoted to Staff Investigator. His work is focused on transgenic mouse models of HIV-induced diseases. Jacek, a native of Lodz, Poland, joined the Lab in 1986 after spending three years at the National Institutes of Health in Bethesda as a visiting fellow.

Gil Morris, Karl Riabowol, Masafumi Tanaka, and Toshiki Tsurimoto, formerly postdocs, accepted positions as Staff Associates. Gil Morris works with Mike Mathews' protein synthesis group in Demerec lab investigating the control of gene expression. Karl Riabowol is with Mike Gilman's group in James studying the mechanisms of signal transductions that result in changes in nuclear transcription. Masafumi Tanaka works with Winship Herr studying the regulation of transcription in mammalian cells. Toshiki Tsurimoto works in Bruce Stillman's lab looking for an understanding of the mechanism and regulation of DNA replication in eukaryotic cells.

Postdoctoral Fellows

Postdoctoral research fellows leaving the Laboratory this year were Dennis Carroll to the State Department, Washington, D.C.; Wai-Kit Chan to Columbia University Law School; Susan Erster to the Biomedical Sciences Research Center, North Shore University Hospital in Manhasset; Joanne Figuerido, East Norwich; Leslie Goodwin to the NYU Medical Center in New York City; Seth Grant to the Howard Hughes Medical Institute, Center for Neurobiology and Behavior, New York City; Gurmukh Johal to Pioneer Hi-Bred International in Johnston, Iowa; Janise Meyertons to Abbott Laboratories in North Chicago; Judith Potashkin to Chicago Medical School, Department of Pharmacology and Molecular Biology in North Chicago; and Richard Sturm to the Center for Molecular Biology and Biotechnology, University of Queensland, Australia.

Graduate students who completed their doctorates included Leonard Brizuela, who returned to his home in Caracas, Venezuela; Lonny Levin, who is at the Howard Hughes Medical Institute, Johns Hopkins Medical School in Baltimore, Maryland; Ian Mohr who is at the Department of Molecular Biology at the Univer-

sity of California at Berkeley; and Brian Ondek who is in the Department of Biology at the University of California at San Diego, La Jolla. Robert Booher is continuing his studies at the University of California School of Medicine in San Francisco.

Employees Recognized for More Than 15 Years of Service

Several new names have been added to the growing list of dedicated Laboratory employees who have worked here for so many years. Fifteen-year awards were presented to Deon Baker, purchasing secretary (now retired); Bob Borruso, equipment maintenance technician; Annette Kirk, Assistant Managing Director of Publications; Michael Mathews, Senior Staff Scientist, and Lane Smith, plumbing supervisor.

M. Mathews, B. Borruso, D. Baker, L. Smith

Making the Business Connection

To create a more tangible bond with the Long Island business community, we have created the Centennial Committee. Co-chaired by Robert R. McMillan of Rivkin, Radler, Bayh, Hart & Kremer and Thomas A. Doherty, Chairman and CEO of Norstar Bank, the committee's main goals are to increase the Laboratory's visibility on Long Island and to sponsor a centennial book. The book will feature a section titled "Partners for the Future," highlighting some of the larger companies on Long Island. It is hoped that this presentation of the strength and depth of research and industry on Long Island will be used by local development agencies to attract new businesses from the U.S. and abroad to the region.

T. Doherty, J.D. Watson, R. McMillan

Laboratory Is Number One among Independent Labs

A recent article in the newspaper *The Scientist* confirmed what we have liked to dream—the Laboratory is number one among the 74 members of the Association of Independent Research Institutes (AIRI). The article based this on "citation impact," a measure of how many times other researchers cite papers from our scientists. Between 1973 and 1987, our scientists' papers were cited an average of 58.7 times, which is more than five times the average impact and 15 more citations than number two on the AIRI list. My thanks and congratulations to all the researchers here over the last two decades who made this possible.

Strong Board of Trustees

For an institution such as ours that is not tied financially to any outside university or foundation, long-term strength is often directly related to the expertise of its governing body. In our case, the sound financial and scientific position we have so far reached heading into our centennial owes very much to the diligent and caring work of all the members of our board of trustees, who encompass a broad spectrum of outstanding achievements. Unfortunately, after their two consecutive three-year terms on the board, we had to bid a partial farewell to Eric R. Kandel and John Klingenstein—"partial" because they are both remaining as advisers on the neurobiology committee. Eric's unmatched experience and wisdom have been, and will continue to be, a guiding force behind the Neuroscience Center and the neurobiology course program. Last year, he helped to organize our lecture course on behavior and this year will organize our October *Aplysia* meeting. John has long been a champion of the neurobiology program and his expertise in investment banking, gained from his many years with Wertheim & Co., has been vital as our operating budget grows and we increase our endowments.

As biotechnology begins to have a real impact on the pharmaceutical industry, we were very fortunate when William R. Miller, Vice Chairman of the Board of Directors of Bristol-Myers Squibb Company, joined our board this past year. He has over a quarter-century of experience under his belt at Bristol-Myers Squibb and is well-known throughout the international pharmaceutical industry. After a one-year hiatus following his past two terms on the board, we welcomed back an old friend, Townsend J. Knight, whose dedication to the Laboratory equals that of his Jones family ancestors. Filling Eric Kandel's position as representative from Columbia University is Thomas M. Jessell, already a familiar face at the Laboratory. Since 1983, he has been a lecturer at summer neurobiology courses, and now, as a member of the neurobiology committee, he will help us to shape the future of the entire program. With so many good scientists and projects here, our concerns often turn from where the next discoveries are to how we will pay for them. John R. Reese of Lazard Freres & Co. has joined the board to help us address these concerns with his knowledge of investment banking. A resident of Cold Spring Harbor and already a LIBA Director, John needs no lessons on how to be a Laboratory supporter. Having not really lost two members and gaining three, our board may indeed be stronger than ever.

More Responsibilities Come with My Directorship of the National Center for Human Genome Research

In October 1988 when I first went down to NIH to help in the build up of its Human Genome Program, I was then the Associate Director for Human Genome Re-

search working under its Director, Jim Wyngaarden. At first, my office did not have the formal powers to give out grants; that role remained the responsibility of the National Institutes for General Medical Sciences. On October 1, 1989, however, my responsibilities became much more operational. A new grant-awarding division within NIH was created to manage NIH's role in our nation's effort to map and sequence the human genome of some three billion base pairs.

I am now the Director of the National Center for Human Genome Research (NCHGR), whose offices are located on the sixth floor of the Lister Hill Building, with fine views over the NIH Bethesda Campus. We have a staff of almost 30 scientists and administrative aides who will function over the more than 100 million dollars that we hope to distribute each year in grants or contracts to scientists working at a large number of universities, medical schools, research institutions, and our biotechnology-oriented companies. Companion to our NIH effort is a Department of Energy Program that plans to distribute roughly half this sum, in large part to those of its National Laboratories such as Los Alamos, Lawrence Berkeley, and Livermore that have initiated major human genome mapping and sequencing efforts.

As the NCHGR Director, I am constantly on the shuttle that flies from LaGuardia Airport to Washington National as well as traveling for talks that explain our genome program to influential university and industrial bodies. I must also attend many of the increasingly large number of highly technical small meetings that bring together key scientists in our program. I'm also spending more time working for NIH here at my Cold Spring Harbor desk than I initially planned, in large part due to the ease with which documents can be transmitted to me from Bethesda over the fax. By now, I suspect that half of my efforts are devoted to NIH, leaving me badly stretched to find the time and energy for my responsibilities here.

Fortunately, ever since my appointment 22 years ago, this institution has run very well without my constant day-to-day interventions. This was most certainly the case prior to 1976 when I simultaneously taught at Harvard, and it is even more true today. This only has been possible because of the high competence of our key scientists and administrative staff. Today, Morgan Browne, our Administrative Director, Richard Roberts, our Assistant Director for Research, and Terri Grodzicker, our Assistant Director for Academic Affairs, know they have to run their own shows and do so well. My functioning in two jobs is also immeasurably aided by Susan Cooper in her capacity as Director of our Public Affairs Department. Particularly important have been her interactions with the many journalists who want to learn more about my job in Washington. My secretary here, Andrea Stephenson, also has been of immeasurable help in presiding over travel plans that often must change at the last moment.

Even with all this help and encouragement, I am just making do and worry what will happen if my genome duties present an even greater call on my time. I do not have the answer and try to keep this potential crisis from affecting my current day-to-day existence.

Our Objectives Must Remain Heroic

I constantly surprise visitors who ask to see my lab by replying that I have never had one since I came here 22 years ago. Initially, that situation reflected the fact that I had an active research lab at Harvard and running two groups was far beyond my capabilities. Later, when we made Cold Spring Harbor our sole residence, I was by then never tempted to strike out again as an experimentalist. In

trying to keep this Laboratory growing in an exciting way, I was never bored and had more than enough anxieties to keep me from either growing complacent or worrying whether my peers viewed me as past my prime and lacking the strengths that led me toward the double helix. The science around me in the labs of our staff was much better than I could have then pulled off through a personal group. By merely walking out of my office, I moved into a scene where the aim was to find the future not follow the past. So I could feel quietly satisfied that my move away from Harvard had not led me into a derivative world lacking the intellectual power of the American Cambridge.

The unique quality of Cold Spring Harbor was allowing us to strive for research objectives that most scientists even at Harvard or MIT inherently shy away from because the odds for success look too low. It has been these heroic purposes that has led to our high relative ranking as measured by the "citation index." That we are judged so well by the outside scientific world obviously reflects the intelligence and high motivation of the so many excellent scientists who have worked here over the past two decades. But these talents would have been lost in the crowd of excellence elsewhere if we had strived for more obviously attainable goals.

Looking Forward to the Next Decade

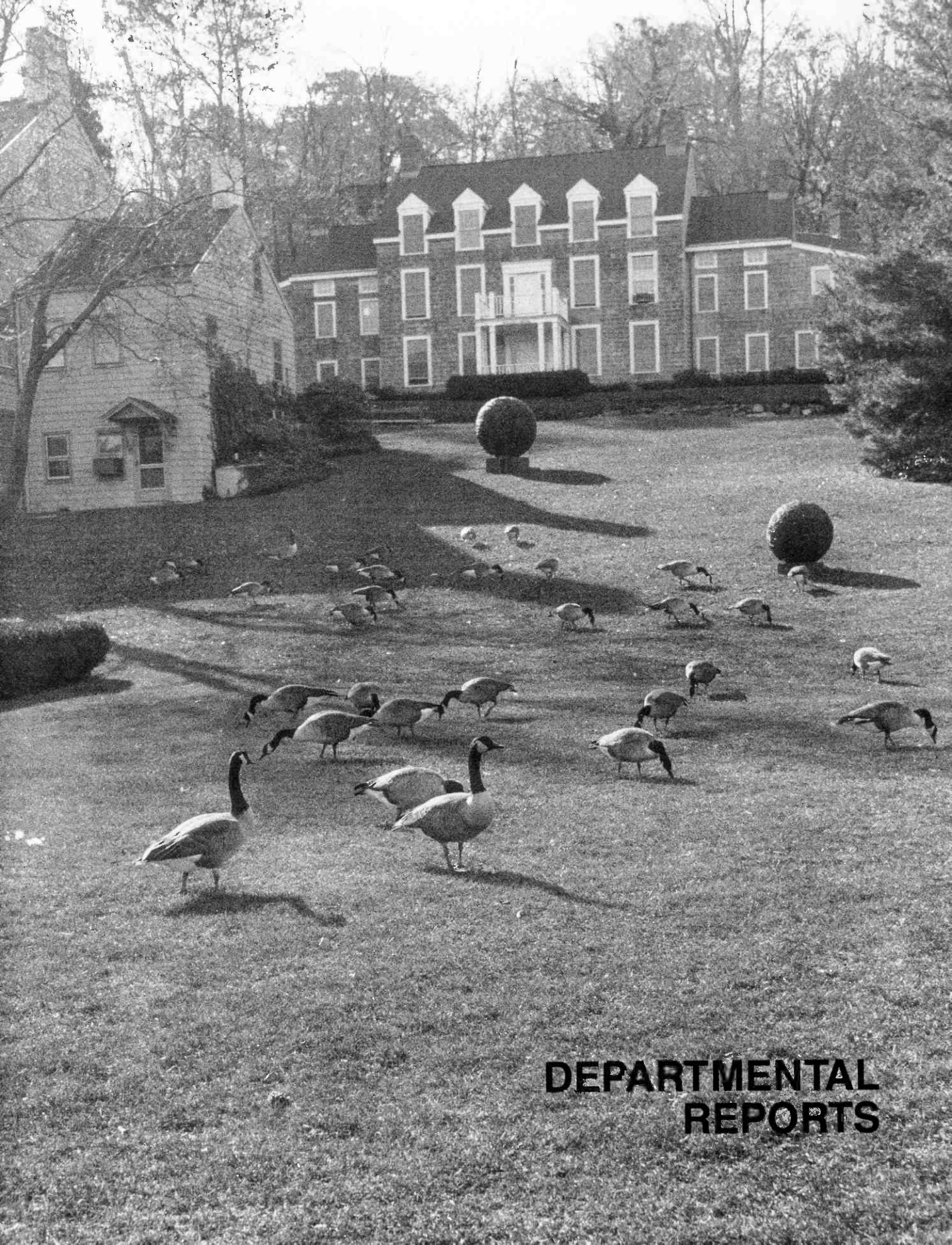
At least until this century ends, we must remain deeply committed to the pursuit of oncogenes and anti-oncogenes. We have come too far to turn back now before we have our quarry. The cancer research facilities in James, Sambrook, Demerec, and McClintock labs should remain so committed until victory. We furthermore must be constantly improving these labs, with the next major upgrade needed being a major refurbishment of our hallowed McClintock lab. So renovated, it will be an ideal site for bringing oncogenes and anti-oncogenes into the world of those who study how normal cells progress through the cell cycle.

We must also set our sights very high as we move seriously into neurobiology. In finally understanding how our brains perceive, memorize, and learn, enormous challenges must be faced and we should have no illusions that anyone soon will find a double helical type of simplicity within the brain that will provide a simple intellectual framework on which to base our ideas and experiments. So, we must be realistic over the short term, while keeping very clear that cognition, not selective neural gene functioning, is what makes the human brain the ultimate gem of all science. Thus, although developmental neurobiology is likely to be the main initial focus of our new Beckman lab, we must have in residence experimentalists who pursue the essences of perception and memory, as well as their more theoretical colleagues who make models for how we may learn. If somehow we can integrate these several worlds together, our ever-growing commitment to neurobiology will make our next century even more exciting than our first one.

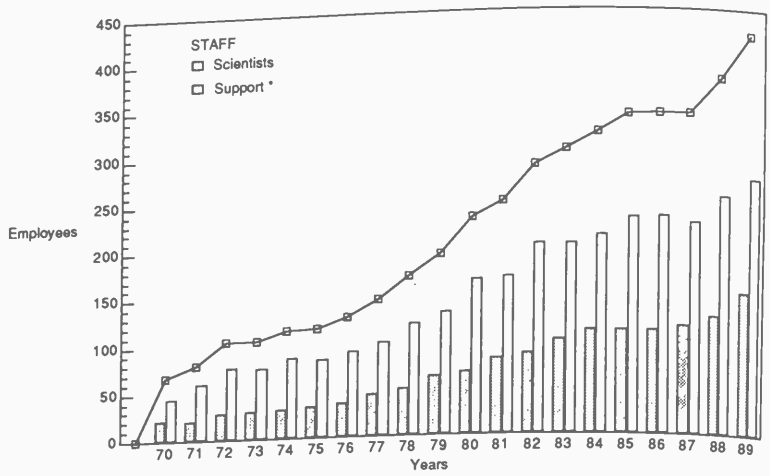
This will indeed be a hard challenge. But in so striving, we shall try hard to maintain the traditions of our past that are so satisfying to look back upon, traditions of scientific honesty, abhorrence of pomposity, and devotion to the teaching that rapidly disseminates the new facts of science to the best of our youths.

April 4, 1990

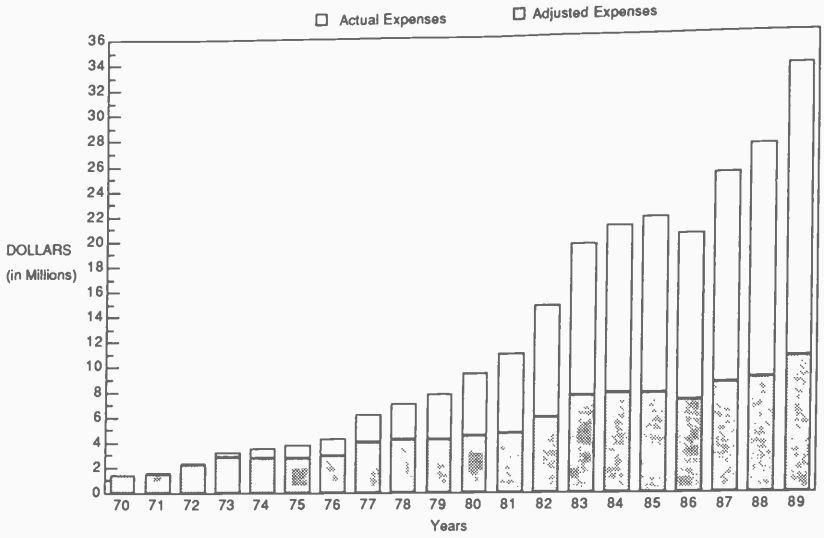
James D. Watson

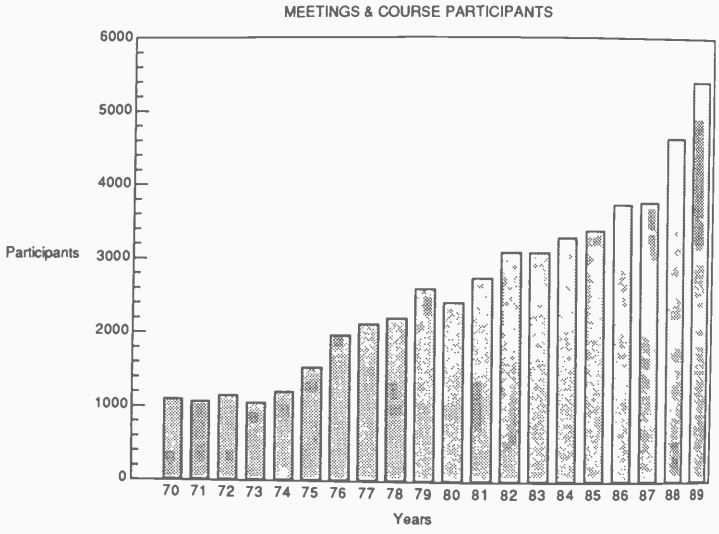


DEPARTMENTAL REPORTS



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





ADMINISTRATION

We were pleased with the Laboratory's financial results for 1989. Revenues exceeded expenditures, and there was a surplus after fully funding depreciation and providing some moderate reserves for future contingencies. These results emphasize the financial health of the Laboratory as it enters its second century and are a reflection of the basic strength of its programs.

This is most clearly demonstrated by the research program. Now is a particularly difficult time for federal funding of basic research, with only about 15% of approved new grant submissions to the NIH being funded nationwide. Yet, scientists here continue to be awarded a good share of available funding, and their extraordinary research is attracting increasing support from private foundations, corporations, and individuals.

Federal funding for meetings and courses is no less difficult to obtain. But again the quality of the education program attracts support from a wide variety of government and private sources, and in 1989, this funding was sufficient to make the year a financial as well as an intellectual success. A record 5417 visiting scientists from academia and private industry attended the meetings, courses, and seminars held on the main campus and at Banbury Center.

The Cold Spring Harbor Laboratory Press achieved record revenues of nearly $4.5 million during 1989. The enormous success of the second edition of the *Molecular Cloning* manual and of Ed Harlow's *Antibodies* manual helped support the publication of a number of less popular but no less worthy books. Moreover, the Journal Department, with substantially reduced investment now needed for *Genes & Development*, found it possible to invest the funds required to launch *Cancer Cells*, our second journal.

Banbury Center and the DNA Learning Center also had a good year. Banbury meetings focus on scientific subjects that have particular social or environmental relevance or are designed to appeal to corporate scientific interests. One result was a record number (31) of Corporate Sponsors who contributed over $500,000 in support of the Laboratory-wide meetings program. Programs of the DNA Learning Center continued to have increasing national impact on high school science education, and the Center made further progress toward financial self-sufficiency after overhead and depreciation.

The financial strength of the Laboratory benefited from another good investment performance of the endowment, consisting primarily of the Robertson and Cold Spring Harbor Funds. As of December 31, 1989, these funds totaled $45 million, a gain of more than 22% over the prior year. Once again, we were able to reinvest sufficient income from the Funds to compensate for inflation and to provide for the future growth of Laboratory programs.

A year as rewarding as 1989 is made possible by the contributions of many individuals and departments. Some, such as Buildings and Grounds, Development, and Public Affairs/Library, are described elsewhere in this report. The Meetings and Food Service Departments continue to astound us with their gracious and efficient registration, housing, and feeding of our many visitors. Other departments, particularly in Nichols Building, should not be overlooked.

Our Controller, Bill Keen, has built an organization that handles the Laboratory's finances with great effectiveness. The operating and capital budgets are managed professionally with a minimum of surprises, and these are most generally of a

positive nature. The addition of Barbara Wang and Alison McDermott to our staff within the past two years has made it possible to provide greater financial assistance to the Cold Spring Harbor Laboratory Press, Banbury Center, and the DNA Learning Center and to maintain the necessary close supervision of now some $75 million of endowment, capital, and operating funds.

Susan Schultz and the Grants Management Department fulfill a particularly vital function. Grants are the lifeblood of the Laboratory. The task of assisting the staff in obtaining and properly managing these grants grows in direct proportion to the number of scientists here. That number increased by some 25% during 1989 and will increase again by at least that amount as the Neuroscience Center is fully staffed.

Such growth also impacts our Purchasing and Human Resources Departments. One of the most important services provided to the scientific staff is fast and efficient response to equipment, supply, and personnel needs. At the beginning of 1990 when Chuck Haibel was promoted to the new position of Contracts and Equipment Manager, Buildings and Grounds, Sandy Chemlev was promoted to Manager of Purchasing and already it is clear that our confidence in her is well placed. In November, Cheryl Sinclair joined the Laboratory as Manager of Human Resources after Tony Napoli accepted a position as Vice President of Human Resources at Tarkett, Inc., part of STORA Group, a large Swedish conglomerate. Cheryl comes to the Lab from Kollmorgan Corporation, where she was Personnel Director of the PCK Technology Division. We are delighted to have her with us.

John Maroney, now successfully beyond the ordeal of the New York State Bar Exam, has been an enormous help in sharing administrative tasks and in managing commercial relations. Licensing of inventions is a small but increasing source of income for the Lab and the scientific staff. John's aim is to familiarize the staff with the procedures and benefits of patenting their discoveries, and he acts as an interface with patent attorneys and private industry.

The Board of Trustees, with its unique mix of prominent scientific and lay members, takes an active role in the affairs of the Laboratory. Roberta Salant responds to their needs with an efficiency and grace that is much appreciated by all.

We look to 1990 and beyond with much anticipation. By this time next year, there will be research and courses under way in Beckman Laboratory; Dolan Hall, along with six additional guest houses, will be providing 108 more overnight accommodations for visiting scientists, bringing the total above 200. A major renovation and expansion of kitchen and dining facilities in Blackford Hall will be under way. It is hoped that Jack Richards and Jim Hope will have figured out how to feed our employees and visitors while Blackford lies in pieces. Refurbishment and expansion of McClintock Laboratory will also be imminent.

During this centennial year, it is good to reflect on the first 100 years of extraordinary achievement at Cold Spring Harbor Laboratory—clearly, the next 100 years will be no less eventful.

G. Morgan Browne

BUILDINGS AND GROUNDS

As I write this report for 1989, I have to think back 20 years ago when I started this job. I had been concerned that we would run out of work in three to four years when all the old buildings were rebuilt. Twenty years later, I could fill volumes for the next few years. So let's go back and see what we did in 1989.

Telephone Service Upgrade

In 1989, we completed a major upgrade to the telephone system, specifically the PBX switch. This switch handles all of the Laboratory telephone traffic and is the heart of the telecommunications system. The original PBX switch was ten years old and operating at full capacity. Because the Laboratory is continually expanding, it was decided to upgrade to a larger, state-of-the-art PBX switch. On Saturday, July 19, the new switch was installed, with a loss of service of only 15 minutes. It was a smooth transition, with minimal disruption to the working Laboratory. Credit for a job well done goes to my assistant, Peter Stahl, who is now keeping all of our phones and switch gear on line with very few problems.

Conversion to Natural Gas Burners

New York State regulations regarding in-ground oil tanks have made it impossible for us to continue to use this form of heating. We have therefore been removing the oil tanks and converting to natural gas burners. Our Plumbing Department worked long and arduous hours designing and installing gas burners in the larger buildings, and a new gas main was installed by LILCO. We expect to complete all conversions to gas within the next two years.

Landscaping and Paving

The Grounds Department had a very busy 1989 as well. We landscaped the area around the new cabins. This required working on a hillside with only picks, shovels, and rakes. We also paved the parking areas north of Nichols and at Waterside next to the Buildings and Grounds Department. Also, we built a pond next to Nichols' parking lot. All of these jobs were held up because of inclement weather—rain, rain, and more rain. Yet we kept the grounds looking great thanks to Buck Trede, our Grounds Foreman, and his crew.

New Construction

Our Carpentry Department had its hands full also. To start the year, we built a mock-up of one of the neurobiology labs scheduled for construction and set it up in the Bush Lecture Hall. It remained there through January for all to see and comment upon. We then went to work on the six new cabins. Off to a slow start due to very cold weather in January and a very wet spring, we were concerned that the cabins would not be completed by the first meeting. A number of other misfortunes also complicated our spring work. Carl Fabrizi, one of our carpenters, fell off the scaffold and could not work for several days; and Jeff Goldblum, our lead carpenter, had a personal emergency and was not his usual energetic self for a time. Lou Jagoda, our electrician, broke his ankle and was also slowed down a bit. And of course there was the rain! In spite of the problems, the cabins were

completed and wood decks and walks were installed, all before the first spring meeting!

Alterations and Renovations

Demerec: After many years of talk about a north addition to Demerec, in 1989 we constructed a small extension to house a seminar room, a secretarial area, and a post-doc office. The extension also provides a new entrance to Demerec and has a landscaped patio area. We completed the work on time and the extension was occupied on November 15.

Harris: Completed in 1982, the Harris Building needed updating and new paint throughout. We rebuilt the cage-washing area, installed a new tunnel washer, put up a new ceiling, and painted the interior of the building from top to bottom.

Wawepex: We gutted the lower floor of Wawepex in preparation for a new development office. We installed new windows and added a new porch, air conditioning, and built-in furniture. We also added a new walk and stairs to Blackford lawn.

DNA Learning Center: A great deal of our time was spent in the DNA Learning Center. In 1989, our last project, the designing and building of two laboratories in the basement area, was completed.

Thanks to a Great Staff

I want to thank my staff for a job well done. We have a crew of workers who do an excellent job despite all the obstacles and they are great mechanics. They are the best, and their efforts are appreciated.

Jack Richards

DEVELOPMENT

In its first complete year as an administrative entity, the Development Department was fully engaged in directing the $44 million Second Century Campaign, the first public capital campaign in the 100-year history of Cold Spring Harbor Laboratory, *and* expanding the annual giving base both in number of donors and in number of dollars.

Unrestricted annual giving to the Cold Spring Harbor Laboratory Associates Program and to the Long Island Biological Association (LIBA) provides strong underpinning for the fiscal health of the Laboratory. These programs are soundly and enthusiastically guided by Chairman George W. Cutting, Jr., and 28 directors. (A complete report of their 1989 activities may be found in *Financial Support of the Laboratory,* later in this Annual Report.) The increase in the numbers of these annual supporters and the success of the Second Century Campaign give testimony to the fact that Cold Spring Harbor Laboratory is no longer "Long Island's best kept secret."

The official announcement of the public phase of the Second Century Campaign occurred in Grace Auditorium on April 22nd at a dinner for more than 100 campaign volunteers and guests. Campaign Chairman and Trustee Treasurer, David L. Luke III, assigned the task of raising the remaining $16 million to the six campaign committees; $28 million had already been raised through the outstanding efforts of Dr. Watson, Dr. Clarkson, Mr. Luke, Morgan Browne, and the trustees. By year's end, another $8.9 million had been raised, making the total of campaign gifts and pledges, $36.8, with $7.2 million left to raise to meet our $44 million goal by December 1991.

Several important areas in the Neuroscience Center have been named as a result of significant campaign gifts: *The Hazen Tower* (Mrs. Lita A. Hazen), *The Oakes Ames Seminar Room* (Mrs. Pauline A. Plimpton and Mr. Amyas Ames), *The Gardner Neuroscience Library* (Mr. and Mrs. Robert B. Gardner, Jr.), and *The Lucy and Edward Pulling Seminar Room* (Mr. and Mrs. George W. Cutting, Jr.).

A request from someone who believes in, works for, and has given to an institution is far more effective than any other type of request. Thanks to the practical labors of our campaign volunteers, most of whom have made their contributions, the Second Century Campaign is progressing well. We are most fortunate to have the assistance of these dedicated people.

Another important element responsible for the progress we have been able to make this year is the Development Department staff. Joan Pesek is a most effective liaison to LIBA and a cordial cultivator of new members; she manages the CSHL Associates Program and is a very knowledgeable tour leader. Debra Mullen has given order to our fledgling department, keeps accurate track of the complexities of our ever-increasing number of gifts (annual and campaign), and manages to satisfy the demanding secretarial needs of the Department. During the year, an important addition was made in the person of Claire Fairman. Claire provides considerable expertise and artistry in the coordination of our campaign committees and the identification and cultivation of new campaign prospects. But, perhaps the Department's greatest asset is George Cutting, a trustee of the Laboratory who, each day, volunteers long development hours, is the Chairman of LIBA, and is one of the co-chairmen of the campaign's Special Gifts Committee. He is a creative and genial campaign leader and we could not function nearly as efficiently without him.

This entire Annual Report attests to the continuing remarkable work being done at the Laboratory and is a great source of pride not only for those who work "on grounds," but also for those who work "off grounds." We are most grateful to those who have helped make such progress toward our development goals. (Campaign contributions are listed under *Financial Support of the Laboratory*.) Only vital and growing institutions need capital campaigns and as we enter the second year of the public phase of the Second Century Campaign, we invite those who have not yet participated to do so. (Methods of contributing, either outright, in trust, or by will, are outlined under *Financial Support of the Laboratory*.) **Come celebrate our past 100 years and make secure our next 100 years!**

Konrad Matthaei

LIBRARY SERVICES

Archives

The Laboratory archives, formerly the library attic, is now a comfortable and active facility, housing the Carnegie collection of historic books and journals, as well as a number of newer books about Long Island and of interest to the Laboratory. In addition, there are files of original papers from the early Carnegie years. Laboratory information and material are continually kept current to ensure accurate records for future generations. Researchers from the media as well as staff members are making use of this facility. Lynn Kasso ably handles organization of archival materials and fulfills outside requests from both publishers and the press.

In connection with the dedication of the new cabins in August, 24 photographs of historic buildings from the archives were selected by Laura Hyman and Elizabeth Watson to be enlarged to 16 × 20″, sepia-toned, and framed. These photographs were hung in each cabin bedroom. The archives also played a major role in the preparation and planning of the Laboratory's first major exhibit of paintings and prints by Jane Davenport Harris De Tomasi, which will take place early in 1990 as part of the Centennial celebration.

Reference Services

The library began its plans to automate several of its reference services. A workstation consisting of a PC and a printer was installed for use by Laboratory patrons. Weekly issues of *Current Contents* on diskette are available for current awareness access by author, journal title, and key word. Users may either print references or download them to floppy disk. In December, a compact disk version of the National Library of Medicine's *Index Medicus* Medline database was set up on a trial basis. It provided scientists with unlimited access to this database at no cost to the individual user. This type of end-user search capacity proved so popular that the library will continue this service in 1990 and add other databases on CDs such as *Biological Abstracts* as they become available. Genemary Falvey, the Head of Library Service, is currently investigating other applications of technology to extend user participation further.

Our computerized search service continues to be Genemary Falvey's first priority. She conducts three times as many searches today as she did five years ago. Wanda Stolen capably handles our computerized interlibrary loan service, which provides faster access to articles requested by staff scientists. Photocopies of the tables of contents of 60 journals are sent as received to 32 scientists.

Storage Facility

The storage facility at West Side School continues to house our older and less-used bound journals. We monitor the requests each year and withdraw unused titles to make room for others and consequently free up space in the main library for newer materials.

Staff Changes

Helena M. (Leigh) Johnson joined the library staff in November, replacing Jeannette Romano—we welcome her aboard.

Space Needs

Library materials and personnel have been rearranged to allow for maximum capacity in the space allotted to us in the Library building. To provide for the future needs of the Laboratory's growing personnel, it will be necessary to plan future expansion in the present building by retrieving our space from the Publications Department, by adding to the present building, or by moving to larger quarters.

Susan Cooper

PUBLIC AFFAIRS

From New Staff to Seasoned Staff

For the first time in its six-year history, the Public Affairs Department has no staff changes to report. This continuity in personnel has contributed to the vast number of materials and events produced by the department in 1989. Margot Bennett, Emily Eryou, Herb Parsons, and Dan Schechter are a dedicated team of professionals whose willingness to work long hours very often is the only reason so much is accomplished in so little space.

For more than ten years, Herb Parsons has served the Laboratory, first as audiovisual technician and now as Audiovisual Director. When he began, Herb handled 9 summer meetings, 5 Banbury meetings, and 12 courses. Today the program has grown to 13 summer meetings, 23 Banbury meetings, and 19 courses. I want to take this opportunity on behalf of the Laboratory to thank Herb for his enthusiasm, dedication, and hard work.

Good Local Press Coverage

The Department issued a dozen press releases and talked to countless reporters and anchor people, eliciting 12 television segments and 145 magazine and newspaper articles mentioning Cold Spring Harbor Laboratory and its scientists. Local coverage has been particularly satisfying this year, laying the foundation for better local support from Long Island communities and businesses. Cablevision's *News 12 Long Island* featured the Laboratory in its new health segment and the Long Island Association's magazine, *Long Island*, featured the Laboratory in its cover story, "Harbor of Discovery." Several items were reported in *Long Island Business News* as well. On the national front, the Laboratory and its DNA Learning Center found themselves touted on ABC, CBS, and PBS and discussed in the *New York Times*, *Washington Post*, and *Christian Science Monitor*, to mention just a few. *Newsday* published a profile on the "Dean of DNA," featuring Dr. Watson's dual role at the Laboratory and at the Office of Human Genome Research in Washington, D.C. Equally compelling was the extensive and personal profile of Dr. Watson in the *Washington Post*—"The Man Behind the Double Helix."

An Array of Available Materials

At a time when information reaches our TV screens and front pages concurrent with discovery, it behooves scientific institutions to describe their research in an accountable and unambiguous manner. It is our obligation to present tools that will nurture, not mystify, our constituency. To this end, *The Good Fight: Cancer Research at Cold Spring Harbor Laboratory* was completed in 1989. Designed for the lay reader, this piece explains the research tools employed by the laboratory scientist to pursue the complexities of the cancer puzzle. *A Vision of Hope*, a new 10-minute video, presents a concise view of the Laboratory from research to education and is used to cultivate new audiences.

Four regular issues of the Laboratory's newsletter, *Harbor Transcript*, were completed, along with one special campaign issue highlighting the kick-off dinner for the public phase of the Second Century Campaign. In its seventh year, the *Harbor Transcript* is now mailed to nearly 6000 neighbors and friends. In addition to its regular features, the newsletter highlighted research in the areas of electron microscopy, cancer and AIDS, and the cell cycle.

Equally important to the Laboratory's public face are its promotional pieces. In 1989, the staff supported the work of other departments by writing, designing, and organizing numerous cultivation tools, publicity pieces, and events. The *McClintock Renovation* and *Infrastructure* brochures were created to marshal funds for the physical plant. A variety of other fund-raising pieces were rewritten, updated, or restructured for use in the Second Century Campaign. Meetings and courses posters, a Nature Study brochure, a LIBA Associates plaque, and a multipurpose Centennial notecard round out the materials available.

The Memorial Program

In 1989, I was approached by a young man whose father was dying of cancer. The older man, Walter Sturm, who had survived Kristallnacht during World War II, waged a heroic battle against the disease for 20 years. Through his son, Robert, the family was searching for a substantive way to honor their father. Over the years, Bob had searched scientific literature for solutions to his father's advancing disease, discovering in the process that papers from the Laboratory were cited most often. The family decided that only quality research would provide the better diagnosis and treatment they had sought unsuccessfully for their father. Before his death on March 24, 1989, Mr. Sturm agreed that any memorial funds resulting from his death should be contributed to the Laboratory. Fittingly, the Sturm Fund has generated over $3500.

In dealing with a mechanism for handling the numerous small contributions from the Sturm Fund, Public Affairs designed a memorial program. Envelopes and pledge cards are now available to anyone through our department.

Special Events

During the final year of our first century, the Public Affairs Department planned and hosted a variety of events. On May 12, marking the completion of the first phase of the Neuroscience Center, we extolled the donors with the placement of the cornerstones, followed by a festive dinner. On August 26, the new cabins were dedicated and named for six of our most esteemed alumni: Bentley Glass, Harry Eagle, Salvador Luria, Gunther Stent, Franklin Stahl, and Herbert Boyer. A

dedication booklet commemorating this event included a history of visitor housing and profiles of the honorees.

Centennial

Peter Orth, one of the most consistently acclaimed pianists of his generation, performed a wonderful concert in honor of our Centennial. LIBA member Mary Lenore Blair was the "angel" who coupled Orth with the Laboratory. Mrs. Blair recruited sponsors who, in addition to making financial contributions, hosted dinners for friends, who in turn purchased tickets to the concert. On August 13, Orth performed to a full house and received the most resounding standing ovation ever experienced here.

To commemorate the Centennial, in December, each member of the staff was presented a lapel pin and *F.A.C.E.S. '89*, a book of photographs of all Laboratory personnel. In addition to snapshots, this rogue's gallery lists date of employment, job title, and resident town for each entry, providing an ever-growing population with the ability to identify co-workers.

Each event adds momentum to, and sets the stage for, our 100th birthday party in July 1990. To ensure its success and mark the day in grand style, Judith Carlson has been retained to create a Centennial book. The book will include a record of Centennial events, historical highlights of Laboratory activities, photographs, and appropriate salutations from the Laboratory's community. A section called "Partners for the Future" will be congratulatory messages from the Long Island business community. A committee of prominent business figures is being organized by Robert McMillan, Publisher, *Economic Times of Long Island*, and Thomas Doherty, Chairman of the Board, Norstar Bank, for the purpose of producing this wonderful tribute.

Susan Cooper


RESEARCH

TUMOR VIRUSES

The Tumor Virus Section presently comprises nine groups, united historically through their use of the DNA tumor viruses to probe cellular functions. The research conducted in this section covers an extraordinarily broad range of topics, and its scope has expanded in recent years as the exploration penetrates more deeply into the molecular machinery of the cell. Although the principal goal remains to develop an understanding of malignancy, a great deal of current effort is concentrated on exposing the mechanisms of macromolecular synthesis that operate in the normal state. Some of the most dramatic advances have illuminated regulatory processes that govern cellular functions in both the normal and transformed states and determine the transition between them. Common regulatory themes that have emerged repeatedly involve the modulation of enzyme function by interaction with other macromolecules or by covalent modification, especially phosphorylation; examples of these two fundamental strategies are described in a number of the reports that follow. In addition to studies of the two traditional workhorses, adenovirus and SV40, several groups have also begun to dissect the control processes exploited by the AIDS virus, HIV-1, and last year also saw the initiation of bovine papillomavirus studies in Dr. Arne Stenlund's laboratory. The year's end sadly brought to a formal close Dr. Yasha Gluzman's long, productive, and ever lively connection with Cold Spring Harbor Laboratory. Related work on SV40 T antigen will continue, however, in Dr. Bruce Stillman's lab, and we hope that Yasha will maintain a vigorous interest, if less frequent presence, in the project in the future.

DNA SYNTHESIS

B. Stillman

E. White
T. Tsurimoto
G. Bauer
S. Brill
J.F.X. Diffley
S.-U. Din
A. Dutta
T. Melendy
F. Bunz
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The replication of DNA is a central step in cell growth and division and is necessarily a precise and highly regulated process. Our goal is to understand the mechanism and regulation of cell chromosomal DNA replication. To achieve this goal, we use as experimental systems, the DNA tumor virus, SV40, and the yeast, *Saccharomyces cerevisiae*. It is therefore satisfying that in the past year, these two avenues of approach have crossed many times, emphasizing the fundamental nature of DNA replication and, at the same time, reinforcing our initial belief that the DNA tumor viruses offer valuable insights into the intricate workings of their host cell.

SV40 DNA Replication

S. Brill, F. Bunz, S.-U. Din, A. Dutta, K. Fien, T. Melendy, T. Tsurimoto, N. Kessler, S. Longinotti, B. Stillman

The DNA tumor virus, SV40, contains a small circular chromosome that replicates in the cell nucleus during the S phase of the cell cycle. The virus encodes a protein, the SV40 T antigen, that plays a central role in the replication of the viral genome. Therefore, the majority of proteins that replicate SV40 DNA are encoded by the host cell. Moreover, the mechanism of SV40 DNA replication reflects the

mechanism of DNA synthesis from a single chromosomal origin of replication. Thus, SV40 is an excellent system to study cellular DNA replication.

Our primary goal over the past five years has been to identify and characterize the cellular proteins required for DNA replication and chromatin assembly, ultimately to use them to study the regulation of DNA replication. A cell-free extract prepared from human cells supports the complete replication of SV40 DNA when purified T antigen is supplied. The mechanism of DNA replication in the cell extract reflects the process that occurs in SV40-infected cells. To identify the cellular components of the replication machinery, we have fractionated the extract into multiple components and have identified a number of novel replication factors. A brief summary of the functions of the known human cellular DNA replication factors follows.

REPLICATION FACTORS

Replication Factor A. In 1988, we reported the purification of a novel replication factor from human cells called replication factor A (hRF-A) and demonstrated that it was a single-stranded DNA-binding protein required for initiation of DNA replication. The human protein contains three protein subunits with molecular weights of 70K, 34K, and 11K. In last year's Annual Report, we noted that hRF-A functions during the presynthesis stage of DNA replication to aid in unwinding of the SV40 replication origin; however, it also functions to stimulate the activity of both DNA polymerase α and DNA polymerase δ.

As a first step toward characterizing the function of RF-A in cellular DNA replication, we identified a homolog of hRF-A in the yeast *S. cerevisiae*. This protein was purified using an origin unwinding assay in which the yeast protein, like its human counterpart, would cooperate with SV40 T antigen to promote extensive unwinding of SV40 origin containing plasmid DNAs. The yeast protein (yRF-A), also a single-strand-specific DNA-binding protein, has a subunit structure similar to hRF-A, containing proteins with molecular weights of 69K, 36K, and 13K. In both cases, the largest subunit was responsible for the DNA-binding activity. The yeast protein, however, would not support complete SV40 DNA replication when it was substituted for the human factor in vitro. This was due to a specific interaction between RF-A and DNA polymerase α. We have also studied the potential role of RF-A in regulation of DNA replication (see below).

Replication Factor C. This year, we reported the purification of a new DNA replication factor, called RF-C, that is required for SV40 DNA replication in vitro. RF-C, which is involved in the elongation stage of DNA replication, is a multisubunit factor containing protein subunits with molecular weights of approximately 140K, 41K, and 37K. The large subunit binds to DNA, whereas the 41K subunit binds to ATP. RF-C functions as a DNA polymerase stimulatory factor; it stimulates DNA polymerase α activity by itself but cooperates with proliferating cell nuclear antigen (PCNA) (see below) and RF-A to greatly stimulate DNA polymerase δ. Subsequent biochemical analysis of RF-C revealed two interesting functions: It binds specifically to single-stranded DNAs containing an annealed primer with a free 3′-OH end and it is a DNA-dependent ATPase. Indeed, analogs of ATP that cannot be hydrolyzed by RF-C (e.g., ATPγS) inhibit the RF-C-dependent activity of DNA polymerase δ.

These results suggest that RF-C plays a critical role in the initiation of DNA synthesis by DNA polymerase δ, the enzyme we have implicated as the leading-strand DNA polymerase. This can now be tested directly with purified DNA polymerase δ (see below).

Proliferating Cell Nuclear Antigen. The first replication factor to be identified using the SV40 system was PCNA, a protein we have described in previous reports. PCNA was shown to be required for synthesis of the leading strand at a DNA replication fork during the elongation stage of SV40 DNA replication in vitro and to be a processivity (stimulatory) factor for DNA polymerase δ. Recent studies have demonstrated that PCNA also stimulates the DNA-dependent ATPase activity of RF-C, suggesting that RF-C, PCNA, and DNA polymerase δ form a multiprotein complex at the replication fork.

Human PCNA has been cloned into an *Escherichia coli* expression plasmid, and large amounts of the protein can be easily purified. In collaboration with P. Fisher's laboratory at SUNY, Stony Brook, we have purified PCNA from *Drosophila* and demonstrated that it can substitute for the human protein. This underscores the extraordinary conservation of the DNA replication apparatus. Antibodies to *Drosophila* PCNA immunostain cells in *Dro-*

sophila tissue that are proliferating, but not those that are terminally differentiated. Therefore, these antibodies should be useful for investigating the relationship between cell proliferation and terminal differentiation in lineages of cells throughout development.

DNA Polymerase δ. Continued fractionation of the human cell extract has revealed several new replication activities that are required for SV40 DNA replication in vitro. Apart from the DNA polymerase α/DNA primase complex, which has long been known to be required for SV40 DNA replication, our studies with PCNA suggested that DNA polymerase δ is required for complete DNA replication. Indeed, genetic evidence from other laboratories, using the yeast *S. cerevisiae*, has demonstrated that DNA polymerase δ (DNA polymerase III in *S. cerevisiae*) is essential during the S phase of the cell cycle. We have now purified to homogeneity another replication factor that is a DNA polymerase that can be stimulated by PCNA. We are now in a good position to test our previous model for coordinated synthesis of both strands at a replication fork.

Conserved Functions in DNA Replication. The elucidation of the functions of the human replication factors RF-A, RF-C, and PCNA has enabled us to compare these activities with the functions of replication factors that operate in replication of the bacterial (*E. coli*) chromosome and replication of bacteriophage T4 DNA. Table 1 summarizes a comparison of some of the known replication proteins from human cells, bacteria, and its phage, T4. Although this list is not complete (e.g., DNA helicase activities and origin recognition proteins are not listed), it emphasizes the highly conserved activities that function in the process of DNA replication.

Of particular interest is that the bacteriophage T4 DNA polymerase is structurally related to the human and yeast polymerase α enzymes and to the yeast polymerase δ enzyme (the gene encoding the human polymerase δ has not been cloned), but the *E. coli* polymerase III is not structurally related. We have also noted sequence similarities between the human PCNA and the phage T4 gene 45 protein, but the β subunit of *E. coli* DNA polymerase III is not structurally related. It therefore appears that the functions have been conserved, but *E. coli* probably has diverged further away from the ancestral form. This may suggest that bacteria have a higher capability for evolutionary change than either eukaryotes or the *E. coli* phages.

TABLE 1 Conserved Functions of DNA Replication Proteins

Human	Phage T4	*E. coli*	Functions
RF-A	gene 32	SSB	single-stranded DNA binding; stimulates DNA polymerase; promotes origin unwinding
RF-C	gene 44/62	γδ.δ′τ	DNA-dependent ATPase; primer-template binding; stimulates DNA polymerase
PCNA	gene 45	β (*dnaN*)	stimulates DNA polymerase; stimulates DNA-dependent ATPase
DNA pol α[a] DNA pol δ	gene 43	DNA pol III	DNA polymerase
DNA primase [a]	gene 61	*dnaG*	primase

[a] The human DNA pol α and primase activities function as a multiprotein complex.

Regulation of DNA Replication. The studies described above on DNA replication using the SV40 system have provided valuable insight into the mechanism of DNA replication. Having isolated these human cell proteins, we are now in a strong position to investigate the regulation of DNA replication, either directly in human cells or indirectly by identifying homologs in yeast and using the power of yeast cell cycle genetics. During the past year, this effort has proven to be productive, and we foresee it as a major part of future experimentation.

The first protein we have studied is RF-A, primarily because it is involved in both the initiation and elongation of DNA replication. A panel of monoclonal antibodies directed against the p70 and p34 subunits of hRF-A was obtained, and polyclonal antisera were raised against the subunits of hRF-A and yRF-A. These antibodies were used to determine that the p34 subunit of hRF-A and the p36 subunit of yRF-A are phosphorylated in a cell-cycle-dependent manner. The protein is not phosphorylated in the G_1 phase of the cell cycle, but it is phosphorylated at the G_1 to S phase transition and throughout the S and possibly G_2 phases. Dephosphorylation occurs late in G_2 or during mitosis, thereby resetting the cycle (see Fig. 1). Because the levels of RF-A do not vary during cell cycle progression and the proteins are stable, the phosphorylation of RF-A at the start of S phase raises the possibility that the phosphorylation may be one mechanism to regulate DNA

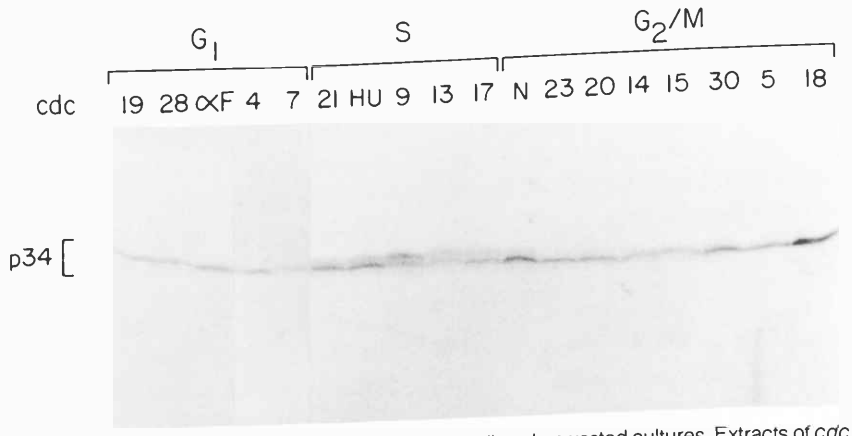

FIGURE 1 Immunoblot analysis of yRF-A p36 from cell-cycle-arrested cultures. Extracts of *cdc* strains arrested at 38 °C for 5 hr or wild-type cells treated with α-factor (αF), hydroxyurea (HU), or nocodazole (N) for 3.5 hr were blotted and probed with affinity-purified anti-p36 antibody. The phosphorylated form of yRF-A p36 is shown as an upper band of the doublet and is present only in the S phase.

replication. Attempts to determine if the phosphorylation of hRF-A may influence its function in the SV40 cell-free replication system have been thwarted by the identification of a potent RF-A kinase activity that is present in the replication reactions. Current efforts are aimed at further characterizing this potentially interesting protein kinase. Finally, a genetic approach to the regulation of RF-A function will be possible with the cloning of the genes encoding the yRFA subunits.

Chromatin Assembly

S. Smith, G. Bauer, B. Stillman

In addition to our studies on DNA replication, we have developed a cell-free system for replication-dependent chromatin assembly and have reported last year the purification of a human cell chromatin assembly factor, CAF-1. The addition of purified CAF-1 to the crude replication extract allows the assembly of the replicating DNA into a chromatin structure containing regularly spaced nucleosomes. Therefore, the mechanism of chromosome assembly in this cell-free system should reflect similar events that occur during replication of cellular chromosomes. To investigate the role of CAF-1 in the replication-dependent chromatin assembly process and to determine if it plays a regulatory role during S phase, we have used the purified protein to raise a panel of monoclonal antibodies that recognize different subunits of the multisubunit CAF-1 complex.

Immunoprecipitation analysis demonstrated that CAF-1 exists as a multiprotein complex in vivo and that the subunits are phosphorylated. Immunostaining of human cells demonstrated that CAF-1 is localized in the nucleus of human cells, but the levels seem to vary from cell to cell. In addition, some antibodies stained a perinuclear structure as well as the nucleus; however, the significance of these observations is not clear and is under investigation. The monoclonal antibodies directed against CAF-1 also immunodeplete the chromatin assembly activity from crude nuclear extracts, confirming that CAF-1 is required for chromosome assembly.

Finally, recent studies have demonstrated that chromosome assembly can be reconstituted in vitro with purified CAF-1, histones, and a partially fractionated replication extract. The purified replication proteins could not support chromatin assembly in the presence of CAF-1 and histones, suggesting the existence of other CAF activities.

Eukaryotic Origins of DNA Replication

J.F.X. Diffley, N. Kessler, Y. Marahrens, B. Stillman

Eukaryotic chromosomal DNA replication initiates from multiple origins during S phase. Little is currently known about either the DNA sequences that function as replication origins or the proteins that interact with these sequences. Seminal experiments by Laskey and co-workers demonstrated that virtu-

ally any DNA tested would replicate in a cell-cycle-dependent fashion when microinjected into *Xenopus* oocytes. This result suggested that specific sequences may not be required for the initiation of DNA replication, at least in early *Xenopus* development when DNA replication proceeds extremely rapidly and presumably from a very large number of origins. Experiments in the yeast *S. cerevisiae*, however, demonstrated an absolute requirement for specific ARSs (autonomously replicating sequences), indicating that DNA replication cannot initiate from any sequence. The number of ARSs in the yeast genome, in fact, is roughly the same as the number of origins of replication, as estimated from fiber autoradiography. Taken with the fact that ARSs have recently been shown directly by two-dimensional gel techniques to function as origins of DNA replication both in plasmids and in the chromosome, this result demonstrates that only very specific sequences can function as origins of DNA replication in yeast.

A resolution of this paradox may come from analysis of ARS sequence requirements and the cellular proteins that bind to these sequences. A number of laboratories including our own have been interested in elucidating the sequences involved in ARS function. Although the picture is not complete, two important generalizations can be made. First, all ARSs contain an 11-bp consensus sequence essential for ARS function. Second, this consensus sequence by itself is insufficient for efficient ARS function. In virtually all cases tested, a flanking sequence is required for ARS function, and we showed several years ago that this sequence is dependent on both its spacing and its orientation relative to the consensus sequence. Although the mechanism by which these auxiliary sequences function is not known and there is little similarity among these sequences, they have been correlated with the presence of degenerate matches (9/11 and 10/11) to the ARS consensus sequence, with nuclear matrix attachment sites, and with structural features such as static DNA bending and ease of unwinding. None of these features, however, correlates completely with flanking sequence function. Thus, all near matches to the ARS consensus sequence can be specifically eliminated from the histone H4 ARS with virtually no effect on ARS function. The C2G1 ARS does not contain bent DNA, and bent DNA can be removed from ARS1 with little effect. Deletions of the H4 ARS exist that are not easily unwound but do function as efficient ARSs; others exist that are efficiently unwound but do not function as ARSs. Flanking sequences at different ARSs may fulfill their replication enhancement functions by different mechanisms, or, alternatively, some unifying mechanism for the function of these sequences may exist.

We continue to characterize two proteins, ABF1 and ABF2, which were identified in our laboratory and interact specifically with the ARS1 sequence in anticipation that such an analysis will lead to an understanding of ARS function. Last year, we described the cloning and sequence analysis of one of these proteins, ABF1. ABF1 binds to a single site within ARS1 and several other ARSs tested. The binding site for ABF1 is important but not essential for the ARS1 flanking sequence function, leading to the proposal that the ARS1 flanking sequence (also known as domain B) can be subdivided into at least two subdomains. In addition to binding to several ARSs, ABF1 is also an important transcription factor in yeast. ABF1-binding sites have been identified as functional elements in controlling the expression of genes encoding ribosomal and mitochondrial proteins, subunits of RNA polymerase C, and glycolytic enzymes. Thus, it is not surprising that the ABF1 gene is essential for viability and suggests that ABF1 may have an important role in coordinating various essential cellular processes—perhaps including DNA replication—during cell growth. We have recently shown that ABF1 is a phosphoprotein and are currently in the process of investigating the possibility that this phosphorylation is regulated during the cell cycle or, perhaps, under different growth conditions.

In addition, we have recently begun characterization of the ABF1 gene product. We showed last year that ABF1 contains an unusually elongated zinc finger DNA-binding motif and that the cysteines in and/or adjacent to this finger are required for sequence-specific DNA binding. Remarkably, mutational analysis has revealed that a second region of the ABF1 protein, located more than 200 amino acids from the zinc finger, is also required for sequence-specific DNA binding and that the spacing between these two domains is not critical for binding. We are currently investigating whether each domain can contact DNA independently with reduced affinity or whether each requires the presence of the other. Furthermore, we are in the process of combining our in-vitro-generated ABF1 mutants with an in vivo approach to map essential domains in the protein.

We have recently succeeded in cloning the gene encoding the second ARS-binding factor, ABF2. ABF2 is a protein with an unusual combination of DNA-binding activities. Most striking is the fact that

ABF2 binds to multiple discrete sites within all ARSs tested and induces a complex series of structural alterations similar to or identical with DNA bends within at least one of these ARSs, ARS1. Although the ABF2 gene is not essential for yeast viability, strains in which the ABF2 gene have been deleted show a marked growth defect that is greatly accentuated at elevated temperatures. The predicted sequence of the ABF2 protein reveals a high level of sequence similarity with the vertebrate high-mobility group proteins HMG1 and HMG2 and the *Schizosaccharomyces pombe* developmental regulator mat1-M_c. The ABF2 sequence is also similar to the human RNA polymerase I transcription factor, UBF (identified in R. Tjian's laboratory). Interestingly, UBF and ABF2 also exhibit great similarities in their mode of DNA binding. Both proteins can bind to many apparently unrelated DNA sequences and induce DNase I hypersensitive sites. The ability of ABF2 to bind to specific sites within the different unrelated flanking sequences of many ARSs may serve to explain how these sequences function and provide some resolution to the paradox concerning the requirement of specific DNA sequences in the initiation of DNA replication. In this regard, we have made a series of linker scan mutations across ARS1 and are in the process of testing these sequences for ABF2 binding and ARS function both on plasmids and in the chromosome using two-dimensional gel technology.

Function of the Adenovirus E1B Oncogene

E. White, R. Cipriani, P. Sabbatini

The adenovirus E1B gene is required for regulation of viral gene expression in productively infected cells and for oncogenic transformation. Last year, we made some interesting discoveries that have greatly enhanced our understanding of E1B protein function. The E1B gene of adenovirus encodes two major tumor antigens, the 19K and 55K proteins. The coding regions for these proteins have been placed under the control of heterologous promoters to ascertain the role of individual E1B proteins in transformation and their primary biological function. These E1B plasmid expression vectors have led to two significant findings. First, both E1B proteins possess transforming activity when cotransfected with a plasmid encoding E1A, but it is the 19K protein that confers anchorage-independent growth. Second, the E1B 19K protein has the unique and unusual ability to associate with and disrupt the organization of two distinct classes of intermediate filaments, cytoplasmic vimentin filaments and nuclear lamin filaments. We propose and are preparing to test that the biological function of the 19K protein in transformation and productive infection is a direct consequence of the perturbation of intermediate filaments.

This year, we have been expanding upon these results in three areas. First, we are preparing to identify those cellular proteins, most likely intermediate filament proteins, that directly interact with the 19K protein. Second, we are expanding our investigation of the role of E1B proteins in transformation and establishing whether or not E1B gene products are capable of cooperating with oncogenes other than E1A. Third, we are performing a mutational analysis of the 19K-coding region to determine whether intermediate filament disruption and the transformation function of the 19K protein are genetically linked and therefore functionally related.

As a first step toward identification of the cellular proteins, which are targets for functional modification by the 19K protein, the 19K protein has been expressed in bacteria and purified. This will provide a source of 19K protein for in vitro binding experiments that will be necessary to demonstrate direct binding of the 19K protein to intermediate filament proteins.

The ability of E1B proteins to cooperate with the products of other viral oncogenes is also well under way. In a collaborative effort with P. Howley at the National Cancer Institute, we have discovered that the E1B 19K protein can enhance the transforming activity of the human papillomavirus type-16 E6 and E7 transforming genes. This is important because it establishes that the transforming activity of the E1B 19K protein is not restricted to cooperation with the adenovirus E1A gene and may represent a more universal function related to the development of human cancer.

Finally, mutational analysis has so far identified an important region of the 19K protein required for both transforming activity and intermediate filament disruption. A single substitution of an acidic glutamic acid residue for the basic lysine at position 44 of the 19K-coding region results in simultaneous loss of transforming activity and the ability to associate with and disrupt intermediate filaments. This is read-

ily apparent in double-label indirect immunofluorescence of transfected cells transiently expressing either the wild-type 19K protein or the mutant protein. Where the wild-type protein localizes to the nuclear envelope and cytoplasm and disrupts intermediate filaments, the mutant protein is defective for nuclear localization and the organization of intermediate filaments is completely unaffected (Fig. 2). Closer inspection of the sequence surrounding this mutation identified a possible homology with nuclear localization signals in polyomavirus large T antigen and a *Drosophila* lamin protein:

Polyomavirus large T antigen	P	K	**K**	A	R	E	D
			:	:	:	:	:
E1B 19K protein	V	C	R	I	**K**	E	D
	:		:	:	:		
Lamin DM_0	V	K	R	L	R	A	V
		:	:		:	:	:
SV40 T antigen	P	K	**K**	K	R	K	V

Those amino acids known to be absolutely required for nuclear localization are indicated in bold type and are usually basic (R or K) amino acids. Whether this sequence of the 19K protein is a bona fide nuclear localization signal is being explored.

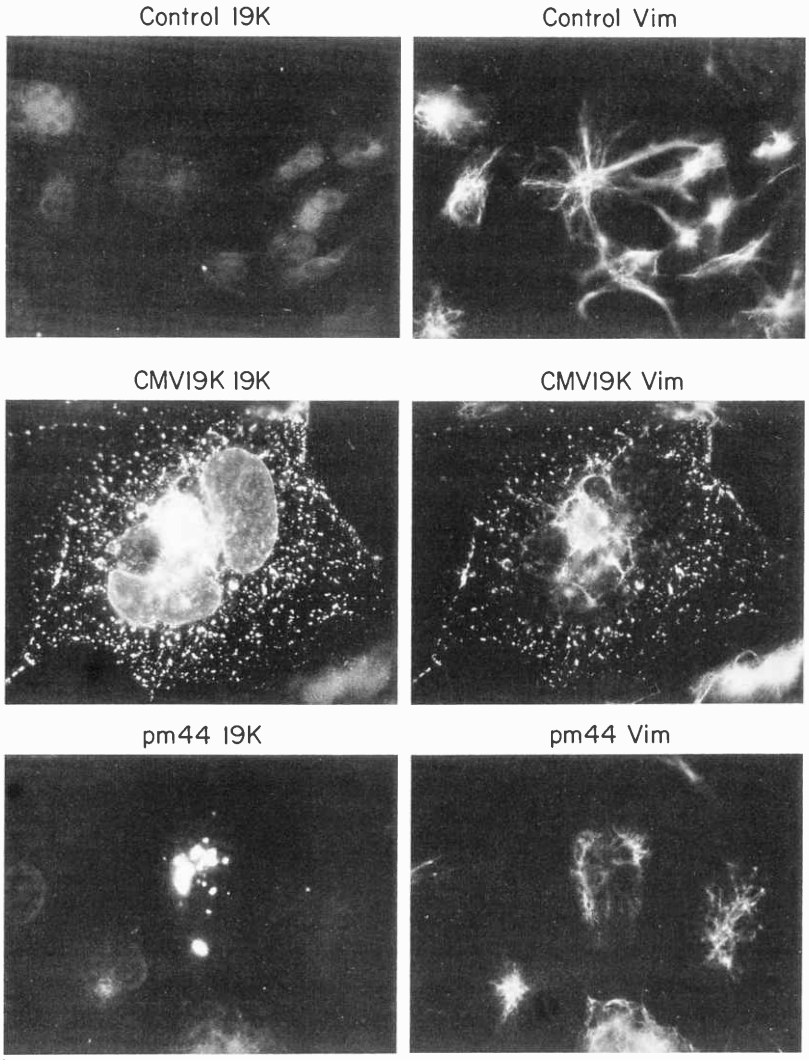

FIGURE 2 Double-label indirect immunofluorescence with anti-vimentin and E1B 19K antisera of HeLa cells transfected with expression vectors producing wild-type 19K protein or the K to E substitution at amino acid 44.

In summary, the E1B 19K protein represents a transforming protein with a novel function that will be useful both for understanding the unique mechanism of oncogenic transformation and for determining the function of those cellular structures with which it interacts, intermediate filaments.

PUBLICATIONS

Brill, S.J. and B. Stillman. 1989. Yeast replication factor-A functions in the unwinding of the SV40 origin of DNA replication. *Nature* **342:** 92–95.

Diffley, J.F.X. and B. Stillman. 1989. Similarity between the transcriptional silencer binding proteins ABF1 and RAP1. *Science* **246:** 1034–1038.

Diffley, J.F.X. and B. Stillman. 1989. Transcriptional silencing and lamins. *Nature* **342:** 24.

Fairman, M.P., G. Prelich, T. Tsurimoto, and B. Stillman. 1989. Replication of SV40 DNA *in vitro* using proteins derived from a human cell extract. *J. Cell Sci.* (suppl.) **12:** 161–169.

Mohr, I.J., M.P. Fairman, B. Stillman, and Y. Gluzman. 1989. Large T antigen mutants define multiple steps in the initiation of simian virus 40 DNA replication. *J. Virol.* **63:** 4181–4188.

Mohr, I.J., Y. Gluzman, M.P. Fairman, M. Strauss, D. McVey, B. Stillman, and R.D. Gerard. 1989. Production of SV40 large T antigen in bacteria: Altered DNA binding specificity and DNA replication activity of underphosphorylated T antigen. *Proc. Natl. Acad. Sci.* **86:** 6479–6483.

Smith, S. and B. Stillman. 1989. Purification and characterization of CAF-1, a human cell factor required for chromatin assembly during DNA replication *in vitro*. *Cell* **58:** 15–25.

Stillman, B. 1989. Initiation of eukaryotic DNA replication *in vitro*. *Annu. Rev. Cell Biol.* **5:** 197–245.

Tsurimoto, T. and B. Stillman. 1989. Multiple replication factors augment DNA synthesis by the two eukaryotic DNA polymerases, α and δ. *EMBO J.* **8:** 3883–3889.

Tsurimoto, T. and B. Stillman. 1989. Purification of a cellular replication factor, RF-C, that is required for coordinated synthesis of leading and lagging strands during SV40 DNA replication *in vitro*. *Mol. Cell. Biol.* **9:** 609–619.

Tsurimoto, T., M.P. Fairman, and B. Stillman. 1989. SV40 DNA replication *in vitro:* Identification of multiple stages of initiation. *Mol. Cell. Biol.* **9:** 3839–3849.

White, E. and R. Cipriani. 1989. Specific disruption of intermediate filaments and the nuclear lamina by the 19-kilodalton product of the adenovirus E1B oncogene. *Proc. Natl. Acad. Sci.* **86:** 9886–9890.

In Press, Submitted, and In Preparation

Din, S., S.J. Brill, M.P. Fairman, and B. Stillman. 1990. Cell cycle regulated phosphorylation of DNA replication factor A from human and yeast cells. *Genes Dev.* (in press).

Ng, L., G. Prelich, C.W. Anderson, B. Stillman, and P.A. Fisher. 1990. *Drosophila* PCNA: Structural and functional homology with its mammalian counterpart. (Submitted.)

Tsurimoto, T. and B. Stillman. 1990. Functions of replication factor C and proliferating cell nuclear antigen: Functional similarities of DNA polymerase accessory proteins from human cells and bacteriophage T4. *Proc. Natl. Acad. Sci.* **87:** 1023–1027.

White, E. and R. Cipriani. 1990. The role of adenovirus E1B proteins in transformation: Altered organization of intermediate filaments in transformed cells that express the 19-kilodalton protein. *Mol. Cell. Biol.* **10:** 120–130.

MOLECULAR BIOLOGY OF SV40

Y. Gluzman D. McVey M. Pizzolato
I. Mohr

The large T antigen encoded by SV40 is a multifunctional protein that possesses numerous posttranslational modifications. Current work is aimed at understanding the role that this protein plays in SV40 DNA replication by evaluating the effects of posttranslational modifications, particularly phosphorylation, on the overall functioning of the protein and by analyzing the domain of the protein involved in the specific recognition of the SV40 origin of replication.

Our previous studies on the origin-specific DNA-binding domain of large T antigen localized the amino- and carboxy-terminal boundaries of this domain between amino acids 132 and 246, respectively. In addition, these studies revealed differences in binding specificity and affinity between proteins isolated from bacteria or mammalian sources. Specifically, we found the following:

1. The amino-terminal 259 amino acids of SV40 T antigen purified from *Escherichia coli* bound inefficiently to the wild-type origin and very ineffi-

ciently to DNA bearing only isolated site I and site II recognition sequences. However, the same protein isolated from HeLa cells bound to the origin much more efficiently, particularly to site II.

2. The amino-terminal 246 amino acids of T antigen contained the highest origin-specific DNA-binding activity of all truncated proteins tested, thus defining the carboxy-terminal boundary of the origin binding domain to between amino acids 246 and 249. The 246-amino-acid protein bound to single-stranded DNA, demonstrating that the single- and double-stranded DNA-binding domains reside in the same or overlapping amino acid sequences. The DNA-binding properties of the 246-amino-acid protein were also affected by the cellular source of the material: T antigen from mammalian sources bound more efficiently to site II than protein isolated from *E. coli.*
3. Analysis of full-size SV40 T antigen isolated from *E. coli* showed that the protein was indistinguishable from its mammalian counterpart in its ability to function as an ATPase and helicase and to bind DNA fragments bearing site I or the wild-type origin. However, it did not bind to site II, an essential *cis*-acting element within the SV40 origin of replication. This observation explains its inability to unwind origin-containing plasmids and to replicate SV40 DNA efficiently in vitro.

Our data, combined with those from other laboratories analyzing T-antigen phosphorylation and its biochemical effects, led us to speculate that phosphorylation of Thr-124 is a critical step in the activation of SV40 T antigen to a replication-competent state. To prove this point, it was necessary to purify a kinase that would phosphorylate Thr-124. The properties of the 259-amino-acid T antigen from *E. coli* presented us with a simple screen for such a kinase. It was anticipated that phosphorylation of Thr-124 would dramatically stimulate binding to the SV40 origin, particularly to site II. Initial experiments employing casein-kinase II and cAMP-dependent kinase, provided by Dr. Marshak (Protein Chemistry, this section), demonstrated that both of these kinases could phosphorylate the 259-amino-acid T antigen. However, the binding activity of the phosphorylated protein was not stimulated, but instead appeared to be inhibited. In contrast, treatment of the 259-amino-acid T antigen with *cdc2* kinase, provided by Drs. D. Beach and L. Brizuela (Genetics Section), resulted in an efficiently phosphorylated protein with a substantially increased ability to bind to site II. Amino acid analysis of this material revealed phosphothreonine as the sole phosphoamino acid present, and direct amino acid sequencing of the phosphorylated protein located the phosphothreonine in a peptide comprising amino acids 112–131. To prove that phosphorylation was occurring on Thr-124, we changed residue 124 to alanine by site-directed mutagenesis. The resulting Ala-124–259 T antigen was not phosphorylated by *cdc2* kinase, and the binding properties of Ala-124–259 T antigen were not affected by exposure to *cdc2* kinase.

Treatment of full-size SV40 T antigen purified from *E. coli* with *cdc2* kinase produced a protein that bound to site II actively and initiated SV40 DNA replication in vivo very efficiently. These data prove that phosphorylation of Thr-124 is a critical step in activation of SV40 T antigen into a competent component of the DNA replication machinery and implicates a *cdc2* kinase as a potential modulator of this activity.

Current studies pursue two directions. First, in collaboration with Dr. Beach, we are trying to prove directly the involvement of *cdc2* kinase in phosphorylation of Thr-124 in vivo by expressing the truncated or wild-type T antigen in *Schizosaccharomyces pombe* (*cdc2* mutant or wild-type parental strain). Second, using a refined DNA footprint analysis, we are exploring the nature of the interactions between the carboxyl terminus of T antigen and its DNA-binding domain and the effect of phosphorylation on this interaction.

PUBLICATIONS

McVey, D., L. Brizuela, I. Mohr, D.R. Marshak, Y. Gluzman, and D. Beach. 1989. Phosphorylation of large tumor antigen by *cdc2* stimulates SV40 DNA replication. *Nature* **341:** 503–507.

Mohr, I.J., M.P. Fairman, B. Stillman, and Y. Gluzman. 1989. Large T-antigen mutants define multiple steps in the initiation of simian virus 40 DNA replication. *J. Virol.* **63:** 4181–4188.

Mohr, I.J., Y. Gluzman, M.P. Fairman, M. Strauss, D. McVey, B. Stillman, and R.D. Gerard. 1989. Production of simian virus 40 large tumor antigen in bacteria: Altered DNA-binding specificity and DNA-replication activity of underphosphorylated large tumor antigen. *Proc. Natl. Acad. Sci.* **86:** 6479–6483.

In Press, Submitted, and In Preparation

McVey, D., M. Strauss, and Y. Gluzman. 1990. Properties of the DNA-binding domain of the simian virus 40 large T antigen. *Mol. Cell Biol.* (in press).

MOLECULAR BIOLOGY OF BOVINE PAPILLOMAVIRUS

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P. Szymanski

The papillomaviruses are a group of small DNA viruses that are characterized by their ability to cause benign tumors (warts) in a variety of animal hosts. In humans, certain types have proven to be clinically important because they have been implicated as a potential causative agent of cervical carcinomas. Over the years, bovine papillomavirus type 1 (BPV-1) has emerged as the prototype for this virus group by virtue of its ability to transform and replicate the viral genome in certain rodent cells in culture. These properties have made it possible to study at least some aspects of the viral life cycle in a tissue-culture system. From the molecular biologist's point of view, two properties of this virus are of particular interest. First, it has recently been demonstrated that the viral genome carries coding information for a group of site-specific DNA-binding proteins, which appear to be key regulators of viral gene expression. One of these is encoded in its entirety from the E2 open reading frame (ORF) and is an activator of viral gene expression that functionally resembles a mammalian transcription factor. This resemblance extends also to the structural level; the carboxy-terminal part of the protein contains a domain that is responsible for dimerization and DNA binding, whereas the amino-terminal part of the protein contains an acidic activation domain. The other members of the E2 family are two polypeptides that share the DNA-binding domain with the activator and therefore have the same DNA-binding specificity. These polypeptides lack the domain responsible for activation and have been described as repressors of transcription. Both the activator and repressor forms exert their various functions through specific binding sites dispersed throughout the viral genome (see Fig. 1). The binding sites that are not clustered in the E2 responsive enhancer are exclusively located close to known promoters, where the specific effect of binding of an E2 molecule appears to be dependent partly on the position of the site.

The second interesting feature of the virus life cycle is the replication properties of the viral genome. It appears that the virus is capable of at least two different modes of DNA replication. In a stably transformed cell, which is the best-studied stage in terms of both DNA replication and gene expression,

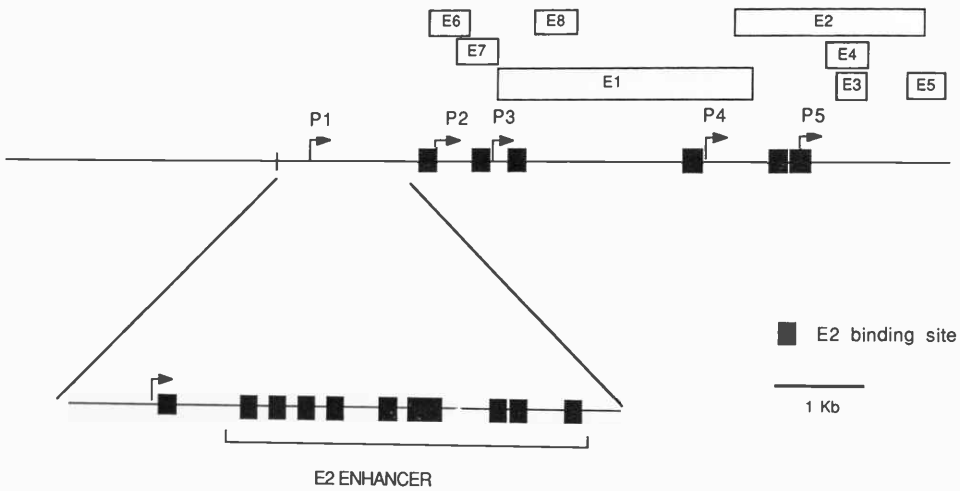

FIGURE 1 Schematic figure showing the organization of the early region of the BPV genome. Open bars represent the early open reading frames E1–E8. Arrows labeled P1–P5 represent the five known promoters that transcribe the early region. Filled boxes show the location of mapped binding sites for the gene products from the E2 open reading frame.

the viral genome replicates as an episome that is stably maintained at an approximate copy number of 100 molecules per cell. Density-labeling experiments have shown that viral DNA replication is regulated at the level of copy number control and that, in a manner reminiscent of cellular DNA replication, each molecule is replicated only once per cell cycle. In the earlier stages (about which little is known), before the viral DNA becomes established as a stably replicating episome, viral DNA replication takes place through a different pathway, which involves amplification to the 100 copies per cell that are then maintained.

We are interested in studying the transition between the two different types of replication in terms of both the direct changes in the mode of replication and the differences in viral gene expression that presumably accompany and cause these changes. Due to the multistep nature of the establishment of stable replication, a genetic analysis is complicated by the fact that mutations that affect an early step in establishment cannot be assayed for their effect on later stages in the absence of conditional mutants. To circumvent this problem, we are trying to dissect the viral genome and its replication machinery into its components for subsequent reconstitution of simplified systems that can execute specific parts of the viral replication cycle in vivo. An important part of this approach has been to develop systems for study of early gene expression and replication, which is something we accomplished this first year. This was possible mainly through development of a highly efficient electroporation procedure that makes it possible to deliver the viral genome at high frequency into the host cell, which is difficult to transfect using conventional methods.

Analysis of Immediate-Early Gene Expression in BPV-1

P. Szymanski, A. Stenlund

For mostly technical reasons, analysis of gene expression and viral gene regulation has so far been performed with subgenomic fragments of viral DNA almost exclusively either in BPV-transformed cells or, for transient expression assays, in a variety of cell types where BPV does not replicate. This means that even though viral gene expression in transformed cells has been extensively studied, virtually nothing is known about immediate-early gene expression or about regulation of viral genes in the context of the whole viral genome. We have begun to study early viral gene expression under replication conditions as well as the response of viral transcription units to the viral *trans*-activator E2. In these experiments, the viral genome has been introduced into recipient mouse C127 cells through electroporation under conditions where replication takes place, followed by RNA extraction at various later timepoints. The assay for expression that we have used is an RNase protection assay with specific probes for the five different promoters in the early region of BPV. So far, we have not been able to detect any activity using replication conditions, which indicates either that the levels of expression are very low (less than 1 molecule of RNA per transfected cell) or that transcription is initiated from start sites other than the five promoters that have been mapped in BPV-transformed cells. In the presence of the cotransfected E2 *trans*-activator, at least two of the five promoters, P2 and P4, are substantially activated in a manner that is dependent on the presence of the E2-inducible enhancer located immediately upstream of P2. It is interesting to note that the level of activation is very similar for the two promoters, even though in the case of P2, the promoter is immediately adjacent to the E2 enhancer, and in the case of P4, a distance of approximately 2.5 kb separates the enhancer and the promoter.

Analysis of Viral Functions Involved in Transient Amplification and Stable Maintenance of BPV Episomes

M. Ustav, A. Stenlund

Replication of the BPV genome in permissive rodent cells appears to take place in two different stages. Initially, amplification occurs where the viral DNA replicates faster than the cellular DNA, resulting in an increase in copy number of the viral DNA on a per cell basis. After this initial stage, replication of the viral DNA proceeds at a constant rate, resulting in maintenance of a constant copy number that is stable over many cell generations. The requirement for viral genes for stable replication has been studied through conventional transfection assays; however, the amplification stage is less well characterized. We have therefore developed a transient replication assay that relies on a highly efficient electroporation procedure to deliver the DNA to a suf-

ficiently large fraction of the cells to allow detection of DNA replication at early times after infection. Low-molecular-weight DNA is then isolated at various times after transfection. The samples are digested with the restriction endonuclease *DpnI*, which cuts only methylated (unreplicated) DNA. The products are then analyzed by gel electrophoresis and Southern transfer. Using this procedure, we have established a tentative time course for early replication, where newly synthesized DNA is first detected between 24 and 48 hours after transfection, after which time, the amount of replicated DNA increases exponentially. Initial results from this type of experiment indicate that genes like E5, which are required for stable replication, are dispensable for this type of transient replication. This is consistent with the idea that the requirements are different for the different modes of replication. On the other hand, functions like the *trans*-activator E2, which has been shown to be required for stable replication, are also absolutely required for transient replication; this shows that some viral functions may be required throughout the viral life cycle. We are now in the process of analyzing a battery of mutants for the involvement of various viral gene products in the early events of replication.

PUBLICATIONS

Choe, J., P. Vaillancourt, A. Stenlund, and M. Botchan. 1989. Bovine papillomavirus type 1 encodes two forms of a transcriptional repressor: Structural and functional analysis of new viral cDNAs. *J. Virol.* **63:** 1743–1755.

Li, R., J. Knight, G. Bream, A. Stenlund, and M. Botchan. 1989. Specific recognition nucleotides and their DNA context determine the affinity of E2 protein for 17 binding sites in the BPV-1 genome. *Genes Dev.* **3:** 510–526.

In Press, Submitted, and In Preparation

Stenlund, A. and M. Botchan. 1990. The E2 *trans*-activator can act as a repressor by interfering with a cellular transcription factor. *Genes Dev.* **4:** 123–136.

ADENOVIRUS GENETICS

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For the past several years of this project, we have been engaged in a genetic analysis of E1A- transforming functions. Genetic analysis has revealed a close biological relationship between the adenoviruses and other classes of DNA tumor viruses, specifically, the papovaviruses, including SV40, and the human papillomaviruses. Each of these viruses contains a transforming gene that encodes a protein containing a highly conserved stretch of about 20-amino-acid residues, designated conserved domain 2 in the E1A products, which is involved in binding the product of the cellular retinoblastoma (RB) tumor suppressor gene (see also Protein Immunochemistry Section). Mutations in these highly conserved regions, with concomitant loss of RB-binding activity, severely impair the transforming activity of the affected genes. The basic structure of the E1A proteins and their relationship with SV40 T antigen are described in Figure 1.

Although domain 2 of E1A is required for E1A-transforming functions, it is not sufficient for these functions. A second region, amino-terminally distal to domain 2, is likewise absolutely required for E1A-transforming function. With the loss of either of these regions, the E1A products lose the ability to induce cell proliferation, establish an extended growth potential in primary cells, or cooperate with a second oncogene such as an activated *ras* gene to induce full morphological transformation. During the past year, we have made a close study of the amino-terminal transforming region. We have approached this (1) by analyzing the biological consequences of loss of E1A amino-terminal functions and (2) by continuing to compare the transforming sequences of adenovirus-2 (Ad2) E1A and SV40 T antigen.

BIOLOGICAL ACTIVITIES ASSOCIATED WITH THE E1A AMINO-TERMINAL FUNCTION

Several lines of evidence indicate that the amino-terminal transforming function is completely independent of domain-2 function and RB-binding activity. Loss of domain 2 does not result in loss of

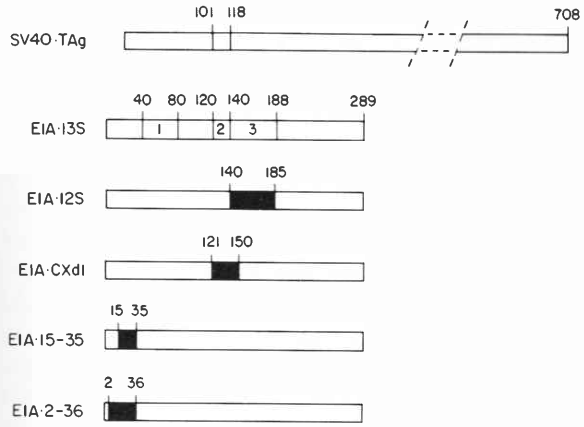

FIGURE 1 E1A and T-antigen protein structure. Several different splice products are made from the E1A gene. The major early products are the 12S and 13S mRNAs. The 12S and 13S protein products are identical except for the presence of an internal region of 46-amino-acid residues unique to the 13S product. The E1A proteins are composed of alternating regions of relatively high or low amino acid sequence identity. There are three highly conserved regions, two of which are common to the 12S and 13S products, and the third comprises the 13S-unique region. The 13S-unique region plays an important role in *trans*-activation of other early virus genes, but E1A-transforming functions do not depend on the 13S-unique *trans*-activation function. The amino-terminal 140 residues of common sequence, containing the two common conserved domains, are sufficient to induce DNA synthesis and rapid proliferation in BRK cells. The numbers above the bars indicate the endpoints of various E1A deletion mutants. The blackened areas represent the regions deleted. All deletions retain the original frame of translation. A representation of the T-antigen product is shown at the top of the figure, with the domain-2 homologous region aligned with E1A domain 2.

all E1A-mediated cell-cycle-regulating activity. Mutants completely deleted for domain 2, and without detectable ability to bind the RB product, retain substantial ability to induce DNA synthesis in quiescent primary baby rat kidney (BRK) cells. An amino-terminal deletion peptide is able to cooperate in *trans* with a domain-2 deletion peptide in a *ras* cotransformation assay, indicating that loss of amino-terminal function can occur without tertiary disruption of domain-2 structure or function. This evidence implies that each biologically active region is functioning autonomously during transformation. If so, then conserved domain 2 and the amino-terminal region may mediate distinctly different biological activities required in transformation.

It is reasonable to assume that the E1A products mediate their cell-growth-regulating effects by directly or indirectly influencing the expression of cellular growth-related genes. Yet, the well-characterized E1A gene *trans*-activation function is unique to the 13S E1A product and is not required for cell-growth-regulating activity. An independent, and negative, gene-regulating function intrinsic to the E1A products has been identified, however, that is more closely linked with E1A-transforming activity, in that it too is independent of the 13S unique region. This is the ability of the E1A products to repress the effects of enhancer-stimulated transcription.

To investigate whether the amino-terminal transforming region of the E1A products mediates biological events distinct from those associated with conserved domain 2, we analyzed amino-terminal mutants for their ability to transform primary BRK cells, induce DNA synthesis, repress insulin-enhancer-stimulated transcription, and bind to E1A-associated cellular proteins. The endpoints of the deletions are indicated in Figure 1.

The mutant plasmids were first assayed for biological activity in a *ras* cotransformation assay. Deletions near the extreme amino terminus, extending from residue 2 to 36, or 15 to 35, showed no activity at all, although they encode stable proteins. Both of these mutants remove only sequences outside of conserved domain 1, whereas a deletion extending from residue 51 to residue 116, described last year, retains substantial transforming activity, suggesting that the strictly essential upstream sequences lie toward the extreme amino terminus of the E1A products, rather than across the whole region of conserved domain 1. To exclude the possibility that loss of function in the amino-terminal mutants is merely a consequence of tertiary effects resulting in impairment of domain-2 function, we determined the activity of the mutant constructs in a *trans*-cooperation assay. The E1A.2-36 and E1A.15-35 mutants can cooperate efficiently with the domain-2 deletion mutant, E1A.CX*dl*, increasing the likelihood that these deletions are affecting a specific active site, rather than broadly disrupting required tertiary structure throughout the E1A protein products.

E1A proteins have the ability to bind a number of cellular proteins, including the p105-RB product and an unidentified 300-kD product. The E1A.2-36 and E1A.15-35 deletions bind the RB gene product efficiently but do not coprecipitate the 300-kD product detectably. These results confirm that binding RB is insufficient for E1A transformation activity. E1A.2-36 (and E1A.15-35) deletion products bind RB both physically and functionally, as determined by their ability to complement a domain-2 deletion mutant; however, these mutants have no transforming activity by themselves. These results support the hy-

pothesis that E1A-mediated transformation is a consequence of the activity of two independently functioning regions within the protein: the amino-terminal region and conserved domain 2. To address this possibility more directly, we compared mutants in these regions for their ability to induce DNA synthesis and repress insulin-enhancer-stimulated transcriptional activity.

We assayed the ability of E1A.2-36 to induce DNA synthesis and virus replication in BRK cells, and we compared its activity with that of two domain-2 mutants, E1A.928 and E1A.CX*dl*. The 928 mutation is a point mutant in residue 124 that affects only domain-2 activity. The CX*dl* mutant (residues 121–150) deletes all of domain 2 and part of domain 3. The transformation defect in the 928 point mutant is as severe as that of the complete deletion mutant. We previously showed that these domain-2 mutants retain an appreciable ability to induce DNA synthesis in BRK cells despite their severe transforming defect. The levels of [^{3}H]thymidine incorporated into viral DNA during infection with Ad5*dl*309 (an E1A.WT virus), E1A.928, and the amino-terminal mutant, E1A.2-36 were compared. The results (Fig. 2A) indicate that the 2-36 deletion significantly impairs the ability of the E1A products to induce DNA replication. Replication of viral DNA in infected quiescent cells requires two E1A functions: the ability to induce cellular DNA synthesis and the ability to *trans*-activate other viral proteins. The latter activity is a function of domain 3. To demonstrate that the impairment in the E1A.2-36 deletion is actually a defect in the ability to induce cellular DNA synthesis, we coinfected E1A.2-36 with a domain-3 defective mutant, E1A.hr3. E1A.hr3 fails to *trans*-activate viral products and therefore fails to replicate viral DNA (Fig. 2B), but it retains the ability to induce cellular DNA synthesis sufficient to support rapid and extensive proliferation of BRK cells. Coinfection of the E1A.2-36 and E1A.hr3 viruses results in near wild-type levels of viral DNA synthesis (Fig. 2B). If the induction of cellular DNA synthesis is indeed independent of both domain-2 and domain-3 activities, we would also expect that E1A.2-36 could be complemented for virus replication by the E1A.CX*dl* mutant that inactivates both domains 2 and 3. Such complementation does indeed occur (Fig. 2B), indicating that induction of DNA synthesis correlates closely with the amino-terminal transforming function and not with the domain-2 transforming function.

Both the 13S and 12S E1A products repress insu-

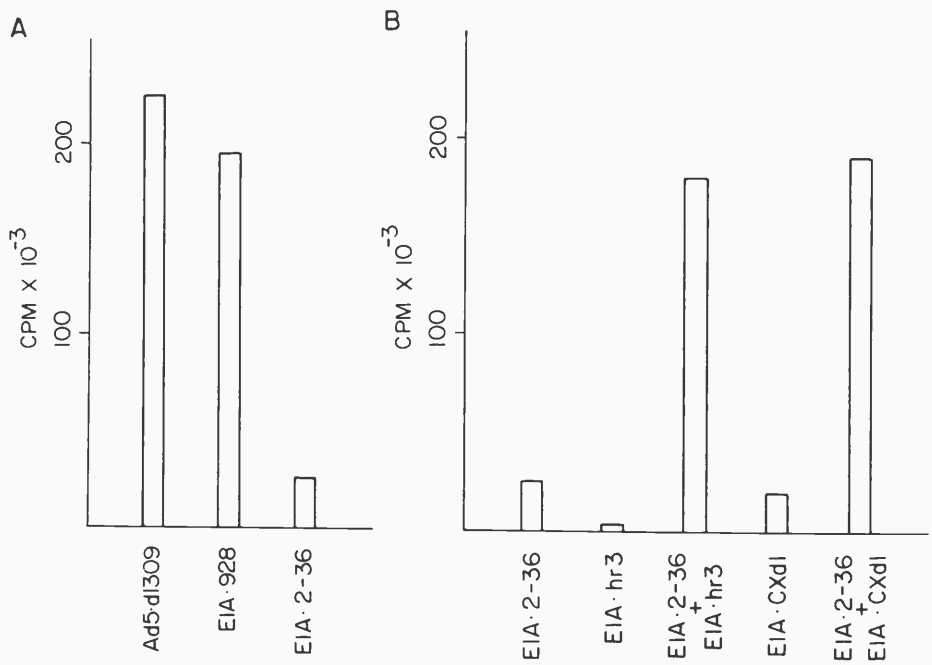

FIGURE 2 Analysis of virus DNA production in infected quiescent cells. Primary BRK cells were infected in the presence of [^{3}H]thymidine at a multiplicity of 10 pfu/cell. Coinfection experiments were done at a total multiplicity of 10, i.e., 5 pfu/cell of each virus. Viral DNA was extracted and counted at 30 hr postinfection.

lin enhancer-stimulated transcription in pancreatic β cells. To identify more precisely the regions in E1A that are required for repression, we cotransfected the deletion mutants into an insulin-producing β cell line, along with p-700 CAT, a plasmid that contains the coding region of the bacterial chloramphenicol acetyltransferase (CAT) gene linked to the rat insulin II gene enhancer promoter. These experiments were done in collaboration with R. Stein at Vanderbilt University. The 2-36 and 15-35 mutants were greatly impaired in their ability to repress −700 CAT expression, whereas the domain-2 deletion mutant, E1A.121-150 (CX*dl*), repressed CAT enzyme levels as efficiently as wild type. These results suggest that enhancer repression, like the ability to induce DNA synthesis, correlates closely with the amino-terminal transforming function and not with the domain-2 transforming function. Enhancer repression and induction of DNA synthesis therefore correlate closely with 300-kD binding and not with Rb binding.

There appears to be a striking corrrelation between binding of the 300-kD product and the specific biological activities of E1A that mediate induction of DNA synthesis and the repression of enhancer-stimulated transcription, both of which are essentially independent of domain-2 function and RB binding. These results are exciting because they reveal possible new steps in the mechanism of cell growth control and point to the 300-kD product and the repression function as playing major roles in this process. Characterization of the 300-kD product and identification of cellular targets of E1A-mediated repression will be a major focus of our work in the upcoming years.

RELATIONSHIPS BETWEEN TRANSFORMING SEQUENCES OF AD2 E1A AND SV40 T ANTIGEN

By last year, we had shown that conserved domain 2 of E1A represents a structural and genetic unit common to the transforming proteins of both adenovirus and SV40. During the past year, we attempted to determine whether SV40 T antigen encodes a function analogous to that encoded in the amino-terminal E1A sequences. We now know more precisely which sequences in the amino terminus are required for E1A-transforming function, but we still cannot recognize any sequences in T antigen that show a close relationship to the required E1A amino-terminal sequences. Nevertheless, given the degree of independence between the two transforming functions in the E1A products, a T-antigen functional homolog need not necessarily be a structural homolog. We have obtained preliminary results this year indicating that T antigen does, indeed, carry a transforming function able to substitute for the amino-terminal function in the E1A products.

We first determined that most sequences in T antigen downstream from the domain-2 homologous region (residues 101–118 in T antigen) and the T-antigen nuclear localization sequence (residues 127–133) are not required for cooperation with *ras* in the cotransformation assay in primary BRK cells. A construct expressing only residues 1–148 is competent for transforming function in this assay. These results suggested that if a function analogous to that encoded in the E1A amino-terminal sequences also exists in T antigen, it will lie within the first 148 residues, probably upstream of residue 100. The amino-terminal and domain-2 E1A functions can complement each other in *trans*. To learn whether sequences in T antigen outside the domain-2 homologous region can substitute for the E1A amino terminus in the *trans*-cooperation assay, we made a T-antigen construct lacking all of conserved domain 2 and tested its transforming function. This construct is negative in the *ras* cooperation assay but can cooperate in *trans* with an E1A construct lacking amino-terminal sequences. We have isolated cell lines stably transformed as a result of cotransfection with *ras*, the E1A deletion peptide, and the T-antigen deletion peptide and have shown that they express the expected mutant products. Although these results are preliminary, and the level of complementing activity is very low, they support the hypothesis that T antigen carries a function analogous to the E1A amino-terminal function. We are currently characterizing a series of chimeric proteins that replace amino-terminal E1A amino acid sequences with sequences from T antigen in order to identify the T-antigen sequences responsible for the complementing activity. Our goals now are to determine the precise required sequences of the active sites and to distinguish whether the active sites encode the same specific biochemical function (perhaps involved with 300-kD binding) or different specific functions that ultimately have a similar effect on cell growth.

PUBLICATIONS

Draetta, G., L. Brizuela, B. Moran, and D. Beach. 1989. Regulation of the vertebrate cell cycle by the *cdc2* protein kinase. *Cold Spring Harbor Symp. Quant. Biol.* **56:** 195–201.

Sturm, R.A. and P. Yaciuk. 1989. DNA cleavage by restriction en-

donuclease *Pfl*M1 is inhibited in recognition sites modified by *dcm* methylation. *Nucleic Acids Res.* **17:** 3615.

In Press, Submitted, and In Preparation

Pietenpol, J.A., R.W. Stein, E. Moran, P. Yaciuk, R. Schlegel, R.M. Lyons, M.R. Pittelkow, K. Munger, P.M. Howley, and H.L. Moses. 1990. TGFβ1 inhibition of c-*myc* transcription in keratinocytes is blocked by DNA tumor virus transforming proteins with intact binding domains for the retinoblastoma gene product. *Cell* (in press).

Stein, R.W., M. Corrigan, P. Yaciuk, J. Whelan, and E. Moran. 1990. Analysis of E1A-mediated growth regulation functions: Binding of the 300 kDa E1A-associated cellular product correlates with E1A enhancer repression function and DNA synthesis inducing activity. (Submitted.)

PROTEIN IMMUNOCHEMISTRY

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The Protein Immunochemistry Group is composed of two sections. The Monoclonal Antibody Facility provides a central site for the production of monoclonal antibodies for Cold Spring Harbor Laboratory scientists. This facility is managed by Carmelita Bautista and is staffed by Margaret Falkowski and Susan Allan. During 1989, this facility produced a total of 873 hybridoma cell lines for the CSHL staff.

The other section of the Protein Immunochemistry Group is a research unit that has been studying the molecular mechanisms used by the adenovirus E1A proteins to transform cells. These studies have concentrated on the physical interactions between the E1A proteins and various cellular polypeptides. There are at least ten cellular proteins that are specifically associated with E1A in virus-transformed or infected cells. These proteins were originally known by their relative molecular weights of 300K, 130K, 107K, 105K, 90K, 80K, 60K, 50K, 40K, and 28K. Recently, several of these proteins have been identified. Probably the most startling was the finding that the 105K protein was the product of the retinoblastoma (RB) tumor suppressor gene. This protein, now known as pRB, is thought to act as a negative regulator of cell proliferation. This interaction, its potential significance, and the other protein/protein complexes are discussed in detail below.

Retinoblastoma Protein and p107 Are Targeted by the Early Proteins of Several Small DNA Tumor Viruses

N. Dyson, K. Buchkovich-Sass, E. Harlow [in collaboration with K. Münger, B. Werness, P. Howley, National Cancer Institute, Bethesda, Maryland; and P. Whyte, Fred Hutchinson Cancer Research Center, Seattle, Washington]

The E1A proteins of adenovirus 5 (Ad5), the E7 proteins of human papillomavirus 16, and the large T antigens of SV40 have several features in common. All are multifunctional proteins that are synthesized by small DNA tumor viruses early in viral infection. All three proteins are believed to play an important role in the ability of these viruses to drive infected cells through the cell cycle to S phase, where efficient replication of viral DNA occurs. A feature of these viral proteins, which is probably closely related to this mitogenic activity, is that each will immortalize primary rodent cells in culture and either transform cells directly (SV40 large T antigen) or cooperate with other oncogenes to produce fully transformed cells (E1A or E7).

Analysis of recombinant adenoviruses containing mutated E1A genes has previously shown that the ability of E1A to associate with three cellular pro-

teins of 105K, 107K, and 300K correlates with the ability of E1A to function as an oncogene in a co-transformation activity (Whyte et al., *Cell 56:* 67 [1989]). Recently, the 105K protein, now known as pRB, has been identified as the product of the RB susceptibility gene (Whyte et al., *Nature 334:* 124 [1988]). Loss of the RB gene has been associated with the generation of tumors of the developing retina as well as in other tissues. Similar to E1A, the SV40 large T antigen has also been shown to associate with pRB, and genetic analysis shows that mutations of large T antigen, which disrupt binding to pRB, inactivate transformation by the viral protein (DeCaprio et al., *Cell 54:* 263 [1988]). It is hypothesized that these viral proteins bind to and inactivate pRB in order to overcome a negative control of cell proliferation, thus mimicking the loss of the RB gene seen in several types of tumor cells.

Two regions of E1A have been shown to be important for binding to pRB, and amino acid sequences homologous to these regions are found in the large T antigens of SV40 and many other polyoma-type viruses. In collaboration with R. Bernards and S. Friend (Massachusetts General Hospital), L. Gooding (Emery University), J. Hassel (McMaster University), E. Major (National Institutes of Health), J. Pipas (University of Pittsburgh), and T. VanDyke (University of Pittsburgh), we have used a coimmunoprecipitation assay to show that the large T antigens of commonly used isolates of mouse, monkey, baboon, or human polyoma-type viruses can all bind to mouse or human pRB. This conservation of function between viral proteins suggests that binding to pRB is an important feature in early viral infection and that pRB binding may be a common component in transformation by these viral oncoproteins.

Although adenoviruses and polyoma-type viruses can cause tumors in rodents, neither class of virus is frequently associated with human cancer. In collaboration with Peter Howley's laboratory at the National Cancer Institute, we have used in vitro assays to demonstrate that the E7 proteins of several types of human papillomaviruses (HPVs) also bind to pRB. HPVs are associated with and are believed to be causative agents for a wide variety of anogenital lesions. More than 60 types of HPVs have been identified, but only certain types are commonly found associated with anogenital lesions. In addition, lesions with certain HPV types (such as HPV-16 and HPV-18) are associated with a high risk of progression to malignancy, whereas other HPVs are almost entirely found in benign lesions (e.g., HPV-6b and HPV-11). E7 proteins from HPV types 6b, 11, 16, and 18 were all shown to be able to bind to pRB in vitro, although the E7 proteins of HPV types 6b and 11 formed complexes with lower affinities. The levels and control of synthesis of the E7 proteins are different with the respective HPV types, and the biological significance of the differences in E7-pRB affinities is unclear. However, these results implicate pRB binding as a potential step in HPV-associated carcinogenesis.

Where known, mutations in Ad5 E1A, HPV-16 E7, or SV40 large T antigen that prevent association with pRB also abolish transformation activity, suggesting a role for pRB binding in the transformation process. The interpretation of the analysis of E1A mutants, however, is complicated by the observation that the regions involved in binding to pRB are also essential for the association of E1A with other cellular proteins. Recently, we have shown that the E1A-associated cellular p107 protein also forms stable protein/protein complexes with the large T antigens of SV40 and JC virus in virus-transformed human cells. Since p107 and pRB share overlapping binding sites on both E1A and SV40 large T antigen, the relative contributions of binding to each protein for transformation have been difficult to assess. However, since binding to both pRB and p107 is conserved by these unrelated viruses, it seems likely that both are cellular targets in a common mechanism for transformation by the viral proteins.

Synthetic Peptides That Bind to the Retinoblastoma Protein

N. Dyson, C. McCall, L. Duffy, E. Harlow

We have used synthetic peptides containing E1A amino acids 37–54 (from conserved region 1) and 115–132 (from conserved region 2) to show that sequences in both of these regions of E1A make physical contact with the RB protein. Peptides from each region alone are able to block the interaction between E1A and pRB, but most effective blocking is seen with peptides containing sequences from both regions. Similarly, when region-1 or region-2 peptides are coupled to Sepharose beads, they are able to form stable complexes with RB polypeptides. As in the blocking experiments, peptides containing

both region-1 and region-2 sequences provide the highest affinity interaction. In cross-blocking experiments, region-1 peptides are unable to block the interaction between region-2 peptide beads and pRB, and the converse is also true, suggesting that the two regions of E1A make distinct and independent contacts with pRB.

Peptides containing regions 1 and 2 were used to raise monoclonal antibodies to the pRB-binding sites on E1A. Approximately 60 hybridomas were produced from two fusions that recognize E1A in immunoprecipitations. Regardless of whether they recognize epitopes in region 1 or region 2, these antibodies have a common characteristic in that they fail to recognize E1A when it is associated with p130, p107, or pRB, and they do not coprecipitate these cellular proteins. Preliminary data suggest that these antibodies inhibit E1A/pRB association in vitro. Several of these antibodies also directly recognize cellular proteins that may be candidates for cellular pRB-binding proteins.

Five of the monoclonal antibodies that were raised against E1A cross-react with the HPV-16 E7 protein. These antibodies all recognize epitopes in region 1 of E1A, confirming that the homologous sequences in E7 contain similar structures. Currently, we are using similar methods to determine whether these regions of E7 also make direct contact with pRB.

Regions of the Retinoblastoma Protein Needed for Binding to Adenovirus E1A or SV40 Large T Antigen Are Common Sites for Mutations

Q. Hu, N. Dyson, E. Harlow

The protein product of the RB gene is thought to function in a pathway that restricts cell proliferation. As described above, the transforming proteins from three different classes of DNA tumor viruses have been shown to form complexes with the pRB. Genetic studies suggest that with all three classes of viruses, these interactions with the RB protein are important steps in transformation. To understand the function of the pRB/viral oncoprotein complexes better, we have mapped the regions of pRB that are necessary for these associations. Mutations were introduced into the pRB-coding region using a polymerase chain reaction strategy. Mutant RB polypeptides were expressed in vitro by translation in rabbit reticulocyte lysates programmed with cRNA prepared from in vitro transcription systems. The resultant RB polypeptides were then mixed with either adenovirus E1A or SV40 large T antigen. If complexes formed, they were detected following immunoprecipitation with monoclonal antibodies specific for the viral proteins.

Two noncontiguous regions of RB were found to be essential for complex formation with adenovirus E1A or SV40 large T antigen. These two regions are located between amino acids 393–572 and 646–772. Interestingly, these binding sites on RB overlap with the positions of naturally occurring, inactivating mutations of the RB gene. These results strongly suggest that these viral oncoproteins are targeting a protein domain that plays an important role in the normal function of the RB protein.

To study further this domain of pRB, we have raised monoclonal antibodies to the E1A/T-binding regions of pRB. A panel of monoclonal antibodies has been isolated that will immunoprecipitate native pRB from mammalian cells. Several of these antibodies recognize subsets of pRB that can be distinguished by their relative levels of phosphorylation. These antibodies are currently being tested to see if they block the interaction of pRB with E1A or T antigen.

Cell Cycle Regulation of the Retinoblastoma Protein

K. Buchkovich-Sass

All RB tumor cells have lost the ability to synthesize pRB, the product of the RB tumor suppressor gene. The correlation between loss of the RB protein and tumor formation has led to the hypothesis that pRB functions as an inhibitor of cellular proliferation, yet pRB is synthesized in most actively proliferating cells. A resolution of this paradox could be the phase-specific control of pRB activity during the cell cycle.

We have shown that pRB is synthesized throughout the cell cycle and that the steady-state level does not change dramatically (within twofold) during the cycle. However, the phosphorylation state of the protein does vary in a phase-dependent manner. In the G_0/G_1 phase of the cell cycle, an unphosphorylated species of the protein is the only detectable form, whereas in the S, G_2, and M phases, multiple phos-

phorylated species of pRB are detected. The earliest phosphorylation events occur late in the G_1 phase, as determined by hydroxyurea treatment of synchronized cells. Hydroxyurea inhibits DNA synthesis but does not block the phosphorylation of pRB. The removal of phosphates from pRB is not complete until after metaphase mitosis, as determined by nocodazole treatment of cells.

A candidate for the kinase(s) controlling pRB phosphorylation is the human homolog of the *Schizosaccharomyces pombe cdc2* product. Partially purified human *cdc2* will phosphorylate pRB in vitro, and a subset of the pRB tryptic phosphopeptides generated after labeling the protein in vivo are the same peptides phosphorylated by *cdc2* in vitro. *cdc2* is known to be a cell-cycle-regulated kinase, with peaks of activity in the S and M phases of the cell cycle. The yeast *cdc2* p34 is required for entry into these phases of the cycle.

There are two clues as to which form of pRB, the phosphorylated or the unphosphorylated, is active in controllng cellular proliferation. First, in their natural hosts, DNA tumor viruses infect cells that are often arrested in a G_0- or G_1-like phase. The early proteins of these viruses are responsible for inducing infected cells to enter S phase, and several of these proteins bind to pRB. These two activities of the early viral proteins may be directly related, suggesting a G_0 or G_1 activity for pRB. Second, Ludlow and colleagues (*Cell 56:* 57 [1989]) have shown that SV40 T antigen binds only to the unphosphorylated form of pRB. If SV40 antigen regulates pRB function via physical association, as has been proposed, then it must be the unphosphorylated form of pRB that is the target of T antigen. Since the unphosphorylated form of pRB is found predominantly in the G_0/G_1 phase of the cell cycle, T antigen must target a G_0/G_1 activity of pRB. Together, these findings provide the first indication that pRB may have an important role in controlling some elements of cell cycle progression.

Is the E1A-associated p107 Protein Related to pRB?

L.-K. Su, C. Herrmann

p107 is a cellular protein that has been found to form complexes with oncoproteins of certain DNA tumor viruses. This protein has several properties similar to those of pRB, the product of the retinoblastoma susceptibility gene (RB-1). Similar to the pRB protein, p107 is a nuclear phosphoprotein. The regions on two viral oncoproteins, the adenovirus E1A and SV40 T antigen, that are required for their interaction with pRB and the p107 have been characterized. The regions of these oncoproteins that are required for their association with p107 overlap with the regions required for binding to pRB. In other experiments, these regions have been shown to be important for the transformation activity of these oncoproteins. In addition, large T antigen will only bind to the unphosphorylated form of either pRB or p107, whereas E1A binds both phosphorylated and unphosphorylated forms.

Although p107 has many properties similar to those of the pRB proteins, it is not an alternative form of pRB. Evidence for this includes the existence of p107 in a cell line that does not have a functional RB-1 gene, different proteolytic patterns of these two proteins, and failure of several antibodies against pRB to recognize p107. The facts that both p107 and pRB share the same binding regions of E1A and that both of them bind to SV40 large T antigen suggest that they may function in a simliar way or in an antagonistic way. In either case, it is interesting to speculate that p107 is involved in the regulation of the cell proliferation as pRB has been suggested to be.

These questions can only be answered by extensive study of p107. To study p107, we are purifying the p107 protein and cloning its complementary DNA. Since p107 can be easily detected by and purified through its association with adenovirus E1A, a cell line that expresses E1A constitutively has been used as the source of p107. Immunoprecipitation and SDS-PAGE have been used to purify the p107. The purified protein has been used for immunization of animals and for amino acid sequencing. To date, the sera of immunized aminals have not shown a response. However, the amino acid sequence of one peptide from p107 has been obtained in collaboration with Dan Marshak (Protein Chemistry, this section). We are in the process of obtaining a cDNA probe by polymerase chain reaction using the synthesized oligonucleotide deduced from the amino acid sequence.

In addition to the studies of the p107 gene, we have also been studying its phosphorylation. Phosphorylation is a common mechanism to alter the activity of a protein. Several years ago, we observed that immune complexes containing E1A and associated cel-

lular proteins possess a protein kinase activity. Recently, we have begun to identify and characterize this kinase. The major endogenous substrates of this kinase are the p107 and p130 proteins; the kinase also phosphorylates exogenous histone. Using E1A mutant viruses or E1A antibodies that coimmunoprecipitate a subset of the complex proteins, we have been able to eliminate the E1A-associated 300K proteins as a potential source of the kinase activity. Lysates precleared of the pRB protein retain the kinase activity, suggesting that the pRB protein is also not required for the kinase activity. It seems unlikely that p107 itself is the kinase, since in several cases, we are able to immunoprecipitate p107 from ^{35}S-labeled cell lysates, and it does not become phosphorylated in a kinase reaction. Therefore, we believe that another associated protein is the kinase. We have begun to purify this activity and are examining its properties during various stages of the cell cycle. We are also measuring the activity of the kinase in a number of tumor cell lines in which the level of p107 is believed to be altered.

p60 E1A-associated Protein also Binds to the Human *cdc2* Protein

A. Giordano, B. Faha [in collaboration with J. Lee, R. Franza, G. Draetta, and D. Beach, Cold Spring Harbor Laboratory]

The adenovirus E1A proteins associate with a group of cellular proteins in virus-transformed or infected cells. Our results show that the cellular p60 protein forms a complex not only with the adenovirus E1A proteins, but also with the product of the *cdc2* gene. The *cdc2* gene encodes a p34 protein that forms the catalytic subunit of a kinase known as MPF (for mitosis-promoting factor). This kinase is involved in regulation of the cell cycle in a wide range of eukaryotes. The p60/*cdc2* complex occurs in both virus-infected cells and uninfected cells. We have shown that the p60/*cdc2* complex has histone H1 kinase activity and that the kinase activity is cell-cycle-regulated, the highest activity being seen in interphase. The time of activation of this complex differs from that seen with MPF, which is most active in mitosis. Differential timing of activation of these two complexes, p60/*cdc2* and MPF, suggests that each might play a distinct role in cell cycle regulation.

We have also investigated the interaction of E1A with p60. An extensive series of E1A deletion mutants have been used to map the binding sites of p60 on E1A. E1A residues between 30–60 and 121–127 form the needed structures for interaction with p60. These same residues are also required, either in total or in part, for the interactions with pRB and p107. Interestingly, mutations in this region destroy the ability of E1A to function as an oncogene.

PUBLICATIONS

Buchkovich, K., L. Duffy, and E. Harlow. 1989. The retinoblastoma protein is phosphorylated during specific phases of the cell cycle. *Cell* **58:** 1097–1105.

Buchkovich, K., N. Dyson, P. Whyte, and E. Harlow. 1989. Cellular proteins implicated in transformation by adenovirus and other DNA tumor viruses. In *Common mechanisms of transformation by small DNA tumor viruses* (ed. L. Villereal), pp. 51–63. American Society for Microbiology Press, Washington, D.C.

Dyson, N., K. Buchkovich, P. Whyte, and E. Harlow. 1989. The 107K cellular protein that binds to adenovirus E1A also binds to the large T antigens of SV40 and JC virus. *Cell* **58:** 249–255.

Dyson, N., K. Buchkovich, P. Whyte, and E. Harlow. 1989. Proteins that are cellular targets for transformation by DNA tumor viruses. In *Current communications in molecular biology: Recessive oncogenes and tumor suppression* (ed. E. Stanbridge et al.), pp. 133–140. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Dyson, N., P. Howley, K. Munger, and E. Harlow. 1989. The human papillomavirus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. *Science* **243:** 934–937.

Giordano, A., P. Whyte, E. Harlow, B.R. Franza, D. Beach, and G. Draetta. 1989. A 60 kd *cdc2*-associated polypeptide complexes with the E1A proteins in adenovirus-infected cells. *Cell* **58:** 981–990.

Horowitz, J., D. Yandell, S. Park, P. Whyte, K. Buchkovich, E. Harlow, R. Weinberg, and T. Dryja. 1989. Point mutational inactivation of the RB anti-oncogene. *Science* **243:** 937–940.

Münger, K., B. Werness, N. Dyson, W. Phelps, E. Harlow, and P. Howley. 1989. Complex formation of the papillomavirus E7 proteins with the retinoblastoma tumor suppressor gene product. *EMBO J.* **8:** 4099–4105.

Whyte, P., N. Williamson, and E. Harlow. 1989. Cellular targets for transformation by the adenovirus E1A proteins. *Cell* **56:** 67–75.

In Press, Submitted, and In Preparation

Buchkovich, K., N. Dyson, Q. Hu, P. Whyte, and E. Harlow. 1990. Cellular proteins that are targets for transformation by DNA tumor viruses. In *Proto-oncogenes in cell development. Ciba Foundation Symposium No. 150* (ed. T. Hunter). (In press.)

Dyson, N., K. Buchkovich, Q. Hu, P. Whyte, and E. Harlow. 1990. Cellular targets for transformation by DNA tumor viruses. In *Genetic basis for carcinogenesis, 20th International Symposium of the Princess Takamatsu Cancer Research Fund* (ed. A. Knudson et al.). (In press.)

Dyson, N., R. Bernards, S. Friend, L. Gooding, J. Hassel, E. Major, J. Pipas, T. VanDyke, and E. Harlow. 1990. The large T

antigens of many polyomaviruses can bind to the product of the retinoblastoma gene. *J. Virol.* **64:** 1353–1356.
Hu, Q., N. Dyson, and E. Harlow. 1990. The regions of the retinoblastoma protein needed for binding to adenovirus E1A or SV40 large T antigen are common sites of mutation. *EMBO J.* (in press).
Lee, C., W. Craigen, D. Munzy, E. Harlow, and T. Caskey. 1990. Cloning and expression of a mammalian peptide chain release factor: Conserved structure to tryptophanyl-tRNA synthetase. *Science* (Submitted.)

PROTEIN CHEMISTRY

D.R. Marshak G.E. Binns, R. Breckenridge, D. Carroll, M. DiDonato, G.L. Russo, M.T. Vandenberg, A. Vongs, I.J. Yu

The Protein Chemistry Laboratory is engaged in research pertaining to the structure and function of proteins involved in fundamental mechanisms of normal and abnormal growth of cells. We use advanced instrumentation such as protein sequencers, mass spectrometers, and peptide synthesizers to take a biochemical approach to the analysis of proteins and particularly in the analysis of modifications on proteins. It is these modifications, such as phosphorylation, and the enzymes that catalyze them, that appear to modulate many of the regulatory mechanisms in cellular growth.

Mapping Phosphorylation Sites on Nuclear Oncoproteins

D. Carroll, M.T. Vandenberg, D.R. Marshak

During the past year, our work on mapping phosphorylation sites has concentrated on SV40 large T antigen (LTag) and the Myc protein. We have also assembled a spectrum of protein kinases to use for in vitro phosphorylation of the overexpressed proteins. These kinases include casein kinases I and II (CK-I and CK-II); protein kinase C (PK-C); cAMP-dependent protein kinase (PK-A); myosin-light-chain kinase (MLCK); calcium-calmodulin kinase II (CaM K-II); *raf* kinase (raf); $p34^{cdc2}$ (p34); glycogen synthase kinase 3 (GSK-3); and phosphorylase *b* kinase. Studies of p34 phosphorylation of LTag were conducted in collaboration with Y. Gluzman (Molecular Biology of SV40 Section) and D. Beach (Genetics Section). The cell-cycle-regulated protein kinase, p34, phosphorylates LTag specifically at Thr-124. This modification affects the specificity of binding of LTag to the origin of replication on SV40. We demonstrated the site of phosphorylation biochemically by isolating and characterizing the phosphorylated peptide from a proteolytic digest of LTag labeled in vitro with [γ-^{32}P]ATP and purified p34. The protein was digested sequentially with trypsin, followed by staphylococcal V8 protease, and subjected to electrophoresis on polyacrylamide gels containing SDS and urea using a Tris-tricine buffer system. Sequence analysis of this peptide gave the amino acid sequence corresponding to residues 102–126, and the phosphorylation site was determined by an increase of ^{32}P radioactivity appearing at the cycle corresponding to Thr-124. These results were confirmed by analysis of a mutant LTag with a threonine-alanine substitution at position 124 (Y. Gluzman, Molecular Biology of SV40 Section).

Further studies on LTag phosphorylation by CK-II have resulted in an interesting phenomenon. First, phosphorylation of LTag by CK-II shows nonlinear kinetics, with a concave upward curvature, indicating that at higher substrate concentrations, the rate of the reaction increases. This is not true for other substrates for CK-II that we have tested. Adding catalytic amounts of LTag to CK-II reactions with other substrate proteins indicates that LTag will accelerate the phosphorylation. The activating domain on LTag has been identified using synthetic peptides, and it appears to consist of residues 116–138. This peptide contains the polybasic nuclear localization

site as well as the p34 phosphorylation site. This domain is adjacent to the CK-II phosphorylation sites at residues 106, 111, and 112. We are currently trying to evaluate the affects of p34 phosphorylation on CK-II phosphorylation in vitro. The effect of LTag on CK-II appears to act in vivo, since extracts of COS cells that have LTag overexpressed have approximately eightfold higher CK-II activity than the parent strain, CV-1. These studies have prompted us to evaluate further the roles of activators and potential inhibitors of CK-II activity in vivo.

Phosphorylation sites on Myc have been determined by in vivo and in vitro labeling experiments. Phosphorylated peptides have been analyzed by electrophoresis on high-resolution, Tris-tricine/urea gels as described above for LTag. The in vivo experiments were done with COLO 320 cells, which are a human colon-carcinoma-derived cell line that overexpresses Myc. The in vitro phosphorylation experiments are performed with Myc protein expressed in and purified from *Escherichia coli*. The patterns of Myc phosphorylation in the cells can be partially accounted for by phosphorylation of the purified protein by PK-A, CK-II, and p34. The sites of phosphorylation were determined by sequence analysis and mass spectral analysis of the purified, phosphorylated peptides. Mass spectrometry allows us to determine the exact stoichiometry and residues that are modified. There appears to be a domain in the protein of approximately 7 kD in the middle of the protein that contains the bulk of the phosphorylation sites. This hyperphosphorylated domain regulates the DNA binding of Myc, since CK-II phosphorylation in this domain abolishes the activity.

Phosphorylation of Transcription Factors

D.R. Marshak, G.L. Russo, I.J. Yu
[in collaboration with E. Chang and M. Gilman, Cold Spring Harbor Laboratory]

Transcription factors refer to a large family of proteins that either bind to regulatory elements of genes or interact with protein complexes that, in turn, promote gene transcription. Many of these transcription factors appear to be phosphorylated, mainly by attachment of phosphate to serine and threonine residues, catalyzed by several candidate nuclear protein kinases. Of these, we have studied casein kinase II, $p34^{cdc2}$, glycogen synthase kinase, protein kinase C, and cAMP-dependent protein kinase. Using this panel of enzymes, our strategy has been to phosphorylate transcription factors in vitro and to test how individual phosphorylation events might alter the ability to bind to the appropriate DNA element or to direct transcription. To this end, we require purified proteins produced in relatively large amounts, unlike the natural products that are in vanishingly small quantities. E. Chang has expressed several factors in *E. coli*, and these are purified to milligram quantities through classical procedures.

We have begun to study two regulatory proteins involved in the control of transcription of the c-*fos* gene. Two regulatory elements have been defined by M. Gilman and his colleagues (Molecular Genetics of Eukaryotic Cells Section) as the serum response element and the cAMP responsive element, and the corresponding proteins that bind to these elements have been termed the serum response factor (SRF) and the cAMP-responsive-element-binding protein (CREB). Both of these proteins have been expressed in *E. coli* and purified to apparent homogeneity. Using our panel of protein kinases, we have begun to examine the phosphorylation sites on the proteins as well as the effects of these phosphorylations on DNA binding. Preliminary evidence suggests that phosphorylation plays a major role in modulating the transcriptional activator functions of these proteins. Further studies will take advantage of our mass spectrometry and protein chemistry expertise to determine the stoichiometry and location of the phosphorylation sites.

Role of Casein Kinase II in Cell Growth

I.J. Yu, D. Carroll, D.R. Marshak

We are continuing our studies of CK-II in cell growth and viral transformation. To this end, we continue to develop reagents that will allow us to investigate changes in enzyme activity, protein levels, and mRNA levels inside cells. Antibodies have been prepared in rabbits against purified bovine liver CK-II. These sera react with the enzyme on nitrocellulose and by immunoprecipitation. Recently, several additional antisera have been prepared by using synthetic peptide antigens raised to various regions of the α and β subunits of the enzyme. We are using

these antibodies to investigate the subcellular localization of CK-II during serum stimulation or virus infection, as well as in cell division cycle experiments. We hope that these antibodies will allow us to distinguish between the catalytic (α) and putative regulatory (β) subunits of the enzyme. We are currently isolating the cDNA clone for the human α subunit using the polymerase chain reaction (PCR). We have constructed oligonucleotide probes based on the published sequence with specific restriction sites. Using total RNA from HeLa cells, we are using reverse transcriptase followed by the PCR reaction to isolate a full-length clone (1.2 kb). If successful, this will allow us to use our cDNA to probe the levels of mRNA in stimulated versus unstimulated cells and in the cell cycle.

Initial studies on cell cycle control of CK-II as compared with p34 have demonstrated a reciprocal relationship between these enzyme activities. CK-II appears to be maximally stimulated in G_1 phase of the HeLa cell cycle, consistent with our previous observations of CK-II stimulation early in serum activation of fibroblasts. The mitotic form of p34, however, is maximally active during the G_2/M phase of the cell division cycle. One interesting region to examine will be S phase, when both enzymes are partially activated and the cell is undergoing profound changes in nuclear activity to replicate DNA. The initiation and control of the DNA synthesis phase of the cell cycle are of particular excitement for continued effort in protein kinase activity measurements.

Synthetic Peptide Substrates for Protein Kinases

D.R. Marshak, M.T. Vandenberg, R. Breckenridge, G.E. Binns, M. DiDonato, D. Carroll

We have been actively involved in the design and synthesis of peptides that are phosphate acceptors on serine or threonine residues. Peptides are synthesized by chemical methods based on the original Merrifield approach using N^{α}-*t*-Boc-protected amino acids and solid supports. Synthesis is done using automated techniques on an Applied Biosystems 430A instrument. The peptides are cleaved from the support in liquid hydrogen fluoride, precipitated with ethyl ether, and purified by reverse-phase high-performance liquid chromatography. The peptides are extensively characterized by mass spectrometry, sequence analysis, and amino acid analysis to ensure that we have generated a pure product. Peptides are analyzed as kinase substrates by carrying out a phosphorylation reaction with radiolabeled ATP and then separating the radioactivity incorporated into the peptide either by immobilization on phosphocellulose paper or by thin-layer chromatography. Both methods have been very successful in quantitative analysis of the products.

Two classes of peptide substrates for protein kinases have been characterized: CK-II substrates and $p34^{cdc2}$ substrates. The CK-II substrates are modeled on the sites of phosphorylation of the nuclear oncoproteins, such as Myc, Fos, adenovirus E1A, and SV40 T antigen. These peptides contain serine or threonine residues followed by a cluster of acidic amino acid residues. The presence of a β-turn generated by proline residues is common but apparently not essential for activity. Substrate peptides for $p34^{cdc2}$ generally contain a serine or threonine residue followed by a proline residue and several basic amino acids. The exact structural features of these substrates and their specificities as substrates is under intense investigation.

Structural Analysis of a Human IGF-II Variant

D.R. Marshak [in collaboration with J. Perdue and W. Burgess, American Red Cross Research Center, Maryland]

During the year, we have been fortunate to be involved in the analysis of several proteins related to growth in human physiology. In collaboration with a group at the American Red Cross Research Center in Maryland, we characterized a new form of the insulin-like growth factor II (GF-II) from human plasma. This protein turned out to be a variant of the more common form, containing a tetrapeptide replacement (Arg-Leu-Pro-Gly) of a serine residue near the amino-terminal end of the protein. The mass of the protein (measured by plasma desorption mass spectrometry) was 7809.4, less than 0.05% from the calculated mass based on classical sequencing techniques. These studies indicate that the mass spectrometer is an invaluable tool in structural analysis of proteins. This variant of IGF-II represents approximately 25% of the plasma IGF-II activity.

Sequence Analysis of Peptides by Mass Spectrometry

D.R. Marshak, G.E. Binns

Sequence analysis of peptides by plasma desorption mass spectrometry (PDMS) has been accomplished for an assortment of peptides. Peptides ranging in molecular weight from 1100 to 3600 amu displayed useful sequence ions under PDMS. Acidic, basic, and neutral peptides all showed sequence fragment ions, and carboxy-terminal sequence information was readily obtained on all peptides (A, B, or C″ ions). The A ion series was most abundant for the basic peptides, and the C″ ion series was most abundant for the acidic peptides. Sequence-specific fragment ion patterns appear to occur. For example, peptide CSH084 showed dehydration (−18 amu) of all the Y″ ions in the internal basic region (residues 10–16). Subsequent examples of peptides containing aromatic side chains indicate that loss of the indole moiety from the side chain is common under PDMS. A similar generation of these w′ ions from histidine residues through loss of imidazole has also been detected. We suggest that PDMS is generally useful for sequence analysis of peptides of various compositions. In particular, PDMS is useful as a complementary tool to classical methods of protein chemistry, and PDMS should be considered an important part of a multidisciplinary approach to the structural analysis of peptides and proteins.

At low sample loads (10 pmoles), the B, C″, and Y″ ions remained detectable, whereas the A and Z ions were not detected. Therefore, sequence analysis of peptides at the low pmole level is possible by PDMS. At higher sample loads, the A ion series was abundant and gave nearly complete sequence information. Because the amino-terminal sequence ion array (A, B, C″ ions) yields carboxy-terminal sequence information, PDMS is quite useful in conjunction with amino-terminal sequence analysis using chemical methods. Sequence fragment ions are more abundant with smaller amounts of nitrocellulose electrosprayed on the targets. Optimal nitrocellulose loading for 1.0 nmole (Val-5) angiotensin I was 10–25 μg. In contrast, the $[M+H]^+$ was more abundant at high loading of nitrocellulose (100 μg). Data acquisition for 4 hours yielded significant abundance of sequence ions for all the peptides studied. Longer acquisition times increased the signal-to-noise ratio for the fragment ions and permitted the assignment of more sequence. These studies demonstrate that

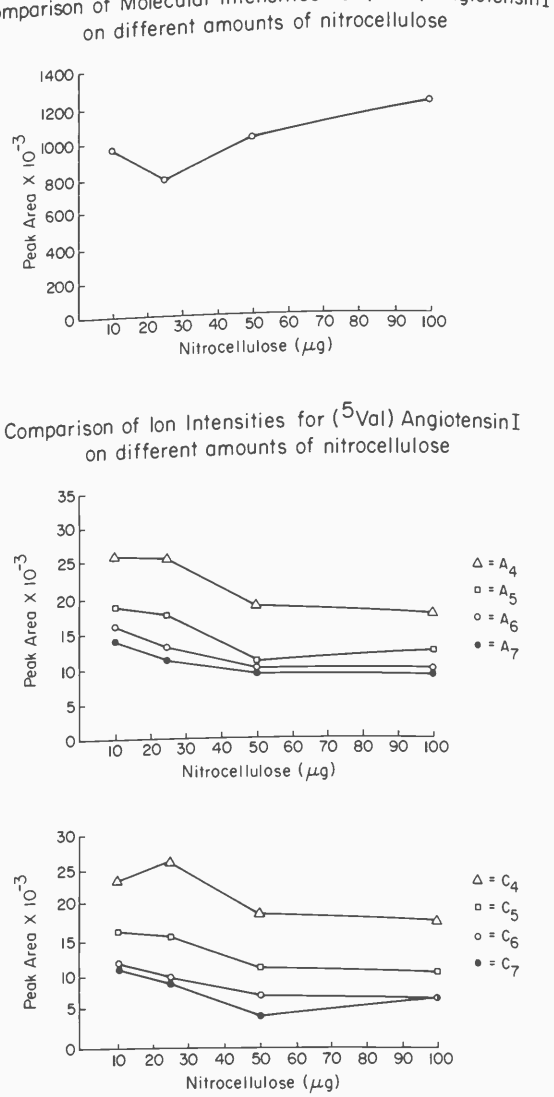

FIGURE 1 (*Top*) Comparison of molecular intensities for (Val-5) angiotensin I on different amounts of nitrocellulose. (*Bottom*) Comparison of ion intensities for (Val-5) angiotensin I on different amounts of nitrocellulose.

PDMS analysis of peptides can provide rapid and accurate sequence information as a supplement to chemical analyses.

Peptide Synthesis

G.E. Binns, M. DiDonato, D.R. Marshak

As part of the core protein chemistry support facility, peptide synthesis is performed to assist researchers in their work throughout the programs at Cold

Spring Harbor Laboratory. In general, there are three uses of peptides: (1) as antigens for antibody production; (2) as enzyme substrates; and (3) for biological studies. Approximately half the peptides synthesized are used as antigens by coupling to carrier proteins or by direct injection into animals. The antibodies produced in this way allow an investigator to prepare a reagent that reacts very specifically with a site on the protein of interest. Such "site-directed" antibodies are of tremendous value in structure-function studies of proteins. To facilitate the rapid synthesis of these peptides, we have employed small-scale (0.1 mmole) rapid-cycle chemistry using an automated synthesis instrument. To optimize these procedures, we developed new cycles that double couple 10 of the 20 common amino acids first in dimethylformamide and then in dichloromethane. This procedure generates very high-yield and high-quality products.

We have performed the synthesis of larger peptides and small proteins using other methods. For larger scale and longer syntheses, we have employed a new solvent system consisting of *N*-methylpyrrolidone and dimethylsulfoxide. This permits the swelling of the resin to increase and boosts coupling yields, although reaction times are increased. Using this technique, we synthesized the full-length protein S100β, consisting of 91 residues, as well as a piece of the protein containing 48 residues. These proteins have been purified by high-performance liquid chromatography and are currently being studied as structural analogs of the native proteins. Many other syntheses have been performed, several resulting in peptides that have high biological activity. This indicates that the amount of racemization and the structural changes in the synthetic species compared to that of the native peptide are minimal. The collection of methods now employed produce peptides of the highest quality and activity available anywhere.

During the last 4 years, the growth of the protein chemistry core services has increased dramatically. Since Dr. Marshak's arrival in 1986, sequence analysis has doubled every year, and peptide synthesis has increased at an even faster pace. We are now seeing the number of procedures performed per year begin to plateau as we reach the physical capacity of the instruments and the limited personnel. At present, scientists have to wait weeks to months for their analyses because of these limitations in resources. In addition, we have instituted several new methods, such as mass spectrometry, high-performance electrophoresis, and quantitative chemical analyses to maintain the facility on the leading edge of new technology. However, this further limits the time available for each procedure. The growth of the use of protein chemistry is illustrated in Table 1. We hope that statistics such as these will help to justify increased resources for protein chemistry.

TABLE 1 Growth of Protein Chemistry

Procedure	1986	1987	1988	1989
Protein sequences	47	87	162	215
Peptide synthesis	6	18	46	64
Amino acid analysis		357	389	197
Chromatography		61	68	35

PUBLICATIONS

Carroll, D. and D.R. Marshak. 1989. Serum-stimulated cell growth causes oscillations in casein kinase II activity. *J. Biol. Chem.* **264:** 7345–7348.

Hampton, B., W.H. Burgess, D.R. Marshak, K.J. Cullen, and J.F. Perdue. 1989. Purification and characterization of an insulin-like growth factor II variant from human plasma. *J. Biol. Chem.* **264:** 19155–19160.

Marshak, D.R. and D.T. Liu, eds. 1989. *Therapeutic peptides and proteins: Formulation, delivery, and targeting*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

McVey, D., L. Brizuela, I. Mohr, D.R. Marshak, Y. Gluzman, and D. Beach. 1989. Phosphorylation of large T antigen by *cdc2* stimulates SV40 DNA replication. *Nature* **341:** 503–507.

In Press, Submitted, and In Preparation

Bischoff, J.R., P.N. Friedman, D.R. Marshak, C. Prives, and D. Beach. 1990. Human p53 is a *cdc2* substrate: Phosphorylation in interphase by p60-*cdc2* and M-phase by cyclin B-*cdc2*. *Proc. Natl. Acad. Sci.* (Submitted.)

Carroll, D., W.-K. Chan, M.T. Vandenberg, D. Spector, and D.R. Marshak. 1990. Phosphorylation of Myc by casein kinase II, cdc-2, and cAMP-dependent protein kinase: Influence on DNA binding properties. (In preparation.)

Chan, W.-K., E. Chang, J. Anderson, D. Carroll, D.R. Marshak, and D.L. Spector. 1990. The structural and functional domains of the Myc oncoprotein. (Submitted.)

Marshak, D.R. and G.E. Binns. 1990. Protein sequence analysis by plasma desorption mass spectrometry. In *Current research in protein chemistry* (ed. J.J. Villafranca). Academic Press, New York. (In press.)

Marshak, D.R. and D. Carroll. 1990. Synthetic peptide substrates for casein kinase II. *Methods Enzymol.* (in press).

Marshak, D.R. and B.A. Fraser. 1990. Structural analysis of synthetic peptides by mass spectrometry. In *High performance liquid chromatography in biotechnology* (ed. W. Hancock). John Wiley & Sons, New York. (In press.)

Marshak, D.R., M.T. Vandenberg, Y.-S. Bae, R. Breckenridge, and D. Carroll. 1990. Synthetic peptide substrates for $p34^{cdc2}$. (In preparation.)

PROTEIN SYNTHESIS

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L. Manche
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We have continued to study the regulation of gene expression at both the transcriptional and translational levels. Our work uses systems drawn from adenovirus and the human immunodeficiency viruses (HIV), as well as cellular genes. During the past year, Gil Morris was promoted to a staff associate position and the group expanded in numbers. We were joined by Odi Echetebu, a visiting scientist, Chitra Kannabiran and Shobha Gunnery, both postdoctoral fellows, and Frank Ma, a graduate student, while Chris Herrmann received her doctorate and moved to Ed Harlow's laboratory in the James building.

Adenovirus VA RNA and Translational Control

K.H. Mellits, T. Pe'ery, Y. Ma, L. Manche,
M. Stoddart, M.B. Mathews

In the mid-1960s, an "unusual" RNA component was discovered in human cells infected with adenovirus type 2 (Ad2) and was termed virus-associated (VA) RNA. Ten years later, it was realized that there are in fact two such RNAs, VA RNA_I and VA RNA_{II}, both about 160 nucleotides in length. These RNA polymerase III transcripts were mapped to two closely spaced genes at approximately map unit 30 on the adenovirus genome. The RNAs accumulate to high concentrations in the cytoplasm of adenovirus-infected cells, and their sequences suggested that they can adopt stable secondary structures. Since then, much has been learned about the role of these RNAs in adenovirus infection. Their participation in translational control was discovered through the study of mutants that contain disruptions of the transcriptional control regions located within the 5′ half of the VA RNA genes. Protein synthesis was depressed in cells infected with an adenovirus mutant that cannot make VA RNA_I. Mutant viruses unable to transcribe VA RNA_{II} showed no such defect, but the defect was exacerbated when both VA RNAs were missing, indicating that VA RNA_{II} can also participate in the translational function. Subsequent work showed that VA RNA_I antagonizes a cellular antiviral defense mechanism by preventing the activation of a cellular protein kinase known as DAI, the double-stranded RNA (dsRNA)-dependent protein kinase. When activated by dsRNA, a process involving autophosphorylation, this enzyme inhibits protein synthesis by phosphorylating the initiation factor eIF-2 and thereby trapping a second initiation factor, GEF (also known as eIF-2B). VA RNA prevents the dsRNA-mediated activation of DAI.

Present work is addressing the nature of the interactions of DAI with RNAs that activate it (dsRNA) or block its activation (VA RNA). On the basis of partial nuclease digestion data, we recently proposed a secondary structure model for Ad2 VA RNA_I that contains two duplex regions, each about 20 base pairs long (believed to be too short to activate the kinase) together with several loop structures (Fig. 1). To assess the validity of this model, we have begun to compare the structures of a variety of VA RNAs. These include the VA RNA_I species of the closely related Ad5 serotype, of two more distantly related human viruses (Ad7 and Ad12), and of monkey and chicken adenoviruses (SA7 and CELO), as well as the VA RNA_{II} species of Ad2 and Ad7. The 5′ halves of these RNAs share pol.III transcription sequences but are otherwise divergent in primary sequence. Computer-assisted structural analysis suggests that they may all possess a region similar to the functionally important central domain of Ad2 VA RNA_I (stippled area in Fig. 1), and direct examination of the structures of Ad7 VA RNA_I and of the CELO and Ad12 VA RNAs confirms that their secondary structures are similar to, but not identical with, that of Ad2 VA RNA_I.

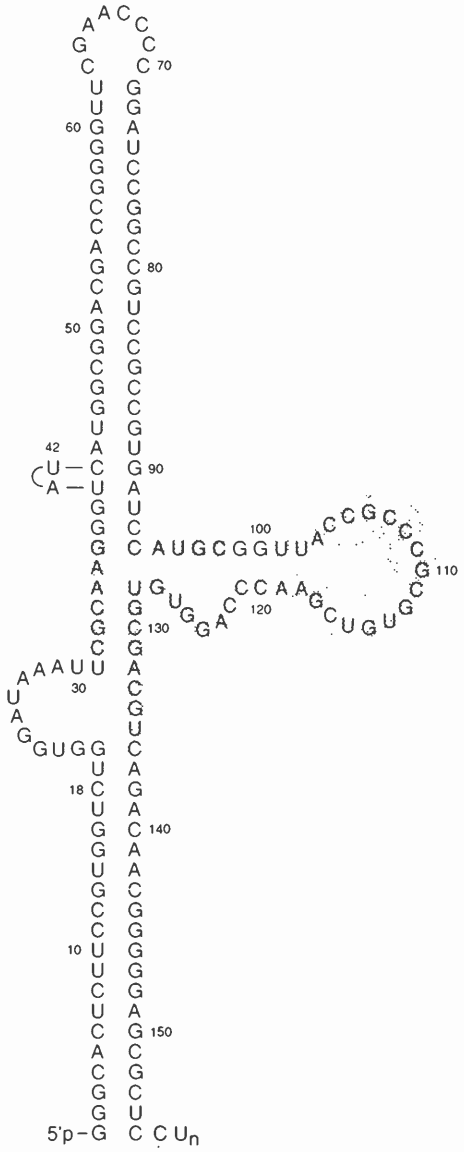

FIGURE 1 Structure of VA RNA. The approximate limits of the central domain (stippled area) are superimposed on the secondary structure model for Ad2 VA RNA_I. (Redrawn from Mellits and Mathews, *EMBO J. 7:* 2849 [1988].)

We have continued to pursue the mutagenic approach described in previous Annual Reports to determine which VA RNA sequences or structures are necessary to prevent the activation of DAI. As a first step, we made a series of deletion and substitution mutations throughout the 3′ half of the VA RNA_I gene and tested the resulting mutant RNAs for their ability to rescue protein synthesis in vivo. The results, coupled with structural information based on partial ribonuclease digestion, defined a small region of duplex RNA (nucleotides 73–84, Fig. 1) that is dispensable for VA RNA_I function and a complex stem-loop structure (the central domain, Fig. 1) that is important for function. Further genetic analysis suggests that secondary structure is more important than primary sequence per se in the regions that flank the central domain. More recently, we have used a vector containing the bacteriophage T7 promoter to make mutant RNAs in the quantities necessary to assay their ability to function in vitro. Thus far, with a single puzzling exception, the wild-type and mutant VA RNAs tested all function in vitro as would be expected from their behavior in vivo. We have gone on to make new mutations in this vector, most notably to elucidate features of the central domain that are important for function. Small deletion and substitution mutations were introduced by oligonucleotide-directed mutagenesis and were tested for their ability to block DAI activation by dsRNA. Deletion or substitution of most of the nucleotides in the loop was tolerated, but a substitution in the neighboring short stem severely impaired the ability of the mutant RNAs to function. Thus, neither the size nor the sequence of the loop is critical; on the other hand, the structure and/or sequence of the short stem is important for function, as are some sequences immediately adjacent to the central domain. We presume that the deleterious mutations perturb the conformation of the central domain, and we are currently probing the secondary structure of these mutants to determine the structural ramifications of the mutations.

Because of its extensive secondary structure, it was proposed that VA RNA_I might block activation of DAI by imitating short duplex RNA and thereby preventing the interaction between dsRNA and DAI. Consistent with this hypothesis, DAI has a single binding site for dsRNA, and this site can be blocked by VA RNA_I. Moreover, VA RNA_I binds to DAI both in vivo and in vitro and can be cross-linked to the enzyme. However, the mutational analysis summarized above ascribes a dominant functional role to the central domain of the molecule rather than to its duplex axis. To explore the relationship between DAI binding and the structure and function of VA RNA_I, we assessed the ability of mutant RNAs to bind to the kinase. In many cases, a mutant's binding efficiency correlated with its function as measured in vivo, but there were several exceptions: A number of mutants that do not function in vivo bound well, and one that functions in vivo did not

bind efficiently. These results indicate that efficient binding is neither necessary nor sufficient for function. Consideration of RNA structures suggested that efficient binding requires a short stem formed at the apex of the molecule rather than the central domain, a deduction that was confirmed by determining the structures of the shortest fragments that retain the ability to bind to DAI. The short duplex structure that promotes efficient binding to DAI is not essential for function, implying that the domains that control these activities are separate and distinct. We postulate that the interaction of DAI with the apical stem occurs at the enzyme's dsRNA-binding site, but does not prevent activation, and that this binding facilitates apposition of the central domain to a sensitive region of the DAI active site, leading to inhibition of DAI autophosphorylation. To flesh out these ideas, we are currently examining the interaction of DAI with RNA duplexes of various lengths and the ability of VA RNA_I to bind to the kinase in the presence of dsRNA.

Regulation of Gene Expression by E1B

C. Herrmann, M.B. Mathews

The adenovirus E1B 19,000-dalton (19K) tumor antigen is essential for the complete transformation of primary rodent cells and is required for the normal infection of permissive human cells. We have used a plasmid cotransfection assay or cell lines that stably express the 19K protein to examine the effect of the 19K protein on expression of a reporter gene product. The results showed that the 19K polypeptide stimulates expression from all promoters introduced into cells by transfection, including a variety of viral promoters and one cellular promoter. In addition, the 19K protein increases expression from an RNA polymerase III-transcribed gene but does not affect the level of expression of an endogenous cellular gene. The effect of the 19K protein appears to be independent of promoter sequence but is strongly influenced by the time course of the experiment, with the greatest stimulation observed at late times.

We have recently shown that the 19K protein stimulates the rate of transcription from transfected promoters, as measured by nuclear run-on assay. Examination of plasmid DNA levels by dot-blot analysis of Hirt extract DNA revealed that the 19K protein increases the stability of plasmid DNA. This stabilization of DNA accounts for the increased transcription from transfected promoters, indicating that the primary role of the 19K protein in increasing expression from transfected promoters is to increase the level of plasmid DNA retained in the cells.

The role of the 19K protein in regulating viral gene expression during infection was also examined. Whereas it has a negative effect on viral gene expression in the presence of the E1A 13S or 12S products (see DNA Synthesis, this section), the 19K protein stimulates viral gene expression in the absence of E1A gene products. The enhanced transcription of viral genes results from increased viral DNA levels. These results demonstrate that in infected cells, as in transfected cells, the 19K protein produces a general and indirect increase in transcription rates by elevating viral and plasmid DNA levels. The stabilization of template DNA is consistent with the role of the 19K protein in preventing the degradation of viral and chromosomal DNAs during infection. Thus, the 19K protein may represent the first example of a viral oncogene product that modulates gene expression by controlling DNA levels.

Regulation of PCNA

G.F. Morris, C. Kannabiran, M.B. Mathews

The proliferating cell nuclear antigen (PCNA), also known as cyclin, is a highly conserved protein that functions in DNA replication by stimulating the processivity of DNA polymerase δ. Sequence homologs for PCNA have been found in animals, plants, insects, insect viruses, and yeast, and a functional analog has been identified in T4 bacteriophage (see DNA Synthesis). The relevance of PCNA to DNA metabolism and the correlation of PCNA expression with cell proliferation prompted us to examine its expression during periods of cell growth in detail. To present, we have focused on the transcriptional response of the PCNA gene to the transforming genes of adenovirus, located in the E1 region. In the long term, we will employ this growth-responsive target gene as a prototype to work toward the mechanism(s) by which other stimuli trigger cells to grow and divide.

We have cloned the promoter for PCNA from human placental DNA and have begun to define sequences within the promoter that are important for its activity. The region from nucleotide −1269 to +60 was excised from a 1.5-kb genomic clone and

fused to the chloramphenicol acetyltransferase (CAT) reporter gene to determine if this DNA fragment can function in transient expression assays. After transfection into HeLa cells, this PCNA promoter was found to drive the synthesis of CAT, albeit 20–40 times less efficiently than the SV40 early promoter. In contrast, in 293 cells (which express E1 products), the PCNA promoter was as active as the SV40 early promoter in transient expression assays. To locate functionally important elements of the PCNA promoter, we prepared a series of upstream deletion mutants of the promoter and fused them at +60 to the CAT reporter gene (Fig. 2). Removal of more than 1000 nucleotides of upstream sequence (nucleotides −1269 to −248) had little effect on the expression of CAT activity in HeLa or 293 cells. Further deletion, removing the region between −1269 and −172, had little impact in 293 cells but reduced activity in HeLa cells by approximately 30–40%. Removal of sequences from −1269 to −88 or −47 reduced the activity of the promoter in both cell lines to levels only slightly higher than the basal levels observed with the parent clone lacking any inserted promoter sequences (pBACAT).

Within this last segment, −172 to −88, lie two potential SP1 sites and two CCAAT motifs. Such elements are found in a number of genes that are constitutively expressed, but it is interesting to note that the organization of these sequences in the PCNA promoter resembles that seen in the human thymidine kinase promoter, possibly reflecting similar regulatory patterns for these two replication-related proteins. The functions of these elements are only conjectural at the moment, but it is possible that the CCAAT motif plays a regulatory role in development and in response to serum stimulation, and the repeated arrangement of SP1 sites may allow for the rapid transcription of these genes during periods of accelerated growth. Although the PCNA promoter lacks an identifiable TATA box upstream of the transcription site, there is some homology near the cap site to the published initiator sequence and to sequences near the transcription initiation site of the thymidine kinase promoter. To assess the possibility that this region specifies the site of transcription initiation in the PCNA promoter, as proposed for the initiator element, we deleted nucleotides −1 to +60 from the promoter-CAT construct containing nucleotides −559 to −60. This deletion reduced the strength of the PCNA promoter in HeLa cells only slightly, but in 293 cells, it reduced expression by about half. Whether this signifies a role for the −1 to +60 region in the E1 response, or some other difference between the two cell types, is not yet clear.

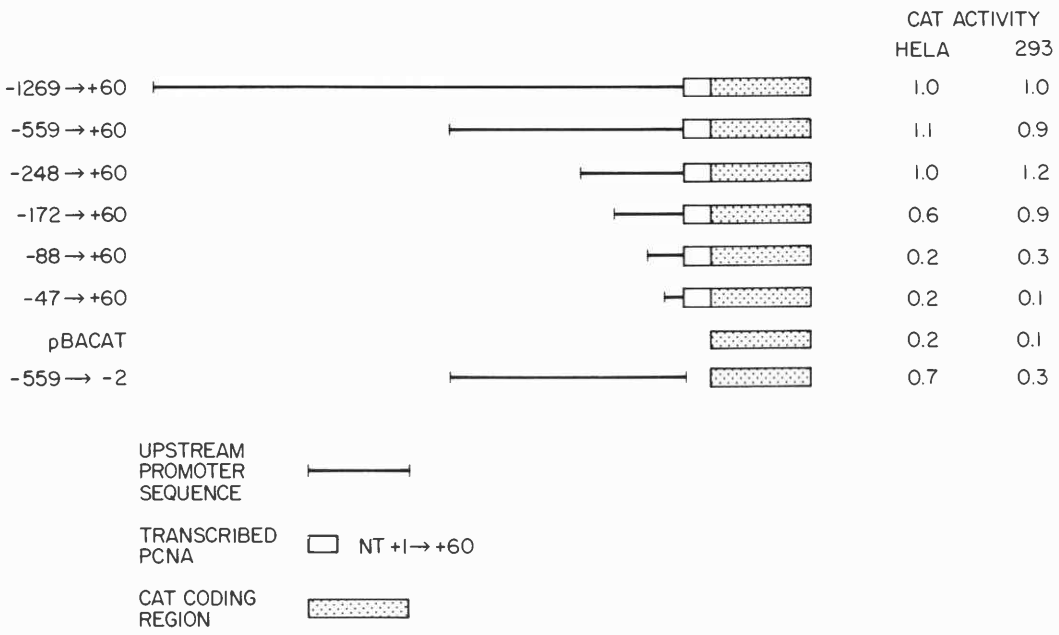

FIGURE 2 PCNA promoter activity in HeLa and 293 cells. A human genomic DNA fragment containing nucleotides −1269 to +60 (relative to the PCNA transcription initiation site at +1) fused to the CAT reporter gene contained in the promoter-less plasmid pBACAT. Each construct was assayed by transfection into HeLa and 293 cells, and relative activities were determined by normalizing the experimentally determined CAT activities to that of the full-length clone.

Prompted by these results, as well as by earlier data showing increased expression of PCNA after infection with E1A-containing adenoviruses, we asked whether the PCNA promoter could be *trans*-activated by E1 products of adenovirus expressed from a plasmid. Cotransfection of HeLa cells with the adenovirus E1 region (encoding both E1A and E1B products) increased the activity of all the promoter-CAT fusion constructs, with the possible exception of the −47 to +60 construct, whose basal activity was so low that it is difficult to be certain that E1 was ineffective. These data imply that at least one E1 responsive element resides between −88 and −47, or possibly between −88 and −2. Within the region from −88 to −2, there are two sequence elements that have homology with known targets for *trans*-activation by the E1A 13S product. All of the adenovirus early gene promoters apart from E1B contain at least one E1A-responsive ATF site, and a sequence that has perfect homology with a CREB/ATF site is located in the PCNA promoter surrounding nucleotide −50. There is also limited homology with E1A-responsive E2F sites at about −20 and −40. Current experiments are designed to determine if these candidates are in fact E1-responsive elements and, if so, whether they are the sole E1-responsive elements in the PCNA promoter.

The E1 region of adenovirus produces several proteins that influence gene expression: One of our goals is to determine which of these proteins affect PCNA and to delineate their roles. To this end, we first tested the E1A and E1B genes separately to discover if either alone could recapitulate the *trans*-activation observed by cotransfection with E1. When cotransfected singly with the PCNA-CAT reporter, neither E1A nor E1B *trans*-activated as well as the intact E1 region, although in combination they were nearly as effective as the E1 plasmid. To analyze matters further, the two major E1A transcripts were placed under the control of the strong cytomegalovirus promoter, and the two major E1B gene products cloned in a similar fashion were obtained from Dr. E. White (DNA Synthesis, this section). These plasmids allow the four major E1A products to be synthesized efficiently and independently.

Preliminary experiments with these clones indicate that the E1A 13S and 12S gene products and the E1B 19K gene product increase the levels of detectable CAT activity upon cotransfection into HeLa cells, whereas the E1B 55K gene product is without effect. Stimulation by E1A 13S and E1B 19K was expected on the basis of the general *trans*-activation and DNA stabilization properties of these two products, but the E1A 12S gene product is best known as a transcriptional repressor. However, its ability to *trans*-activate the PCNA promoter is fully consistent with observations, made previously in this laboratory by Dr. E. Moran, that PCNA synthesis is induced in baby rat kidney cells by infection with an adenovirus expressing the 12S E1A gene product. Experiments are under way to discover whether the same promoter sequences are required for response to each of these three E1 products and to more physiological stimuli such as serum.

Mechanism of *trans*-Activation by HIV-1 Tat Protein

M.F. Laspia, M. Kessler, S. Gunnery,
A.P. Rice, M.B. Mathews

The human immunodeficiency viruses, HIV-1 and HIV-2, have been implicated in the pathogenesis of acquired immunodeficiency syndrome (AIDS). HIV-1 is prevalent in isolates from patients with AIDS. It infects helper T cells, monocytes, and nervous system cells, and following a prolonged latency, it enters a period of active viral growth characterized by severe depletion of the helper T-cell population. This renders the host immunocompromised and susceptible to infection by opportunistic pathogens as well as to neurological disorders.

In addition to the structural genes *gag*, *pol*, and *env*, common to all nondefective retroviruses, HIV-1 encodes several novel regulatory genes. One of these, *tat*, is an essential gene that greatly stimulates the expression of genes directed by the long terminal repeat (LTR) of the virus. Tat acts via the TAR sequences located in the LTR downstream from the site of transcription. The 5′-untranslated leader transcribed from TAR is capable of forming a stem-loop structure, and mutations that disrupt this structure greatly reduce *trans*-activation by Tat. The mechanism of Tat action remains controversial, as regulation has been proposed to occur at several levels, but in our hands, Tat appears to act principally at the transcriptional level.

To explore the molecular mechanism of Tat action, we employed a recombinant adenovirus containing an HIV-1 LTR-CAT gene fusion in place of the E1 region. This virus provides an efficient means to deliver an HIV-1 LTR-driven reporter gene into Tat-expressing or control cells. As described last year,

analysis of HIV-1-promoted cytoplasmic RNA revealed two classes of correctly initiated transcripts in HeLa cells. One class contains long, predominantly polyadenylated RNA and corresponds to full-length transcripts; the other class contains short, exclusively poly(A)$^-$ RNA, approximately 55–59 nucleotides in length, corresponding to terminated or processed RNAs. Tat increases the accumulation of full-length RNA at least 100-fold but does not affect the accumulation of short transcripts. The general *trans*-activator encoded in the adenovirus E1A gene, on the other hand, increases the accumulation of both full-length and short transcripts. These results are consistent with Tat, as well as E1A, acting to increase HIV-1-promoted transcription. This is unlikely to be the only effect, however, since Tat increases the level of full-length RNA without increasing the short transcripts, whereas E1A increases accumulation of both transcript classes.

To establish these conclusions more firmly, we performed direct measurements of RNA synthesis using nuclear run-on assays. The products were hybridized to short single-stranded DNA probes corresponding to various regions of the gene, providing an estimate of transcription rates and RNA polymerase distribution along the gene. In the absence of Tat, transcription in the promoter proximal region is low and decreases sharply in the CAT gene: This polarity suggests that the density of RNA polymerases on the template declines with increasing distance from the promoter. Tat produces a large increase (over 15-fold) in transcription rate in the HIV-1 leader and also increases transcription of CAT sequences. Transcriptional polarity also occurs in the presence of Tat, although to a lesser degree: About 30% of the RNA polymerases in the leader region transcribe into the CAT gene in the presence of Tat, and only 10% do so in its absence. E1A also produces a large increase in promoter proximal transcription and it increases transcription in the CAT gene sequences as well, but the sharp polar effect on transcription is quantitatively similar to that seen without *trans*-activators. The increase in transcription rates brought about by Tat or E1A can be observed within the first 24 nucleotides of the leader, suggesting strongly an increase in transcriptional initiation. Thus, as depicted in Figure 3, we conclude that Tat and E1A both stimulate initiation and that Tat additionally suppresses transcriptional polarity.

Studies with deletion mutants demonstrate that TAR is required for the stimulation of HIV-1 transcription by Tat but not by E1A, confirming that TAR is not indispensable for a high level of HIV-1 transcription. Since the basal level of transcription is neither increased nor decreased by the deletions, TAR is neither a negative element (e.g., a terminator) nor a positive element required for basal levels of transcription. Although we do not fully understand the nature of the interaction between Tat and TAR, our findings are most readily consistent with the hypothesis that Tat interacts with TAR at the DNA level to promote the efficient formation of an initiation complex capable of stable elongation. In light of genetic evidence suggesting that the effect of Tat is mediated by TAR RNA, it is also possible that one or both of Tat's activities result from a novel regulatory interaction (direct or indirect) with an RNA structure formed immediately downstream from the site of transcription initiation.

To explore further the mechanism of *trans*-activation by Tat, we are currently studying the combined effects of Tat and E1A on HIV-1 LTR-directed gene expression. At the level of CAT enzyme and mRNA accumulation, these two *trans*-activators produce a much greater than additive effect on reporter gene expression. Synergy between Tat and other *trans*-activators has been observed before, and the interaction between Tat and E1A may serve as paradigm for such interactions. Consistent with the model proposed above (Fig. 3), the effect of Tat on E1A-stimulated RNA accumulation is similar to its effect on basal transcription in that accumulation of full-length RNA is increased but short transcripts are unaffected. Interestingly, preliminary results from run-on transcription assays suggest that the combined effect of Tat and E1A produces no more than an additive increase in promoter proximal transcription. This observation implies that the synergistic *trans*-activation is due primarily to stabilization of transcriptional elongation rather than to an additional stimulation of initiation, possibly because the latter process is operating at maximal efficiency. We are currently examining the effect of Tat and E1A together on transcription rates in the 3′ end of the gene to assess the role of stabilization of transcription elongation in the synergy produced by these two *trans*-activators. These studies will be extended to an analysis of the basis of the synergy between Tat and other transcriptional activators, such as phorbol esters.

An earlier study from Peterlin's laboratory (Kao et al., *Nature 330:* 489 [1987]) reported that Tat increases the efficiency of transcription elongation without any effect on the level of transcription ini-

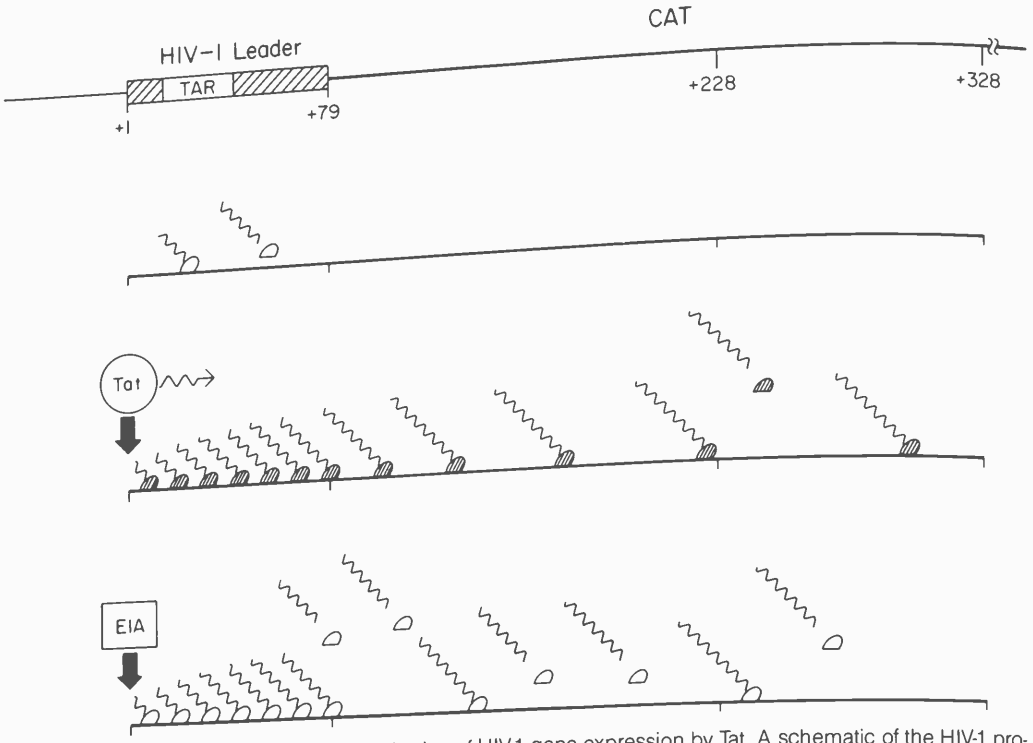

FIGURE 3 A model for transcriptional activation of HIV-1 gene expression by Tat. A schematic of the HIV-1 promoter fused to the CAT reporter gene is shown on the top line. In the absence of *trans*-activators (the second line), very few RNA polymerases (depicted as half ellipses) initiate transcription, and those that do disengage from the template after transcribing a short distance. On the third line, the HIV-1 *trans*-activator Tat interacts with the TAR element to promote the formation of a modified RNA polymerase complex (shaded ellipses) which initiates transcription efficiently and elongates stably, resulting in a large increase in expression of the reporter gene. In the presence of a general *trans*-activator, the adenovirus E1A protein (the fourth line), RNA polymerases initiate efficiently but undergo transcription termination, resulting in a small stimulation of CAT expression. (Reproduced, with permission, from Laspia et al., *Cell 59:* 283 [1989].)

tiation. This study differed from ours in several notable respects, any or all of which could account for the discrepant conclusions. First, whereas we used a recombinant adenovirus to deliver HIV-1 LTR-CAT template into human cells via viral infection, Kao and co-workers introduced plasmids containing the LTR-CAT sequences into monkey COS cells by transfection techniques. Second, the transfected plasmid contained an SV40 origin and replicated to high copy number in the COS cells, whereas the recombinant adenovirus experiments were conducted under nonreplicating conditions. Third, the plasmid construct and the adenovirus recombinant were derived from different sources (ARV2 and pU3RIII, respectively): They exhibit sequence differences in the promoter region and encode different lengths of HIV leader (186 and 83 nucleotides, respectively). Finally, there were of course variations in procedural details.

To assess the significance of these differences in a systematic fashion, we are conducting run-on transcription assays using nuclei isolated from COS cells transfected with replication-competent plasmids following the protocol of Kao et al. Preliminary data suggest that cotransfection of a Tat-expressing plasmid with the pU3RIII-based construct results in a substantial increase in transcription initiation and a detectable, but lesser, increase in elongation. This finding resembles that obtained with the recombinant adenovirus/HeLa cell system, although the basal level of HIV-directed transcription (observed in the absence of Tat) is higher in COS cells. However, a different result was obtained with the ARV2-based construct, where Tat functions primarily by increasing the efficiency of transcription elongation. Removal of the additional 3′ leader sequences (corresponding to parts of the R and U5 segments of the viral LTR) present in the ARV2-based construct but not in the pU3RIII construct did not change the result. This suggests that the source of the discrepancy resides in the promoter or the TAR region,

and current work is aimed at pinpointing the sequences responsible. Bearing in mind the results obtained in the adenovirus system, it is possible that the ARV2 promoter can reach its maximal initiation rate in the absence of Tat and the pU3RIII promoter cannot.

Structural Analysis of HIV Tat Proteins

A.P. Rice, C.O. Echetebu, F. Carlotti, M. Sullivan, R. Packer

We are interested in gaining an understanding of the structural features of the Tat protein that are important for its function. Such an understanding is crucial for the rational design of inhibitors of the protein that could be used in treating AIDS. Tat is essential for HIV replication, and the molecular mechanisms involved in Tat *trans*-activation appear to be unique to HIV-1, HIV-2, and some closely related lentiviruses. Inhibitors of Tat function may therefore specifically block HIV replication. The Tat protein from HIV-1 isolate HXB2 consists of 86 amino acids and has three regions with notable sequence features (Fig. 4A). The amino terminus is proline-rich, containing five prolines within the first 18 residues. This region is followed by a cysteine-rich region, with seven cysteines from residue 22–37. The cysteine-rich region is capable of binding metal ions, either Zn^{++} or Cd^{++}; Tat expressed and purified from *Escherichia coli* exists as a homodimer with four metal ions bound per dimer. The cysteine-rich region is followed by a basic region, with eight lysines or arginines between residues 49 and 57. The basic region serves as a nuclear localization signal, similar

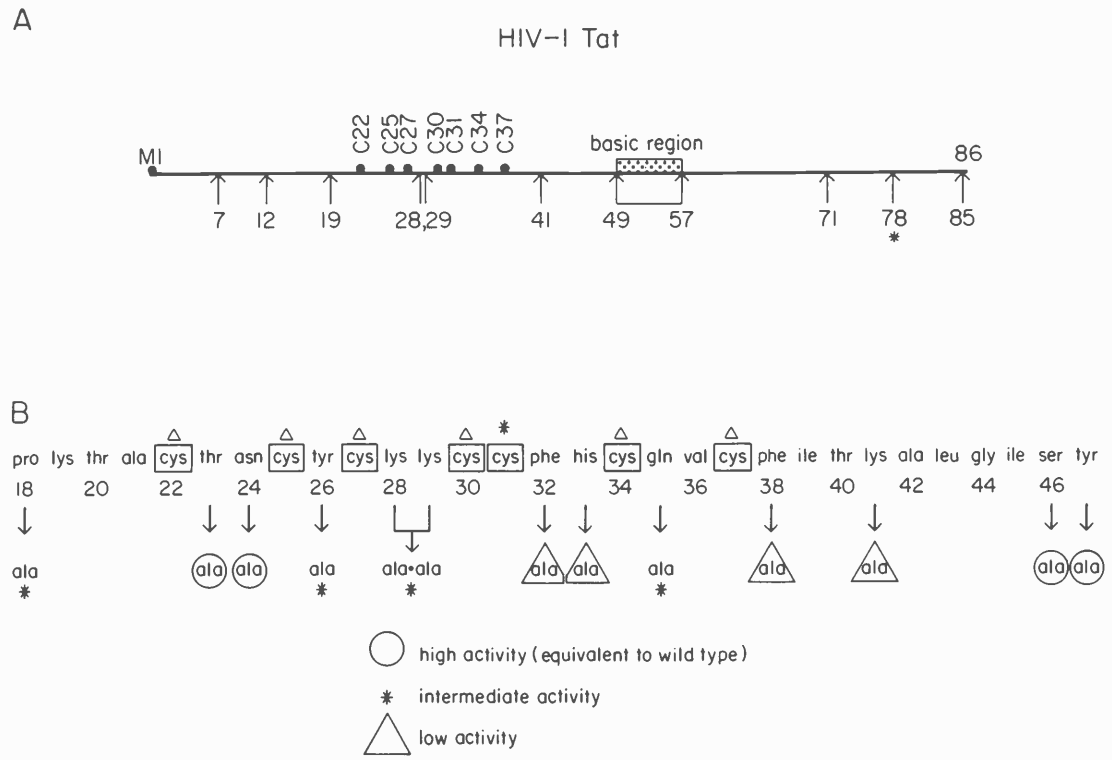

FIGURE 4 HIV-1 Tat protein. (*A*) The Tat protein from HIV-1 isolate HXB2 is shown. The initiator methionine and seven cysteine residues are indicated by closed circles; the basic region, a nuclear localization signal, is indicated by the box. Predicted sites of cleavage by trypsin are indicted by arrows: Note that the basic region contains eight cleavage sites. The trypsin cleavage site at residue 78 is indicated by an asterisk; a single cleavage of wild-type Tat at this site results in a molecule that is relatively resistant to subsequent cleavage. (*B*) The *trans*-activation activities of Tat proteins possessing single mutations between residues 18 and 47 are summarized. Those mutants indicated by circles are equivalent to wild type in activity, those indicated by asterisks possess 10–30% wild-type activity, and those indicated by triangles possess less than 10% wild-type activity. The cysteine mutants, analyzed by ourselves and other groups, contain substitutions of glycine or serine for cysteine. The noncysteine mutants contain substitution of alanine for the wild-type residue.

to that first observed and characterized in SV40 large T antigen.

Last year, we reported the construction and initial characterization of a collection of 24 mutant Tat proteins. Because of the small size of the Tat protein, the collection represents mutations throughout the entire molecule. In most cases, the mutant proteins contain substitutions of a single wild-type amino acid. The mutant proteins were constructed by site-directed mutagenesis, and their *trans*-activation activities were measured by plasmid DNA transfection experiments. Results of this analysis are summarized in Figure 4B. To investigate the structure-function relationships of the Tat protein, we expressed wild-type and mutant Tat in analytical amounts in a wheat germ cell-free translation system. Wild-type and 15 selected mutant cDNAs were cloned into a riboprobe vector, allowing the production of pure mRNAs by in vitro transcription. These mRNAs were translated in the wheat germ system, giving a single radiolabeled protein for structural analysis.

We initially employed trypsin as a probe for the structure of Tat protein, based on the rationale that, under limiting conditions, proteases digest proteins preferentially in unstructured regions. Trypsin first cleaves wild-type Tat near its carboxyl terminus, most likely at residue 78, and the singly cleaved Tat is then relatively resistant to further digestion. Some mutant Tat proteins with low *trans*-activation activities do not display this relative resistance to trypsin, being up to 100-fold more sensitive than wild-type Tat. Therefore, these mutant Tat proteins are significantly less structured than the wild-type protein when expressed in the wheat germ system. Their altered structure probably explains these mutants' low *trans*-activation activities in human cells. A second class of mutant Tat proteins also exhibit low *trans*-activation activity but display trypsin sensitivity identical to that of wild-type Tat. The mutations in this class may identify regions, or even individual amino acids, that are directly involved in protein-protein or protein-nucleic acid interactions that are important in the *trans*-activation process. Interestingly, to date, all the members of this second class of mutations, low in *trans*-activation activity but wild-type in overall structure, are located between residues 18 and 29.

Metal ions can be manipulated in the wheat germ system, and the effects on Tat structure can be analyzed. Pretreatment of wheat germ-expressed Tat with EDTA results in a more than tenfold increase in sensitivity to trypsin. Presumably, EDTA removes endogenous Zn^{++} or Cd^{++} bound to Tat and causes destructuring of the molecule. Addition of excess Cd^{++} after EDTA treatment results in partial restoration of Tat to its trypsin-resistant state. Initial experiments demonstrate that some mutant proteins are deficient in these apparent interactions with metal ions. We are currently assaying our collection of mutants to correlate *trans*-activation activity with the ability to interact with metal ions. Future work will also use wheat germ-expressed Tat to investigate direct binding of the Tat protein with TAR RNA.

We have initiated similar studies with the Tat protein of HIV-2, a human immunodeficiency virus isolated from western Africa. HIV-2 is closely related to HIV-1 both in genetic organization and in nucleotide sequence, but displays significant biological differences. It appears that a much lower percentage of individuals infected with HIV-2 develop AIDS. The HIV-1 and HIV-2 Tat-TAR systems share interesting cross-*trans*-activation properties: The HIV-1 Tat protein can fully *trans*-activate both the HIV-1 and HIV-2 LTRs; however, the HIV-2 Tat protein can fully *trans*-activate its cognate HIV-2 LTR but can only partially *trans*-activate the HIV-1 LTR.

To gain insight into structural features of Tat proteins important for the *trans*-activation process, we are analyzing similarities as well as differences between the HIV-1 and HIV-2 Tat proteins. As with Tat-1, the first coding exon of Tat-2 is fully competent for *trans*-activation activity. The first coding exon of Tat-2 encodes 99 amino acids, and the first coding exon of Tat-1 encodes 72 amino acids. The sequences of the two proteins are highly conserved in the center of the molecules, including the cysteine-rich and basic regions (residues 22–57 of Tat-1 and residues 49–84 of Tat-2), but their termini show considerable divergence. To define structural features of the Tat-2 protein that are important for activity, we are using site-directed mutagenesis to construct a collection of mutant proteins that will be assayed for *trans*-activation activity following the procedure described in last year's Annual Report for analysis of the HIV-1 Tat protein. We have inserted the first coding exon of the HIV-2 Tat gene into an expression vector, pSVL, that has three important features: (1) Tat is under control of the SV40 late promoter; (2) the plasmid contains the SV40 origin of replication, allowing high-level expression in COS cells; (3) the plasmid contains the bacteriophage f1 origin, allowing production of single-stranded DNA for mutagenesis and DNA sequence analysis. Preliminary assays have shown that the pSVL/Tat-2 expression

vector *trans*-activates the HIV-2 LTR by more than 20-fold and that the pSVL/Tat-1 and pSVL/Tat-2 expression vectors fully *trans*-activate the HIV-2 LTR, whereas only the pSVL/Tat-1 vector fully *trans*-activates the HIV-1 LTR.

We will use the pSVL/Tat-2 vector to construct and analyze mutant Tat-2 proteins. We will construct proteins truncated at the carboxyl terminus to determine the maximum deletion that retains activity. We expect, if Tat-1 can be used as a paradigm, that the Tat-2 protein truncated after residue 84 will retain partial activity, but further truncations will abolish activity. In addition, the amino terminus of Tat-2 is relatively acidic, containing 9 acidic residues within the first 49 amino acids, and is predicted to exist in an α-helical structure. The amino terminus of Tat-2 is therefore reminiscent of the activating domains of transcription factors that are sequence-specific DNA-binding proteins. To determine if the acidic residues in the amino terminus of Tat-2 are important for activity, we will substitute alanine for the wild-type residues in individual proteins. We will also construct chimeras between the Tat-1 and Tat-2 proteins in an attempt to define regions of the proteins involved in full *trans*-activation of the HIV-1 and HIV-2 LTRs.

PUBLICATIONS

Herrmann, C.H. and M.B. Mathews. 1989. The adenovirus E1B 19K protein stimulates gene expression by increasing DNA levels. *Mol. Cell. Biol.* **9:** 5412–5423.

Kostura, M. and M.B. Mathews. 1989. Purification and activation of the double-stranded RNA-dependent eIF-2 kinase DAI. *Mol. Cell. Biol.* **9:** 1576–1586.

Laspia, M.F., A.P. Rice, and M.B. Mathews. 1989. HIV-1 Tat protein increases transcriptional initiation and stabilizes elongation. *Cell* **59:** 283–292.

Mathews, M.B. 1989. The proliferating cell nuclear antigen, PCNA, a cell-cycle regulated DNA replication factor. In *Growth control during cell aging* (ed. E. Wang and H.R. Warner), pp. 89–120. CRC Press, Boca Raton, Florida.

Morris, G.F. and M.B. Mathews. 1989. Regulation of proliferating cell nuclear antigen during the cell cycle. *J. Biol. Chem.* **264:** 13856–13863.

O'Malley, R.P., R.F. Duncan, J.W.B. Hershey, and M.B. Mathews. 1989. Modification of protein synthesis initiation factors and shut-off of host protein synthesis in adenovirus-infected cells. *Virology* **168:** 112–118.

Rice, A.R., M. Kostura, and M.B. Mathews. 1989. Identification of a 90 KD polypeptide which associates with adenovirus VA RNA_I and is phosphorylated by the double-stranded RNA dependent protein kinase. *J. Biol. Chem.* **264:** 20632–20637.

In Press, Submitted, and In Preparation

Mellits, K.H., M. Kostura, and M.B. Mathews. 1990. Interaction of adenovirus VA RNA_I with the protein kinase DAI: Nonequivalence of binding and function. (Submitted.)

Morris, G.F. and M.B. Mathews. 1990. Response of the PCNA promoter to adenovirus E1 products. (In preparation.)

Rice, A.P. and F. Carlotti. 1990. Mutational analysis of the conserved cysteine-rich region of the human immunodeficiency virus type 1 Tat protein. *J. Virol.* (in press).

NUCLEIC ACID CHEMISTRY

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A.R. Krainer D. Kozak M. Wallace
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Isolation of the Spliceosome

G.C. Conway

Multiple lines of evidence indicate that during the process of pre-mRNA splicing, many factors remain stably bound to the RNA substrate, forming a macromolecular complex called the spliceosome. Although density gradient sedimentation and native gel analysis can be used to identify this stable complex, these techniques fail to purify the complex sufficiently to allow the identification of its protein constituents; in addition, after these techniques are used, the complex is not amenable to ultrastructural anal-

ysis. In an effort to isolate native spliceosomes, we have developed a new isolation technique.

Splicing extracts contain endogenous complexes that copurify with spliceosomes formed on exogenously added pre-mRNA substrates. Unlike several purification schemes previously used by others, our purification method involves the removal of these contaminating particles from extracts prior to the addition of exogenous RNA substrate. Splicing extracts are first incubated under splicing conditions without the addition of exogenous RNA substrate. We have found that during this preincubation, several splicing factors, including the small nuclear ribonucleoprotein particles (snRNPs), are released from large complexes. This pretreated extract is then subjected to sucrose gradient fractionation, and material less than 30S is collected. These upper gradient fractions contain all of the factors necessary for in vitro pre-mRNA splicing. If this extract is then used for in vitro splicing with the addition of the exogenous pre-mRNA substrate and fractionated by sucrose gradient sedimentation, the spliceosomes formed on the exogenous substrate sediment as a 45S complex into a clean portion of the gradient.

Using these pre-cleared extracts, we have investigated the kinetics of spliceosome formation using native gel techniques. With the pre-cleared extract, complex formation is accelerated, perhaps due to the prior release of splicing factors from endogenous complexes. Cleared extracts have also allowed us to analyze the snRNA composition of the spliceosome, and, as noted by others, we find that the abundant nucleoplasmic snRNAs U1 through U6 are present in the spliceosome. In agreement with other investigators, we have found that the U4 snRNA is underrepresented in the spliceosome, consistent with the model that U4 snRNP leaves the spliceosome prior to the first step of splicing. We are presently conducting ultrastructural studies of splicing complexes in collaboration with David Spector (Molecular Genetics of Eukaryotic Cells Section). Our approach is to use biotinylated pre-mRNA substrates in splicing reactions with the pre-cleared extracts. Purified splicing complexes are being tagged using 1-nm streptavidin gold particles. It is hoped that the substantial purification which we achieve using cleared extracts coupled with gold particle tagging will allow us to identify splicing complexes unambiguously. This should open the door to more-detailed ultrastructural characterizations such as epitope mapping using monoclonal antibody probes.

Biochemistry of Mammalian Pre-mRNA Splicing

A.R. Krainer, D. Kozak, N. Pecina

The long-term aim of our research is to obtain a detailed understanding of the mechanism of pre-mRNA splicing in mammalian cells. In particular, we are interested in determining how the RNA cleavage-ligation reactions are catalyzed and how the specificity of splice-site selection is achieved. As part of this effort, we are purifying several of the nucleoprotein and protein factors that are necessary for cleavage of the pre-mRNA at the 5′ splice site and for lariat formation. Our general strategy is to develop complementation assays for individual activities, such that one or both cleavage-ligation reactions are strictly dependent on the presence of the active component in question. The factors responsible for these activities are purified and then characterized to determine their mode of action. The identification and detailed characterization of splicing factors should provide crucial insights into the mechanism of pre-mRNA splicing, the specificity of splice-site selection, and the origin and evolution of the splicing machinery and of pre-mRNA introns.

Purification and Characterization of Splicing Factors

A.R. Krainer, D. Kozak [in collaboration with G. Conway, Cold Spring Harbor Laboratory]

SF2 PURIFICATION

Our previous work demonstrated that the cytoplasmic S100 fraction obtained during the preparation of nuclear extracts contains active small nuclear ribonucleoproteins (snRNPs) and other splicing factors, but it is usually deficient in at least one protein activity, termed SF2, which is necessary for the first cleavage-ligation of pre-mRNA splicing. The reason for the selective retention of SF2 in the nuclear fraction in a hypotonic buffer is not known. It may be that the polypeptides responsible for SF2 activity are tightly bound to the nuclear membrane or to a subnuclear structure. Alternatively, SF2 is part of large RNP complexes, which cannot traverse the nuclear pores in their intact form. In fact, sedimentation analysis showed that SF2 and other splicing factors are associated with large endogenous RNP com-

plexes in nuclear extracts. To facilitate the purification of SF2, we developed a different extract preparation procedure, which maximizes the recovery of SF2, as judged from titrating the extracts against a constant amount of complementing S100 fraction. From this extract, SF2 has been purified to apparent homogeneity by conventional chromatography and FPLC, using complementation of the S100 extract as a functional assay.

The polypeptide composition of the fractions from the last column, Phenyl Superose, was analyzed by SDS-PAGE to try to identify the polypeptide(s) responsible for SF2 activity (Fig. 1). In the first active fraction (Fig. 1A, lane 3), only a doublet of 33 kD is detectable by Coomassie blue (Fig. 1B, lane 3) or silver staining. Complementing activity peaks in the adjacent fraction (lane 4), which also corresponds to the 33-kD doublet peak. This fraction contains additional polypeptides. Complementing activity was also detectable in the next three fractions, although it did not correlate well with the presence of the 33-kD doublet. A second peak of activity was detected in fraction 16, which contains, among other bands, a polypeptide that comigrates with the top band of the 33-kD doublet. On the basis of these data, in particular the presence of complementing activity in fraction 3, we conclude that SF2 activity is most likely encoded by one or both of the 33-kD polypeptides, although there may also be other polypeptides with SF2 activity. At this stage, we cannot rule out the existence of very minor polypeptides that are responsible for activity. We have shown that the 33-kD polypeptides are distinct from the heterogeneous nuclear RNP (hnRNP) A1 and U1 snRNP A polypeptides, which are similar in size. A small amount of endogenous hnRNA copurifies with the SF2 polypeptides, but SF2 activity does not appear to require an essential RNA component, as judged from its resistance to micrococcal nuclease treament.

RNA-BINDING PROPERTIES OF SF2

To determine whether purified SF2 binds RNA, several types of analyses were carried out. Ultraviolet cross-linking demonstrated binding to pre-mRNA and to mRNA. In this type of analysis, when the cross-linked material is extensively digested with ribonuclease A, the protein-oligoribonucleotide adducts usually comigrate or migrate slightly behind the untreated polypeptides on SDS-PAGE. In the case of SF2, the labeled adducts comigrate with the 33-kD doublet, as expected if both polypeptides bind RNA. No RNA binding was detected by Northwestern blotting, but this could be due to poor renaturation after SDS-PAGE. We have not been able to separate the two polypeptides by nondenaturing techniques, and thus we cannot presently determine whether only one or both are responsible for the observed activities. However, amino acid analysis of the individual electrophoretically separated polypeptides suggests that they are highly related. The above experiments showed more efficient cross-linking to mRNA than to pre-mRNA, which may reflect the higher-order structure of these RNAs or perhaps efficient binding or cross-linking to the spliced exon borders.

Footprinting experiments were carried out by partial modification of globin pre-mRNA with dimethylsulfate, followed by primer extension, and by partial digestion of end-labeled pre-mRNA with ribonucleases. No specific footprint was observed, suggesting that at least in the absence of other components, SF2 does not bind pre-mRNA in a sequence-specific manner. However, since we do not know what fraction of the SF2 molecules was active in these experiments, and since detection of a footprint requires efficient binding, sequence-specific RNA binding by SF2 cannot be completely ruled out.

SF2 IS REQUIRED FOR SPLICEOSOME FORMATION

The complementation assay employed for the purification of SF2 indicates that this activity is required for cleavage of the pre-mRNA at the 5′ splice site and lariat formation. This reaction is normally preceded by the assembly of specific pre-spliceosome and spliceosome complexes, in which pre-mRNA interacts in a stable manner with multiple components of the splicing apparatus, such as the U snRNPs. To determine the requirement for SF2 in this assembly pathway, RNP complexes were analyzed by a gel-retardation assay. Under the electrophoretic conditions employed, three specific complexes are normally detected. When pre-mRNA is incubated with the S100 extract under splicing conditions, no specific complexes are detected. Incubation of pre-mRNA with purified SF2 also fails to generate specific complexes of discrete mobility. A smear is detected under these conditions, which is consistent with nonspecific RNA binding by SF2, as detected with the assays described above. In contrast, when pre-mRNA is incubated with purified SF2 and S100,

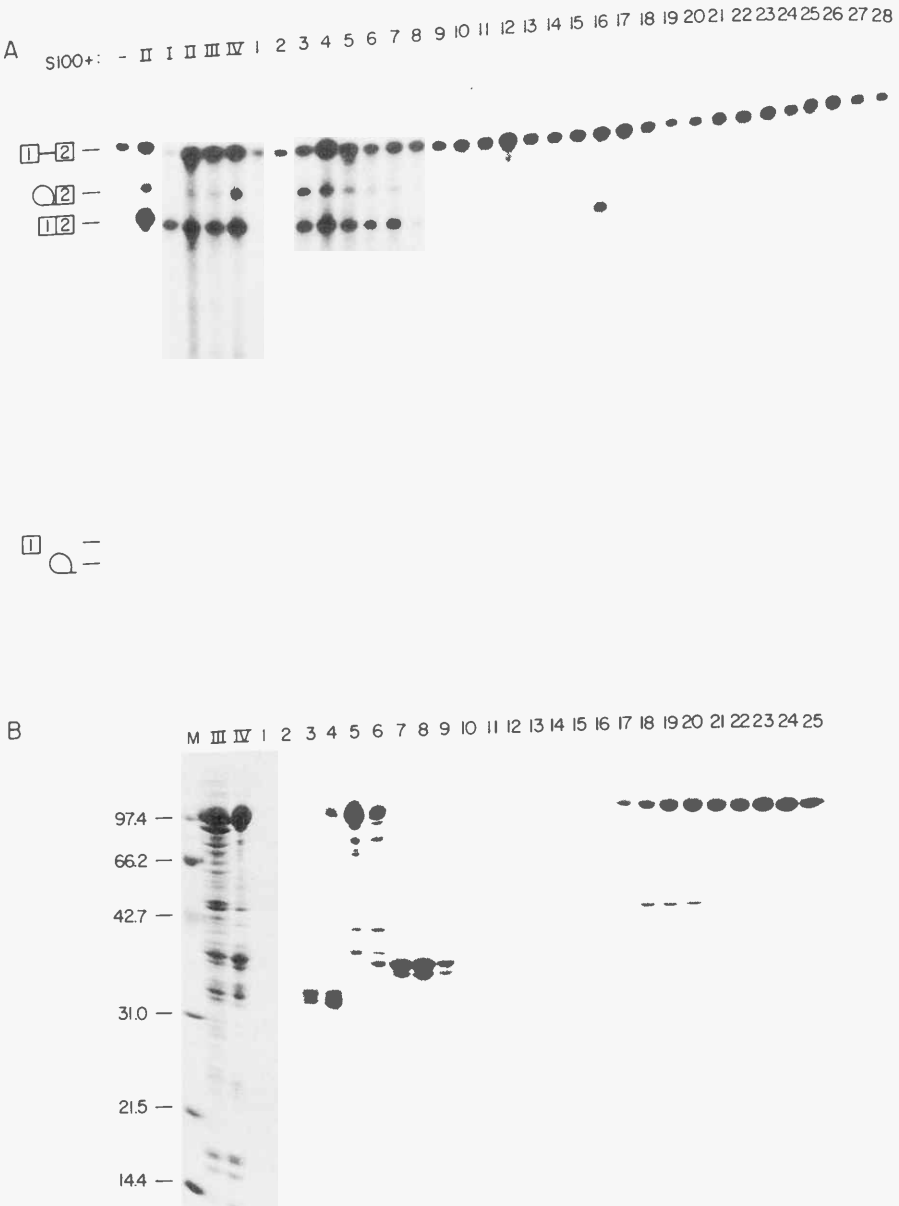

FIGURE 1 Phenyl Superose column profile. (*A*) SF2 complementation assay. Splicing reactions were carried out with the S100 extract plus the indicated chromatographic fractions. Roman numerals indicate fractions from preceding chromatographic steps. Numbers 1 through 28 correspond to the Phenyl Superose fractions. The pre-mRNA, intermediates, and products of splicing are indicated at the left of the autoradiogram. (*B*) Polypeptide composition. The proteins in the indicated fractions were analyzed by SDS-PAGE, followed by Coomassie blue staining.

three discrete complexes are detected, which have the same mobility as the specific complexes obtained with the nuclear extract. We conclude that SF2 is required for the assembly or the stabilization of the earliest specific complex in the spliceosome assembly pathway, which is thought to be the A complex.

SF2 HAS AN RNA ANNEALING ACTIVITY

We have tested purified SF2 for possible RNA helicase and/or annealing activities (Fig. 2). Complementary ^{32}P-labeled RNAs were incubated with purified SF2 in splicing buffer in the presence or absence of ATP. After incubation, digestion with

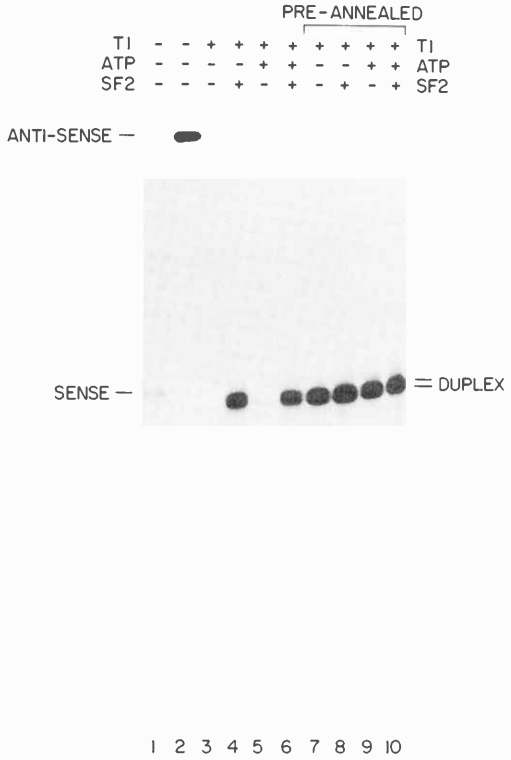

FIGURE 2 RNA annealing and helicase assays. Individual or pre-annealed complementary RNAs were incubated as described in the text and figure diagram and digested with ribonuclease T1. The digestion products were analyzed by denaturing PAGE and autoradiography.

ribonuclease T1 was carried out, and nuclease-resistant double-stranded RNAs were detected by denaturing PAGE. In the absence of protein, complementary RNAs do not anneal under these buffer conditions at 30°C, due to the higher-order structures of the individual strands (Fig. 2, lanes 3 and 5). Annealing requires an initial incubation at high temperature to eliminate these intramolecular helices. However, in the presence of SF2, rapid annealing can take place at 30°C, with or without ATP (Fig. 2, lanes 4 and 6). When the complementary strands are pre-annealed, SF2 does not melt them, even when ATP is present (Fig. 2, lanes 8 and 10). In summary, SF2 lowers the activation energy for intermolecular RNA duplex formation, and thus behaves as an RNA annealing activity. The 35-kD hnRNP A1 protein has been shown to have helix destabilizing properties on double-stranded RNA. Under some conditions, this lowering of the melting temperature by hnRNP A1 can promote RNA annealing (S. Munroe, pers. comm.). We therefore tested purified A1 (kindly provided by S. Wilson, National Institutes of Health) to see if it could substitute for SF2 in the splicing complementation, exon skipping (see below), and RNA annealing assays. Concentrations of A1 that were active in RNA annealing did not substitute for SF2 in the other assays. These observations suggest that if RNA annealing activity is integral to SF2 and necessary for splicing, it is not sufficient, implying that specific protein-protein or protein-RNA interactions are involved in mediating the role of SF2 in splicing and splice-site selection.

Further experiments are necessary to elucidate the precise substrate specificity, if any, of the SF2 RNA annealing activity. However, we can envisage three types of complementary RNA targets upon which the SF2 RNA annealing activity could act to mediate its effects on splicing and splice-site selection. First, at least two snRNA–pre-mRNA helices are known to be formed during splicing and to contribute to splice-site selection; these involve base-pairing interactions between the 5′ terminus of U1 snRNA and pre-mRNA 5′ splice sites and between an internal region of U2 snRNA and pre-mRNA branchpoint sequences. Second, U4 and U6 snRNAs coexist in a single snRNP particle held together by intermolecular base pairs, and there is some evidence that the U4 subunit may be released from the spliceosome during the course of splicing, possibly to be recycled. Third, pre-mRNA may be recognized as a proper splicing substrate when it adopts a defined secondary and tertiary structure. Some of the intramolecular RNA helices may preferentially expose the splice sites and branchpoint.

SF2 INFLUENCES 5′ SPLICE SITE SELECTION

U1 and U2 snRNAs play important roles in splice-site selection, by virtue of their ability to interact by intermolecular RNA-RNA base pairing with 5′ splice sites and branchpoint sequences, respectively. However, these interactions are not sufficient to account for the specificity of splice-site selection. Splice sites are often surrounded by cryptic splice sites that are only activated upon mutation of the natural splice sites. It is not known whether the same set of factors that recognize natural splice sites are also capable of recognizing the cryptic sites when the natural sites are mutated. Furthermore, although 5′ and 3′ splice sites from different introns are usually compatible, the splicing machinery normally avoids exon skipping, by an unknown mechanism. Many genes are capable of expressing multiple protein isoforms by using alternative 5′ and/or 3′ splice sites, often

in a regulated manner. Again, it is not known how this type of mechanism is controlled. Finally, it appears that in addition to the conserved 5′, 3′, and branchpoint sequence elements, additional, poorly defined sequences in exons and introns can also influence splice-site selection and splicing efficiency. It is not known how these context elements exert their effects. We have investigated whether SF2 plays a role in splice-site selection, in addition to its more general role in the splicing reaction.

The in vitro splicing system derived from HeLa nuclear extracts generally follows the same hierarchy rules that are followed in the cell, with respect to the recognition of bona fide versus cryptic splice sites. For example, a β-thalassemic allele of human β-globin that contains a G→A transition at position 1 of the first intron cannot express normally spliced mRNA in detectable quantities, either in vivo or in vitro. Instead, three cryptic 5′ splice sites are activated (Fig. 3, top). The predominant spliced mRNA detected in vitro corresponds to use of the first upstream cryptic 5′ splice site, whereas the second upstream cryptic 5′ splice site and the downstream cryptic 5′ splice site are used only in a small proportion of the pre-mRNA molecules (Fig. 3, bottom, lane 11). It is noteworthy that these cryptic sites are completely silent in the context of the wild-type allele.

When wild-type β-globin pre-mRNA is spliced in the reconstituted in vitro system consisting of crude S100 extract and pure SF2, the intermediates and products are virtually indistinguishable from those obtained with the nuclear extract (Fig. 3, lanes 2 and 4). When pre-mRNA derived from the thalassemic allele is spliced in the same system, no wild-type mRNA is generated, and two of the three cryptic sites are activated (Fig. 3, lane 13). Surprisingly, the downstream cryptic 5′ splice site is now the preferred site, whereas the first upstream cryptic is used at a low level, and the second upstream cryptic is not used at a detectable level (compare lanes 13 and 11). To determine whether this unique pattern reflects differences in the concentration of SF2 and/or other differences between the two extracts, we carried out titrations of SF2 while holding the amount of S100 extract constant. The extent of utilization of the downstream cryptic site was directly proportional to the concentration of SF2 (Fig. 3, lanes 12–16). In the absence of SF2, the S100 alone was unable to support any splicing reaction with either the wild-type or the mutant pre-mRNA (not shown). With the mutant substrate, the first upstream cryptic site was used at a roughly constant low level over a broad range of SF2 concentration. Remarkably, the highest concentration of SF2 inhibited all splicing with the mutant but not with the wild-type pre-mRNA (Fig. 3, lanes 3 and 12).

We next tested whether addition of excess purified SF2 to the nuclear extract would affect splice-site selection. No effect was observed with the wild-type pre-mRNA (Fig. 3, lanes 9 and 10); in contrast, with the thalassemic pre-mRNA, addition of SF2 again activated the downstream cryptic 5′ splice site (Fig. 3, lanes 18 and 19). The first upstream cryptic site was still used at a high level, but the second upstream cryptic site was inactive. These results indicate that the concentration of SF2 can affect the use of cryptic 5′ splice sites. In particular, the internal cryptic site is favored. We also infer that there are other differences between the nuclear extract system and the S100 plus SF2 system. The nuclear extract already contains some SF2, which is an essential splicing factor. Even when further supplemented with purified SF2, the nuclear extract still efficiently used the first upstream cryptic splice site, whereas under all conditions employed, the S100 system could only use this site inefficiently.

Previous studies have shown that the sequence context surrounding splice sites could influence their use by the splicing apparatus. *cis*-competition studies employing duplicated splice sites have demonstrated this context effect and have also suggested that when comparable splice sites are present in *cis*, the splicing machinery favors the use of the internal sites. However, if the internal sites are weaker, due, for example, to an unfavorable sequence context, then the external sites are preferred. We have employed several pre-mRNAs containing duplicated 5′ or 3′ splice sites (kindly provided by R. Reed, Harvard Medical School) to analyze the mode of action of SF2 (Fig. 4). Substrate 2 contains a short duplication of the β-globin first intron 5′ splice site and surrounding sequences. Substrate 3 contains a longer duplication of this site. As shown previously, the external 5′ splice site is used when substrate 2 is spliced in nuclear extracts (Fig. 4, lane 2, NE), due to an insufficient sequence context surrounding the internal duplicated site. In the case of substrate 3, which contains a larger duplication, the internal 5′ splice site is used exclusively (Fig. 4, lane 3, NE). When the same substrates are spliced by S100 extract plus purified SF2, the internal splice site is used exclusively, even in the case of substrate 2, which contains the short duplication (Fig. 4, lanes 2 and 3, S100 + SF2). When the nuclear extract is supplemented with SF2, there is a par-

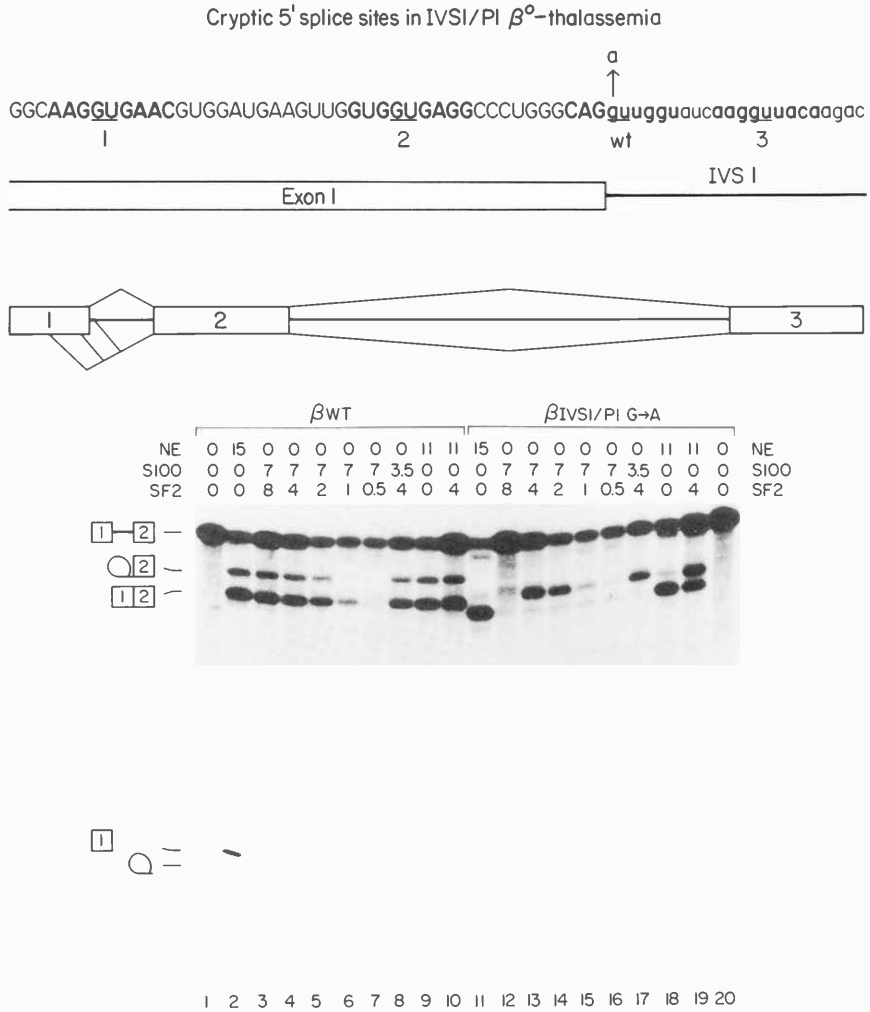

FIGURE 3 Effect of SF2 on cryptic 5′ splice-site utilization. (*Top*) The structure of human β-globin pre-mRNA is shown at the bottom of the diagram, with all potential splices indicated. The nucleotide sequence of the region surrounding the first intron 5′ splice-site is shown at the top. The natural and cryptic 5′ splice-site sequence elements are indicated in boldface, with the invariant GU dinucleotides underlined. The single-base β-thalassemia mutation that results in cryptic 5′ splice-site activation is shown. The exon-intron boundary is shown schematically below the sequence. (*Bottom*) Splicing reactions (25 μl) were carried out with the indicated microliter amounts of nuclear extract (NE), S100 fraction, and purified SF2. The substrates were truncated pre-mRNAs containing the first two exons and first intron of wild-type (lanes *1–10*) or thalassemic (lanes *11–20*) human β-globin alleles. The electrophoretic mobilities of wild-type pre-mRNA, intermediates, and splicing products are indicated schematically at the left of the autoradiogram. Wild-type mRNA is 367 nucleotides; mRNA spliced via cryptic splice site 1 is 329 nucleotides; mRNA spliced via cryptic splice site 2 is 351 nucleotides; and mRNA spliced via cryptic splice site 3 is 379 nucleotides.

tial switch to the internal duplicated 5′ splice site in the case of substrate 2 (Fig. 4, lane 2, NE + SF2). Thus, high concentrations of SF2 can compensate for the poor context surrounding the internal duplicated 5′ splice site.

Two additional substrates containing different size duplications of the human β-globin first intron 3′ splice site were also tested in the different extracts. In nuclear extract, substrate 4 is spliced exclusively via utilization of the external duplicated site. In contrast, substrate 5, which contains a larger duplication, uses predominantly the internal site, although, in a minor proportion of the pre-mRNA molecules, the external 3′ splice site is chosen. Identical splic-

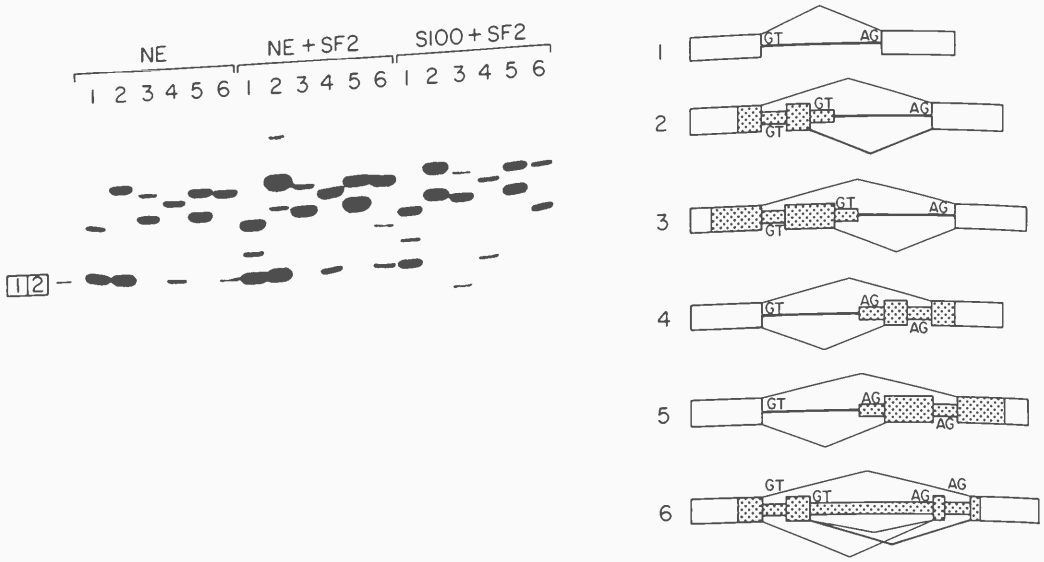

FIGURE 4 Effect of SF2 on artificial alternative 5′ splicing substrates. The six pre-mRNA substrates are shown schematically at the right of the autoradiogram. Shaded areas represent the duplicated exon and intron sequences. Substrate 1 is a wild-type β-globin truncated pre-mRNA. Substrates 2 and 3 contain duplications of the first intron 5′ splice site and surrounding exon and intron sequences. Although not shown in the diagram, pre-mRNA 2 is longer than pre-mRNA 3 due to the presence of non-β-globin sequences at the duplication junction. Substrates 4 and 5 contain duplications of the first intron 3′ splice site and surrounding exon and intron sequences. Substrate 6 contains duplications of both the 5′ and 3′ splice sites and their respective surrounding sequences. The angled lines represent all the possible splicing events. The bold angled lines represent splicing events that are promoted by high concentrations of SF2. Splicing reactions were carried out with nuclear extract, nuclear extract supplemented with additional purified SF2, and S100 extract plus purified SF2, as indicated at the top of the autoradiogram.

ing patterns were obtained with the S100 extract supplemented with SF2. With nuclear extract supplemented with SF2, there was slightly less efficient use of the external 3′ splice site. These results indicate that SF2 does not strongly influence the selection of competing 3′ splice sites. Finally, a construct containing duplications at both 5′ and 3′ splice sites was processed in the different in vitro systems (substrate 6). In nuclear extract, the external 5′ and 3′ splice sites were recognized, as expected from previous studies. However, in the S100 + SF2 system, or, to some extent, in SF2-supplemented nuclear extract, the internal 5′ splice site and the external 3′ splice site were selected.

In summary, SF2 promotes the utilization of proximal 5′ splice sites and has no effect on 3′ splice-site selection. The molecular mechanism responsible for this effect remains unknown, but its elucidation should help clarify our understanding of splice-site selection. Since the observed effect is targeted at 5′ splice sites, it is tempting to speculate that it relates to the U1 snRNA-5′ splice-site interaction, perhaps involving the intermolecular RNA-RNA annealing activity described above. On the other hand, the U2 snRNA-branchpoint interaction could also have an indirect effect on 5′ splice-site selection, since the branchpoint A and the 5′ terminus of the intron are joined to form a lariat. It is unclear, however, and of great importance why high concentrations of SF2 promote selection of the proximal 5′ splice sites. We plan to examine more substrates to determine whether this is simply a coincidence and thus whether in some cases, distal sites are favored.

The striking effect of SF2 on in vitro 5′ splice-site selection in mutant and artificial constructs suggests a plausible mechanism for the in vivo regulation of alternative splicing in at least some cases. A variety of transcription units are alternatively spliced in a tissue-specific or developmentally controlled manner. In those cases involving alternative 5′ splice-site utilization, regulation might be accomplished by controlling the levels of activity of SF2 or a similar factor. Regulation of SF2 activity could be achieved by posttranslational modification. Analogous mechanisms could operate to regulate 3′ splice-site selection. Our observations show that the concentration of an essential splicing factor can affect splice-site selection. It seems likely that this also applies in the

case of other splicing factors as well. In collaboration with D. Helfman (Molecular Genetics of Eukaryotic Cells Section), we are currently analyzing whether SF2 can influence alternative splicing of rat tropomyosin pre-mRNAs in vitro.

CLONING OF SF2 AND OTHER BIOCHEMICAL STUDIES

We are presently trying to elucidate the structural relationships between the two SF2 polypeptides. This task has proved difficult due to our inability so far to obtain antibodies that react by a Western blot assay. Antibodies generated against native SF2 immunoprecipitate a doublet of 33K polypeptides and a 55K polypeptide from ^{35}S-labeled HeLa cells. We do not know at present if the 55K band represents a subunit of SF2 or a cross-reacting polypeptide. The purified 33K polypeptides have been isolated electrophoretically, and their amino acid composition was determined by the Protein Chemistry Facility. By this criterion, the polypeptides appear to be related. The SF2 polypeptides also yielded a single, limited amino-terminal amino acid sequence. This sequence is identical to that of a slightly smaller polypeptide, which is inactive, and may arise by partial proteolysis. On the basis of the latter amino acid sequence, we used the polymerase chain reaction (PCR) to generate a specific probe from first-strand HeLa cell cDNA. This unique probe was used to isolate a cDNA clone. We are currently determining the nucleotide sequence of this cDNA, as well as generating antibodies against a fusion protein expressed in *Escherichia coli*. These experiments should help clarify the structural relationships among the various polypeptides and allow a more precise definition of the structure and function of SF2.

Another project is being done in collaboration with G. Conway (Tumor Viruses Section), to continue the purification of SF4B, another polypeptide factor necessary for 5′ splice-site cleavage and lariat formation. When the purification is completed, SF4B will be used together with SF2 and our purified snRNPs to attempt to reconstruct the prespliceosome and spliceosome assembly pathway and to determine whether these factors are sufficient to catalyze the first cleavage-ligation reaction.

PUBLICATIONS

Conway, G.C., A.R. Krainer, D.L. Spector, and R.J. Roberts. 1989. Multiple splicing factors are released from endogenous complexes during *in vitro* pre-mRNA splicing. *Mol. Cell. Biol.* **9:** 5273–5280.

In Press, Submitted, and In Preparation

Conway, G.C., A.R. Krainer, and R.J. Roberts. 1990. RNA splicing in ribonucleoprotein depleted nuclear extracts. (Submitted.)

Conway, G.C., A.R. Krainer, and R.J. Roberts. 1990. Spliceosome formation in splicing extracts depleted of large endogenous RNP complexes. (In preparation.)

Krainer, A.R., G. Conway, and D. Kozak. 1990. Purification and characterization of SF2, a human pre-mRNA splicing factor. (Submitted.)

Krainer, A.R., G. Conway, and D. Kozak. 1990. The essential pre-mRNA splicing factor SF2 influences 5′ splice site selection by activating proximal sites. (Submitted.)

TRANSCRIPTIONAL CONTROL

W. Herr
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J. Ambro, R. Aurora, M. Cleary, G. Das, J.-S. Lai, L. Leo, V. Meschan, B. Ondek, W. Phares, V. Shick, S. Stern, R. Sturm, W. Thomann, B. Whelan, K. Zito

Regulation of transcription in mammalian cells is best characterized as combinatorial and modular. Unlike the more streamlined and highly evolved bacterial regulatory networks, eukaryotic transcription, particularly in multicellular organisms, is controlled by baroque arrays of *cis*-acting binding sites for *trans*-acting factors. This complexity is increased by the propensity of proteins that activate transcription to bind to grossly divergent DNA sequences and for multiple different transcription factors to bind to the

same site. These transcription factors are frequently modular, consisting of separate domains for DNA binding (e.g., homeodomains and zinc fingers) and transcriptional activation (e.g., acidic and glutamine-rich domains). To probe transcriptional regulation in mammalian cells, we study the bidirectional SV40 transcriptional regulatory region. In past years, we analyzed the arrangement of *cis*-acting elements; more recently, we have characterized *trans*-acting factors that regulate transcription.

Our analysis of transcription factors has been concentrated on understanding differential transcriptional activation by the ubiquitous and lymphoid-specific octamer motif-binding proteins Oct-1 and Oct-2. The recent discovery that these two activators are homeodomain proteins and therefore related to proteins that direct development of the fruit fly *Drosophila melanogaster* has integrated our transcriptional studies with studies of development. One of the challenges in the field of *Drosophila* development is to understand how homeodomain proteins that bind to very similar DNA sequences can have very different effects on development. Because Oct-1 and Oct-2 bind to the same DNA sequence but are known to activate transcription differentially, they serve as examples of how developmental regulation may occur in *Drosophila*. Last year, we discovered that protein modification and protein:protein interactions involving Oct-1 and Oct-2 can be used to distinguish between homeodomain proteins that bind to the same sequence.

Transcriptional Activation by SV40 Enhancer Elements

B. Ondek

During past years, much of our effort was devoted to understanding the underlying structure of the SV40 enhancer. These studies, together with studies by P. Chambon and colleagues (Strasbourg), showed that the SV40 enhancer is composed of many different individual units or *cis*-acting elements that come together in different ways to create an enhancer. The individual units, which we refer to as enhansons, represent protein-binding sites and can be classified into different groups by their intrinsic ability to enhance transcription. Thus, certain enhansons (class A) can cooperate with copies of themselves or other enhansons to activate transcription, but only when appropriately juxtaposed in pairs. The appropriately juxtaposed binding sites are referred to as protoenhancers because duplication of these elements, without strict spacing requirements, can create a potent enhancer. Enhansons of the second class, class B, cannot enhance transcription alone or as duplicates of themselves but instead must cooperate with a class-A enhanson to be active. The third class of enhansons (class C) does not require precise juxtaposition with a second enhanson for activity, and thus these enhansons individually display protoenhancer activity. The octamer motif (ATGCAAAT) found within the SV40 enhancer is an example of a class-C enhanson. The large number and multiple activities of SV40 enhansons help explain the complex structure of this enhancer.

We have now compared the activity of enhansons and protoenhancers both near and far from the transcriptional start site and in the context of either an extended upstream promoter region or a truncated promoter containing only a TATA box. The first series of studies were designed to test whether individual class-A enhansons, which are inactive as enhancers when positioned at a distance, can activate transcription when positioned near the start site. Analysis of individual or inappropriately duplicated coreA enhansons revealed, however, that they cannot activate transcription even when positioned near the transcriptional start site. Thus, a functional protoenhancer is required for transcriptional activation even when distance is not an issue.

The second series of studies were directed at the question of how the transcriptional activation potential of an enhancer is targeted to a specific transcriptional start site. There must be considerable DNA sequence information required to target enhancer function effectively, because enhancers can be positioned at large distances from the transcriptional start site without activating many closer cryptic promoters. Use of two truncated β-globin promoters, one containing CCAAT and CACCC proximal promoter elements ($p\beta\Delta^{127}$) and the other truncated to the TATA box ($p\beta\Delta^{36}$) showed that only the extended promoter could respond effectively to a distal enhancer. The TATA-box-only promoter was very sensitive to the distance between the enhancer and the TATA box. Thus, an enhancer with two copies of a highly active coreA/coreA protoenhancer, although virtually inactive at a distance, was a potent activator close to the TATA box. A single coreA protoenhancer, however, was relatively inactive in a proximal position. Although the single protoenhancer element is a weak activator positioned near the TATA

box, it confers on the TATA box the ability to respond effectively to a distal enhancer. Thus, the requirements for autonomous activation (two protoenhancers) and for targeting enhancer activity (one protoenhancer) apparently differ. By testing the ability of a series of truncation mutants of the herpes simplex virus *trans*-activator VP16 to activate transcription and target enhancer function, we have shown that mutants that do not effectively activate transcription alone because they lack an acidic activation domain can still target enhancer activity. Thus, these results suggest that targeting enhancer activation and direct activation itself are different, albeit perhaps overlapping, properties of *trans*-acting factors.

Structure and Function of Oct-1

R. Aurora, G. Das, J.S. Lai, V. Shick,
R. Sturm, K. Zito

We have been using the mammalian octamer motif (ATGCAAT)-binding proteins Oct-1 and Oct-2 as models to understand how two proteins can bind to the same DNA sequence and differentially activate transcription. Oct-1 and Oct-2 are members of a new class of homeodomain proteins called POU (for *P*it, *O*ct, *U*nc). The founding members of the POU class of homeodomain proteins include the mammalian pituitary-specific factor Pit-1/GHF-1 and the nematode developmental gene product *unc-86*. This new class of homeodomain proteins is distinguished by the presence of an extended region of sequence similarity approximately 160 amino acids long. The carboxy-terminal portion contains the 60-amino-acid-long homeodomain, and the amino-terminal region contains a POU-specific sequence of about 75 amino acids. A nonconserved region of 15–27 amino acids separates these two conserved domains. Deletion analysis and point mutagenesis showed that both of the POU subdomains (POU-specific and POU-homeo) are involved in sequence-specific DNA binding, whereas the exact structure of the nonconserved region is not critical for binding.

We are now examining the contribution of the POU-specific and POU-homeodomain regions in sequence-specific DNA binding. By using large amounts of the entire Oct-1 POU domain and the homeodomain alone isolated from genetically engineered *Escherichia coli*, we have shown that the homeodomain retains sequence-specific DNA-binding activity but at greatly reduced affinity compared to the POU domain.

The two POU proteins Oct-1 and Pit-1 share related but distinct DNA recognition properties. To understand the contribution of the POU-specific and POU-homeodomain regions for recognition of the different DNA-binding sites by Oct-1 and Pit-1, we have constructed a series of chimeric Oct-1/Pit-1 POU domains in which the two different segments of the POU-specific region (A and B boxes), the linker, and the homeodomain of Oct-1 are exchanged for Pit-1 sequences. The chimeric proteins reveal that both the POU-specific and POU-homeodomain regions contribute to sequence-specific DNA recognition. Curiously, however, the relative contribution of each region differs among different binding sites. We are now examining whether the POU-specific region contributes to sequence-specific DNA binding by making contact with the DNA or instead by modulating the conformation of the homeodomain bound to DNA.

Following the initial isolation of a cDNA clone encoding Oct-1, we have continued our analysis of the *oct-1* gene by isolating multiple cDNA and genomic clones and performing an expression analysis in different adult mouse tissues. Analysis of the *oct-1* expression pattern in adult mice showed that as is the case in cell culture, Oct-1 is ubiquitously expressed. Analysis of 16 different human Oct-1 cDNAs revealed one cDNA encoding an alternatively spliced form of *oct-1* mRNA that can encode an Oct-1 protein in which the carboxyl terminus of the protein is truncated and replaced by a short 12-amino-acid sequence. This alternatively spliced form, called *oct-1B*, is expressed at 5- to 20-fold lower levels than the original *oct-1* mRNA in all human cell lines tested. We continue efforts to detect the truncated Oct-1 protein, but it is apparently present in low amounts or modified. Nevertheless, when translated in vitro, the truncated Oct-1 protein, which retains the DNA-binding domain, can bind to the octamer motif and may therefore be able to alter the activation patterns of the larger Oct-1A protein in vivo.

Analysis of human genomic clones reveals that the *oct*-1 gene is very large. To date, we have isolated over 70 kb of genomic DNA but have not yet isolated the entire gene. The large size of the gene is reminiscent of some *Drosophila* homeobox genes such as *Ultrabithorax* and *Antennapedia* (which are very large) and distinguishes this homeobox gene from that of many of the vertebrate homeobox genes analyzed to date, which are considerably smaller in

size. Chromosome mapping of the *oct-1* gene by C.-L. Hsieh and U. Francke (Stanford University) shows that this gene is located on human and mouse chromosomes 1. These are regions of synteny between the human and mouse genomes. The region in which the *oct-1* gene maps contains a number of genes that are related to genes that lie on human chromosome 19, where the *oct-2* gene is located (Ko et al., *Cell 55:* 135 [1988]). This relationship between multiple genes near the *oct-1* and *oct-2* chromosomal loci suggests that the *oct-1* and *oct-2* genes may have arisen by an ancient chromosome duplication. The similarities between the region surrounding *oct-1* in mouse chromosome 1 and a region of mouse chromosome 7 suggest that the *oct-2* gene will lie on mouse chromosome 7.

Oct-1 and Oct-2: Protein Modification and Protein:Protein Interactions in Transcriptional Activation by POU Homeodomain Proteins

M. Cleary, W. Herr, L. Leo, S. Stern,
M. Tanaka, W. Thomann

The DNA recognition sequence of the Oct proteins, the octamer motif (ATGCAAAT), is found in the promoters of both ubiquitously expressed genes and lymphoid-specific genes. In the SV40 enhancer, the octamer motif confers lymphoid-specific activation of transcription. The different activities of the octamer motif correlate with the ubiquitous and lymphoid-specific expression patterns of the Oct-1 and Oct-2 proteins, respectively. Separate studies, done last year in collaboration with N. Hernandez (Genetics Section), indicated that the ability of the Oct proteins to activate transcription is promoter-context-dependent. Thus, the Oct-1 protein is dedicated to activation of transcription of the ubiquitously expressed small nuclear RNA and histone H2B genes, whereas Oct-2 can activate the more typical mRNA genes such as immunoglobulin genes.

We have used the cloned cDNA copies of the genes for Oct-1 and Oct-2 (the latter obtained from P. Sharp and D. Baltimore, MIT) to study their differential activation functions. We find that to activate transcription, Oct-2 relies on two interdependent domains, an amino-terminal glutamine-rich region and a carboxy-terminal serine-, threonine-, and proline-rich region. Neither of these sequences is acidic and therefore do not conform to the acidic activation domains found in the yeast activators GAL4 or GCN4. Analysis of Oct-1/Oct-2 chimeras reveals that Oct-1 also contains a functional glutamine-rich region but fails to activate transcription of an mRNA promoter because the Oct-1 carboxyl terminus is inactive, indicating that differential activation by Oct-1 and Oct-2 is determined by the combination of multiple activation domains.

To show that wild-type, mutant, and chimeric proteins were expressed appropriately during the activation assays, we showed that in each case these octamer motif binding proteins can interfere with the activity of the SV40 enhancer Sph motifs that overlap the SV40 enhancer octamer motif. We also assayed expression of the Oct proteins directly by fusing an epitope from the influenza virus hemagglutinin protein to the amino terminus of selected proteins. Using a monoclonal antibody directed at the fused epitope, we could specifically immunoprecipitate the ectopically expressed Oct proteins. Figure 1 shows the results of such an analysis of transiently expressed wild-type, chimeric, and mutant Oct proteins. The results reveal that Oct-2 and transcriptionally active Oct-1/Oct-2 chimeras produce a heterogeneous set of proteins. The heterogeneity is due to phosphorylation, which induces a shift in electrophoretic mobility. This phosphorylation-induced shift in electrophoretic mobility is largely or entirely absent from molecules lacking one or the other activation domain and inactive Oct-1/Oct-2 chimeras. Thus, in Oct-2, nonacidic activation domains may stimulate transcription by inducing protein phosphorylation, which could activate transcription by causing a conformational change or perhaps by creating an acidic domain.

Although Oct-1 does not normally activate an mRNA promoter, it can activate such a promoter in the presence of the herpes simplex virus (HSV) *trans*-activator VP16. VP16 is an HSV late gene product that is incorporated into the virion tegument and, upon infection, activates expression of the HSV immediate early genes. VP16 does not bind to DNA directly but instead binds to its target sites by forming a multiprotein-DNA complex with Oct-1. We have shown that the Oct-1 homeodomain is responsible for directing formation of the complex with VP16. Oct-2 fails to direct formation of a similar complex, and this is because the Oct-2 homeodomain differs from Oct-1 at 7 out of 60 possible positions.

Sequence comparisons and a structural analysis indicate that homeodomains contain a tri-α-helical

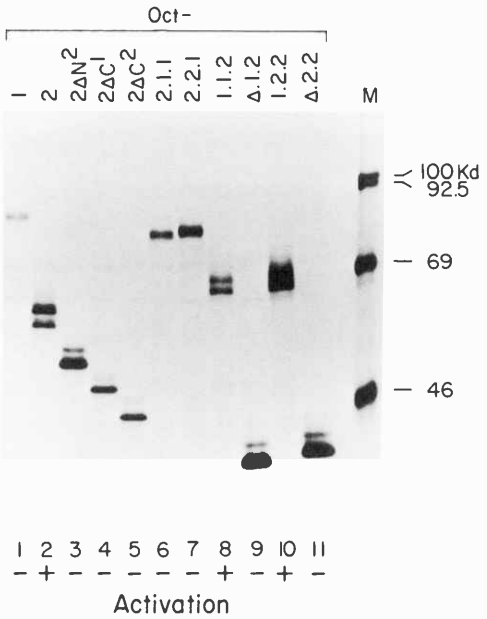

FIGURE 1 Immunoprecipitation of recombinant Oct proteins. HeLa cells were transfected with the Oct expression vector indicated above each lane, and the cells were metabolically labeled with [^{35}S]methionine for 4 hr. The cells were lysed and subjected to immunoprecipitation with a monoclonal antibody directed at the amino-terminally fused epitope from the influenza virus hemagglutinin protein. The immunoprecipitates were analyzed by 8% SDS-PAGE and fluorographed. The Oct-2 deletions span the amino-terminal glutamine-rich domain (Oct-2ΔN^2) or the carboxyl terminus (Oct-2ΔC^1 and C^2). The chimeric Oct proteins are divided into three regions, amino terminus, POU domain, and carboxyl terminus, and the origin of each region is indicated by 1 (Oct-1), 2 (Oct-2), or Δ (deleted). Thus, Oct-2.1.1 contains the amino terminus of Oct-2 fused to the Oct-1 POU domain and carboxyl terminus. The +/– designation under each lane indicates the potential of each Oct protein to activate the β-globin promoter in our assay. (Reprinted, with permission, from Tanaka and Herr, *Cell 60:* 375[1990].)

structure (helices 1–3) similar to the DNA-binding domains of the bacteriophage λ and 434 repressors. Figure 2 shows a diagram of the DNA-binding domain of λ repressor. In the tri-α-helical structure, helices 1 and 2 lie over helix 3; this latter helix makes the major contacts with the DNA and thus has been coined the "DNA recognition" helix. In contrast, the external surfaces of helices 1 and 2 face away from the DNA. When the Oct-1 and Oct-2 homeodomains are modeled into the structure of the λ repressor DNA-binding domain, the positions of the seven residues differing between Oct-1 and Oct-2 lie in (or in one case adjacent to) helices 1 and 2 on the surface of the homeodomain facing away from the DNA. Three of the differences lie in helix 2, where positive control mutations in λ repressor lie.

The λ positive-control (pc) mutations affect the ability of the λ repressor to activate transcription but do not affect DNA-binding activity. In λ repressor, pc mutations lie in or adjacent to helix 2 in a region that probably contacts promoter-bound RNA polymerase directly. Changing in Oct-1 the three residues in helix 2 that differ between Oct-1 and Oct-2 into the Oct-2 amino acids is sufficient to disrupt formation of the complex with VP16 without obviously affecting the DNA-binding activity of the protein (see Fig. 2). These results suggest that the tri-α-helical DNA-binding motif is an ancient target for protein-protein interactions mediating transcriptional control. We now plan to extend these studies by performing detailed structural analyses of the VP16/Oct-1 interactions.

Regulatory Elements of the HIV Promoter

W. Phares, B. Whelan [in collaboration with B.R. Franza, Cold Spring Harbor Laboratory]

In 1988, we initiated a study of the human immunodeficiency virus (HIV) promoter as a part of a new program project at the Laboratory to study activation of HIV in infected cells. There are a number of striking similarities between the SV40 early promoter and the HIV promoter: They both contain TATA box motifs, multiple binding sites for the transcription factor Sp1, and two copies of the 10-bp κB motif in inverse orientation, GGGACTTTCC (called the enhancer cores ECI and ECII) in HIV or GGAAAGTCCC in SV40. In SV40, the κB motif lies within the SV40 C protoenhancer element, which was identified in our studies of the SV40 enhancer and is active in many different cell types.

To dissect the HIV promoter, we are focusing our attention on the sequences upstream of the Sp1-binding sites, which by analogy to the SV40 early promoter probably contain multiple enhancer elements. We showed last year that the κB motifs of HIV could functionally replace the SV40 enhancer to allow growth of SV40 in CV-1 cells. To extend this finding further, we have compared the activities of reiterated copies of the HIV ECI and ECII elements and the SV40 C element in transient expression assays of transfected DNA. The results show that multiple copies of both the HIV-derived and SV40-derived elements can independently enhance transcription to similar levels in CV-1 cells, human H9 T cells, and HeLa cells, whereas single (*spm5*) and

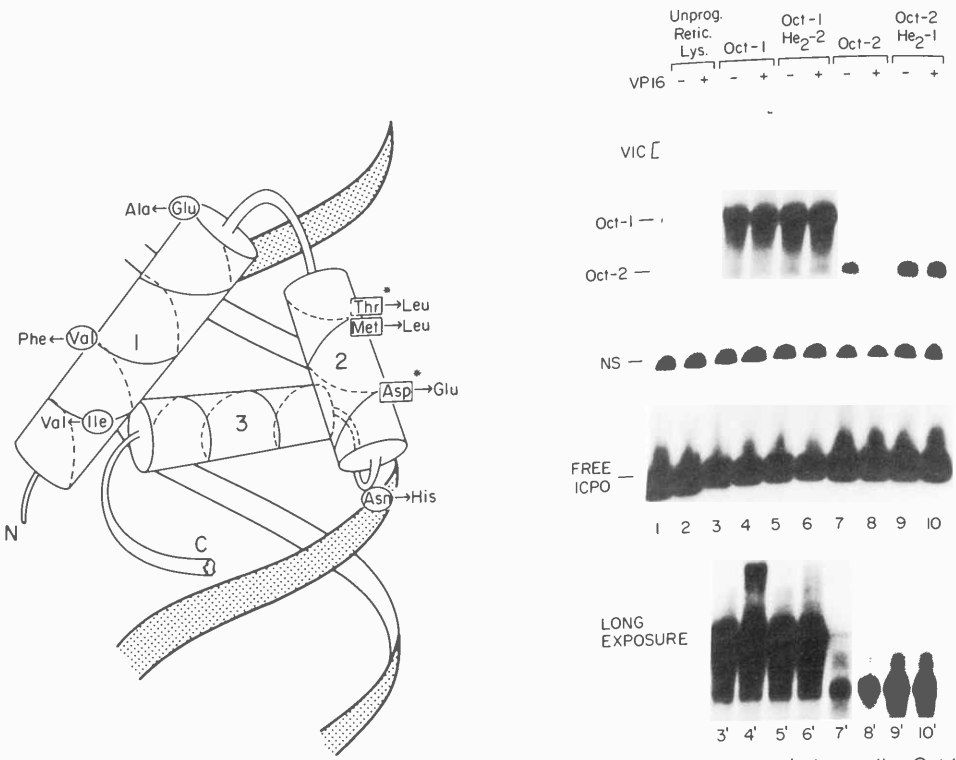

FIGURE 2 λ repressor DNA-binding domain model with the positions of differences between the Oct-1 and Oct-2 homeodomains indicated and VP16-induced complex formation by Oct proteins with exchanged helix 2. (*Left*) Diagram of the tri-α-helical DNA-binding motif of λ repressor and DNA (behind) as a model for the POU homeodomain (S. Harrison, pers. comm.). The three α helices are drawn as cylinders, with the α-carbon backbone indicated by solid (facing viewer) and dashed (hidden from viewer) lines. The seven amino acid positions that differ between Oct-1 and Oct-2 are circled or boxed, with the Oct-1 residues shown inside and an arrow pointing to the Oct-2 residue. Boxed positions were exchanged in the helix-swap experiment, and asterisks indicate those positions in the homeodomain that are analogous to λ repressor *pc-2* and *pc-3* mutations. (*Right*) Gel-retardation assay for VP16-induced complex formation by wild-type Oct-1 (lanes *3,4*), Oct-1 carrying Oct-2/helix-2 (Oct-1/He_2-2; lanes *5,6*), wild-type Oct-2 (lanes *7,8*), and Oct-2 carrying the Oct-1/helix-2 (Oct-2/He_2-1; lanes *9,10*). Each protein was assayed without (lanes *1,3,5,7,9*) and with (lanes *2,4,6,8,10*) VP16 protein. The positions of Oct-1 and Oct-2 complexes and VP16-induced complexes (VIC) are shown at the left. (*Bottom*) Longer exposure of the Oct complexes and VICs. (Reprinted, with permission, from *Nature 341:* 624[1989].)

double (*dpm*10) point mutations that mutate either one or the other of the terminal nucleotides of the κB motif *G*GGACTTTC*C* abolish or drastically reduce activity in these cell lines. In Jurkat T cells, these elements are all relatively inactive in unstimulated cells, but the activity of the wild-type constructs is strongly stimulated by treatment of the cells with PMA and PHA. Taken together with the SV40 revertant analysis, these results indicate that the enhancer core elements of HIV are functionally equivalent to the SV40 C element. Thus, if NF-κB is responsible for the activity of the κB motif, its activity is unlikely to be lymphoid-specific but may instead be a general target for activation by mitogenic signals.

In collaboration with B. Robert Franza (Molecular Genetics of Eukaryotic Cells Section), we have examined the proteins that bind to the multimerized wild-type and mutant HIV and SV40 κB motifs. The multimerized enhancer constructs used in vivo provide a matched set of active and inactive elements that only differ by one or a few point mutations. The multimerized copies of each element were used in DNA affinity precipitation assays after biotinylation. Analysis of the precipitated proteins from stimulated Jurkat T cells shows that several families of proteins coprecipitate with each of the wild-type ECI, ECII, and SV40 C element sites. Curiously, although in a triple point mutation, these proteins fail to precipitate, in the inactive single point mutant *spm5*, these proteins still bind and instead a new series of pro-

teins appear. An interesting possibility is that this mutation is inactive because it promotes the binding of an inappropriate set of proteins instead of merely preventing the activators from binding.

We are now expanding our studies of HIV regulatory elements by examining the promoter sequences in different isolates of HIV from AIDS patients. These studies will involve the use of the polymerase chain reaction (PCR) to amplify directly the regulatory region from HIV isolates from single individuals at different times of infection and in different cell types (i.e., T cells and macrophages) without passage of the virus in cell culture. We have already observed a large degree of heterogeneity within the long terminal repeat of different HIV isolates. This heterogeneity is likely to uncover rearrangements of enhancer elements that are important for HIV pathogenesis and that may display different cell type-specific activities.

PUBLICATIONS

Stern, S., M. Tanaka, and W. Herr. 1989. The Oct-1 homeodomain directs formation of a multiprotein-DNA complex with the HSV *trans*-activator VP16. *Nature* **341:** 624–630.

Sturm, R.A. and P. Yaciuk. 1989. DNA cleavage by restriction endonuclease *Pfl*MI is inhibited in recognition sites modified by *dcm* methylation. *Nucleic Acids Res.* **17:** 3615.

In Press, Submitted, and In Preparation

Hsieh, C.-L., R. Sturm, W. Herr, and U. Francke. 1990. The gene for the ubiquitous octamer-binding protein Oct-1 is on human chromosome 1, region cen q32, and near *Ly-22* and *Ltw-4* on mouse chromosome 1. (Submitted.)

Tanaka, M. and W. Herr. 1990. Differential transcriptional activation by Oct-1 and Oct-2: Interdependent activation domains induce Oct-2 phosphorylation. *Cell* **60:** (in press).

MOLECULAR GENETICS OF EUKARYOTIC CELLS

Research in this section touches on broad problems in eukaryotic biology, from the control of cell proliferation to the regulation of gene expression; from the simple eukaryotic organism, the yeast *Saccharomyces cerevisiae*, to the mouse. Our approaches are equally eclectic: genetic, biochemical, and ultrastructural. Two unifying themes are the mechanism of action of oncogenes and the process of signal transduction. Dr. Franza's laboratory studies early changes in transcription patterns, particularly in human T cells. Dr. Field's laboratory utilizes transgenic approaches to study problems in cardiovascular physiology, development, and neoplasia. Dr. Skowronski's laboratory studies pathogenesis of HIV-1, the human immunodeficiency virus that causes AIDS, and the transforming potential of papillomavirus oncogenes using transgenic mouse models. Dr. Bar-Sagi's laboratory studies the involvement of the *ras* proteins and phospholipase A_2 in signal transduction and ligand-stimulated exocytosis. Dr. Spector's laboratory studies the functional compartmentalization of the nucleus using immunoelectron microscopy. Dr. Helfman's group studies the family of tropomyosin genes and the alteration of expression of these genes seen upon cellular transformation and development. Dr. Garrel's group continues to improve protein database collection. Dr. Gilman's laboratory studies nuclear events that result in changes in transcription. Dr. Wigler's laboratory continues studies on *ras* and other oncogenes, and the control of cell growth and proliferation.

TRANSGENIC MICE

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The advent of transgenic mouse technology has provided new and exciting experimental inroads toward the analysis of complex biological systems. Using this technology, it is possible to introduce chimeric genes directly into the mouse germ line. Once integrated, the foreign DNA is retained and transmitted to subsequent generations in a Mendelian fashion. This method of mammalian gene transfer provides an ideal model system with which to study complex biological problems at the organismal level in that the consequences of a single, defined genetic trait can be studied in intact animals. The Mouse Genetics Group at Cold Spring Harbor Laboratory has developed several model systems that have been designed to address a broad range of biologically relevant issues. These studies include characterization of selected genetic elements of the human immunodeficiency virus long terminal repeat (LTR), characterization of the transforming activity of the bovine papillomavirus E2 protein, identification of genes that regulate myocardiocyte proliferation, and transgenic models of hypertension. Our models illustrate the potential utility of the transgenic approach, especially with regard to the ability to study complex disease processes.

Transgenic Models of Viral Pathogenesis

L. Usher, G. Yenikolopov, J. Skowronski

Transgenic mice provide a system whereby various aspects of viral host range and pathogenicity can be studied in the absence of viral infection. Direct introduction of viral DNA into mouse embryos and its subsequent germ-line transmission result in its

presence in every cell of the body. This circumvents the limitations of host range and tissue tropism associated with the infection process itself. Our effort has been concentrated on two viruses, the human immunodeficiency virus type 1 (HIV-1) and bovine papillomavirus type 1 (BPV-1). Both viruses, upon introduction of their complete genetic information into the mouse germ line, induce lesions resembling those arising in natural hosts. Thus, different aspects of pathogenicity of these two viruses can be addressed in transgenic mice.

In Vivo Specificity of the HIV-1 LTR

J. Skowronski

The disease spectrum displayed by viral pathogens is restricted, among other factors, by tropism of viral infection and specificity of viral transcriptional control elements. HIV-1 preferentially infects cells expressing the CD4 surface antigen, which is a receptor for the virus mediating its entry through specific interaction with the HIV-1 *env* gene product. The major cellular targets in HIV-1 infections are helper/inducer T lymphocytes. CD4 antigen is also expressed at variable, although reduced, levels on subsets of glial cells, cells of the monocyte/macrophage lineage, and B lymphocytes, all of which are infectable by HIV-1. Surprisingly, however, some $CD4^-$ cells (e.g., fibroblasts) can also be infected in vitro by HIV-1. It is possible that in addition to CD4-mediated viral entry, other mechanisms may be operational.

As one approach to define the in vivo tissue specificity of the HIV-1 transcription control information, hybrid reporter genes composed of the HIV-1 LTR positioned to direct expression of the SV40 early region were introduced into the germ line of inbred mice. Three lines of transgenic mice resulting from three independent integration events of the HIV-1 T-antigen gene were generated. Although integration of foreign DNA microinjected into mouse embryos and integration of proviral DNA in infected cells are not mediated by the same mechanisms, a common feature of both is that integrations can target many different chromosomal locations. The transcriptional activities of the HIV-1 T-antigen transgenes residing in three independent chromosomal sites of the HIV-1 T-antigen transgenic lines were studied in detail.

The HIV-1 promoter preferentially targets expression of the SV40 reporter gene to the lymphoid tissue, as transgenic transcripts are consistently observed in thymuses, lymph nodes, and spleens of mice of all three transgenic lines. Skin is another organ reproducibly targeted by the HIV-1 T-antigen construct. In addition to expression of the HIV-1 T-antigen transgenes in lymphoid tissue and skin, which is consistently observed in mice of all the three lines, each transgenic line reproducibly shows a characteristic, but unique, pattern of additional sites of transgene expression. Levels of expression in these additional sites vary greatly between different transgenic lines. Patterns of these additional sites of transgene expression are heritable within lines of transgenic mice and thus reflect a dominant transcriptional effect of the particular chromosomal locus where transgenes become integrated. There are many well-characterized examples of activation of cellular transcription units by murine retroviruses integrating in their vicinity. It also appears that retroviral promoters may be targets of similar interference and that some chromosomal loci can confer high constitutive levels of expression on resident proviruses.

HIV-1 LTR reproducibly targets expression of reporter genes into lymphoid organs, all of which contain major populations of T lymphocytes, a major target of HIV-1. However, analysis of cell-type specificity revealed that transgene expression in T cells is relatively low. This is reminiscent of the inactivity of the viral promoter and virus itself in resting human T cells and in T-cell lines. The striking observation is that cell types other than T cells are permissive for the HIV-1 promoter and contribute the majority of expression detectable in thymuses and spleens. Permissive cell types, which appear to provide a better environment for expression of the HIV-1 LTR than resting T cells, include splenic B lymphocytes and an unidentified stromal element in the thymus. These data, at least with regard to B cells, are in agreement with results of experiments performed by other investigators with human cells isolated directly from infected individuals or with permanent cell lines. Human B lymphocytes, under some conditions, are infectable with HIV-1 and do support expression of the HIV-1 LTR more efficiently than cultured T cells. It is intriguing that B lymphocytes, macrophages, and thymic epithelial cells, all of which support transcription directed by the HIV-1 LTR in vivo, are by the virtue of performed functions involved in direct interactions with CD4-positive T cells. Such direct interactions may provide

a microenvironment that facilitates otherwise infrequent events of infection of these cell types. Given transcriptional inactivity of HIV-1 in latently infected T cells, such events of infection of constitutively permissive cell types, although possibly infrequent and thus not necessarily obvious, may contribute to increased virus replication in natural infections.

Benign Fibroepitheliomas in BPV-1 E2 Transgenic Mice

G. Yenikolopov, J. Skowronski [in collaboration with I. Seidman, New York University]

BPV-1, a circular double-stranded DNA tumor virus, induces benign fibropapillomas, composed of proliferative epidermal cells and fibroblasts. BPV-1, or a subgenomic fragment comprising the viral early region open reading frames (ORFs E1 through E8), can also transform selected murine cell lines in vitro. Results of experiments with mutant viruses have implicated three of the early ORFs, E2, E5, and E6, as necessary for the transforming activity of the virus. Overexpression of the E5 or E6 ORF alone, but not the E2 ORF, results in transformation of permissive cell lines, which indicates that the former two encode proteins with the oncogenic activity. E2 ORF encodes a set of transcription factors that interact directly with the BPV-1 enhancer to regulate expression of the BPV-1 early genes. Although in vitro experiments failed to prove a direct involvment of E2 in BPV-1 transformation of cultured cells, E2 proteins, as transcription factors, have a potential to perturb expression of cellular genes and may act synergistically with the E5 and E6 oncogenes to effect cellular transformation or tumorigenesis.

As one way of addressing this issue, we have constructed transgenic mice expressing the BPV-1 E2 gene in a variety of tissues. To achieve a broad pattern of expression, the E2 ORF, followed by the SV40 splicing and polyadenylation signals, was placed under the transcriptional control of the chicken β-actin promoter (pCBAE2). Eight founder mice harboring the pCBAE2 construct were generated and used to establish seven independent lines of pCBAE2 transgenic mice. Of mice from each of four transgenic lines (pCBAE2 5, 6, 7 and 8), 10–20% develop overt kidney lesions at 6–15 months of age. These lesions are characterized by abnormal retention of urine in the renal pelvis and, as a consequence, atrophy of the kidney tissue (hydronephrosis). Such lesions usually result from obstruction of urinary tracts, which can be caused by tumors of the urogenital organs (or those located in immediate vicinity). Histological analyses of the urinary tracts collected from several affected animals revealed focal hyperplastic growth in the upper portions of urethras. Polypoidal projections into the lumen of urethras were caused by abnormal proliferation of both the stromal (fibroblastic) and epithelial (transitional epithelium of the urethra) components of the urethra wall. These hyperplastic changes were histologically benign in most cases examined. Interestingly, in one case, a histologically malignant focal growth of epithelial cells (transitional cell carcinoma) was identified within the polypoid structures. This spectrum of lesions arising in pCBAE2 transgenics has never been observed in nontransgenic mice or mice of various transgenic lines that were maintained and autopsied in a CSHL colony (>100 mice at 1–2 years of age).

Expression of the pCBAE2 transgene was assessed at the RNA level. pCBAE2 transcripts, correctly initiated at the β-actin cap site, are detectable in several tissues, including kidney, urethra, bladder, testis, vagina, intestine, and skin. The highest levels of expression were consistently detected in the urinary tract, specifically bladders and urethras prior to development of overt macro- or microscopical lesions. These observations suggest that E2 gene products can, under certain conditions, induce abnormal proliferation of fibroblasts and epithelial cells. However, the identity of E2-expressing cells, and thus the mechanism of concomitantly induced proliferation of both fibroblastic and epithelial components of affected urethras, is at the present time unknown. Sporadic occurrence of transitional cell carcinomas in polypoid proliferations suggests that transitional epithelium may be a primary target of E2 in pCBAE2 transgenic mice. The localization of lesions in pCBAE2 transgenic mice is different both from that observed in natural infections with BPV-1 in cattle and from what is seen in transgenic mice harboring the complete BPV-1 genetic information. Interestingly, experimental infection of cattle and laboratory rodents with BPV-1 induces lesions in the urinary tracts that very closely resemble those of E2 transgenic mice. This particular location of stromal/epithelial hyperplasia in E2 mice may be due to unusually high susceptibility of the target tissue, transgene expression pattern, or other features of these particular transgenic models.

The BPV-1 E2 gene encodes a family of transcrip-

tion factors, which have a common DNA-binding specificity and the properties of both positive and negative regulators of transcription. At the present time, we do not know which forms of E2 are expressed in the target tissues of transgenic mice, and therefore phenotypes observed in pCBAE2 mice may result from either of these activities. E2 proteins and their DNA-binding specificities are conserved among different members of papillomavirus family, which includes several viruses implicated in human skin and genital premalignant and malignant lesions. The ability of E2 to induce hyperplastic growth in transgenic mice suggests that this conservation of E2 may be important not only in the context of viral functions, but also in the context of the virus-host interactions resulting in deregulation of growth control in target cells.

Cardiac Tumors in Transgenic Mice

M. Steinhelper, P. Weinberg, S. Teplin, L. Field

Mammalian adult cardiac muscle is incapable of growth by proliferation of existing muscle cells. This inability to proliferate has important implications for individuals who suffer from myocardial infarcts and related trauma. The failure of adult cardiomyocytes to divide results in the replacement of electromechanically functional muscle with electrically inactive fibrous (scar) tissue that is unable to contract. Such electrical and mechanical dysfunction places individuals suffering heart attacks at risk for further cardiac disease. A resource of proliferating adult heart muscle cells may provide insight into the mechanisms by which myocardiocyte growth is regulated during development and disease states. In addition, the ability to induce controlled myocardial growth in vivo would have therapeutic implications for the treatment of heart disease. As a first step toward these goals, we have generated several lineages of transgenic mice whose hearts express the SV40 T-antigen oncogene. Expression of this transforming gene in myocardiocytes induces cell division, leading to the formation of heart tumors. We have analyzed the structural and functional properties of the cells constituting the tumors and have investigated whether cardiac muscle cells derived from the heart tumors can divide in culture, with the goal of isolating a stable cardiac heart muscle cell line. In separate experiments, we have identified a genetic locus that regulates both the temporal pattern and morphology of cardiac tumorigenesis. This locus, when identified and cloned, may provide insight into the molecular events that regulate myocardiocyte proliferation. Descriptions of these studies are considered separately below.

PROPAGATION OF SUBCUTANEOUS TUMOR TRANSPLANTS DERIVED FROM ANF-TAG ATRIA

This work was carried out in collaboration with N. Lanson, J. DelCarpio, and W. Claycomb (LSU Medical Center, New Orleans). A transgenic model was developed in which expression of T antigen was targeted to the cardiac atria using the atrial natriuretic factor (ANF) promoter. Transgenic mice carrying this fusion gene (designated ANF-TAG) express T antigen in both atria. However, these animals exhibit unilateral right atrial hyperplasia. The differential capacity of right and left atrial cardiomyocytes to respond to T antigen with right unilateral hyperplasia is most likely a result of differential asymmetrical expression of genes in cardiac tissue. This hypothesis is founded on the observation that when prehyperplastic neonatal transgenic atria were transplanted to a physiologically neutral environment (i.e., subcutaneously in nude mice), only transgenic right atria proliferated. This observation suggests that the unilateral right atrial hyperplasia observed in the ANF-TAG mice is unlikely due to a trivial consequence related to environmental cues that differ between the atria.

The tumorigenic capacity of hyperplastic right atria was also demonstrated in histocompatible syngeneic animals. Injection of minced transgenic atria subcutaneously into numerous mice yielded tumors composed of well-differentiated cardiomyocytes. Subsequent passage of these tumors yielded tumors with enhanced growth properties as assessed in vivo and in tissue culture. Analysis of tumors by electron microscopy, indicates that the majority of tumor cells retain several differentiated characteristics, such as well-formed sarcomeres, abundant transverse tubules, atrial-specific electron-dense granules (which contain ANF immunoreactivity), and intercalated discs. Virtually all tumor cells, with the exception of vascular tissue (presumably arising from the host), contain both sarcomeric myosin and T antigen. The tumor cells proliferate rapidly as documented by the observation that 20–30% of cells incorporate [^{3}H]thymidine within an 18-hour period. Somewhat surprisingly, our analysis of the expression of additional cardiac determinants at either

the protein level or the RNA level revealed few differences between normal cardiac tissue, the primary atrial tumors, and the subcutaneous transplant tumors.

Studies of cardiac cells are hindered presently by the absence of a cell line that displays any significant degree of differentiation. The well-differentiated cardiac phenotype displayed by the cells constituting our tumors, coupled with their rapid proliferation in vivo, suggested that these cells might be an ideal resource for cell-culture experiments. Thus, cells dissociated enzymatically from either primary atrial tumors or the subcutaneous syngeneic transplants were tested for their ability to proliferate in culture. Cells isolated from several primary atrial tumors essentially do not divide in culture; however, cells isolated from the transplant tumors grew quite rapidly. Cells from both tumor types were well differentiated and contracted spontaneously in culture. Intact cell-cell communication was evident from the synchronous contraction of neighboring cells observed under light microscopy. Structural analysis by electron microscopy has revealed that the transplant tumor cells are highly differentiated. Furthermore, the contracting cells retained a high degree of cardiac-specific electrophysiological activity. For example, the resting membrane potentials and the action potentials are similar to those of normal atrial cells. Cultures of spontaneously contracting tumor cells can be obtained following passaging or recovery from frozen stocks. The characteristics displayed by these cells suggest that they are a unique and renewable resource of proliferating and highly differentiated cardiomyocytes.

GENETIC LOCI AFFECTING ATRIAL HYPERPLASIA IN ANF-TAG MICE

ANF-TAG transgenic mice were produced in (C57BL/6J × DBA/2J)/F_2 embryos, abbreviated (B6 × D2)/F_2. Consequently, subsequent breeding of the transgenic animals must result in segregation of alleles arising from one or the other progenitor strains (namely, B6 alleles or D2 alleles). Given this segregation, there is a distinct possibility that polymorphic alleles which differentially affect atrial tumorigenesis in ANF-TAG mice may be identified. To address this issue systematically, we have generated sublines by repeatedly backcrossing ANF-TAG mice to either the B6 or D2 progenitor animals. Over the past year, we have monitored the survival of these two populations of ANF-TAG mice.

We observe that backcrossing to B6 yields an apparent homogeneous population of mice that die from atrial tumorigenesis at 46.5 ± 1.6 weeks ($n=30$). In contrast, mice of the D2 subline appear to consist of two statistically distinct populations; approximately one half of the animals in the D2 subline die at 6.6 ± 0.7 weeks ($n=17$), whereas the other half die at 24.6 ± 0.8 weeks ($n=14$). Unfortunately, the mice from the 6.6-week group die around the time of sexual maturity and are unproductive breeders. Thus, the D2 subline must be propagated from the slower dying population. Such crosses yield transgenic offspring that fall into each of the two populations (i.e., a 6.6-week group and a 24.6-week group). The simplest interpretation of these results is that a single genetic locus (designated X) differs between the two progenitor inbred strains. Mice with an X^D/X^D genotype would die rapidly and mice with an X^B/X^B genotype would live longer. Heterozygous mice (X^D/X^B) would die at an intermediate rate, assuming codominance. The strongest evidence in favor of this working hypothesis is that presumptive X^D/X^B heterozygotes (slower D2 population) when crossed to D2 (X^D/X^D) yield two populations, fast and intermediate, in roughly equal proportions. We are presently generating informative backcross populations to confirm that the transition from the slow to intermediate population is determined by a single locus.

Associated with the differences in the rate of tumorigenesis is an intriguing morphological difference between atrial tumors in the two sublines. Mice from the D2 subline show a gross hyperplasia involving essentially all right atrial myocytes. Moreover, unilateral left atrial involvement has never been observed. In contrast, atrial tumors in the B6 subline appear to arise from a focal event involving the proliferation of only a portion of the right atrial cells. The delayed onset and focal nature of the atrial tumors arising in the B6 subline suggest that additional genetic events are required for T-antigen-induced proliferation. Were this the case, one might expect that this putative cooperative event could occur at finite frequency in the left atrium and consequently give rise to left atrial tumors. Indeed, such events do occur as illustrated by three mice (all animals from the B6 subline) that had left atrial tumors in the absence of right atrial involvement. Finally, our observations regarding genetic background have been observed in two ANF-TAG lineages in which the chromosomal location of the transgene differs. This observation effectively rules out triv-

ial explanations related to the site of transgene integration.

In conclusion, these traditional genetic backcross experiments have revealed at least one and at most two genetic loci that regulate both the temporal pattern and eventual end-stage morphology of T-antigen-induced cardiac pathology. We hope to identify genetically these loci by utilizing traditional linkage studies as well as recombinant inbred lines of mice.

TRANSGENIC MODELS OF VENTRICULAR CARDIOMYOCYTE PROLIFERATION

The initial microinjections of the ANF-TAG construct yielded several transgenic animals that died at very early ages. At necroscopy, these animals exhibited gross right atrial hyperplasia similar to that seen in the established ANF-TAG lineages. Further histological analyses revealed that these mice also developed ventricular pathology; the left wall and the septum of the ventricles were grossly hyperplastic, whereas the right ventricular wall and left atrium are relatively normal in appearance. Immunohistological analyses indicated that T-antigen expression is seen throughout the hyperplastic septum and left ventricle. As the endogenous ANF gene is expressed in ventricles during late embryological development, those ANF-TAG mice exhibiting ventricular oncoprotein expression presumably carried transgenes that integrated in a chromosomal site compatible with normal developmental expression of ANF. These observations demonstrate that T antigen can induce embryonic or neonatal ventricular cardiomyocytes to divide.

To extend these observations to adult ventricular tissue, we have generated transgenic mice that express T antigen when fused to different cardiac-specific promoters. Our initial results are from mice expressing a rat α-myosin heavy-chain–T-antigen (MHC-TAG) fusion gene. We have obtained three founder mice that contain the MHC-TAG fusion gene. Two of the mice died prematurely of cardiac tumorigenesis without transmitting the fusion gene to progeny. The third founder mouse has transmitted the fusion gene successfully and has a fractionated ECG typical of that seen in ANF-TAG mice with atrial hyperplasia. Transgenic progeny of this MHC-TAG founder inherit similar electrical abnormalities and heart pathology. Preliminary immunohistochemical analysis indicates that in this lineage, both atria and ventricles contain the oncoprotein and exhibit hyperplasia. This pattern of T-antigen expression obtained from the rat α-myosin heavy-chain gene promoter is consistent with the cell-type specificity for the endogenous MHC gene. In summary, our preliminary observations in the MHC-TAG lineages indicate that adult ventricular myocytes can proliferate in response to T antigen. These animals will provide a useful resource to assess the pathophysiological consequences of ventricular tumorigenesis and may also provide the basis for the generation of a ventricular myocyte cell line.

Transgenic Models of Hypertension Research

K. Cochrane, M. Steinhelper, S. Teplin, P. Weinberg, L. Field

Blood pressure and electrolyte homeostasis is largely dependent on an integrated, multi-organ response to numerous hormonal signals. The inappropriate regulation of these hormones often has overt pathophysiological consequences, as exemplified by the malignant hypertension that can accompany adrenal medullary tumors and reninomas. The ability to generate animal model systems with specific, known alterations in this hormonal regulatory cascade would be of considerable utility. During the last three years, our laboratory has been using the transgenic approach to study the molecular biology and physiology of ANF, a peptide hormone that is synthesized and stored in the cardiac atria. The circulating form of the hormone is a 28-amino-acid peptide derived from the carboxyl terminus of a 152-amino-acid preprohormone precursor by proteolytic cleavage. ANF is secreted into the circulation in response to volume overload, presumably as a consequence of atrial wall stretch. ANF exhibits profound effects on the cardiovascular and renal systems upon acute administration. These include a prompt and pronounced natriuresis and diuresis, an increase in glomerular filtration rate, inhibition of angiotensin-II-stimulated proximal tubular sodium and water reabsorption, inhibition of the renin-aldosterone axis, as well as potent vasodilatory and smooth muscle relaxant properties. Although the physiological consequences of acute ANF administration are well known, the role of the hormone in the chronic regulation of blood pressure and electrolyte homeostasis has remained elusive. In an effort to address this issue directly, we have generated a transgenic model that exhibits chronically elevated levels of ANF in the

systemic circulation. These transgenic animals are hypotensive, i.e., they have lower blood pressure, as compared to the nontransgenic littermates. Our molecular and physiological analyses of this model are described below.

GENERATION OF A TRANSGENIC MODEL WITH ELEVATED ANF LEVELS

To generate transgenic animals that would exhibit elevated levels of ANF in the systemic circulation, we constructed a fusion gene composed of the transthyretin (or TTR) promoter fused to the mouse ANF structural gene. The transthyretin-ANF fusion gene (abbreviated TTR-ANF) was designed such that the TTR promoter would target expression of ANF to the liver. The mouse TTR gene was isolated from a BALB/cCr bacteriophage λ genomic library, and the promoter (localized on a 3-kb restriction fragment of the 5′-flanking region) was subcloned into Bluescribe vector. The mouse ANF sequences were also isolated from a BALB/cCr genomic library. The promoter and a region of the 5′-untranslated leader of the ANF gene were removed by BAL-31 exonuclease, and a *Sal*I restriction site was introduced. This fragment was ligated into the clone carrying the TTR sequences such that transcripts arising from the TTR promoter would encode pre-pro-ANF. The TTR-ANF fusion gene was linearized at a unique *Nde*I site and microinjected into one-cell embryos obtained from C3HeB/Fe inbred mice using standard methodologies. Ten independent TTR-ANF transgenic mice were obtained from our initial series of microinjections. Of these, four animals were genetically mosaic and did not transmit their fusion genes, and two animals were sterile. However, the remaining four animals have successfully transmitted their fusion genes, and transgenic lineages have been established. These lineages have been designated TTR-ANF 2, 4, 5, and 6, respectively.

To characterize expression of the TTR-ANF transgene, RNA was isolated from transgenic animals from the four TTR-ANF lineages and subjected to both Northern blot and RNase protection analyses. Our experiments indicate that the TTR-ANF 2 and 4 lineages express the fusion gene in the liver and the TTR-ANF 5 and 6 lineages do not. Developmental studies in the TTR-ANF 4 lineage indicate that the transgene is active at day 15 of development and is essentially at adult levels by birth. RNase protection tissue surveys for TTR-ANF fusion gene expression in the TTR-ANF 2 and 4 lineages have revealed that the fusion gene is expressed only in transgenic liver. To characterize the effect of transgene expression on hormone levels in the systemic circulation in the TTR-ANF 2 and 4 lineages, an ANF-specific radioimmunoassay (RIA) was performed. These studies revealed that the transgenic animals have approximately eightfold elevated plasma levels of immunoreactive ANF as compared to their nontransgenic littermates. This difference is observed at every age assayed (3–25 weeks), and no differences in expression are seen between male and female transgenic animals. Several studies have indicated that plasma polypeptides can nonspecifically interfere with the ANF RIA. For this reason, we also performed the RIA on plasma that had been extracted. Given the small blood volume of a mouse, it was necessary to pool plasma from three animals for each assay. Analysis of the extracted pooled plasma samples revealed that the transgenic animals have approximately 20-fold elevated levels of immunoreactive ANF as compared to their nontransgenic littermates. Sephadex G-75 column chromatography was employed to assess the molecular form of the immunoreactive ANF present in the plasma of transgenic animals. The study showed that approximately 65% of the immunoreactive plasma ANF in the transgenic animals comigrates with pro-ANF, and 35% comigrates with the processed hormone.

CHRONIC CARDIOVASCULAR REGULATION IN TRANSGENIC ANIMALS THAT OVEREXPRESS ANF

The molecular and biochemical data described above attest that mice expressing a TTR-ANF fusion gene in their liver have elevated concentrations of immunoreactive ANF in the systemic circulation. Our initial assessment of the physiological consequences of chronically elevated ANF levels has relied on blood pressure determination. Direct measurements provide the most reliable assessment of blood pressure. Although direct pressure is easily measured by cannulation of femoral arteries in anesthetized mice, ideal recordings would be obtained from conscious animals with indwelling catheters. To this end, we have implanted indwelling cannulas into the aorta of nontransgenic and TTR-ANF mice. These analyses revealed that TTR-ANF 4 transgenic mice have a MAP (mean arterial blood pressure) of 75.5 ± 0.9 mm Hg ($n=9$) and age-matched nontransgenic littermates have a MAP of 103.9 ± 2.0 mm Hg ($n=9$). Similarly, the TTR-ANF 2 transgenic mice are also hypotensive (78.9 ± 1.4 mm Hg). The observation that

two independent TTR-ANF transgenic lineages are hypotensive provides compelling proof that the decrease in blood pressure arises as a consequence of transgene expression. Examination of water balance, plasma and urinary electrolytes (sodium and potassium), and heart rates revealed no significant differences between the TTR-ANF 4 mice and their nontransgenic littermates.

We have characterized several tissues in the TTR-ANF transgenic lineages to determine whether ANF overexpression elicits any histopathological changes. The initial experiments simply entailed harvesting tissue and processing for standard hematoxylin and eosin histological analyses. We have observed a reactive hyperplasia in the zona glomerulosa of the adrenal gland of the TTR-ANF 4 transgenic mice. The zona glomerulosa in the transgenic animal is markedly hyperplastic, as evidenced by its increased thickness and by an increase in the ratio of nuclear density to cytoplasm density. That this pathology involved cell division was confirmed in [^{3}H]thymidine incorporation studies. The hyperplastic response in the adrenal zona glomerulosa is somewhat surprising as several studies have shown that ANF effectively inhibits aldosterone production. Thus, one would not expect zona glomerulosa hyperplasia (which has previously been observed in animals maintained low-salt diets). However, it is possible that the signals to retain salt (i.e., aldosterone production) circumvent the inhibitory effect of ANF on zona glomerulosa cells. Alternatively, the zona glomerulosa hyperplasia may arise as a consequence of ACTH stimulation. These issues will be resolved when we determine the levels of aldosterone, ADH, and ACTH in the transgenic mice.

The ability to generate transgenic mice that carry defined modifications of the ANF structural gene provides a unique opportunity to correlate specific genetic alterations with their pathophysiological consequences in intact animals. Our initial study conclusively demonstrates that chronically elevated levels of ANF can exert a sustained hypotensive effect. In addition, the study attests to the potential utility of the transgenic mouse model for the study of cardiovascular physiology. Whether the lower MAP observed in transgenic mice results from direct actions of ANF on the heart and vasculature or from indirect actions on other cardiovascular regulatory systems, such as the renin-angiotensin-aldosterone system or the sympathetic nervous system, is currently under study. Undoubtedly, the TTR-ANF transgenic mice will facilitate investigations of such regulatory mechanisms.

ACUTE CARDIOVASCULAR REGULATION IN TRANSGENIC MICE THAT OVEREXPRESS ANF

These studies were carried out in collaboration with A. Veress and H. Sonnenberg (University of Toronto). The studies described above indicate that chronically elevated levels of ANF can induce a sustained hypotensive response. However, the observation that other physiological parameters remain normal indicates that the animals are at physiological homeostasis. In an effort to characterize cardiovascular homeostasis in the TTR-ANF mice further, we have initiated experiments to assess the capacity of these animals to respond to acute physiological stress. As indicated above, ANF is a major effecter of the renal response to intravascular or extracellular fluid volume expansion. In rats, plasma levels increase about fivefold after a 25% increase of circulating volume, and volume natriuresis is markedly impaired or prevented either by atrial appendectomy or by prior ANF antibody injection. To determine whether chronically elevated ANF concentration would affect the renal volume response, kidney function was studied in TTR-ANF transgenic mice and their nontransgenic littermates before and during acute intravascular expansion. Animals were anesthetized and prepared for clearance measurements. After a 1-hour control period, 4.5% albumin in Ringers was infused intravenously over the next 20 minutes to raise calculated blood volume by 25%. A further 40 minutes was allowed before taking a blood sample for determination of immunoreactive ANF. Plasma ANF averaged 5185 ± 514 pg/ml in the transgenic group, compared to 577 ± 83 in controls, confirming earlier results. Similarly, average blood pressure was about 20 mm Hg lower in transgenics. Despite this relative hypotension, baseline urine volumes and sodium excretions were not different from those of nontransgenic controls. After blood volume expansion, both diuresis and natriuresis were markedly elevated compared to control, even though blood pressure remained relatively depressed. Glomerular filtration rates were not statistically different between groups, either before or after volume expansion. Since baseline hematocrits also were not different, these data suggest that the ANF-induced decrease in arterial and therefore renal perfusion pressure counteracts the direct natriuretic effects of the pep-

tide, thus allowing maintenance of salt balance. However, when volume is expanded acutely, the relatively elevated ANF levels in the transgenic group exaggerate the renal response, even to the point of volume depletion. It will be instructive to determine volume responses on different sodium diets, as well as the nephron segments affected by the primary lesion and compensatory mechanisms.

Transgenic Mice with Cardiac Arrhythmias

M. Steinhelper, L. Field [in collaboration with K. Dresdner and A. Wit, Columbia University]

A progressive increase in both the frequency and severity of cardiac conduction abnormalities accompanies the formation of cardiac tumors in both ANF-TAG and MHC-TAG mice. These pathological perturbations in cardiac conduction should have a basis at the cellular and molecular levels. In an effort to identify such mechanisms, we have examined certain electrophysiological properties, such as the resting membrane potential, action potential waveform, and automaticity, as atrial hyperplasia proceeds. In addition, application of multiple electrode recording techniques has allowed us to map the three-dimensional changes in intercellular electrical coupling during atrial hyperplasia. Evidence acquired to date indicates that atrial hyperplasia is accompanied by the formation of multifocal pacemakers. In humans, such multifocal pacemakers are responsible for a significant portion of cardiac dysrhythmias. Additional characterization of the electrophysiological perturbations during the development of atrial hyperplasia should enhance our understanding of the molecular mechanisms by which cardiac arrhythmias arise and develop.

The consistent observation of arrhythmias in transgenic mice expressing the T-antigen oncoprotein in cardiac tissue has led us to consider alternative approaches for producing a heritable model of cardiac arrhythmia in the absence of cardiac hyperplasia. Although a molecular basis for the initiation and maintenance of cardiac arrhythmias is not known, it is highly likely that inappropriate intercellular propagation of electrical depolarizations is involved. In cardiac tissue, conduction of the electrical current from cell to cell is facilitated by structures within the cell membrane called intercalated disks. The disks have low resistance compared with the surrounding lipid bilayer, and it is thought that the velocity and pattern of electrical propagation may reflect the number and distribution of the disks in the cardiac membrane, respectively. The cardiac intercalated disks are related to the gap junctions present in electrically inactive tissues such as the liver. Gap junctions comprise a single protein (called a connexin) that is arrayed as a homohexamer within the plasma membrane. Recently, cDNA clones for the liver and cardiac connexins were obtained from a rat heart cDNA library. Analysis of the predicted protein sequences revealed that connexin 43 (heart) and connexin 32 (liver) are highly conserved, with the notable exception that the cardiac connexin has a 11-kD carboxy-terminal extension.

Several groups have assessed the ability of the connexins to induce cell-cell coupling by injecting RNA encoding these proteins into frog oocytes. Expression of both the liver and cardiac connexins induced large voltage-insensitive conductance between pairs of oocytes. However, expression of both connexins within the same cell type yielded gap junctions composed of nonfunctional heterohexamers. These observations provide formal proof that connexins can mediate intercellular electrical communication. Furthermore, these observations suggest that expression of the liver gene in the heart would lead to the formation of nonfunctional intercalated disks. To determine whether inappropriate intercellular communication can induce cardiac arrhythmias, we have created two fusion genes that should facilitate and inhibit electrical coupling between cardiac muscle cells. Our initial fusion gene constructs have exploited the ANF promoter so as to limit fusion gene expression to the atria. In the past few months, we have obtained several independent lineages of ANF-C43 and ANF-C32 transgenic mice and will initiate transgene expression analysis shortly.

PUBLICATIONS

Fabian, J.R., L.J. Field, R.M. McGowan, J.J. Mullins, C.D. Sigmund, and K.W. Gross. 1989. Allele-specific expression of the murine Ren-1 genes. *J. Biol. Chem.* **264:** 17589–17594.

Lindgren, V., M. Sippola-Thiele, J. Skowronski, E. Wetzel, P.M. Howley, and D. Hanahan. 1989. Specific chromosomal abnormalities characterize fibrosarcomas of bovine papillomavirus-1 transgenic mice. *Proc. Natl. Acad. Sci.* **86:** 5025–5029.

Hanahan, D., C. Jolicour, and J. Skowronski. 1989. Alternative self or non-self recognition of an antigen expressed in a rare

cell type in transgenic mice: Implications for self-tolerance and autoimmunity. *Cold Spring Harbor Symp. Quant. Biol.* **54:** 821–835.

Mbikay, M., S.G.N. Grant, F. Sirois, H. Tadros, J. Skowronski, C. Lazure, N. Seidah, D. Hanahan, and M. Cretian. 1989. cDNA sequence of neuroendocrine protein 7B2 expressed in β cell tumors of transgenic mice. *Int. J. Pep. Protein Res.* **33:** 39–45.

Skowronski, J., C. Jolicoeur, and D. Hanahan. 1989. Modulation of tolerance and autoimmunity in transgenic mice. In *Current communications in molecular biology: Perspectives on the molecular biology and immunology of the pancreatic β cell.* (ed. D. Hanahan et al.), pp. 187–195. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Steinhelper, M.E. and L.J. Field. 1989. SV40 large T-antigen induces cardiocyte proliferation in transgenic mice. In *The development and regenerative potential of cardiac muscle* (ed. John Oberpriller). Harwood Academic Publishers, New York.

In Press, Submitted, and In Preparation

Cochrane, K.L. and L.J. Field. 1990. Assessment of baroreceptor gain in hypotensive transgenic mice which over express ANF. (In preparation.)

Dresdner, K., A. Wit, M. Steinhelper, and L.J. Field. 1990. Change in myocardial electrophysiological properties accompanies atrial tumorigenesis in transgenic mice expressing an ANF-T antigen fusion gene. (In preparation.)

Field, L.J., A.T. Veress, M.E. Steinhelper, and H. Sonnenberg. 1990. Enhanced renal response to acute volume expansion in transgenic mice which overexpress ANF. (Submitted.)

Hanahan, D., E. Wetzel, J. Skowronski, M. Sippola-Thiele, and V. Lindgren. 1990. Tumorigenic latency and separable stages during fibrosarcoma development in transgenic mice carrying papillomavirus genomes. In *Proceedings of the International Symposium of The Princess Takamatsu Cancer Research Fund.* (In press.)

Skowronski, J. 1990. In vivo specificity of HIV-1 LTR. (In preparation.)

Skowronski, J., C. Jolicoeur, S. Alpert, and D. Hanahan. 1990. MHC and other determinants of autoimmunity toward a transgenic b cell autoantigen. (Submitted.)

Steinhelper, M.E. and L.J. Field. 1990. A genetic variant which regulates cardiomyocyte proliferation in transgenic mice expressing atrial natriuretic factor-SV40 T antigen fusion genes. (In preparation.)

Steinhelper, M.E., K. Cochrane, and L.J. Field. 1990. Transgenic mice expressing a transthyretin-atrial natriuretic factor fusion gene are hypotensive. (Submitted.)

Steinhelper, M.E., K. Cochrane, and L.J. Field. 1990. Adrenal zona glomerulosa hyperplasia in transgenic mice with elevated ANF levels. (In preparation.)

Steinhelper, M.E., N. Lanson, K. Dresdner, W. Claycomb, and L.J. Field. 1990. Proliferation in vivo and in vitro of differentiated cardiocytes derived from atrial tumor transplants. (Submitted.)

Yenikolopov, G. and J. Skowronski. 1990. Fibroepitheliomas in BPV-1 E2 transgenic mice. (In preparation.)

GENETICS OF CELL PROLIFERATION

M. Wigler J. Field, D. Young, J. Colicelli, K. Ferguson, T. Michaeli, G. Bolger, Y. Wang, R. Ballester, E. Chang, J. Gerst, G. Heisermann, M. Kawamukai, A. Vojtek, I. Wieland, H.-P. Xu, C. Nicolette, G. Asouline, K. O'Neill, M. Riggs, L. Rodgers, J. Brodsky, J. Douglas

Our laboratory focuses on two general areas: signal transduction and growth control. Much of our work centers about the RAS proteins, small guanine-nucleotide-binding proteins, which reside on the inner surface of the plasma membrane, that have been highly conserved in evolution and play a critical role in mediating signals that control cellular growth and other cellular events. We study the RAS proteins in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* and in mammalian cells. In addition to RAS, we also study the families of mammalian cAMP phosphodiesterases, which play important roles in modulating the response of cells to cAMP. We plan to continue our studies of *ROS*, an oncogene that is expressed specifically in glioblastomas. Finally, we are developing a method for genomic difference cloning, which should enable scientists to discover new genetic abnormalities in cancer cells and search more effectively for new pathogenic organisms.

RAS in *Saccharomyces cerevisiae*

J. Field, R. Ballester, E. Chang, K. Ferguson, C. Nicolette, A. Vojtek, J. Colicelli, J. Gerst, T. Michaeli, M. Riggs, L. Rodgers, H.-P. Xu

There are two RAS proteins in *S. cerevisiae*, encoded by the *RAS1* and *RAS2* genes. They are highly ho-

mologous to the mammalian *RAS* genes. These yeast proteins are essential for growth and control the activity of adenylyl cyclase, the product of the *CYR1* gene. Activation of *RAS2* by point mutation (e.g., $RAS2^{val19}$) leads to activation of the cAMP signaling pathway and a consequent cluster of cellular phenotypes, including loss of tolerance to heat shock and sensitivity to nitrogen starvation. We have previously described the cloning of many genes that function along the RAS pathway, including *CDC25*, which encodes a factor that probably functions to regulate RAS by controlling guanine nucleotide exchange; *CYR1; BCY1*, which encodes the regulatory subunit of the cAMP-dependent protein kinase (cAPK); *TPK1*, *TPK2*, and *TPK3*, which each encode catalytic components of the cAPK; and *PDE1* and *PDE2*, which encode the low- and high-affinity cAMP phosphodiesterases, respectively. Using these genes, we have demonstrated a powerful feedback inhibition that maintains this system in homeostasis. We have demonstrated that the mammalian RAS proteins have been sufficiently conserved in evolution that they can function in yeast and stimulate yeast adenylyl cyclase in in vitro biochemical reactions. We have further demonstrated that RAS proteins must bind guanosine triphosphate in order to stimulate adenylyl cyclase. Finally, we have demonstrated through genetic analysis that it is highly likely that RAS proteins have additional functions in yeast (Wigler et al., *Cold Spring Harbor Symp. Quant. Biol. 53:* 649 [1988]).

Many of the most essential questions about RAS in yeast remain unsettled: How is RAS itself controlled? How does RAS control adenylyl cyclase? Are there intermediate proteins required for this function? What are the other functions of RAS in yeast? We have made progress on these questions.

RAS in *S. cerevisiae*: How Is It controlled?

R. Ballester, E. Chang, K. Ferguson,
C. Nicolette, A. Vojtek

We have previously shown that RAS can stimulate its yeast effector only when bound to GTP. Thus, RAS may be controlled by factors that influence nucleotide binding. Two genes have been discovered in *S. cerevisiae* that are likely to encode proteins involved in this type of control of RAS. The first is *CDC25*, which encodes a product that probably catalyzes guanine nucleotide exchange on RAS. The second is *IRA1*, which encodes a protein that probably catalyzes GTP hydrolysis by RAS (Tanaka et al., *Mol. Cell. Biol. 9:* 757 [1989]). *IRA1* has slight homology with a mammalian gene called *GAP* (Trahey et al., *Science 242:* 1697 [1988]). GAP has been shown to induce GTP hydrolysis by RAS (Trahey and McCormick, *Science 238:* 542 [1987]). It has been proposed by other investigators that GAP is the effector for RAS (Adari et al., *Science 240:* 518 [1988]). In contrast, genetic analysis strongly suggests that IRA1 protein is involved with the down regulation of RAS (Tanaka et al., *Mol. Cell. Biol. 9:* 757 [1989]), and more specifically with its feedback control (Wigler et al., *Cold Spring Harbor Symp. Quant. Biol. 53:* 649 [1988]; Ballester et al., *Cell 59:* 681 [1989]; Tanaka et al., *Mol. Cell. Biol. 9:* 757 [1989]). We have found that mammalian GAP, when expressed in yeast, does indeed down-regulate RAS (Ballester et al., *Cell 59:* 681 [1989]). It down-regulates both wild-type RAS2 and mammalian Ha-*ras*, when expressed in yeast, but not the activated $RAS2^{val19}$ protein, and can genetically complement *ira1*$^-$ yeast. These results suggest to us that mammalian GAP may be involved in the feedback inhibition of RAS in mammalian cells.

cAMP levels in yeast are regulated by glucose (Thevelein and Beullens, *J. Gen. Microbiol. 131:* 3199 [1985]). Other nutrient effects may be mediated through the cAMP effector pathway. We do not know the chain of events by which these signaling events occur. Other gene products besides those of *CDC25* and *IRA1* may be involved. To explore this area, we have begun searching for genes that, when overexpressed in yeast, can suppress the loss of *IRA1* or can suppress the loss of *CDC25*. Several new candidate yeast genes have been identified, and they will be characterized by sequence, genetic, and biochemical analyses. To perform biochemical analysis, we must first find conditions under which some component can affect the nucleotide bound to RAS. We are in the process of purifying CDC25 protein from yeast to determine under what conditions it can catalyze nucleotide exchange. We have shown that dominant interfering mutants of RAS can block function of wild-type RAS (Powers et al., *Mol. Cell. Biol. 9:* 390 [1989]). Interference is suppressed by overexpression of CDC25. We therefore proposed that these interfering RAS mutants form stable complexes with CDC25. We are in the process of testing this hypothesis by biochemical studies.

Our focus on the control of RAS is CDC25. For

reasons stated below, we believe it is likely that CDC25 has a mammalian homolog. Our studies in yeast thus will help guide experiments in mammalian cells. The fundamental question, both in yeast and in mammals, is the nature of the ultimate initiating signal for the pathway. In yeast, this initiating signal may originate from within the cell, perhaps a consequence of the metabolic state of the cell. There may be a similar initiating signal in mammalian cells.

RAS in *S. cerevisiae*: Its Interactions with Adenylyl Cyclase

R. Ballester, J. Colicelli, K. Ferguson, J. Field, J. Gerst, T. Michaeli, M. Riggs, L. Rodgers, A. Vojtek

We showed several years ago that the major effects of RAS proteins in yeast were explainable by their stimulation of adenylyl cyclase and that RAS proteins do indeed stimulate adenylyl cyclase in vitro. It has remained an open question whether this interaction is direct or whether it requires the presence of intermediary proteins. To help resolve this question, we are using extensive genetic and biochemical approaches. *S. cerevisiae* adenylyl cyclase made in *Escherichia coli* is neither full length nor RAS-responsive (unpublished results). To purify the adenylyl cyclase complex from yeast, we developed a method of epitope fusion and utilized immunoaffinity chromatography. This epitope fusion method is widely applicable for studying stable protein complexes. The adenylyl cyclase purified by this method copurifies with a tightly associated subunit with an apparent molecular weight of 70K (Field et al., *Mol. Cell. Biol. 8:* 2159 [1988]). We call this protein CAP for cyclase-associated protein. We have raised polyclonal antisera to CAP and identified the cDNA encoding CAP (Field et al., *Cell* [1990] in press) by screening an *S. cerevisiae* cDNA expression library (a generous gift from J. Kuret, Structure Section) with this antisera.

We have found that *CAP* is identical to a gene we had previously isolated, called *SUPC. supC* was a mutation that suppressed the phenotypes of activated *RAS*. In fact, cells that have disruptions of *CAP* do not respond to activated $RAS2^{val19}$, and the adenylyl cyclase purified from such yeast does not respond to RAS proteins in vitro.

We are currently testing if the coexpression of CAP and adenylyl cyclase in *E. coli* is sufficient to generate a RAS-responsive complex. At present, we know that these two proteins do form a complex when coexpressed in *E. coli*, but we do not yet know if other factors are required to form a RAS responsive complex. We are continuing the search for such factors by both biochemical and genetic means.

CAP is itself an interesting protein. Preliminary data suggest that it is conserved in evolution (see section on *S. pombe*, below). Disruptions of *CAP* lead to a severe phenotype that, at present, can best be explained by a failure to manage amino acid metabolism. We are in the process of testing this hypothesis and are looking at the interaction of CAP with GCN4, a protein involved in general amino acid metabolism (Hinnebusch, *Mol. Cell. Biol. 5:* 9 [1985]). The primary structure of CAP indicates that it contains two domains, separable by a stretch of prolines. Work in progress suggests that each domain has a distinct function: The amino-terminal domain is required for RAS responsiveness and the carboxy-terminal domain is required for amino acid response. We will continue to explore the function of CAP in an attempt to understand its role in mediating RAS effects, its role in mediating other signal transduction pathways in yeast, and its role in the evolution of signal transduction pathways.

We have also investigated the domains of adenylyl cyclase that appear to be required for interactions with RAS by assaying the RAS responsiveness of mutant adenylyl cyclase molecules in vitro. Figuring prominently in this work is a domain of cyclase that we call the leucine-rich repeat. The leucine-rich repeat occurs in the middle third of the adenylyl cyclase molecule and is composed of about 25 units of a consensus motif 23 amino acids in length. This motif is punctuated by proline and asparagine and contains leucine or an aliphatic amino acid every two to three residues. Similarly organized repeats have now been noted in a number of mammalian and insect proteins that form stable complexes with other proteins (Field et al., *Science 247:* 464 [1990]). Our work has shown that an adenylyl cyclase molecule with an amino-terminal deletion within 100 amino acids of the leucine-rich repeat is still fully RAS-responsive (Colicelli et al., *Mol. Cell. Biol.* [1990] in press). Deletions within the remaining molecule destroy RAS responsiveness. Small inframe insertion mutations reveal a different picture. Such mutations are generally without effect, except within the leucine-rich repeat. These data indicate that large-scale spacing of the adenylyl cyclase molecule is critical to its ability to respond to RAS, and the struc-

tural requirements within the leucine-rich repeat are especially rigorous.

We have discovered a second approach to studying RAS/target interactions. Mutant forms of adenylyl cyclase can actually interfere with the function of RAS, as evidenced by their ability to restore heat-shock resistance to strains carrying the *RAS2^{val19}* mutation (Field et al., *Science 247:* 464 [1989]). This observation led us to map the minimum region of the adenylyl cyclase molecule capable of interfering with RAS to the leucine-rich repeat itself. These studies also led to the design of genetic screens for the mammalian effectors of RAS (see below).

RAS in *S. cerevisiae*: Its Other Functions

T. Michaeli, A. Vojtek, H.-P. Xu

Although most of the functions of RAS in *S. cerevisiae* can be explained by effects on the cAMP signaling pathway, not all of its functions can be so readily explained (Wigler et al., *Cold Spring Harbor Symp. Quant. Biol. 53:* 649 [1988]). First, *ras1$^-$ ras2$^-$* spores are not viable, whereas *cyr1$^-$* spores are. Second, cells that are *ras1$^-$ ras2$^-$* and suppressed by high-copy kinase genes, such as *TPK1* and *SCH9*, are temperature sensitive, whereas cells that are *cyr1$^-$* and suppressed by these kinase genes are not temperature sensitive.

There is a third effect of RAS that is not readily explained by its effects on adenylyl cyclase. We have found that high levels of expression of an Ha-*ras* protein that has lost its carboxy-terminal processing site (e.g., Ha-*ras^{ter186}*) can suppress the phenotype of the *RAS2^{val19}* gene in yeast (Michaeli et al., *EMBO J. 8:* 3039 [1989]). Our biochemical and genetic analyses suggest that these types of Ha-*ras* mutants do not interfere with the processing of *RAS2^{val19}* or its interaction with adenylyl cyclase. Moreover, the mutant Ha-*ras* proteins must be bound with GTP to interfere. Our results are most readily explained if the mutants of Ha-*ras* bind to a second RAS effector, effectively competing for its binding with membrane-bound RAS. We think that this function has been conserved in evolution, since similar interfering effects of defective Ha-*ras* proteins are seen in *Xenopus* oocytes (Gibbs et al., *Proc. Natl. Aad. Sci. 86:* 6630 [1989]). Several candidate yeast suppressor genes of these mutant Ha-*ras* genes have been found and are being analyzed. These studies may lead to the discovery of RAS effector pathways that are truly conserved in eukaryotes.

RAS in *S. pombe*

M. Kawamukai, M. Riggs, L. Rodgers, Y. Wang, H.-P. Xu, D. Young

We have initiated the study of RAS in *S. pombe*, a fission yeast that is very diverged from *S. cerevisiae*. At the nucleotide level, *S. pombe* appears as diverged from *S. cerevisiae* as it is from mammals. In this organism, there is a single known homolog of RAS, called *ras1* (Fukui and Kaziro, *EMBO J. 4:* 687 [1985]). *ras1$^-$* cells are viable, round, sporulation-defective, and mating-deficient (Fukui et al., *Cell 44:* 329 [1986]). *ras1* does not appear to operate through adenylyl cyclase. These differences between *S. pombe* and *S. cerevisiae* make the study of *ras1* in *S. pombe* particularly attractive.

We have focused on the function of *ras1* and have utilized two approaches. First, we are in the process of analyzing the adenylyl cyclase system of *S. pombe*. Knowledge of this system will allow us to compare and contrast *S. pombe* and *S. cerevisiae* RAS-responsive systems. We will be able to test *S. cerevisiae* components in *S. pombe* to learn more about their function. In particular, we will be able to learn if these components are RAS-specific or adenylyl-cyclase-specific. Second, we are in the process of a genetic analysis of suppressors of *ras1*-deficient cells, in the expectation that some suppressors will encode proteins in the *ras1* effector pathway. Progress has been made in both of these approaches. We have identified the *S. pombe* gene encoding adenylyl cyclase (Young et al., *Proc. Natl. Acad. Sci. 86:* 7989 [1989]). This gene encodes a protein that is highly similar to *S. cerevisiae CYR1*, including the carboxy-terminal catalytic domain and the leucine-rich repeats. Amino-terminal to the repeats is complete divergence. Overexpression of *S. pombe CYR1* leads to sterility in that organism. We have begun to look for suppressors of this phenotype. It is our expectation that *S. pombe* contains another RAS-like protein that controls its adenylyl cyclase activity.

The availability of the *S. pombe* system will enable us to examine the functions of *S. cerevisiae* genes more clearly. For example, we have already found that antibodies to *S. cerevisiae* CAP can be used to precipitate *S. pombe* adenylyl cyclase activity. A homolog to CAP must exist in this organism, and

we have begun searching for it in *S. pombe* expression libraries. By isolating this homolog, we will learn about the evolution of the domains of CAP, and this knowledge will help us to search for CAP homologs in higher organisms.

Our genetic analysis of the RAS pathway in *S. pombe* has centered around the search for genes that, when overexpressed, can suppress the phenotype resulting from *ras1* deficiency. To do this, we have created *S. pombe* strains lacking *ras1* or containing dominant interfering mutants in *ras1*, of the type that we found in *S. cerevisiae* which block CDC25 function (Powers et al., *Mol. Cell. Biol. 9:* 390 [1989]). Such cells are defective in sporulation. One gene was isolated from a high-copy chromosomal library that could suppress the interfering *ras1*, and we called it *sir1*. The nucleotide sequence of *sir1* indicates that it encodes a serine/threonine kinase. Genetic analysis indicates that the *sir1* product acts downstream from Ras1 protein and perhaps upstream of the product of *byr1*, another gene encoding a protein kinase that is capable of suppressing *ras1* deficiency (Nadin-Davis and Nasim, *EMBO J. 7:* 985 [1988]). Thus, it is likely that the *sir1* kinase is closer to the root of the *S. pombe ras1* pathway than is the *byr1* kinase. We are testing if *sir1* copurifies as part of a *ras1* responsive complex. In yeast, Sir1 protein appears to be exclusively membrane bound. We have succeeded in expressing Sir1 protein in *E. coli*.

Mammalian RAS

R. Ballester, E. Chang, J. Colicelli, K. Ferguson, J. Field, C. Nicolette, M. Riggs, L. Rodgers, A. Vojtek, H.-P. Xu

The parallels between the mammalian and yeast RAS pathways are striking. First, mammalian RAS can function in a yeast host and can stimulate yeast adenylyl cyclase in vivo and in vitro. Second, yeast RAS can function in mammalian cells to induce transformation (DeFeo-Jones et al., *Science 228:* 179 [1985]). Third, mammalian GAP can complement yeast deficient in *IRA1*. Fourth, we and other investigators have shown that Ha-*ras* mutants that are dominant interfering in yeast are dominant interfering in mammalian cells (Powers et al., *Cell 36:* 607 [1984]; Feig and Cooper, *Mol. Cell. Biol. 8:* 3235 [1988]). Most recently, we have shown that the yeast *SCD25* gene, a homolog of *CDC25* (Boy-Marcotte et al., *Gene 77:* 21 [1989]), can transform animal cells. Despite these similarities, the targets of RAS action appear to be different in yeast and vertebrates.

We have applied a genetic approach to identifying mammalian components that interact with RAS. We constructed mammalian cDNA libraries in yeast expression vectors and have searched these libraries for genes that can suppress mutations in the RAS pathway in yeast (Colicelli et al., *Proc. Natl. Acad. Sci. 86:* 3599 [1989]). We have utilized three types of screens, using both *S. cerevisiae* and *S. pombe* as hosts.

The first cDNA screen has been for genes that can suppress the heat-shock phenotype of cells with the *RAS2*^{val19} mutation. The rationale for this screen is that, like the defective adenylyl cyclase gene, the mammalian effector of *RAS* might well interfere with activated RAS. Several candidates have emerged from this screen. One set encodes cAMP phosphodiesterases (see below). The second set is under evaluation. Genes from the second set do not appear to encode cAMP phosphodiesterases and bear no homology with previously identified nucleotide sequences in the data banks. At least one member of this set encodes a product that interferes with activated *ras1* in *S. pombe*. We expect that genes from this set either encode true inhibitors of RAS function (e.g., by blocking nucleotide exchange) or, more likely, are effectors of RAS that are not functional in yeast hosts.

In the second cDNA screen, we have sought cDNA genes that can suppress the loss of *ras1* in *S. pombe*. Several mammalian cDNAs have been isolated this way, and they are still under investigation. Among the genes so isolated have been Ha-*ras*, *RAP1*, and *RAP1A*. The latter two genes are members of the *RAS* superfamily (Pizon et al., *Oncogene 3:* 201 [1988]). *RAP1* was also isolated as Ki-*rev*-1, a gene capable of suppressing Ki-*ras* function in mammalian cells (Kitayama et al., *Cell 56:* 77 [1989]). Our results demonstrate that *RAP* genes and *RAS* genes can share effectors.

The third cDNA screen is designed to search for genes that can suppress *S. cerevisiae* with temperature-sensitive alleles of *CDC25*. We hope that this screen uncovers mammalian homologs of *CDC25*.

The *ROS* Oncogene

C. Birchmeier, K. O'Neill, M. Riggs, S. Sharma

The *ROS* oncogene was first discovered as the transforming principle of the avian retrovirus UR-2 (Neck-

ameyer and Wang, *J. Virol. 53:* 879 [1985]). We first encountered it as *MCF3*, an oncogene arising by rearrangement following a DNA transfer experiment. Subsequent studies revealed ROS to be expressed in a high proportion of human glioblastomas, but in very few other kinds of tumors, and not at all in normal glial cells. In glioblastomas, the predominant mRNA is about 8.0 kb. We have cloned cDNA to this mRNA from SW1088 and have completed the nucleotide sequence (Birchmeier et al., *Cell 43:* 615 [1985]). We have also cloned a cDNA to a 4.0-kb *ROS* mRNA found in another cell line, U118 (Sharma et al., *Oncogene Res. 5:* 91 [1989]). *ROS* can encode a transmembrane tyrosine kinase that shares homology with the product of the *Drosophila melanogaster sevenless* gene, both in the intracellular kinase domain and in the extracellular domain. Studies of *ROS* have shown that in two out of two glioblastomas, it has undergone mutation, consistent with the idea that the aberrant expression of ROS contributes to the malignancy of tumors of glial origins. We plan to continue these studies, examining the expression of ROS in primary brain tumors, using antisera to a ROS protein that we have developed. It is our hope that ROS protein will prove to be a useful cell surface marker for the diagnosis and treatment of tumors of glial origin.

Mammalian cAMP Phosphodiesterase Genes

G. Bolger, J. Colicelli, T. Michaeli, M. Riggs, L. Rodgers

In the course of analysis of mammalian cDNAs that can suppress the effects of activated RAS in yeast, we discovered several cDNAs encoding high-affinity cAMP phosphodiesterases (Colicelli et al., *Proc. Natl. Acad. Sci. 86:* 3599 [1989]). These phosphodiesterases are of the class inhibited specifically by rolipram, and they comprise a family of enzymes similar in structure to the product of the *dunce* locus in *D. melanogaster* (Chen et al., *Proc. Natl. Acad. Sci. 83:* 9313 [1986]). We have begun identifying and enumerating the species of phosphodiesterases expressed in human brain. The number of such genes expressed is surprisingly high. We are pursuing this project in the expectation that the characterization of the phosphodiesterases may be of medical value and in the hope that an understanding of the pattern of gene expression may lead to insights into the organization and evolution of neuronal cell populations.

A New Method for Genomic Difference Cloning

I Wieland, G. Bolger, K. O'Neill, G. Asouline

One common and fundamental problem of molecular biology confronts us when two similar genomes differ and we desire to understand the difference. One simple form of this problem can occur when a genome becomes deleted for sequences present in another due to germ line mutation, as can happen in genetic disease, or due to somatic mutation, as can happen during the development of cancer. Differences can also be acquired by infection with a DNA-based pathogen. Methods for identifying and isolating sequences present in one DNA population that are absent or reduced in another are called "difference cloning." Methods for difference cloning of cDNA populations have been widely described. Only one method for the difference cloning of genomic DNA is reported in the literature. This method was first described by Lamar and Palmer (*Cell 37:* 171 [1984]), who used it to clone sequences from the Y chromosome. Kunkel and co-workers used a variation of this method to clone fragments of the Duchenne's muscular dystrophy locus (Kunkel et al., *Proc. Natl. Acad. Sci. 82:* 4778 [1985]), which becomes deleted in some afflicted individuals. We have developed a different method for genomic difference cloning that is potentially more powerful (Wieland et al., *Proc. Natl. Acad. Sci.* [1990] in press). Although our method is not yet sufficient to isolate and define the small differences in genomes that would make it enormously useful as a tool for the study of neoplasia or infectious disease of viral origin, improvements are under way that will bring our method into that range. In its present state, our method is useful for the analysis of some genetic diseases and infectious diseases of unknown origin.

In our procedure for genomic difference cloning, sequences present in one genomic DNA population ("tester") are isolated that are absent in another ("driver"). By subtractive hybridization, a large excess of driver is used to remove sequences common to a biotinylated tester, enriching the "target" sequences that are unique to the tester. After repeated subtractive hybridization cycles, tester is separated from driver by avidin/biotin affinity chromatogra-

phy, and single-stranded target is amplified by the polymerase chain reaction, rendering it double-stranded and clonable. We have successfully modeled two situations: the gain of sequences that result from infection with a pathogen and the loss of sequences that result from a large hemizygous deletion. We obtain 100–700-fold enrichment of target sequences. We are in the process of improving the method by exploiting the second-order kinetics of self annealing. We are hopeful that we can soon begin to identify very small sequence differences between chromosomes.

PUBLICATIONS

Ballester, R., T. Michaeli, K. Ferguson, H.-P. Xu, F. McCormick, and M. Wigler. 1989. Genetic analysis of mammalian GAP expressed in yeast. *Cell* **59:** 681–686.

Colicelli, J., C. Birchmeier, T. Michaeli, K. O'Neill, M. Riggs, and M. Wigler. 1989. Isolation and characterization of a mammalian gene encoding a high-affinity cAMP phosphodiesterase. *Proc. Natl. Acad. Sci.* **86:** 3599–3603.

Michaeli, T., J. Field, R. Ballester, K. O'Neill, and M. Wigler. 1989. Mutants of Ha-*ras* which interfere with *RAS* effector function in *S. cerevisiae*. *EMBO J.* **8:** 3039–3044.

Powers, S., K. O'Neill, and M. Wigler. 1989. Dominant yeast and mammalian *RAS* mutants that interfere with the *CDC25*-dependent activation of wild-type *RAS* in *S. cerevisiae*. *Mol. Cell. Biol.* **9:** 390–395.

Sharma, S., C. Birchmeier, J. Nikawa, K. O'Neill, L. Rodgers, and M. Wigler. 1989. Characterization of the *ros1*-gene products expressed in human glioblastoma cell lines. *Oncogene Res.* **5:** 91–100.

Young, D., M. Riggs, J. Field, A. Vojtek, D. Broek, and M. Wigler. 1989. The adenylyl cyclase gene from *Schizosaccharomyces pombe*. *Proc. Natl. Acad. Sci.* **86:** 7989–7993.

In Press, Submitted, and In Preparation

Birchmeier, C., K. O'Neill, M. Riggs, and M. Wigler. 1990. Characterization of *ROS1* cDNA from a human glioblastoma cell line. *Proc. Natl. Acad. Sci.* (in press).

Colicelli, J., J. Field, R. Ballester, N. Chester, D. Young, and M. Wigler. 1990. Mutational mapping of RAS-responsive domains of the *Saccharomyces cerevisiae* adenylyl cyclase. *Mol. Cell. Biol.* (in press).

Field, J., A. Vojtek, R. Ballester, G. Bolger, J. Colicelli, K. Ferguson, J. Gerst, T. Kataoka, T. Michaeli, S. Powers, M. Riggs, L. Rodgers, I. Wieland, B. Wheland, and M. Wigler. 1990. Cloning and characterization of *CAP*, the *Saccharomyces cerevisiae* gene encoding the 70 kDa adenylyl cyclase associated protein. *Cell* (in press).

Field, J., H.-P. Xu, T. Michaeli, R. Ballester, P. Sass, M. Wigler, and J. Colicelli. 1990. Mutations of the adenylyl cyclase gene that block *RAS* function in *Saccharomyces cerevisiae*. *Science* **247:** 464–467.

Wieland, I., G. Bolger, G. Asouline, and M. Wigler. 1990. A method for difference cloning: Gene amplification following subtractive hybridization. *Proc. Natl. Acad. Sci.* (in press).

RAS ONCOGENES AND SIGNAL TRANSDUCTION

D. Bar-Sagi N. Gale, L. Graziadei, S. Kaplan, S. Palmer, A. Samatar, Y. Yeh

During the past year, our research has continued to focus on two areas: (1) the cellular function of *ras* proteins and (2) the molecular properties of phospholipase A_2 (PLA_2). Both *ras* proteins and PLA_2 have been implicated in the transduction of signals across the plasma membrane, and earlier studies from our laboratory indicate a possible functional link between PLA_2 activation and *ras*-induced cell transformation. Our goal is to understand the role that these proteins play in the control of cell proliferation.

Molecular Cloning of Phospholipase A_2

N. Gale, S. Palmer, A. Samatar, D. Bar-Sagi

During the past several years, evidence has been accumulating in support of a role for membrane PLA_2 ($mPLA_2$) in the transduction of mitogenic signals. A major aim in our laboratory has been the cloning of $mPLA_2$. As an initial approach, we have carried out immunological screening of rat pan-

creatic and rat brain λgt11 libraries using rabbit polyclonal anti-PLA_2 antibodies. These antibodies recognize $mPLA_2$ in rat cells, as determined by immunocytochemical and functional assays, and therefore constitute a potentially powerful tool for use in screening cDNA expression systems. However, exhaustive screening of a λgt11 cDNA expression library from rat brain tissue has not yielded positive clones. Presumably, the low abundance of PLA_2 message within cells and/or the toxic effects of its overexpression in the *Escherichia coli* system has made the cloning of PLA_2 refractory to this method. In an attempt to circumvent these difficulties, we are utilizing the polymerase chain reaction (PCR). Alignment of the peptide sequences of all the known mammalian PLA_2s reveals a significant sequence divergence among these enzymes. However, several regions of high conservation can be identified, including the calcium-binding loop and regions comprising the enzyme's active site. Using the information generated by these alignments, we have designed several slightly degenerate pairs of synthetic oligonucleotide primers to be used to amplify the rare PLA_2 sequence from rat brain cDNA. Although this research is still in progress, pilot studies suggest that this method will yield success in the cloning of intracellular PLA_2.

To learn more about the structure, function, and evolution of PLA_2, we have also sought to clone the genes for other members of the PLA_2 family from *Drosophila melanogaster* and *Saccharomyces cerevisiae*. Toward this end, we are using a full-length cDNA clone of the secreted pancreatic form of the enzyme that was isolated in our laboratory. Southern analysis reveals several hybridizing bands when using the pancreatic cDNA probe at low stringency. To date, a *Drosophila* genomic DNA library has been screened, and several positive phages have been isolated. Characterization of these clones from *Drosophila* and the pursuit of similar clones from yeast will be the focus of continuing effort in the laboratory.

Role of Phospholipase A_2 in Exocytosis

L. Graziadei, D. Bar-Sagi

Last year, we demonstrated that microinjection of the *ras* oncogene induces exocytotic degranulation in rat peritoneal mast cells. This exocytotic response involves the translocation of histamine-containing granules to the plasma membrane, fusion of the granule and plasma membranes, and subsequent release of the granule contents to the extracellular space. Cellular PLA_2 is a candidate for a mediator of this response as the enzyme's activity results in the release of lysophospholipids, which are potent membrane fusogens.

We have used our polyclonal anti-PLA_2 serum to analyze the participation of PLA_2 in ligand-induced exocytotic degranulation. Affinity-purified preparations of these anti-PLA_2 antibodies recognized a 16-kD band on immunoblots of total cellular proteins from mast cells. Furthermore, incubation of mast-cell membranes with the anti-PLA_2 antibodies resulted in a significant inhibition of PLA_2 activity, as assayed by thin-layer chromatography. These results indicate that the anti-PLA_2 antibodies recognize a population of the mast-cell membrane-associated PLA_2. Compound 48/80 and the calcium ionophore A23187 are each capable of inducing mast-cell degranulation. Compound 48/80 acts via a cell surface receptor, whereas A23187 bypasses the receptor and activates the exocytotic response by postreceptor signals. Microinjection of affinity-purified anti-PLA_2 antibodies inhibited degranulation induced by compound 48/80, and that induced by calcium ionophore was unaffected. These observations suggest a regulatory role for PLA_2 in ligand-induced exocytosis.

The anti-PLA_2 antibodies were employed in an immunocytochemical localization study utilizing indirect immunoperoxidase and immunogold techniques. Resting mast cells show a low level of immunoperoxidase staining primarily on the cell membrane and, infrequently, on intracellular membranes, including those of the histamine-containing granules. Enhanced staining was detected on membrane ruffles. Stimulation of cells with compound 48/80 or calcium ionophore induced a dramatic redistribution of staining. Heavy, discrete patches of staining were evident over the points of apposition between the plasma membrane and granule membrane that appear prior to membrane fusion. Surrounding areas of membrane were depleted of staining. To refine the localization of immunoreactive material, immunogold labeling was performed. A sporadic gold labeling of the granule matrix was observed in resting and stimulated cells. Granule membranes showed little labeling, whereas most of the gold particles were localized to the plasma membrane.

Taken together, our observations indicate that catalytic activity of PLA_2 is required for some types of ligand-induced exocytosis in rat peritoneal mast cells. The spatial redistribution of this enzyme during exocytosis is consistent with a role for PLA_2 as a catalytic membrane fusogen. Our future studies will focus on the functional interrelation between PLA_2 and *ras* proteins in the exocytotic response.

Role of *ras* Proteins in Transmembrane Signaling in B Lymphocytes

L. Graziadei, D. Bar-Sagi [in collaboration with K. Riabowol, Cold Spring Harbor Laboratory]

Signal transduction in B lymphocytes is marked by a well-characterized set of morphological changes: surface immunoglobulin (Ig) receptors cross-linked by antibody or appropriate multivalent antigens form aggregates called patches that then collect, via interaction with the cytoskeleton, into a single cap-like assembly at one pole of the cell. Receptor aggregation constitutes the initial signal for a process known as B-cell activation, which includes growth factor receptor expression, clonal proliferation, and Ig class switching. The movement of cross-linked surface Ig provides a useful experimental system for the identification of other cell membrane components that may be involved in B-cell activation. In particular, using double-labeling techniques, it is possible to monitor the movement of surface Ig induced by a specific labeled ligand while visualizing any associated changes in the localization of a second, specifically labeled membrane component.

We have used double immunofluorescence staining to monitor the distribution of *ras* proteins in mouse splenic B cells during various stages of patching and capping. Surface Ig was stained with fluorescein-conjugated goat anti-mouse IgG. *ras* proteins were stained using affinity-purified rabbit anti-*ras* antibodies, followed by rhodamine-conjugated goat anti-rabbit IgG. The specificity of the anti-*ras* antibodies was established by immunoprecipitation and immunofluorescence staining. Resting cells displayed a uniform membrane staining of IgG and *ras*. However, stimulation of B cells with anti-mouse Ig was accompanied by the redistribution of *ras* proteins. At each stage of patching and capping, *ras* redistribution paralleled that of surface IgG, with *ras* becoming directly localized under each patch and cap (Fig. 1). The kinetics of redistribution of each protein were identical.

We have initiated studies aimed at understanding the mechanisms underlying the redistribution of *ras* proteins and the functional significance of this redistribution event. Treatment of B cells with meta-

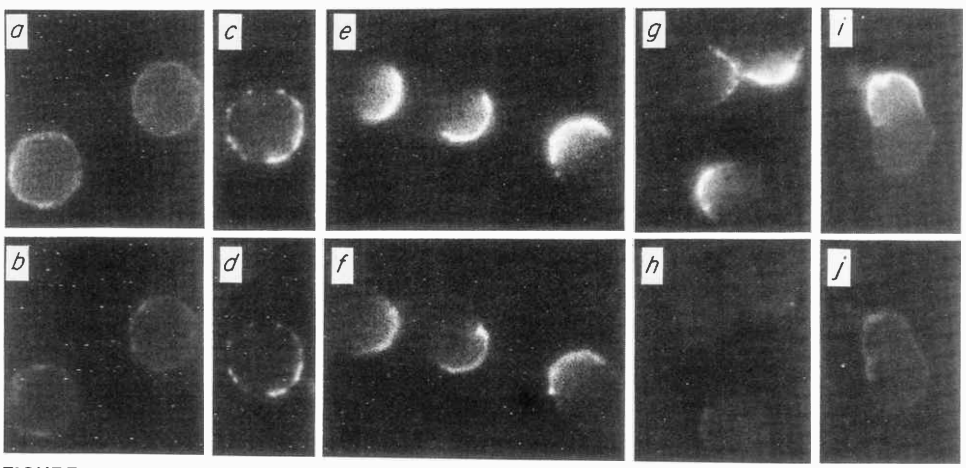

FIGURE 1 Redistribution of surface IgG and $p21^{ras}$ in mouse splenic B lymphocytes treated with anti-mouse IgG. Induction of patching and capping was carried out using fluorescein-conjugated goat anti-mouse IgG or fluorescein-conjugated Con A. Upper panels show the fluorescence staining pattern of surface IgG (*a*,*c*,*e*,*g*) and Con A receptors (*i*), and lower panels show the fluorescence staining pattern of $p21^{ras}$. (*a*,*b*) Uniform distribution prior to stimulation with goat anti-mouse IgG or Con A; (*c*,*d*) patching after 5 min of stimulation; (*e*,*f*) capping after 15 min of stimulation: (*g*) IgG caps at 15 min of stimulation; (*h*) same cells as in panel *h* stained with secondary antibody alone; (*i*) Con A cap after 30 min of stimulation. Magnification, 3312×.

bolic inhibitors or with cytoskeletal disrupting agents prevents surface Ig capping. Under these conditions, IgG is arrested in patches and *ras* remains in a corresponding patchy distribution. Furthermore, we have demonstrated the specificity of *ras* capping by using concanavalin A (Con A), a nonmitogenic lectin, to induce the capping of Con-A receptors. Although Con-A-induced caps are morphologically indistinguishable from those induced by IgG, *ras* displays a resting, uniform membrane distribution in these cells (Fig. 1). Thus, the redistribution of *ras* proteins appears to be a signal-specific event. Overall, these findings illustrate for the first time the dynamic aspects of the association of *ras* with the plasma membrane and provide evidence that the distribution of *ras* proteins can be controlled by receptor-ligand interaction. We are now investigating the functional significance of this redistribution event. Two possibilities are being specifically examined: (1) *ras* protein as a component of the mechanism for the ligand-induced capping process or (2) *ras* protein as a component of the signaling pathway triggered by the aggregation of ligand-receptor complexes.

Association of *ras* Proteins with Cellular Polypeptides

S. Kaplan, D. Bar-Sagi

We are using polyclonal anti-*ras* antibodies to identify cellular proteins that interact with $p21^{ras}$. In addition to $p21^{ras}$ proteins, the anti-*ras* antibodies precipitate from A431 cell lysates several polypeptides with relative molecular weights of approximately 150K, 120K, 105K, and 50K. Two-dimensional gel electrophoresis of the immunoprecipitates reveals two additional, although less prominent, "coprecipitates" with relative molecular weights of 50K and 60K. Several lines of evidence suggest that these proteins are cellular polypeptides that interact with *ras*: (1) These polypeptides are not precipitated from cells when the antibody is preincubated with an excess of purified *ras* protein. (2) After denaturation, the $p21^{ras}$ proteins, but not the other polypeptides, are specifically recognized by the antibodies. (3) In immunoblots of A431 cells, the anti-*ras* antibodies did not detect the *ras*-associated polypeptides. Thus, it is unlikely that the 150K, 120K, 105K, and 50K polypeptides are recognized directly by the anti-*ras* antibodies due to epitopes shared with $p21^{ras}$ proteins. Cellular fractionation reveals that the 150K polypeptide is found exclusively in the membrane (P-100) fraction, whereas the 120K and 105K polypeptides are found in both the membrane and soluble (S-100) fraction. The 120K and 105K are phosphorylated on serine and threonine residues. We are currently characterizing the association of these proteins with $p21^{ras}$ in normal and *ras*-transformed cells. Our aim is to establish whether the cellular proteins constitute functional targets for *ras* proteins.

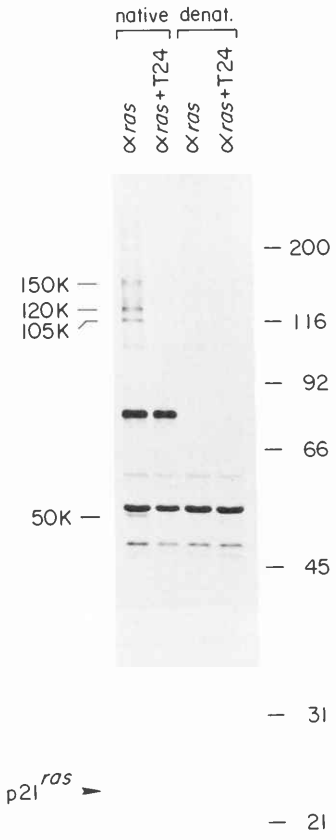

FIGURE 2 Immunoprecipitation of native or denatured proteins from A431 cells. A431 cells were labeled with [^{35}S]methionine, and cell lysates were prepared in RIPA buffer with or without 1% SDS. The SDS-treated lysates were boiled for 5 min and then diluted tenfold with RIPA buffer. Aliquots from the native and the denatured samples were immunoprecipitated with the anti-*ras* (α-*ras*) antibodies or with the anti-*ras* antibodies preincubated with 1 μg of purified Ha-*ras* protein (T24). The immune complexes were collected on protein A–Sepharose, washed, and separated on a 12.5% SDS-polyacrylamide gel.

PUBLICATIONS

Bar-Sagi, D. 1989. Phospholipase A_2 in *ras*-transformed cells. In *Cell activation and signal initiation: Receptor and phos-*

pholipase control of inositol phosphate, PAF and eicosanoid production, UCLA Symposium, pp. 331–335. Alan R. Liss, Inc.

Bar-Sagi, D. 1989. *Ras* proteins: Biological effects and biochemical targets (Review). *Anticancer Res.* **9:** 1427–1438.

Kim, D., D. Lewis, L. Graziadei, E.J. Neer, D. Bar-Sagi, and D.E. Clapham. 1989. G protein βγ subunits activate the cardiac muscarinic K^+ channel via phospholipase A_2. *Nature* **337:** 557–559.

In Press, Submitted, and In Preparation

Bar-Sagi, D. 1990. Phospholipase A_2: Microinjection and cell localization techniques. *Meth. Enzymol.* (in press).

Cockcroft, S. and D. Bar-Sagi. 1990. Effect of H-*ras* proteins on the activity of polyphosphoinositide phospholipase C in HL60 membranes. *J. Cell. Signalling* (in press).

Graziadei, L. and D. Bar-Sagi. 1990. Control of exocytosis by cellular phospholipase A_2. (In preparation.)

Graziadei, L., K. Riabowol, and D. Bar-Sagi. 1990. *Ras* proteins co-cap with surface immunoglobulin in B lymphocytes. *Nature* (Submitted.)

NUCLEAR ONCOGENES AND SIGNAL TRANSDUCTION

M. Gilman R. Attar, L. Berkowitz, D. Girgenti, R. Graham, K. Riabowol, W. Ryan

Our major interest is how extracellular signals are communicated to the nucleus. Our focus is the proto-oncogene c-*fos*, which is a critical crossroads for information flow to the nucleus. The c-*fos* gene is rapidly activated at the transcriptional level by triggering of any one of several intracellular signal transduction pathways. Sequences within the c-*fos* gene thus constitute physical targets for these incoming signals. Several projects in our laboratory are concerned with identifying these target sites and tracing the signaling chains back toward the cell surface.

The product of the c-*fos* gene is a nuclear protein that participates in the formation of a heterogeneous set of sequence-specific DNA-binding proteins that are presumed to act on the next set of genes activated in the transcriptional cascades triggered by growth and differentiation factors. Thus, the c-*fos* gene is itself part of the cell's signal transduction machinery, acting to convert short-term signaling information (on the order of minutes) to long-term cellular responses (on the order of hours and days). Other projects in the laboratory are aimed at understanding the role of Fos in this process and especially how the biological specificity of growth factors might be reflected in the make-up of Fos protein complexes.

Functional Analysis of the Serum Response Element

M. Gilman, R. Graham

A major DNA target in the c-*fos* gene for incoming signals is the serum response element (SRE). The SRE was first identified as a sequence required for induction of c-*fos* transcription by whole serum. Subsequently, the SRE was shown to consist of a 20-bp dyad symmetry element that is necessary and sufficient for the response of c-*fos* to serum, many purified growth factors, and at least two distinct intracellular signal transduction pathways. More recently, the SRE has also been shown to be the target for repression of c-*fos* transcription in unstimulated cells and for the rapid repression that follows induction by serum. In the latter case, the Fos protein itself is believed to play a role. Thus, the SRE is a multifunctional element. As described below, the SRE is a binding site for at least three cellular DNA-binding proteins. At least one of these proteins is required for activation of c-*fos* transcription in response to serum, because microinjection of an SRE oligonucleotide into quiescent cells blocks subse-

quent induction of c-*fos* expression by serum. Other proteins may be required for repression.

To attempt to assign specific functional roles to each of the DNA-binding proteins that interact with the SRE, we performed saturation mutagenesis on a portion of the SRE that is contacted by all three proteins. Our goal was to generate a collection of mutant sites that bound distinct combinations of the three proteins. Of particular interest, we isolated several single-nucleotide substitutions in the SRE that bound one protein, SRF, at wild-type levels but failed to form a ternary complex with a second protein ($p62^{TCF}$) that specifically recognizes the SRE-SRF complex (Fig. 1). This ternary complex has been reported to be required for serum activation of c-*fos* transcription. However, each of these mutants was found to be inducible by serum at at least wild-type levels. Our current hypothesis is that the SRF-$p62^{TCF}$ is required for activation by some but not all signaling pathways.

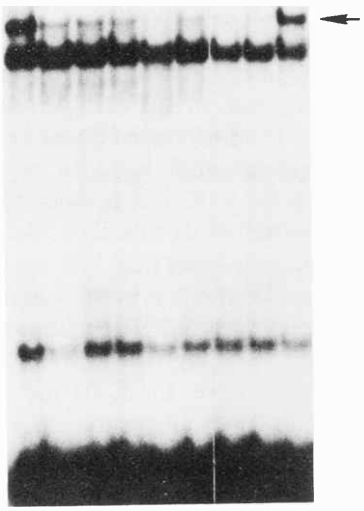

FIGURE 1 DNA-binding assay of mutant serum response element derivatives. Nine SRE probes, each containing a single nucleotide substitution, were used as substrates in a mobility-shift assay. Each probe was assayed with partially purified nuclear fractions enriched for SRF and p62TCF. Arrow points to the ternary complex containing SRE DNA and both proteins. The lower band contains only SRF.

Structural and Functional Studies of SRE-binding Proteins

W.A. Ryan, Jr., M. Gilman [in collaboration with D. Marshak and E. Chang, Cold Spring Harbor Laboratory]

The c-*fos* SRE is a control element that mediates c-*fos* activation by several distinct intracellular signal transduction pathways and c-*fos* repression by one or more distinct pathways. To understand how these different activities are regulated, we are studying the cellular DNA-binding proteins that interact at the SRE. Until recently, only a single SRE-binding protein was known. This protein is SRF, a 67-kD protein that binds symmetrically to the SRE. Mutations that interfere with SRF binding abolish both induction and repression of c-*fos* transcription. However, it has not been possible to demonstrate any biochemical changes in SRF activity or structure that accompany c-*fos* induction or repression, so the specific function of SRF has remained obscure. To identify additional DNA-binding proteins that might participate in the complex regulatory events at the SRE, we fractionated human cell nuclear extracts and used two different DNA-binding assays to look for novel SRE-binding proteins. We found two. One protein binds directly to the SRE in asymmetric fashion distinguishable from SRF. Its apparent molecular mass is 62 kD, as determined by DNA-affinity precipitation and by UV cross-linking, and it is chromatographically separable from SRF. We call this protein $p62^{DBF}$ (direct binding factor). A second protein, chromatographically distinct from the other two proteins, does not bind directly to the SRE, but rather specifically recognizes and binds the SRF-SRE complex, forming a biochemically distinguishable ternary DNA-protein complex. This protein complex makes additional contacts with SRE DNA on the 5′ side of the dyad not obtained with SRF alone. By UV cross-linking, we have determined the size of this protein to be 62 kD, and we call it $p62^{TCF}$ (ternary complex factor). The two 62-kD proteins are biochemically and chromatographically distinct, but the possibility remains that they are related forms of the same polypeptide.

The specific roles played by each of these proteins in the c-*fos* transcription cycle remain to be established. At present, we have been unable to demonstrate any biochemical modifications to these proteins associated with induction or repression of c-*fos* transcription. The DNA-binding activities of the pro-

teins do not appear to change, and although these are phosphoproteins in vivo, we have detected no changes in the phosphorylation state of the proteins. More sensitive assays are being used to examine these proteins in vivo.

To carry out structural and functional studies of SRF, we have obtained an SRF cDNA from Richard Treisman (ICRF) and expressed the protein at high levels in *Escherichia coli*. *E. coli* SRF has an apparent molecular mass of 62 kD. It binds specifically to the SRE in vitro, but its specific activity in these assays is substantially lower than SRF purified from human cells. In addition, it is inefficient at forming a ternary complex with $p62^{TCF}$. Preliminary results suggest that *E. coli* SRF is efficiently phosphorylated in vitro by casein kinase II (CK-II) and that phosphorylation significantly augments its DNA-binding activity. Ongoing experiments are aimed at understanding the role of CK-II phosphorylation in SRF function.

Cloning of a Novel SRE-binding Protein

R. Attar, M. Gilman

As described above, the SRE is a target for multiple signals that activate and repress c-*fos* transcription. The SRE is a binding site for at least three cellular DNA-binding proteins. One of these proteins, $p62^{DBF}$, binds directly to the SRE in a manner distinguishable from that of SRF. To determine the structure and function of this protein and its possible relationship to the other 62-kD SRE-binding protein, $p62^{TCF}$, we have purified this protein by DNA-affinity chromatography and have attempted to isolate cDNA clones encoding it.

We screened a HeLa cell cDNA expression library for phage expressing proteins that specifically bound an SRE oligonucleotide. For this study, we used a variant SRE sequence that binds $p62^{DBF}$ with high affinity and binds SRF with low affinity. We isolated a phage that encoded a *lacZ* fusion protein that specifically bound this SRE oligonucleotide, but not a mutant site that does not bind the human protein in vitro. The fusion protein binds specifically to the SRE in both a Southwestern blot and a mobility-shift assay (Fig. 2).

Analysis of the partial DNA sequence of this clone revealed that it was a previously unidentified gene that belongs to the family of zinc-finger-containing proteins related to the *Drosophila Kruppel* gene. The clone contains seven tandem repeats in the carboxyl terminus that match the zinc finger consensus for this gene family. In the zinc finger region, the clone shares 65% sequence identity with several human and mouse *Kruppel*-related genes. The presence of zinc fingers in this clone strongly suggests that it is a DNA-binding protein and probably a transcription factor. Experiments currently under way are aimed at determining whether this clone indeed encodes $p62^{DBF}$ and what its effector function is.

Regulation of c-*fos* Transcription by cAMP

L. Berkowitz, M. Gilman

Among the signal transduction pathways that activate c-*fos* transcription is the well-characterized cAMP pathway. We had found previously that induction of c-*fos* transcription by cAMP was independent of the SRE. To locate the sequences in the c-*fos* promoter responsive to cAMP, we tested a series of promoter deletions and point mutants for their ability to respond to cAMP in a transient expression assay. We found that the c-*fos* promoter carries multiple elements that contribute the cAMP responsiveness of the promoter. The relative strengths of these sites as CREs correlated with their affinity for CRE-binding factors in vitro. Thus, their strengths reflect their intrinsic activities and not their relative positions in the promoter.

The elements we mapped in the transient expression assay are functional as CREs in the endogenous c-*fos* gene. This was demonstrated by microinjection competition assays. We microinjected into quiescent fibroblasts double-stranded oligonucleotides corresponding to the major CRE at −65 and to the SRE and stimulated the cells with cAMP or serum. Treatment with cAMP failed to induce c-*fos* expression in cells injected with the CRE oligonucleotide but induced c-*fos* normally in cells injected with SRE DNA. Conversely, induction of c-*fos* expression by serum was blocked by the SRE oligonucleotide but not by the CRE. We therefore concluded that binding of proteins to elements related to the CREs mapped in the transient expression assays is also required for activation of the endogenous c-*fos* gene

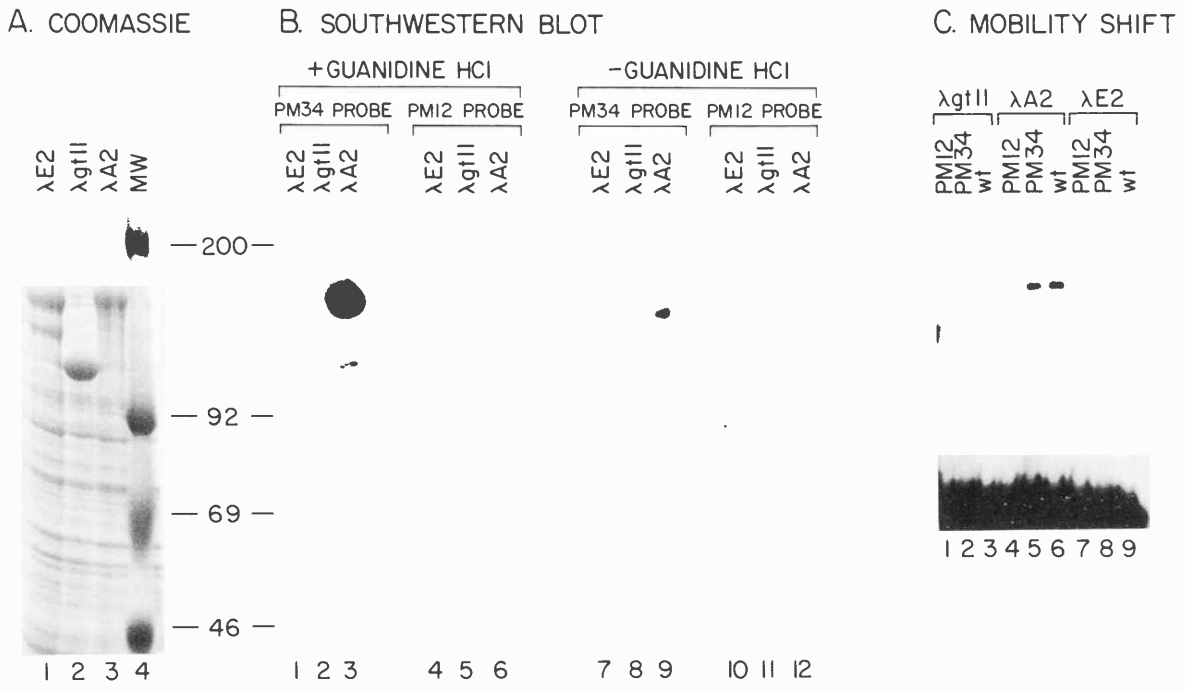

FIGURE 2 DNA-binding assays of novel SRE-binding protein. Cultures of *E. coli* were infected with phage vector or two insert-containing recombinants. A2 is the clone described in the text; E2 is an independently isolated clone carrying a different insert. Portions of the infected cells were lysed and loaded onto an SDS/polyacrylamide gel. (*A*) Portion of the gel stained with Coomassie blue; (*B*) identical gel lanes were blotted to nitrocellulose. In lanes *1–6*, proteins on the filters were denatured in guanidine HCl and renatured by dilution; lanes *7–12* were not treated in this way. The filters were incubated in solution containing radiolabeled oligonucleotide encoding an SRE derivative (PM34) that binds p62DBF or a derivative that does not bind (PM12). (*C*) Mobility-shift assay of solubilized fusion proteins with the same probes as in panel *B* and with a true wild-type SRE probe.

in its natural chromatin configuration. Moreover, unrelated elements such as the SRE do not play a significant role in regulating c-*fos* expression in response to cAMP in these cells.

Microinjection experiments carried out previously in the lab demonstrated that activation of c-*fos* expression by cAMP was due to the catalytic activity of cAMP-dependent protein kinase (pkA) and that the action of pkA also required CRE-binding proteins. These observations suggested a model in which activation of gene expression by cAMP occurs via phosphorylation of cellular proteins by pkA that eventually act on CREs. The simplest form of this model is that this interaction is direct, i.e., that pkA directly phosphorylates and activates CRE-binding transcription factors. A candidate for such a factor has recently been described by M. Montminy and colleagues. This protein, termed CREB, is a high-affinity CRE-binding protein that is phosphorylated in vitro by pkA. To determine whether direct phosphorylation of CREB by pkA could account for regulation of gene expression by cAMP, we isolated cDNA clones encoding human CREB. We recovered two distinct clones that differed by a 14-amino-acid peptide. Both CREB mRNAs are widely expressed in many tissues. Both encode proteins that bind as dimers to the c-*fos* CRE in vitro (Fig. 3). Both function as cAMP-regulated transcription factors in vivo. When the site of phosphorylation by pkA was mutated, cAMP-regulation of CREB activity was lost. This observation is consistent with direct phosphorylation of CREB by pkA. The two forms of CREB differ in their transcriptional activities when the pkA phosphorylation site is destroyed. This observation suggests a regulatory role for the inserted peptide in the larger form of CREB.

Current experiments are directed at learning whether CREB or other CRE-binding proteins participate directly in the regulation of c-*fos* expression in vivo. A second issue we would like to address is

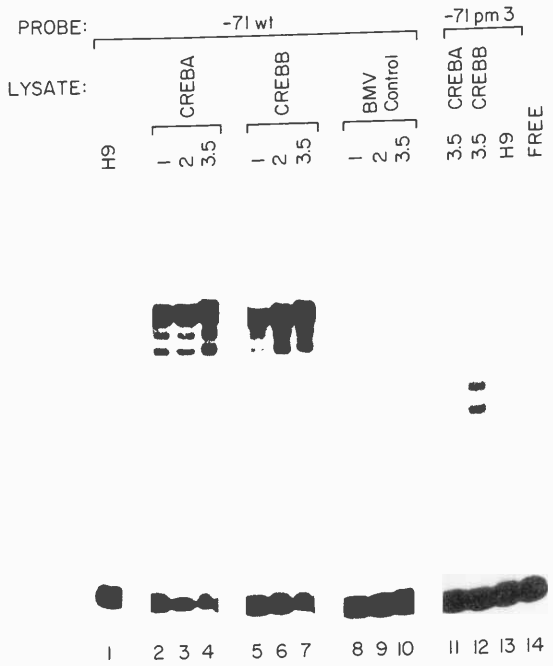

FIGURE 3 DNA-binding assay of in-vitro-translated CREB proteins. CREB cDNAs were transcribed into mRNA in vitro using T7 RNA polymerase, and the resulting RNA was translated in a reticulocyte lysate. Shown is a mobility-shift assay of the reticulocyte lysates programmed by CREB-A RNA (lanes *2–4*, *11*), CREB-B RNA (lanes *5–7*, *12*), brome mosaic virus RNA (lanes *8–10*). The probes were c-*fos* promoter fragments containing either a wild-type CRE (located at −65 in the promoter) (lanes *1–10*), or a similar probe carrying mutations that inactivate the CRE (lanes *11–13*).

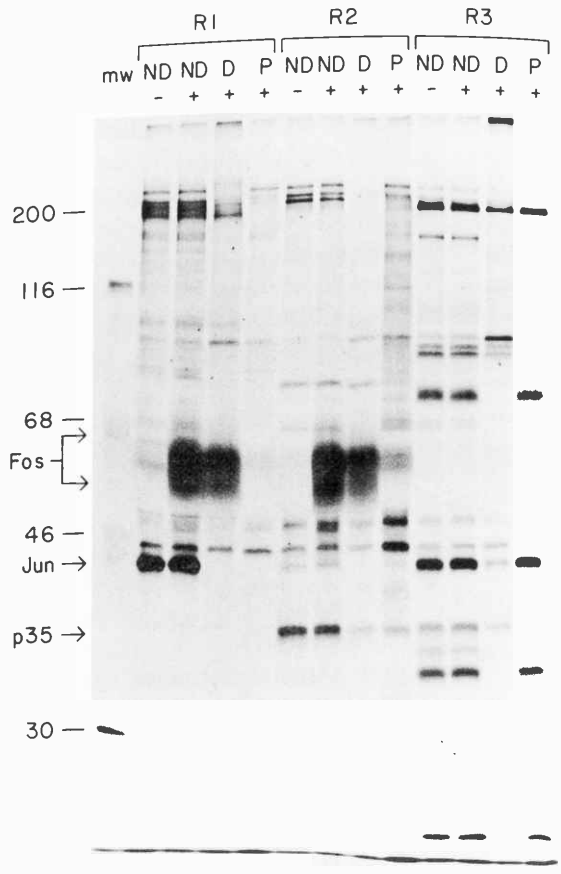

FIGURE 4 Immunoprecipitation of T-cell lysates with antisera raised against Fos and Fos-associated proteins. [^{35}S]Methionine-labeled H9 cell lysates were prepared from ionomycin-stimulated (+) or unstimulated (−) H9 cells under denaturing (D) or nondenaturing (ND) conditions. Lysates were immunoprecipitated with antiserum from rabbits immunized with TrpE-Fos fusion protein (R1, R2) or with total proteins from preparative Fos immunoprecipitations. Immunoprecipitations from stimulated cells were also done in the presence of unlabeled Fos fusion protein (P) to determine if antisera from these rabbits directly recognized the proteins they precipitated under nondenaturing conditions.

where in the cell phosphorylation of CREB by pkA takes place and how nuclear translocation of either the kinase or phosphorylated CREB is regulated.

Generation of Antibodies against Fos-associated and Fos-related Proteins

K. Riabowol, D. Girgenti, M. Gilman

The Fos protein associates with a subset of proteins in the nucleus, which includes the protein product of the c-*jun* proto-oncogene. Like Fos, Jun is a nuclear transforming protein and both are members of a set of DNA-binding proteins collectively termed AP-1, which specifically bind activator protein-1 (AP-1) sites. These sites are also referred to as TPA-response elements (TREs). The Fos protein is able to bind DNA by itself, but both specificity and avidity of binding are conferred when Fos is complexed with Jun. For example, in one set of experiments in which c-Jun homodimers were observed to bind a TRE sequence, Fos:Jun heterodimers bound 30-fold more efficiently, whereas c-*fos* failed to bind at detectable levels.

Fos, Jun, and the protein products of several other genes including FosB, FRA1, JunB, and JunD contain a highly conserved basic motif believed to bind DNA, and a structure termed the leucine zipper. The leucine zipper consists of a heptad repeat of leucine residues that are oriented toward one "face" of an α-helical polypetide. Leucine residues on an α-helical region of one protein are proposed to interdigitate with those of a second protein, resulting in dimer

formation through strong hydrophobic interactions. Recent evidence suggests that the sequences of the leucine zipper regions control the stability and therefore the composition of protein complexes that form in vivo. This, in turn, would determine which complexes could subsequently associate with subsets of specific DNA regulatory regions, thereby regulating gene expression through more amino-terminal activation domains. These observations suggest that the diverse effects upon cellular physiology seen when various cell types are treated with different extracellular stimuli might be due to the expression of defined subsets of the Fos/AP-1 complex proteins.

To determine if such cell-type-specific or stimulus-specific expression of Fos complex proteins occurs in vivo, we have taken a two-step immunological approach. The first step involves identification of cell-type-specific or stimulus-specific proteins of the Fos complex. As shown in Figure 4, antisera from two rabbits immunized with TrpE-Fos fusion proteins (R1 and R2) directly recognize [^{35}S]methionine-labeled Fos protein from stimulated T cells under both denaturing (D) and nondenaturing (ND) conditions. However, when antisera were preincubated with excess Fos fusion protein, no Fos protein was recovered (lanes marked P). Antisera from these two rabbits also independently recognize two other prominent proteins, but only under nondenaturing conditions. This suggests that these proteins associate with the Fos/AP-1 complex by binding to discreet regions of the Fos protein. One of these proteins migrates on one- and two-dimensional gels similarly to the previously characterized Fos-associated oncoprotein Jun (antiserum from R1). The other protein called p35 in Figure 4 migrates with an M_r of 35 kD, has an isoelectric point similar to that of the characterized Jun proteins, and, like the putative form of Jun recovered by R1 antiserum, is synthesized in the absence of stimulation in T cells. Since we have been unable to detect the presence of p35 in a wide variety of human and rodent cell lines, p35 likely represents a T-cell-specific member of the Fos protein complex. As a first step toward cloning p35 and other uncharacterized members of the Fos protein complex, large amounts of Fos complex proteins were isolated using affinity-purified Fos antibodies that had been cross-linked to protein A–Sepharose beads. A rabbit was inoculated several times to generate antibodies that directly recognize proteins which associate with Fos. Reactivity against several proteins including Jun and p35 was obtained after three injections. Unlike the case for antisera raised against the Fos fusion protein, preincubation of the antiserum from rabbit 3 (R3) with Fos fusion protein failed to prevent the immunoprecipitation of p35, Jun, or the majority of other proteins recognized by this antiserum (Fig. 4, compare R3 lanes ND$^+$ and P$^+$). This antiserum and monoclonal antibodies also generated by boosting animals with proteins of the Fos complex will be used to screen protein expression libraries to isolate cDNA clones of these proteins.

PUBLICATIONS

Berkowitz, L.A., K.T. Riabowol, and M.Z. Gilman. 1989. Multiple sequence elements of a single functional class are required for cyclic AMP responsiveness of the mouse c-*fos* promoter. *Mol. Cell. Biol.* **9:** 4272–4281.

Ryan, W.A., Jr., B.R. Franza, Jr., and M.Z. Gilman. 1989. Two distinct cellular phosphoproteins bind to the c-*fos* serum response element. *EMBO J.* **6:** 1786–1792.

In Press, Submitted, and In Preparation

Berkowitz, L.A. and M.Z. Gilman. 1990. Two distinct forms of transcription factor CREB. (In preparation.)

Riabowol, K.T., D.Q. Girgenti, and M.Z. Gilman. 1990. A T-cell-specific member of the Fos protein complex. (In preparation.)

Riabowol, K.T., D.Q. Girgenti, and M.Z. Gilman. 1990. Biochemically and immunologically distinct Fos-containing complexes. (In preparation.)

CELL BIOLOGY OF THE NUCLEUS

D.L. Spector S. Huang G. Lark
R. Derby

Our research program has continued to focus on the structural-functional organization of the mammalian cell nucleus. During the past year, we have had several changes in personnel. Dr. Wai-Kit Chan left the laboratory to attend Columbia University Law School. Michael Delannoy, a technician in the

laboratory, has left to assume a new position at Johns Hopkins University, where he is responsible for the operation of the electron microscopy laboratory; Joe Suhan has left for an electron microscopy position at Carnegie-Mellon University.

Dr. Sui Huang joined the laboratory as a post-doctoral fellow, and she will be working on mRNA transport. Gayle Lark joined the laboratory as an electron microscopy technician, and she is concentrating on projects relating to nuclear localization of proteins involved in pre-mRNA processing. The use of the electron microscopy core facility has continued to expand, and a large number of collaborations are under way with the excellent technical expertise of Robert Derby, who has also recently joined the laboratory.

Reorganization of snRNP-enriched Nuclear Regions upon Alterations in the Level of Transcription

D.L. Spector, M. Sovak [Undergraduate Research Program]

In a series of previous studies, we have shown that small nuclear ribonucleoprotein particles (snRNPs) are distributed in a nuclear network that extends between the nucleolar surface and the nuclear envelope-lamina. In addition, we have shown that the bulk of nuclear DNA is not contained within this nuclear region. In the present study, we are interested in determining the effects of alterations in the level of transcription on the distribution of snRNPs. Cells treated with 0.04 μg/ml of actinomycin D (for 2 hr), a drug concentration that inhibits RNA polymerase I activity, showed no alteration in the distribution pattern of snRNPs (Fig. 1d) as compared to control cells (Fig. 1b). Cells treated with 0.04 μg/ml actinomycin D showed little to no autoradiographic grains over the nucleoli, since this dose of drug specifically inhibits RNA polymerase I. However, cells incubated with a higher dose of actinomycin D (10 μg/ml for 2 hr) known to inhibit all RNA polymerase activity exhibited a marked alteration in the distribution pattern of snRNPs (Fig. 1f). snRNPs were diffusely distributed throughout the nucleoplasm, excluding the nucleoli. Cells treated with this higher dose of actinomycin D, which inhibits all RNA polymerases, showed no autoradiographic grains in the nucleoplasm. The alteration in the snRNP distribution pattern upon incubation of cells with concentrations of actinomycin D that inhibit all RNA polymerases is similar to what we have previously shown to occur to the distribution of snRNPs after heat shock, which inhibits transcription of most genes and processing of their transcripts.

Since RNA polymerase II, which is involved in the synthesis of pre-mRNA, is highly sensitive to α-amanitin, whereas RNA polymerase I is insensitive and RNA polymerase III is sensitive only to high concentrations, we were interested in the effect of this drug on the snRNP distribution pattern. Cells were treated with a concentration of α-amanitin that would specifically inhibit RNA polymerase II (5 μg/ml) for 5, 9, 11, 18, and 24 hours. Cells were examined by immunofluorescence microscopy to determine the effect on the snRNP distribution pattern and by in situ autoradiography to determine the effect on transcription. Cells incubated in drug from 5 hours (Fig. 2d) to 9 hours (Fig. 2f) exhibited snRNP clusters that were more rounded in appearance than those observed in control cells (Fig. 2b). At 9 hours of drug incubation, the level of transcription by RNA polymerase II was decreased as determined by significantly fewer autoradiographic grains within the nucleoplasm due to [^{3}H]uridine incorporation. After 11 hours of incubation with the drug, the snRNPs appear in larger clusters (Fig. 2h), which are fewer in number as compared to control cells (Fig. 2b). By 18–24 hours, each cell contains one large immunoreactive region plus several rounded snRNP clusters (Fig. 2j,l). These data show that the snRNP network, characteristic of actively growing mammalian cells, reorganizes upon inhibition of transcription by RNA polymerase II and is therefore sensitive to the transcriptional level of genes transcribed by RNA polymerase II.

Localization of Single Copy Genes in the Interphase Cell Nucleus

D.L. Spector

Recent developments that enhance the efficiency of hybridization and the sensitivity of hybrid detection have made it possible to identify and localize specific cellular or viral DNA and RNA sequences even when present in low copy number. Although the genes transcribed by RNA polymerase I are highly organized within a nuclear region known as the

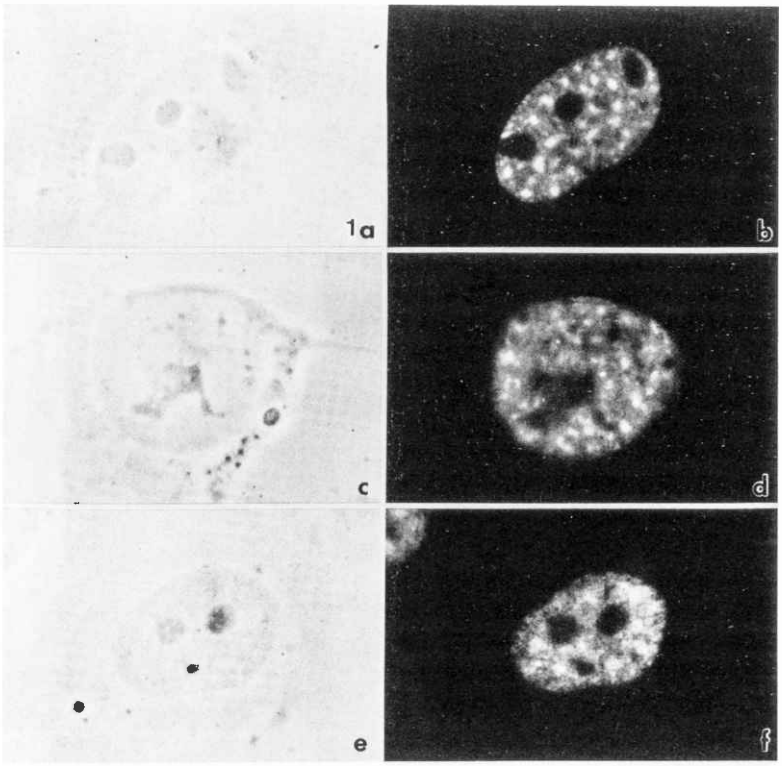

FIGURE 1 CHOC 400 cells immunostained with anti-Sm antibodies exhibit a speckled staining pattern throughout the nucleoplasm excluding the nucleoli (*b*). Cells incubated with 0.04 μg/ml actinomycin D for 2 hr show no change in the distribution of snRNPs (*d*). Cells incubated with 10 μg/ml actinomycin D for 2 hr exhibit a diffuse distribution of snRNPS (*f*). Magnification, 830×.

nucleolus, little information is available with regard to the nuclear organization of genes transcribed by RNA polymerases II or III. We have begun to study the distribution of several genes that have different transcription units to evaluate their intranuclear spatial organization. This study represents the beginning of a long-term goal to determine whether there is an underlying spatial organization to the interphase genome. These initial studies, which evaluate the distribution of interphase genes based on the type of transcription unit, will lay the groundwork for future studies that will evaluate whether cell-cycle-regulated genes, housekeeping genes, structural genes, or developmentally regulated genes localize in clustered patterns in the interphase nucleus. Such studies combined with antibody localization studies of proteins involved in such processes as transcription and RNA processing (snRNPs) will provide important data on the organization of nuclear function. Our studies on gene localization have started out by examining the localization of the dihydrofolate reductase (*dhfr*) gene, a housekeeping gene, in the interphase nucleus of Chinese hamster ovary cells. The mammalian *dhfr* gene is a 26–29-kb gene that is transcribed by RNA polymerase II and whose transcription unit contains introns and codes for a poly(A) signal. We have obtained clones inserted into pBR322 for several regions of the CHO *dhfr* gene from Dr. Larry Chasin (Columbia University). We have selected several of these clones, subcloned them, and inserted them into the Gem4Z plasmid for use in in vitro transcription reactions using SP6 and T7 polymerases. We have prepared subclones of the *dhfr* gene that range in size from 1.4 kb to 2.1 kb (Fig. 3) and represent unique sequences.

Probes pMB5-*Eco*RI and pB13-7 represent intron-plus exon-containing probes, whereas probe pB61H1 represents an intron-containing probe. Using T7 polymerase, we have made single-stranded biotinylated RNA probes that are complementary to the DNA sense strand and can be used for DNA localization. We have used a mixture of our probes

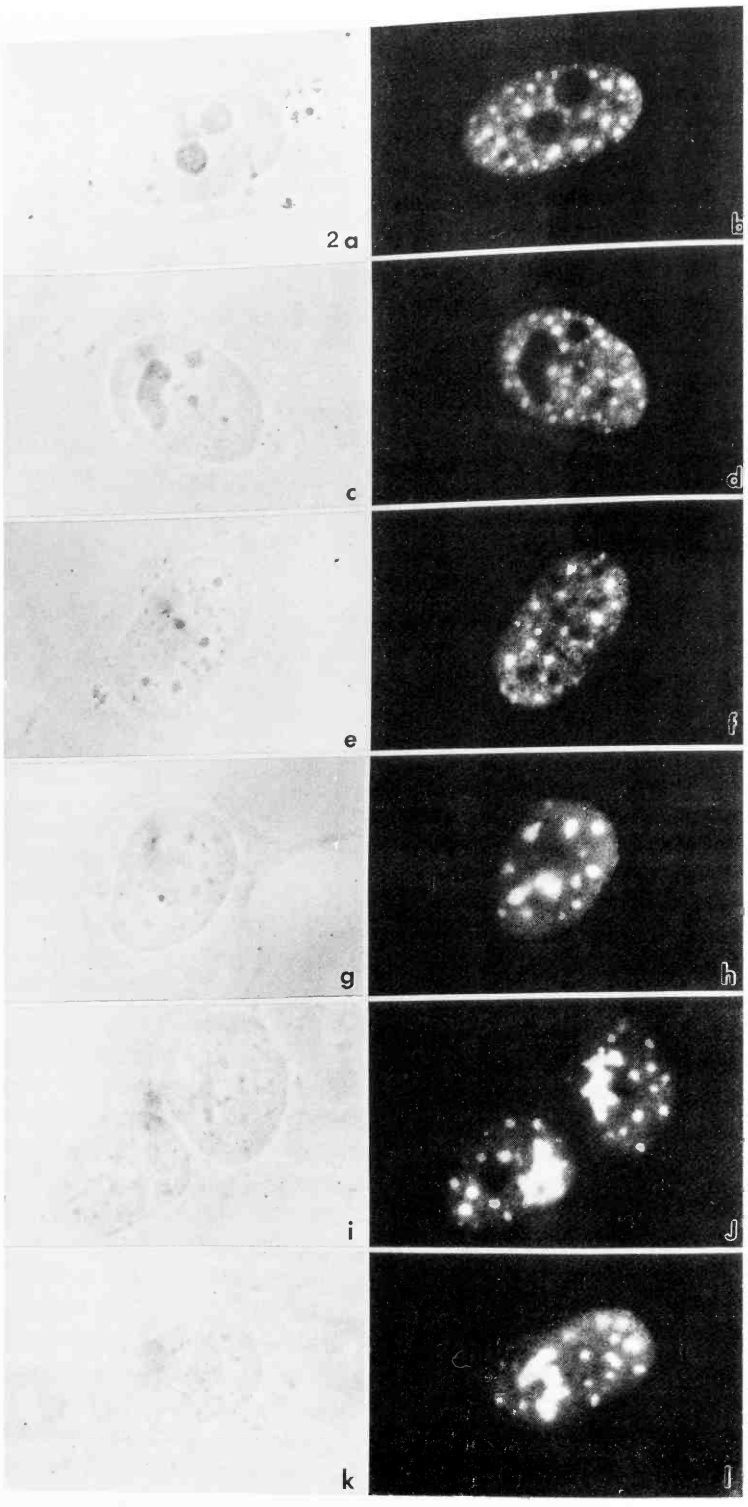

FIGURE 2 Cells incubated with 5 μg/ml α-amanitin for 5 hr begin to show a rounding up of snRNP clusters (*d*) as compared to control cells (*b*). This change is more apparent after 9 hr of drug incubation (*f*). By 11 hr of drug incubation, snRNPs appear to accumulate in large clusters (*h*), which increase in size by 18 and 24 hr of drug incubation (*j,l*). Magnification, 800×.

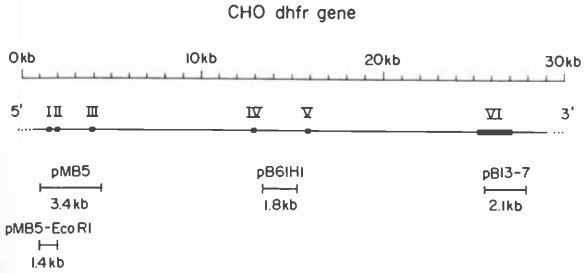

FIGURE 3 Diagram of the *dhfr* gene and probes used for in situ hybridization.

pMB5-*Eco*RI, pB61H1, and pB13-7 (Fig. 3) to achieve a combined hybridization length of >5 kb (total length = 5.3 kb) to localize the *dhfr* gene in nonamplified CHO cells, where it is present as two copies per diploid genome. We have been able to localize the two single copies of the *dhfr* gene in unamplified CHO cells by using Streptavidin-FITC. Each cell in Figure 4 shows two spots, representing the two copies of the gene in these diploid cells. Each spot represents the signal from the hybridization of biotinylated riboprobes spanning 5.3 kb of the gene. We are now preparing probes to localize a second gene in these cells. Double-label gene localizations (4 spots) in the same cell will allow us to map the three-dimensional localization of a specific gene copy within the interphase nucleus.

Differential Distribution of Factors Involved in Pre-mRNA Processing in the *Schizosaccharomyces pombe* Cell Nucleus

D.L. Spector, R.J. Derby [in collaboration with J.A. Potashkin, Cold Spring Harbor Laboratory]

IMMUNOFLUORESCENT LOCALIZATION OF snRNAs

We have localized the abundant snRNAs in a wild-type strain of the fission yeast *Schizosaccharomyces pombe* by indirect immunofluorescence and immunoelectron microscopy using an anti-m_3G antibody (obtained from Adrian Krainer, Cold Spring Harbor Laboratory) that recognizes the fission yeast homologs of U1–U5 snRNAs. The snRNAs were localized to the nonchromatin-containing portion of the nucleus (Fig. 5b,e,h,k), as determined by double-labeling with the fluorochrome DAPI, which specifically binds to DNA (Fig. 5c,f,i,l). This portion of the *S. pombe* nucleus has been referred to as the nucleolus. To confirm that the immunolabeled snRNAs were present in the nucleolar portion of the nucleus, a nucleolar-specific antibody was used on another series of samples. D77 is an antibody (obtained from John Aris, Rockefeller University) that recognizes a nucleolar protein of 38 kD in *Saccharomyces cerevisiae*, and it has been shown to cross-react with *S. pombe*. D77 is thought to recognize the yeast homolog of fibrillarin, which is a protein found in the fibrillar region of the metazoan nucleolus and is associated with the nucleolar-specific U3 snRNP. Immunofluorescense microscopy of cells stained with the D77 antibody showed immunoreactivity in the nonchromatin-enriched portion of the *S. pombe* nucleus (Fig. 5n,q,t,w), confirming its nucleolar composition. To rule out the possibility that the chromatin-enriched region of the nucleus is inaccessible to antibodies, we used a DNA-specific antibody, 2C10 (obtained from David Stollar, Tufts University), for immunofluorescence staining. As shown in Figure 5, z, cc, ff, and ii, the DNA antibody recognizes the same region of the nucleus as the fluorochrome DAPI (Fig. 5aa,dd,gg,jj), which clearly demonstrates that antibodies can penetrate the chromatin-enriched portion of the nucleus.

IMMUNOELECTRON MICROSCOPY

When observed by electron microscopy, the *S. pombe* nucleus contains a light staining region and a dark staining region (Fig. 6a). The percentage of the total nucleus occupied by each of these regions in each section varied depending on the plane of section through the nucleus. The light staining region has previously been shown to contain chromatin. The chromatin appears to be fairly uniform in appearance, with little to no condensed or heterochromatin present (Fig. 6a). The dark staining region of the nucleus contains nucleolar components. In our preparations, there appear to be several electron lucid zones (between 1 and 5) within the nucleolar region (Fig. 6a). These regions resemble fibrillar centers of mammalian cell nucleoli and are often surrounded by a region of greater electron density, which resembles the fibrillar component of mammalian cell nucleoli. To determine if there are any snRNAs in the chromatin region that were not abundant enough to be detected by immunofluorescence, we used immunoelectron microscopy. To visualize the snRNAs at the electron microscopic level, we used monoclonal m_3G antibody in combination with secondary

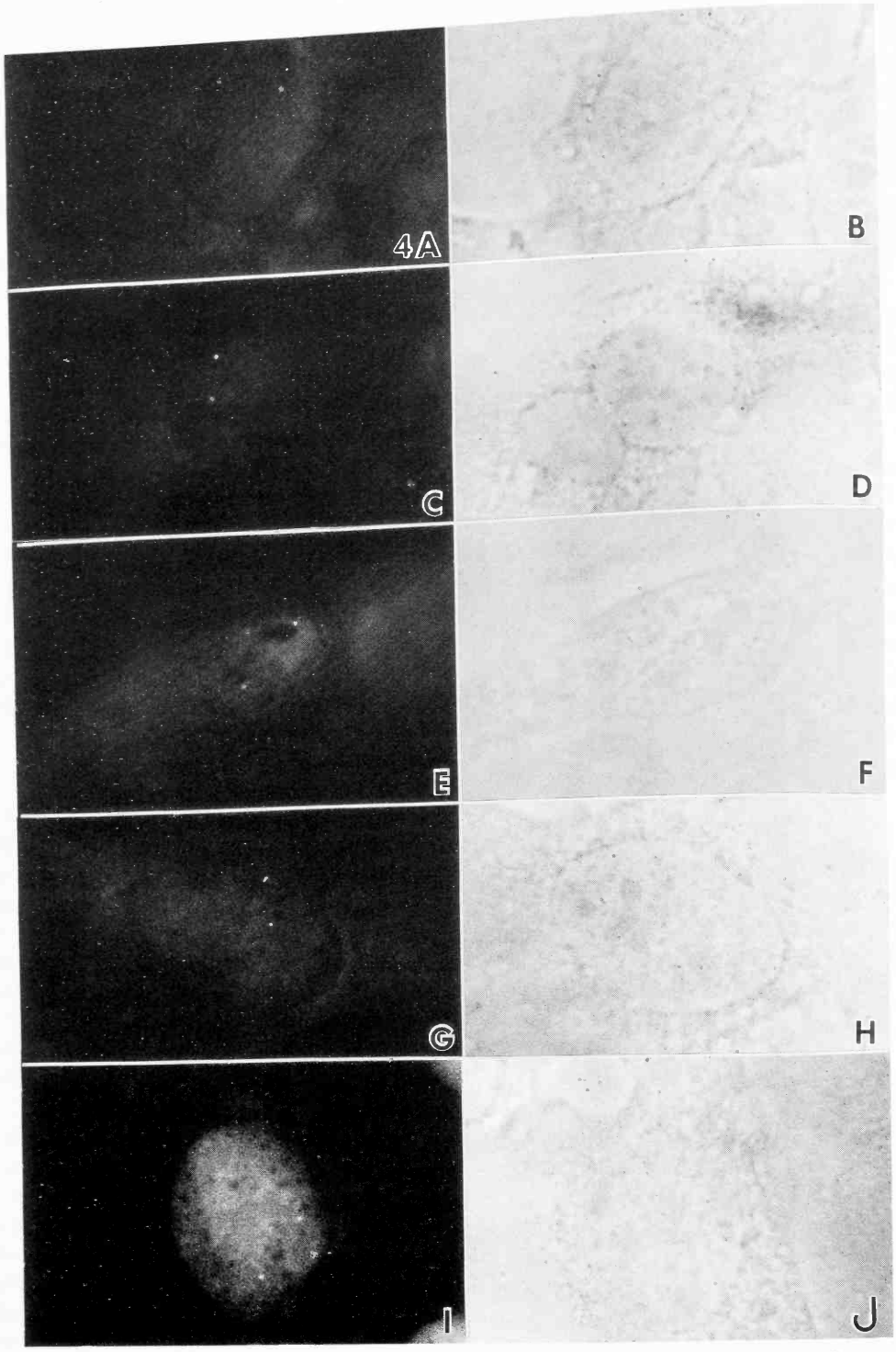

FIGURE 4 In situ hybridization to the dihydrofolate reductase gene in interphase CHO cells. The two single copies of the gene have been localized in each cell (*A,C,E,G,I*) using biotinylated riboprobes spanning 5.3 kb of the gene. Visualization is accomplished by FITC-labeled avidin. (*I*) Stained with the fluorochrome Hoechst 33258 to show the total distribution of DNA. Magnification, 885×.

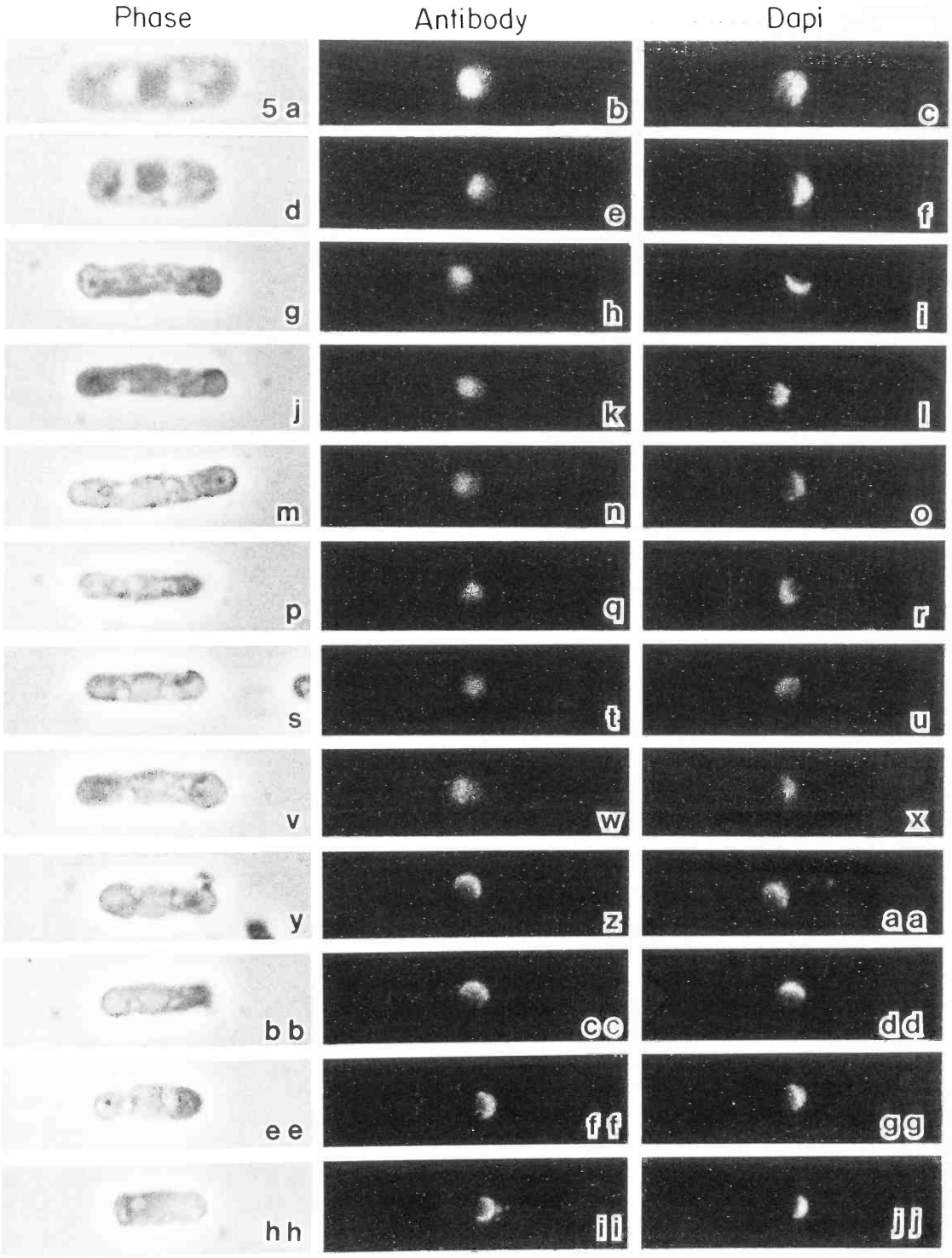

FIGURE 5 Indirect immunofluorescence localization of snRNAs, nucleoli, and DNA in the fission yeast *S. pombe*. Immunofluorescence was performed with spheroplasts prepared from a wild-type strain of *S. pombe* using a fluorescein-conjugated anti-mouse secondary antibody. Phase-contrast views (*a,d,g,j,m,p,s,v,y,bb,ee,hh*), immunfluorescence micrographs of cells labeled with anti-m_3G (*b,e,h,k*) to localize snRNAs, D77 (*n,q,t,w*) to localize nucleoli or anti-DNA antibody (*z,cc,ff,ii*), and counterstained with DAPI to show the distribution of chromatin (*c,f,i,l,o,r,u,x,aa,dd,gg,jj*).

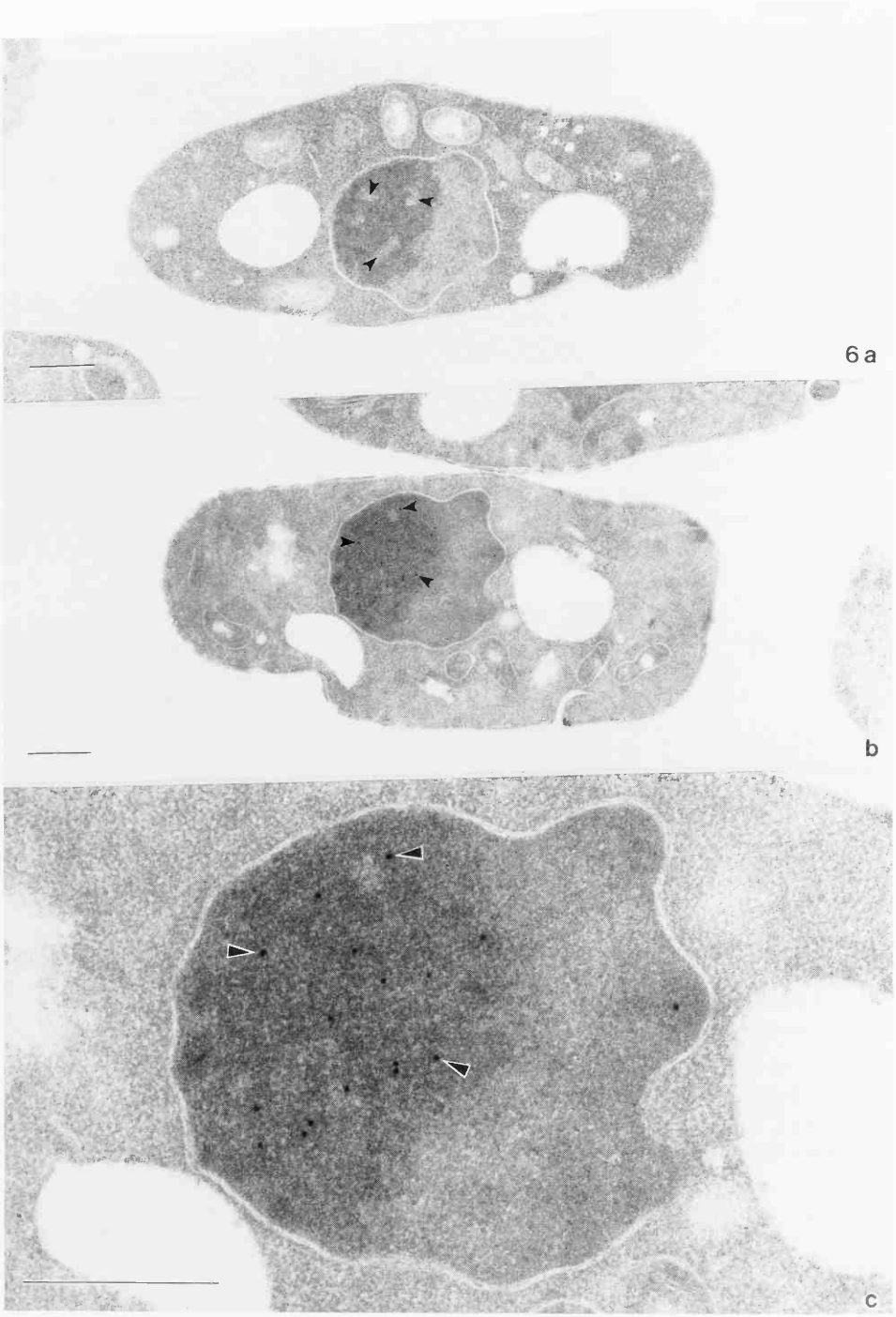

FIGURE 6 Transmission electron micrograph of a cell section of *S. pombe* (*a*) showing the electon-dense and less-electron-dense portions of the cell nucleus. Within the electron-dense portion of the nucleus, irregularly shaped electron lucid regions are observed (arrowheads). These regions resemble fibrillar centers of mammalian cell nucleoli. Ultrathin sections of fission yeast were incubated with anti-m_3G primary antibody followed by 15-nm colloidal gold-conjugated secondary antibody and poststained for electron microscopy. The control cells that were not incubated with primary antibody show no gold labeling (*a*). Yeast cells stained with both primary and secondary antibody show the majority of the gold particles in the nucleus (*b*). An enlarged view of the nucleus shows that the majority of the gold particles were present in the electron-dense portion of the cell nucleus (*c*). Arrowheads in *b* and *c* point to representative gold particles. Bar, 0.5 μm.

antibodies coupled to colloidal gold. Control sections that were labeled only with secondary antibody showed no gold staining (Fig. 6a). Sections of spheroplasts labeled with both primary and secondary antibodies showed the majority of the gold particles in the nucleus and minimal background labeling in the cytoplasm (Fig. 6b). The gold particles located in the nucleus were concentrated in the electron-dense nucleolar region (Fig. 6c). The gold particles were counted in 50 individual spheroplasts to quantitate the distribution of snRNAs in yeast cells. The results showed that 85% of the gold particles were localized in the nucleolar region, 11% were present in the chromatin-enriched region, and 4% were present in the cytoplasm. To confirm further the nucleolar origin of the electron-dense nucleolar region, cells were immunolabeled with the nucleolar-specific antibody D77, and 20 cells were counted to quantitate the distribution of this protein within the cell nucleus; 78% of the gold particles were located in the electron-dense nucleolar region, 10% were present in the chromatin-enriched region, and 13% were present in the cytoplasm.

The results from these experiments showed that the majority of the snRNAs are present within the more electron-dense portion of the nucleus, which has been referred to in the literature as the nucleolus. These findings are in contrast to the results obtained with mammalian cells, where the majority of the abundant snRNAs are present in a nonnucleolar network within the nonchromatin-containing regions of the nucleoplasm. Our data suggest that this dark-staining region of the *S. pombe* nucleus contains both nucleolar components and nuclear particles involved in pre-mRNA processing. Clearly, further research is needed to define which nuclear processes are occurring in the two parts of the yeast nucleus and the relationship between components within each of these nuclear regions as well as between the regions themselves. Such studies will depend on the availability of new nuclear-specific probes. In addition, the yeast system provides for powerful genetic approaches to define associations between nuclear structure and function.

PUBLICATIONS

Conway, G., A. Krainer, D.L. Spector, and R.J. Roberts. 1989. Multiple splicing factors are released from endogeneous complexes during *in vitro* pre-mRNA splicing. *Mol. Cell. Biol.* **9:** 5273–5280.

In Press, Submitted, and In Preparation

Potashkin, J.A., R.J. Derby, and D. L. Spector. 1990. Differential distribution of factors involved in pre-mRNA processing in the *Schizosaccharomyces pombe* cell nucleus. *Mol. Cell. Biol.* (Submitted.)

Powers, A.C., S. Efrat, S. Mojsov, D. Spector, J.F. Habener, and D. Hanahan. 1990. Pancreatic alpha-like cell line derived from a transgenic mouse tumor processes proglucagon similarly to normal islets. *Diabetes* (in press).

Spector, D.L. 1990. Higher order nuclear organization: Three-dimensional distribution of small nuclear ribonucleoprotein particles. *Proc. Natl. Acad. Sci.* (in press).

CELLULAR *TRANS*-ACTIVATORS OF GENE EXPRESSION

B.R. Franza J.-H. Lee G. Mak
Y. Li J. Ross

We study cellular proteins involved in the control of growth of mammalian cells. The mammalian cell we study most comprehensively is the human T lymphoblast. When an interesting event is observed in the T lymphoblast, we then compare other mammalian cells, including lymphoblasts and fibroblasts from different species, to determine the generality or specificity of the proteins involved in the change. The QUEST protein database system is the tool that permits such comparative, qualitative and quantitative observations.

During the past several years, we have focused on three processes. One is the effect expression of oncogenes has on the entire complement of proteins present in a particular cell type. The second process is the induction of protein alterations when quiescent cells are stimulated to proliferate. These studies are directed at determining the molecules involved in conveying signals to the genome and the earliest responses of the genome to these signals. The third process is the control of gene expression at the level of regulation of transcription of mRNA encoding genes.

A coalescence of several observations of each of

the above processes led to the recognition that proteins whose expression was altered within moments of the quiescent (resting) cell receiving certain signals were among the proteins involved in modulating transcription of mRNA. Included in the resultant set of proteins were the products of two cellular genes whose abnormal expression leads to oncogenesis. The goal, implicit in these studies, to construct a molecular map of the networks involved in regulating transcription as well as other genome responses to a change in growth status is in its infancy. The results of the initial studies appear to justify a comprehensive application of the strategy underlying these early investigations.

The strategy employs several tools. Among them are recombinant or synthetic fragments of DNA containing genetically defined transcriptional control elements; antibodies to proteins identified either to associate with the control elements or to respond to stimuli that activate quiescent cells; high-resolution two-dimensional protein gels; a computer-accessible protein database; and an in vitro assay of transcriptional activation/repression to determine biological function of identified proteins. Because of the formidable complexity of a living cell, the strategy is designed to enable multiple investigators to make observations that ultimately can be linked. Several projects, utilizing or contributing necessary components to the strategy, were initiated or further developed during 1989. These will be discussed in individual sections.

Further Studies of the Interaction of Cellular Proteins with Single-strand Transcription Regulatory Control Sites

B.R. Franza [in collaboration with F. Rauscher, Wistar Institute]

During the past year, we have characterized further the interaction of the Fos and Jun proteins with single-strand oligonucleotides that contain the AP-1 (ssAP-1) sequence element. One of the studies involved the introduction of ssAP-1 or a synthetic mutant (mssAP-1) into lysates of whole cells. The cells used for these studies are the human T lymphoblast line, Jurkat. Prior to lysing the cells, they were stimulated with agents that we know induce the expression of the Fos and Jun proteins. Unstimulated cells serve as a control for the level of induction. The microscale DNA affinity precipitation (DNAP) assay coupled with two-dimensional gels are the methods used to study the protein-DNA interactions for these studies and others discussed below. As seen in Figure 1 (panel B vs. E), a significant induction of Fos and Jun occurred, and the proteins associate with ssAP-1 oligonucleotides. No detectable Fos or Jun associated with the mutant ssAP-1 oligonucleotides (see Fig. 1, panel C vs. F). We conclude from this study that a specific single-stranded piece of DNA is the target for a unique interaction of the Fos/Jun complex. In this case, several thousand cellular proteins were presented to the single-strand AP-1 site, and yet only a very small number of proteins apparently associated with it in a specific interaction. All other proteins in the figure are nonspecifically interacting with the insoluble matrix used to recover the DNA (compare Fig. 1, panels A and D with the others).

A different approach to the issue of a specific interaction of the Fos/Jun complex with ssAP-1 involved the expression of mutant forms of either the Fos or Jun protein in vitro, followed by DNAP assays of their ability to interact with the ssAP-1 site cooperatively. Several mutant forms of Fos and Jun had already been shown to abrogate cooperative interactions with the double-strand AP-1 site (T. Curran and colleagues, Roche Institute, Nutley, New Jersey). The same proteins showed significant reductions in interactions with single-strand AP-1 sites. These studies suggest that a possible function for the Fos/Jun complex, localized to the AP-1 site, may involve an alteration of DNA structure in which single-strand-specific binding is a requisite or consequence.

Development of Antipeptide Antibodies to Proteins That Associate with the Transcription Control Elements AP-1 and CRE: Two-dimensional Protein Gel Characterization

B.R. Franza, G. Mak

Among the transcription regulatory elements we study are the AP-1 site and the cAMP response element (CRE) site. We demonstrated that a subset of Fos and Jun proteins along with other cellular proteins preferentially associated with either site. Since these studies were initiated, numerous groups have isolated several different genes whose products interact with these sites and in some cases are genetic

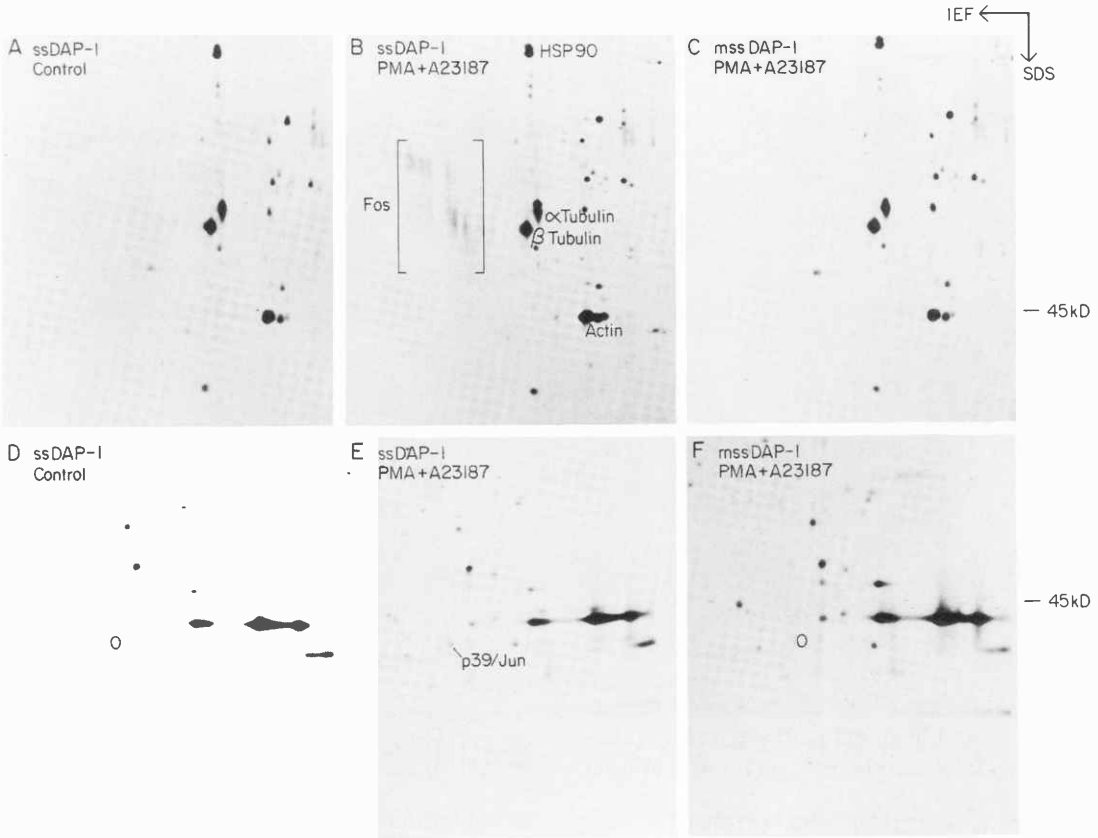

FIGURE 1 Single-strand AP-1 site DNAP binding assays of cellular extracts. Jurkat cells were used for these assays. Cells were either not stimulated or stimulated with PMA plus the calcium ionophore A23187. Stimulation was for 30 min, at which point [^{35}S]methionine was added for an additional 30 min. Cells were extracted, DNAP assays were performed, and proteins were resolved on two-dimensional gels. (*A*, *D*) Proteins from the unstimulated cells that are recovered using the single-strand probe ssDAP-1; (*B*, *E*) proteins from stimulated cells that bind to the DAP-1 probe from cells that were stimulated; (*C*, *F*) proteins from stimulated cells that associated with the mutant single-strand probe, mssDAP-1.

relatives of either Fos or Jun. To pursue further the types of studies that interest us regarding these two regulatory elements, we needed good immunologic reagents.

To produce these reagents, we decided to choose regions of each protein that are not conserved among its genetic relatives. Peptides were synthesized by Dan Marshak and colleagues at Cold Spring Harbor Laboratory. We then injected the peptides, without any modification, into the lymph nodes of rabbits. Following two additional lymph node injections, we detected a very significant response to the first peptide used. We are familiar with the proteins that antibodies to this peptide immunoprecipitate from mammalian cells. T. Curran designated it the M peptide—amino acids 127 to 152 of the Fos protein—several years ago. Anti-M-peptide sera were used in our original studies of the Fos complex and Fos-related antigens (FRA). We used the same peptide to verify whether immunization by lymph node injection of unmodified peptide approach would work. It did, and subsequently we have isolated substantial amounts of antisera to one of the CRE-binding proteins to FosB and Jun. Combined with more extensive DNAP analysis, we envision using these and other antisera to study protein-DNA interactions at each control element as cells are activated to proliferate.

In Vitro Demonstration of Transcriptional Activation of the HIV-1 LTR

Y. Li, J. Ross

The strategy for elucidating the molecular network that controls alterations in transcription in response

to changes in cellular growth status incorporates an in vitro assay to assist in determining the biochemical function of the participating proteins. We have modified the traditional format for preparing extracts of cells for transcription assays. These extracts retain the ability to transcribe mRNA in vitro. The modifications permit the extraction of small numbers of cells. The time from extraction to performance of the transcription reaction has been reduced to 20 minutes. This approach permits the simultaneous analysis of multiple samples of cells exposed to transcriptional activating agents for different intervals. In addition, several different experiments can be compared with relative ease to determine the reproducibility of the temporal course of activation for different agents. Represented in Figure 2 are samples derived from Jurkat cells treated for different periods with the mitogenic lectin, phytohemagglutinin (PHA). The transcripts produced in the reaction are detected and quantified by using the primer extension method. A second primer to an endogenous cellular transcript for the U2 snRNA gene is used to control for sampling and/or gel-loading errors. Clearly, a significant induction of transcription from the HIV-1 LTR template occurs in cells exposed to PHA.

We have also determined that biotinylation of the template does not interfere with the production of mRNA transcripts. This will permit the isolation of the template from the reactions and determination of proteins associated with templates using the DNAP procedures. Initially, this approach allows the correlation of induction of transcription with the proteins associated with control regions within the HIV-1 LTR template. Biochemical fractionation of activated extracts combined with this approach will enable simultaneous assay of transcriptional activity and the proteins enriched by the separation procedures. We may learn whether the relative abundance of several proteins in a cell at different moments in its growth cycle will determine the transcriptional activity of a gene. It may be that purification of a transcription factor to homogeneity and subsequent addition of it to an assay, although productive of a response, may not mimic its activity in the cell. In the cell, it is in context relative to all other transcription factors. Determining abundances of the several proteins capable of interacting at a specific control element may therefore be essential to understanding the contribution they each make to the transcription of specific genes. Coupling the DNAP assay, two-dimensional gels, and in vitro assay of transcriptional activity may contribute to elucidation of these issues.

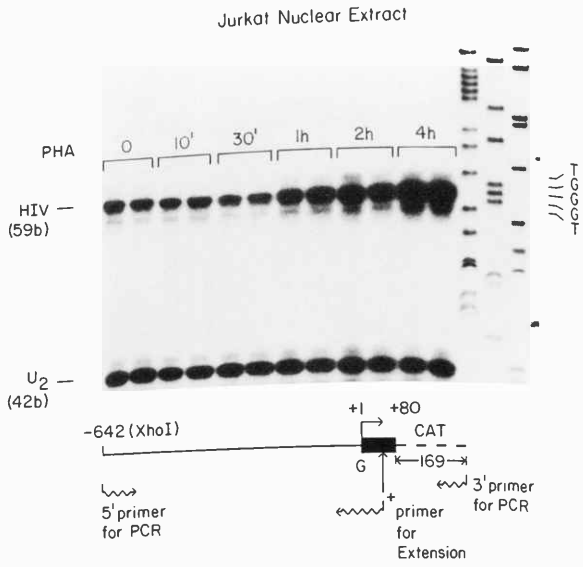

FIGURE 2 In vitro transcription of Jurkat nuclear extracts. This figure demonstrates an analysis of the transcriptional activity of Jurkat cells stimulated for the indicated intervals with PHA. The HIV-1-LTR template was used in the in vitro transcription assays. Primer extension analysis was used to demonstrate the quantitative change in in vitro transcription activity of each extract. Duplicate reactions for each time point were performed. A sequence reaction is shown to the right of the assay products to indicate the site of initiation of transcription.

Identification of the Large Subunits of RNA Polymerase II

B.R. Franza

Since we study mRNA transcription and since two-dimensional gels and the resultant protein databases are central to the strategy, we are beginning to acquire antibodies to known proteins involved in transcription. Using antibody provided by Dr. M. Dahmus (University of California, Davis), we have successfully identified a set of proteins, including several isoforms of the large subunit of RNA polymerase II, the enzyme that catalyzes the synthesis of mRNA (see Fig. 3). Several other proteins were specifically enriched using the rabbit antisera provided by Dahmus. It may be that one or more of these proteins is functionally associated with the large subunit. Studies are under way to characterize these proteins further, to identify all the members of the RNA polymerase II complex, and to begin

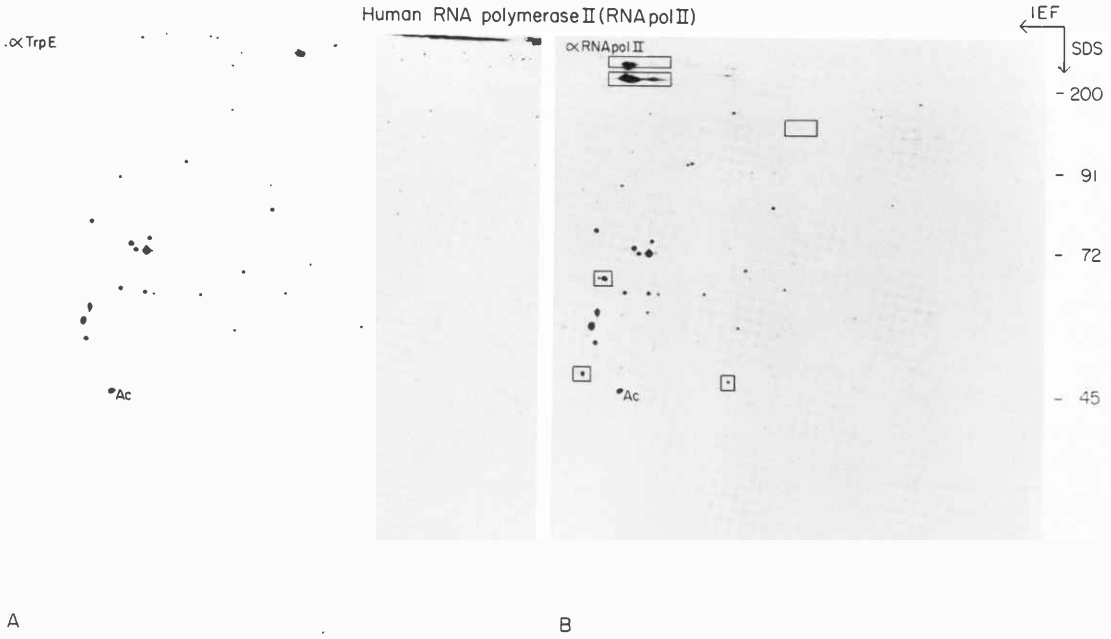

FIGURE 3 Two-dimensional gel analysis of Jurkat cellular proteins immunoprecipitated by an anti-human RNA polymerase II antibody. (*A*) Cellular proteins immunoprecipitated by a control antibody; (*B*) proteins immunoprecipitated by an anti-RNA pol II large-subunit antibody. Jurkat cells were labeled for 2 hr with [^{35}S]methionine and then extracted and immunoprecipitated. Proteins were then resolved on two-dimensional gels.

characterizing how these proteins are modulated as the cell responds to signals that alter its growth status.

Characterization of *cdc2* and Associated Proteins in Human T Lymphoblasts

B.R. Franza [in collaboration with G. Draetta and D. Beach
Cold Spring Harbor Laboratory]

Resting T lymphoblasts are cells capable of being induced to synthesize DNA and then divide. Activation of these cells is accomplished by their being exposed in vivo to antigenic challenge, cytokines (growth factors), other cells, or combinations of the above. The cells not only divide, but mature (differentiate). The responses involve transcriptional activation (and repression) of specific cellular genes. These events can be mimicked in tissue culture dishes by exposing less mature cells to different growth factors, mitogenic lectins, tumor promoters, other cells, antigens, or combinations thereof. In addition to cellular genes being transcriptionally activated in response to these signal-inducing events, viral genes integrated in the cellular genome are also activated. One of these viral genes is the proviral form (DNA copy) of the AIDS virus, HIV-1.

The induction of transition from resting (G_0) to DNA synthesis and proliferation has been to some extent genetically dissected. Mutant cells have been identified that will arrest at different stages under certain growth conditions. Genes have been identified to overide (suppress) the defect(s). One consequence of mutation of the cell-division cycle gene, *cdc2*, in *Schizosaccharomyces pombe* is a block of progression of the cell through the G_1 stage to the S stage (DNA synthesis interval). It was therefore of interest to us to identify the *cdc2* products in T lymphoblasts so that we could monitor changes in *cdc2* as resting cells are induced to exit their resting state, activate transcription of specific genes, and commit to DNA synthesis.

The first immunoprecipitation of a T-lymphoblast cellular protein extract with anti-*cdc2* antibody and the resultant two-dimensional gel image contained several bits of information (see Fig. 4). It showed the diversity of isoforms of *cdc2* in asynchronously dividing T lymphoblasts. It also revealed several other proteins associated specifically with *cdc2*. Figure 4 shows the initial two-dimensional gel image of the *cdc2* complex in the human T-lymphoblast cell

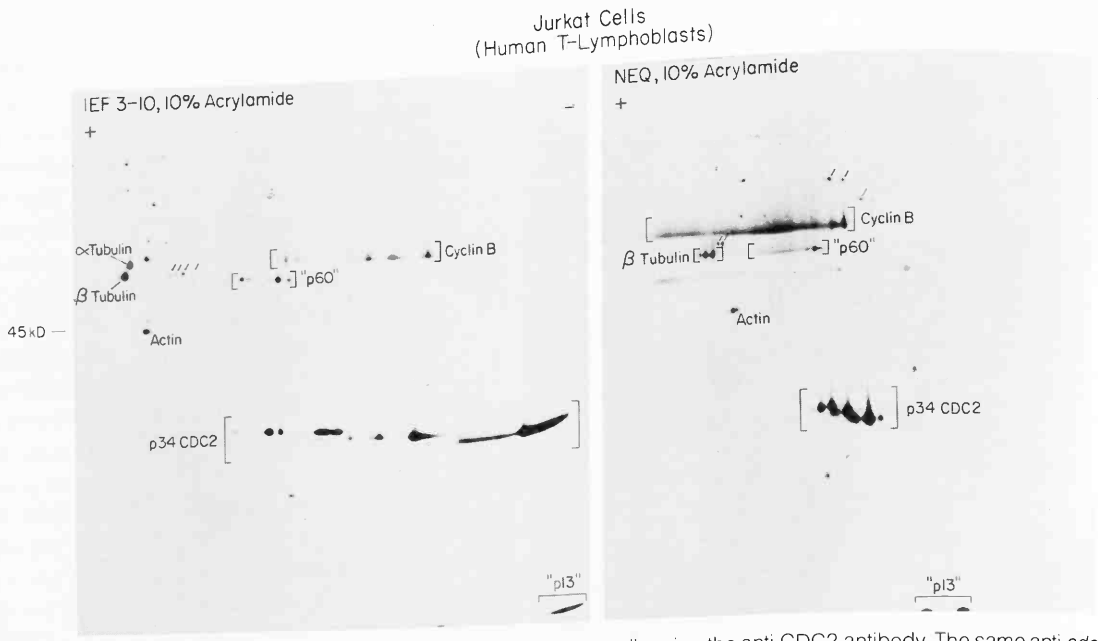

FIGURE 4 A complex of cellular proteins is recovered from Jurkat cells using the anti-CDC2 antibody. The same anti-*cdc2* immunoprecipitated proteins were split into two equal samples and resolved on two types of two-dimensional gels. (*Left*) Proteins resolved by isoelectric focusing. (IEF); (*right*) proteins resolved on a gel type referred to as noneqiulibrium gel (NEQ) electrophoresis. NEQ gels are used to detect proteins that are too basic to be resolved by isoelectric focusing. Cellular proteins were labeled for 2 hr with [^{35}S]methionine.

line, Jurkat. One of these proteins subsequently has been identified as cyclin B (antibody provided by J. Pines and T. Hunter, Salk Institute). Another protein, p13, was also identified to be associated with *cdc2*, analogous to the situation in *S. pombe*.

A surprise was the identification of a cellular protein known from previous two-dimensional gel images (collaborative study with E. Harlow, Tumor Viruses Section) to be associated with the adenovirus E1A gene product. This protein, designated p60, although its migration in two-dimensional gels is faster than vimentin (known molecular size, 55,700), was shown by P. Whyte and E. Harlow to be recognized by a monoclonal antibody, C160. Use of this antibody in immunoprecipitation assays followed by two-dimensional gel electrophoresis showed that specific isoforms of *cdc2* were complexed to p60. The data presented in Figure 5 are the result of collaborative studies involving A. Giordano, E. Harlow, D. Beach, and G. Draetta (Cold Spring Harbor Laboratory). Another human cell line, HeLa, was used in these studies. Of note is the absence of the spots just below cyclin B (compare Fig. 5 with Fig. 4) in the lymphoblast lines immunoprecipitated with anti-*cdc2* antibodies. Otherwise, the components of the *cdc2* complex are similar (details of the figure are found in the legend).

Our interest in the p60 protein was further heightened by observations described in the next section. In addition, G. Draetta established that the C160 antibody immunoprecipitated a kinase activity toward the histone H1 protein, a previously identified substrate for the *cdc2* kinase. The question we are most interested in is p60, either in its H1 kinase form or in some other form, a contributor to the activation process when resting T lymphoblasts are stimulated?

Association of p60 with a Major, Immediate Substrate of Protein Kinase C

B.R. Franza, J.-H. Lee, G. Mak

Using the monoclonal anti-p60 antibody, I identifed several other proteins, in addition to *cdc2*, that associate with p60. One of these proteins is now referred to as MARCKS. MARCKS was orginally identified as a substrate for protein kinase C. Agents that activate protein kinase C induce an extremely rapid

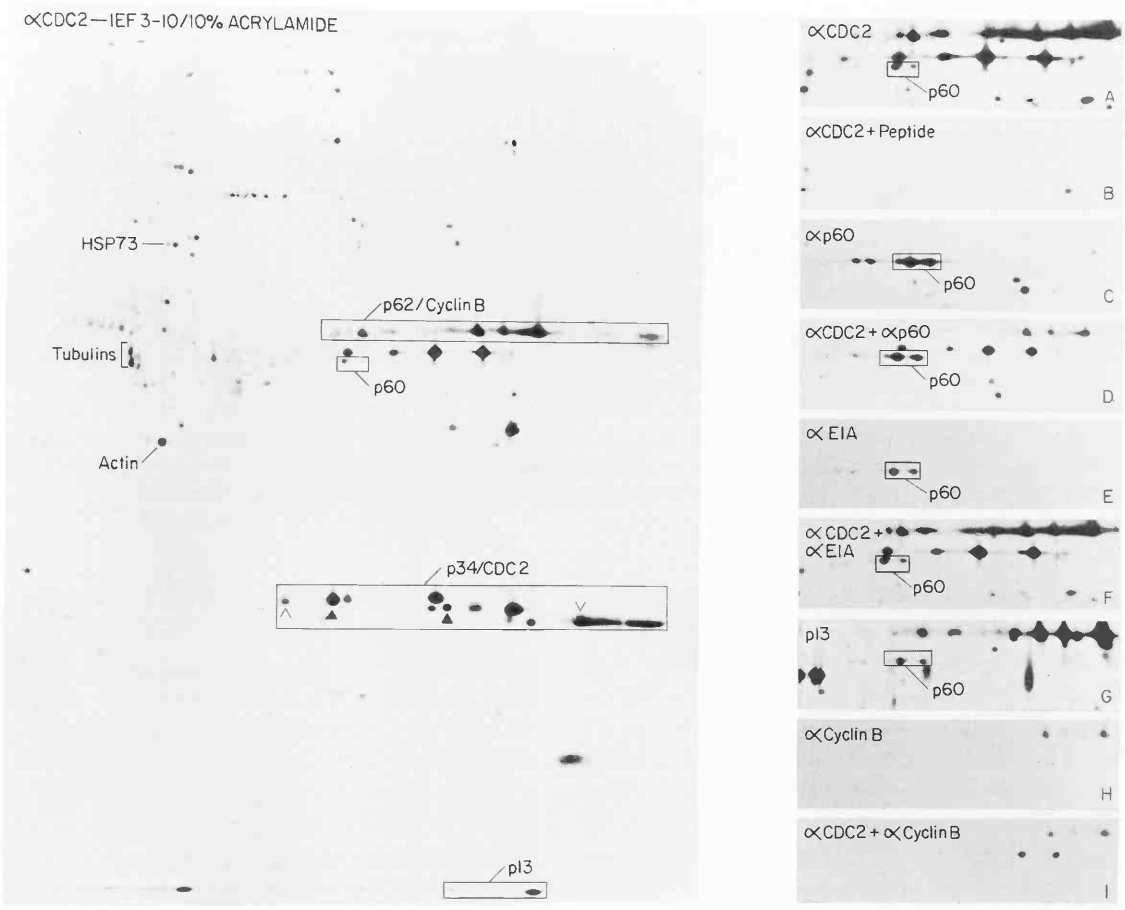

FIGURE 5 Two-dimensional gel of *cdc2*- and E1A-associated proteins. The left panel represents proteins detected in an anti-*cdc2* (G6) immunoprecipitate. *A* through *I* are regions of comparable two-dimensional gels in which p60 is resolved. (*A*) Anti-*cdc2*; (*B*) anti-*cdc2* precipitation performed in the presence of 100 nmoles of competing antigenic peptide; (*C*) anti-p60 precipitation with the C160 monoclonal antibody; (*D*) mixture of proteins from immunoprecipitates shown in *A* and *C*; (*E*) anti-E1A precipitation using the M73 monoclonal antibody; (*F*) mixture of proteins from immunoprecipitates shown in *A* and *E*; (*G*) proteins recovered in a p13-Sepharose precipitation; (*H*) precipitation with anti-cyclin B; (*I*) mixture of proteins in *A* and *H*. Open arrowheads in the p34/CDC2 box indicate isoforms that associate with p60 in C160 monoclonal antibody precipitates. Filled arrowheads show the primary form of *cdc2* in anti-cyclin B precipitates. Actin, tubulins, and the 73-kD heat-shock protein (HSP73) are indicated for orientation purposes. p62/cyclin B, p60, and p13 isoforms are indicated in the appropriate boxes. All samples were resolved in pH 3–10 isoelectric focusing gels (first dimension) followed by separation in 10% polyacrylamide gels (second dimension) (Garrels 1983).

phosphorylation of this protein. Its two-dimensional gel *signature* is unique. As shown in Figure 6, antibody to MARCKS (kindly provided by Dr. P. Greengard, J. Wang, and colleagues, Rockefeller University, New York) immunoprecipitates the same cluster of proteins as found associated with p60. In addition to p60, several other proteins are associated with p60 (A through D in Fig. 6). The technique for identifying these proteins is simple. Cells are lysed, the immunoprecipitation is accomplished, the immune complex is equilibrated in a kinase-permissive buffered solution, and [γ-^{32}P]ATP is added. After 10 minutes at 30°C, the immune complex is solubilized and the proteins are electrophoretically separated. The addition of ^{32}P to proteins is nonspecific, i.e., protein phosphorylation is apparently only a function of the mass amount of each particular polypeptide present in the immune complex. Proteins that nonspecifically associate with the insoluble matrix used to capture the immune complexes are detected. Therefore, many of the proteins seen in each image are the same and are confirmed as nonspecific

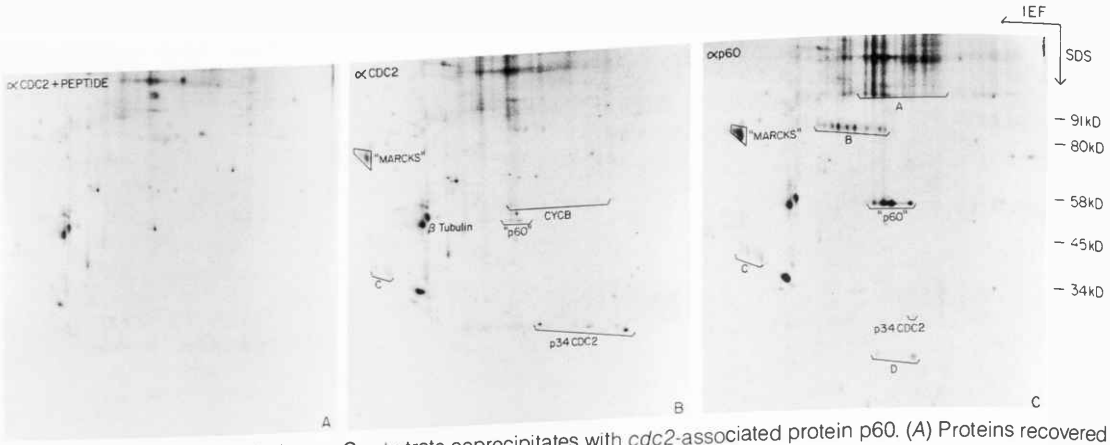

FIGURE 6 MARCKS protein kinase C substrate coprecipitates with *cdc2*-associated protein p60. (*A*) Proteins recovered with an anti-*cdc2* antibody in the presence of the synthetic peptide to which the antibody was raised; (*B*) proteins immunoprecipitated by the anti-*cdc2* antibody; (*C*) proteins immunoprecipitated by the anti-p60 antibody (C160).

by their equivalent presence in the control reaction in which the antigenic peptide is mixed with the extract prior to addition of the anti-*cdc2* antibody (Fig. 6, panel A).

It is interesting that at least one of the proteins associated with p60, i.e., MARCKS, is an immediate target of a kinase activated at the earliest moments of cellular activation. It is also interesting that an apparently unique p60-associated protein, not MARCKS, is found in lymphoblasts. In fact, in the Jurkat T-lymphoblast line, MARCKS is not detected, but the unique p60-associated proteins are. It may be that signals converge on the p60/*cdc2* complex via MARCKS or the other p60-associated proteins. p60 may thereby be used to convey signals to *cdc2*. It is possible that the equivalent to the "start" function of *cdc2* in *S. pombe* involves a functional connection of MARCKS and the other p60-associated proteins with *cdc2*. Having the gene for p60 will be essential to studying such questions. Dr. J.-H. Lee is attempting to clone the gene for the *cdc2*-associated p60 protein.

PUBLICATIONS

Ballard, D.W., E. Bohnlein, J.A. Hoffman, H.P. Bogerd, E.P. Dixon, B.R. Franza, and W.C. Greene. 1989. Activation of the interleukin-2 receptor α gene: Regulatory role for DNA-protein interactions flanking the κB enhancer. *The New Biologist* **1:** 83–92.

Bohnlein, E., M. Siekevitz, D.W. Ballard, J.W. Lowenthal, L. Rimsky, H. Bogerd, J. Hoffman, Y. Wano, B.R. Franza, and W.C. Greene. 1989. Stimulation of the human immunodeficiency virus type 1 enhancer by the human T-cell leukemia virus type 1 *tax* gene product involves the action of inducible cellular proteins. *J. Virol.* **63:** 1578–1586.

Cohen, D.R., P.C.P. Ferreira, R. Gentz, B.R. Franza, and T. Curran. 1989. The product of a Fos-related gene Fra-1 binds cooperatively to the AP-1 site with Jun: Transcription factor AP-1 is comprised of multiple protein complexes. *Genes Dev.* **3:** 173–184.

Garrels, J.I. and B.R. Franza. 1989. The REF52 protein database: Methods of database construction and analysis using the QUEST system, and characterization of protein patterns from proliferating and quiescent REF52 cells. *J. Biol. Chem.* **264:** 5283–5298.

Garrels, J.I. and B.R. Franza. 1989. Transformation-sensitive and growth-related changes of protein synthesis in REF52 cells: A two-dimensional gel analysis of SV40-, adenovirus-, and Kirsten murine sarcoma virus-transformed rat cells using the REF52 protein database. *J. Biol. Chem.* **264:** 5299–5312.

Giordano, A., P. Whyte, E. Harlow, B.R. Franza, D. Beach, and G. Draetta. 1990. A 60 kD c*dc2*-associated polypeptide complexes with the E1A proteins in adenovirus-infected cells. *Cell* **58:** 981–990.

Lowenthal, J.W., E. Bohnlein, Y. Wano, M. Siekevitz, B.R. Franza, D.W. Ballard, and W.C. Greene. 1989. Coregulation of mitogen induced interleukin-2 receptor (TAC) and human immunodeficiency virus type 1 (HIV-1) gene expression. In *Mechanisms of action and therapeutic applications of biologicals in cancer and immune deficiency disorders*, pp. 361–370.

Ryan, W.A., B.R. Franza, and M.Z. Gilman. 1989. Two distinct cellular phosphoproteins bind to the c-*fos* serum response element. *EMBO J.* **8:** 1785–1792.

In Press, Submitted, and In Preparation

Franza, B.R. 1990. The p60/*cdc2* complex is physically associated with at least one substrate of protein kinase C: MARCKS. (In preparation.)

Franza, B.R. 1990. The microscale DNA precipitation assay. In *Methods in Molecular Biology*, volume 5 (ed. G. Goodwin). Humana Press, Clifton, New Jersey. (In preparation.)

Franza, B.R. 1990. Complex protein interactions at the HIV-1 direct repeat enhancer structure: A paradigm for database analysis of protein-nucleic acid interactions. *UCLA Symp. Mol. Cell. Biol. New Ser.* **119:** 181–191.

Franza, B.R. and F.J. Rauscher III. 1990. Sequence-specific single-strand deoxyribonucleic acid recognition by the *Fos/Jun* protein complex. (In preparation.)
Giordano, A., C. Schley, B.R. Franza, and E. Harlow. 1990. Identification of the adenovirus early region 1A domain required for association with the p60 *cdc2*-associated protein. *J. Virol.* (In preparation).
Li, Y. and B.R. Franza. 1990. *In vitro* analysis of the transcriptional activation of the HIV-1 long terminal repeat. (In preparation.)
Phares, W., B.R. Franza, and W. Herr. 1990. Functional similarities between HIV-1 and SV40 enhancer elements. (In preparation.)

MOLECULAR BIOLOGY OF THE CYTOSKELETON

D.M. Helfman K. Galactionov, J. Kazzaz, G. Mulligan, M. Leonard, L. Goodwin, A. Kistler, M. Pittenger, R. Roscigno, W. Guo, J. Lees-Miller, L. Finn

The cytoskeleton is involved in a multitude of cellular functions such as cell shape and motility, organelle movement, chromosome movement, and cytokinesis. The cytoskeleton of all eukaryotic cells is composed of three major filamentous systems: actin filaments, intermediate filaments, and microtubules. Each of these filamentous systems contain a number of different protein components, although different cell types and tissues express specific protein isoforms that comprise these structures. The research in our laboratory is focused on two related problems in molecular and cell biology: (1) the mechanisms responsible for tissue-specific and developmentally regulated patterns of gene expression and (2) the functional significance of cell-type-specific protein isoform expression. Our laboratory has been interested in understanding the regulation and function of tropomyosin gene expression in muscle and nonmuscle cells. Tropomyosins are a diverse group of actin-binding proteins with distinct isoforms present in striated muscle (skeletal and cardiac), smooth muscle, and nonmuscle cells. We now know that 12 different tropomyosin isoforms are expressed from three separate genes in rats. The α-tropomyosin (α-TM) gene encodes nine isoforms, the β-tropomyosin (β-TM) gene encodes two isoforms, and the tropomyosin-4 (TM-4) gene encodes only a single isoform. We have been studying the expression of these genes with particular attention to understanding the mechanisms of their regulation at the posttranscriptional (alternative RNA splicing) and the transcriptional levels. In addition, the expression of a diverse group of tropomyosin isoforms in a highly tissue-specific manner via alternative promoters and alternative RNA processing strongly suggests that each isoform is required to carry out specific functions in conjunction with the actin-based filaments of various muscle and nonmuscle cells. The function of these different isoforms is not known and is under study in our laboratory. We are also interested in the relationship between transformation and tropomyosin gene expression. Transformed cells exhibit dramatic alterations in the patterns of tropomyosin isoform expression. How these changes in tropomyosin expression are related to the transformed phenotype is currently being investigated. Finally, we have begun to study the role of p34 *cdc2* in the process of differentiation. Below is a summary of our present studies.

Alternative RNA Splicing in the Control of Gene Expression

D.M. Helfman, R. Roscigno, G. Mulligan, L. Finn, M. Leonard, W. Guo

An ever-growing number of cellular and viral genes have been characterized that encode multiple protein isoforms via the use of alternatively spliced exons. In many cases, alternative splicing contributes to developmentally regulated and tissue-specific patterns of gene expression. At present, little is known about the mechanisms that are responsible for the selection of alternative splice sites in complex transcription units and how the splicing signals in alternatively spliced exons differ from those in constitutively spliced exons. We are using the rat β-TM gene as a model to investigate developmental

and tissue-specific alternative splicing. The gene spans 10 kb with 11 exons and encodes two distinct isoforms, namely, skeletal muscle β-TM and fibroblast TM-1 (Fig. 1). Exons 1 through 5, 8, and 9 are common to both mRNAs expressed from this gene. Exons 6 and 11 are used in fibroblasts as well as smooth muscle, whereas exons 7 and 10 are used exclusively in skeletal muscle. In addition, during myogenesis, expression of these two isoforms is subject to developmental control. From this gene, myoblasts express exclusively the fibroblast TM-1 isoform, but upon differentiation, they express only the skeletal muscle β-TM isoform. Our previous studies of tropomyosin pre-mRNA splicing revealed an ordered pathway of splicing in which either of the internal alternatively spliced exons (exons 6 or 7) must first be joined to the downstream common exon before they can be spliced to the upstream common exon (Helfman et al., *Genes Dev. 2:* 1627 [1988]). We subsequently characterized the branchpoints formed during the use of these alternatively spliced exons (Helfman and Ricci 1989). These studies revealed that the splicing of exon 5 to exon 7 (skeletal-muscle-type splice) is accompanied by the selection of multiple branchpoints, which are located an unusually long distance (144, 147, and 153 nucleotides) from the 3′ splice site of exon 7. These results were found to be different from those of most branchpoints mapped previously, in which a single adenosine residue located 18–40 nucleotides from a 3′ splice site is used during lariat formation. In addition, these studies suggested that the use of branchpoints located at long distances (i.e., >40 nucleotides) from a 3′ splice site may be an essential feature of some alternatively spliced exons.

We have now investigated the functional role of the intron sequences between the unusual branchpoints and the 3′ splice site of the skeletal muscle exon (exon 7) in splice site selection of the rat β-TM gene. These studies have identified two distinct elements in the intron, upstream of exon 7, involved in splice site selection (Helfman et al. 1990). The first element is composed of a polypyrimidine tract located 89–143 nucleotides upstream of the 3′ splice site, which specifies the location of the lariat branchpoints used, 144–153 nucleotides upstream of exon 7. The 3′ splice site AG dinucleotide has no role in the selection of these branchpoints. The second element is composed of intron sequences located between the polypyrimidine tract and the 3′ splice site of exon 7. It contains an important determinant of alternative splice site selection, because deletion of these sequences results in the use of the skeletal-muscle-specific exon in nonmuscle cells. We propose that the use of lariat branchpoints located far upstream of a 3′ splice site may be a general feature of some alternatively excised intron, reflecting the presence of regulatory sequences located between the lariat branch site and the 3′ splice site. The data also indicate that alternative splicing of the rat β-TM gene is regulated by a somewhat different mechanism from that described for the rat α-TM gene and the *transformer-2* gene of *Drosophila melanogaster* (see below). Work is currently under way to explore further the role of specific intron sequences in alternative splice site selection and to identify cellular factors that may interact with specific regions of the pre-mRNA to regulate alternative RNA splicing (see below).

Analysis of *cis*-Acting Elements Involved in Alternative Splice Site Selection of β-TM Pre-mRNA

W. Guo, D.M. Helfman

As described above, intron sequences upstream of exon 7 act as a negative regulatory element, preventing the use of this exon in nonmuscle cells. The mechanism by which these intron sequences play a role in alternative splice site selection in not known. One

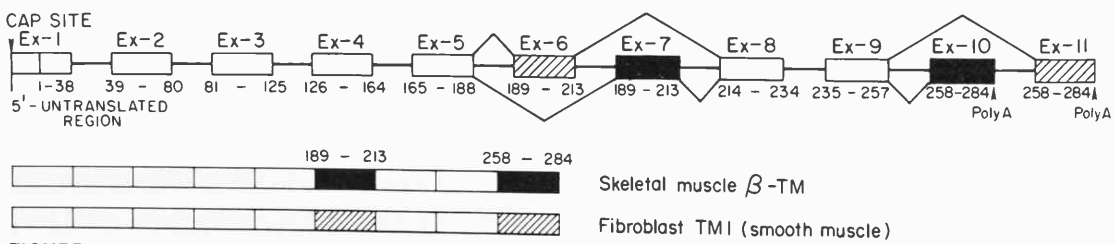

FIGURE 1 Schematic diagram of the rat β-TM gene and the two different isoforms expressed from this gene. The amino acids encoded by each exon are indicated. The cap site and polyadenylation sites are also indicated.

way in which these sequences could regulate splice site selection is by binding to factors in nonmuscle cells that inhibit the use of this 3′ splice site. This "blockage" mechanism has been proposed for a number of alternatively spliced genes, including the *transformer-2* gene and the *suppressor of white apricot* gene of *D. melanogaster.* However, the blockage model cannot explain the regulation of the rat β-TM gene, because using precursors without deletions in intron 6, we are able to detect splicing of exon 5 to exon 7 both in vitro and in vivo if exon 7 is first joined to exon 8 (Helfman et al., *Genes Dev. 2:* 1627 [1988]). Therefore, it is unlikely that a negative factor simply binds to the 3′ splice site of exon 7 and prevents its use in nonmuscle cells. On the other hand, a factor bound to the 3′ splice site of exon 7 might function by preventing the use of the 5′ splice site of this exon. Such a mechanism would be consistent with the observation that splicing of exon 5 to exon 7 in nonmuscle cells requires that exon 7 first be spliced to exon 8 (Helfman et al., *Genes Dev. 2:* 1627 [1988]). A second way in which these sequences may regulate splice site selection is by participating in the formation of RNA structures that sequester this exon and prevent its utilization in nonmuscle cells. Computer analysis of possible RNA secondary structures involving intron sequences upstream and downstream from the skeletal muscle exon reveals that this exon may be sequestered in a stable hairpin structure (Helfman et al. 1990). Although the use of computer algorithms is not without limitations, analysis of the same gene from chicken reveals a similar secondary structure that could sequester this exon. Thus, on the basis of computer analysis, there is phylogenetic conservation of these structures. Additional data consistent with this hypothesis come from our previous studies which demonstrated that splicing of exon 5 to exon 7 in nonmuscle cells required that exon 7 first be spliced to the downstream common exon. Joining exon 7 to exon 8 and thereby removing the flanking intron may have prevented the formation of RNA secondary structures that interfere with the interaction of splicing factors with the splice site of exon 7.

To determine whether intron sequences upstream of exon 7 act either by interacting with cellular factors that block the use of this exon or through the formation of RNA structures that sequester this exon, we are carrying out a more a detailed analysis of these *cis*-acting sequences. We are introducing a series of point mutations in specific regions of the pre-mRNA and analyzing their effects in vitro and in vivo. In addition, to determine if alternative splicing is the result of RNA secondary structure, we are introducing mutations in intron sequences upstream as well as downstream from exon 7 that should destabilize any secondary structure. These studies should provide important information about the mechanism responsible for alternative splice site selection of β-TM pre-mRNA. In addition, these studies should have broad implications for the numerous biological systems that involve alternative RNA processing as a mechanism to regulate the expression of cell-type-specific proteins.

Identification of Pre-mRNA Binding Proteins

G. Mulligan, D.M. Helfman

Regulation of alternative splice site selection may be achieved through blockage of splice sites or facilitation of exon usage, perhaps via alterations in RNA structure. We are presently defining factors that bind to β-TM pre-mRNAs which may act to regulate alternative splicing. Using pre-mRNA uniformly labeled with ^{32}P-labeled nucleotides, we are determining whether muscle and nonmuscle cells express proteins that interact with specific regions of the pre-mRNA. These studies involve UV cross-linking, gel shifts, and direct binding assays. In both HeLa and myogenic splicing systems, we have identified a protein of 60–65 kD that binds to the long polypyrimidine sequence within intron 6. This pyrimidine tract is a *cis*-element critical for the use of the adjacent upstream lariat branchpoints associated with the splicing of exon 5 to 7 (skeletal-muscle-type splice). Recently, a 62-kD protein was reported that binds to extended polypyrimidine sequences thought to be involved in pre-mRNA splicing (Garcia-Blanco et al., *Genes Dev. 3:* 1874 [1989]). It remains to be determined how these interactions contribute to the splicing of tropomyosin pre-mRNAs. In addition, we are pursuing proteins whose interactions exhibit tissue-specific differences.

Structure and Evolution of the Tropomyosin Genes

J. Lees-Miller, D.M. Helfman

Tropomyosin is a simple dimeric protein that forms an α-helical coiled-coil along its entire length. Its

structure is stabilized by a hydrophobic core formed by nonpolar residues in the first and fourth positions of a repeated seven-amino-acid pattern. The early evolution of genes encoding tropomyosins and a host of other coiled-coil proteins appears to have occurred through duplication of seven-amino-acid units and multiples there of. The tropomyosin genes from existing organisms frequently possess exons encoding 21 or 42 amino acids. These values correlate well with the presence of actin-binding sites, which is one function that all tropomyosins have in common. A comparison of the *D. melanogaster* TMII gene and the vertebrate genes indicates that the positions of the intron-exon splice junctions relative to the final transcript are identical, demonstrating evolutionary conservation of gene structure over several hundred million years. Another common feature of most tropomyosin genes is alternative splicing of the primary transcript.

Our recent completion of the structure of the three rat tropomyosin genes has allowed us to examine the evolution of the vertebrate tropomyosins (Fig. 3). Sequence comparison of several vertebrate tropomyosins indicates that four genes evolved from a common ancestor. The rat α-gene is the most complex of these transcription units (Fig. 2). It possesses alternative promoters, and alternative exons 2a and 2b, 6a and 6b, and 9a, 9b, 9c, and 9d. The rat β-TM gene is less complex, with a single promoter and two sets of alternative exons 6a and 6b and 9a and 9d (Fig. 1). The TM-4 gene is not alternatively spliced, unlike all the other tropomyosin genes from multicellular organisms that have been described (Fig. 3). Two exon-like sequences that are highly similar to alternatively spliced exons 2b and 9a of the rat α-TM gene, the rat β-TM gene, and the human TMnm gene have been located in the appropriate region of the gene encoding rat fibroblast TM-4. However, several mutations in these sequences render them nonfunctional as tropomyosin-coding exons. We have termed these exon-like sequences, vestigial exons. It appears that most vertebrates do not require all the possible tropomyosin isoforms that could be encoded by four complex tropomyosin genes. Many of the alternative splices and in some cases entire genes have therefore been selectively lost.

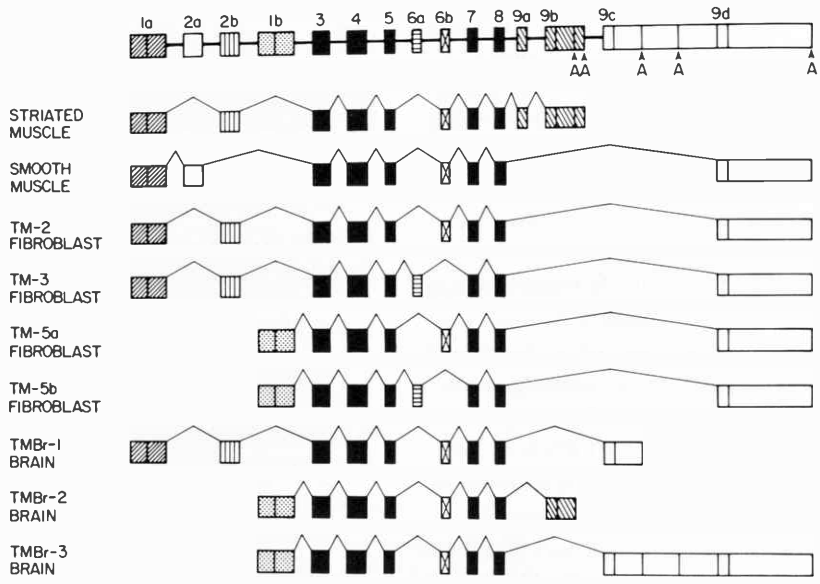

FIGURE 2 Schematic diagram of the rat α-TM gene and nine different isoforms expressed from this gene. Boxes represent exons and horizontal lines represent introns; they are not drawn to scale. The gene contains two alternative promoters that result in expression of two different amino-terminal coding regions (exons 1a and 1b), two internal mutually exclusive exon cassettes (exons 2a and 2b and 6a and 6b), and four alternatively spliced 3′ exons that encode four different carboxy-terminal coding regions (exons 9a, 9b, 9c, and 9d). The different polyadenylation signals are also indicated (*A*).

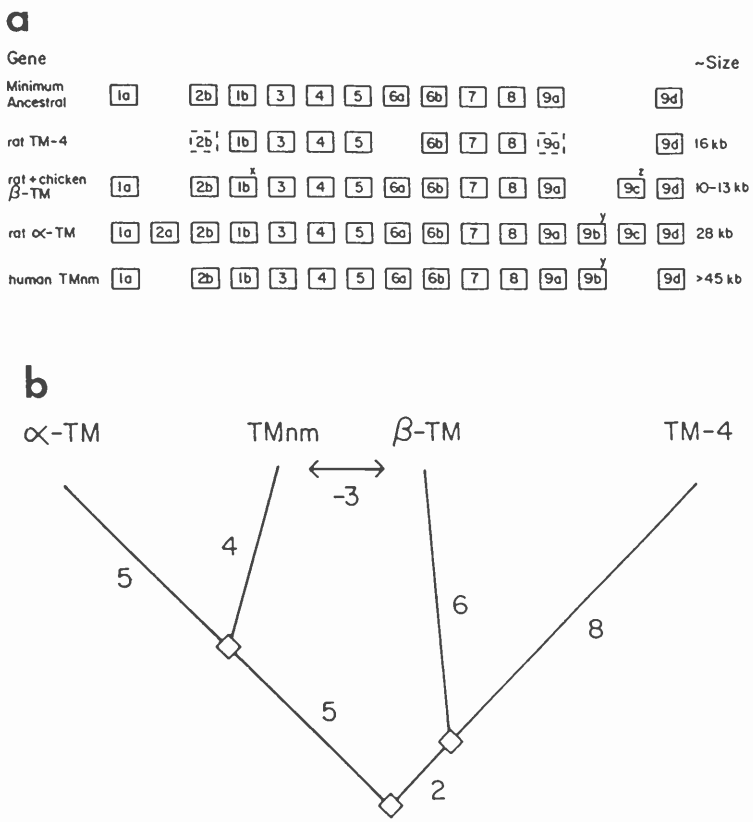

FIGURE 3 Organization and evolution of the vertebrate tropomyosin genes. (*A*) The intron-exon structure of the vertebrate tropomyosin genes. We have tentatively identified an exon-9c-like sequence in intron 9a of the chicken β-TM gene. No similar sequence is found in the β-TM gene of the rat. (*B*) A possible evolutionary relationship between four vertebrate tropomyosin gene is presented. The sequence of 13 vertebrate tropomyosins were compared over the 176-amino-acid region encoded by exons 3, 4, 5, 6b, 7, and 8. Gene duplications are indicated at diamonds (◇). The numbers represent a best approximation to the encoded amino acid differences between the genes, where species-specific differences are ignored. The figure overestimates by three the number of amino acid substitutions between the TMnm gene and the β-TM gene. Double arrows indicate the difference. It should be noted that exons 6a and 6b and exons 9a and 9d correspond to exons 6 and 7 and 10 and 11, respectively, presented in Fig. 1 for the rat β-TM gene.

The Brain Tropomyosins

J. Lees-Miller, D.M. Helfman

Tropomyosins are thought to be involved in the regulation of the structure and motility of cells through their interaction with cytoskeletal actin filaments. Within the nervous system, the cytoskeleton is essential for the formation and maintenance of cell contacts both within neural networks and between neurons and their support cells. We have found that a set of tropomyosins specific to brain (termed TMBr-1, TMBr-2, and TMBr-3) is expressed through the use of alternative promoters and alternative splicing from the α-TM gene (Fig. 2). The alternative splicing results in three brain tropomyosins with unique carboxy-terminal sequences. This region of tropomyosin is known to be critical for its interaction with actin and with Ca^{++}-sensitive regulatory components such as troponin. Our goals are to determine the functional significance of the brain isoforms and to understand the regulatory mechanisms

involved in their expression. As a first step, we have developed a high-yield bacterial expression system and a rapid purification procedure that produce near-gram quantities of pure protein. This protein, along with a brain tropomyosin-specific synthetic peptide, is being used to raise polyclonal and monoclonal antibodies. We now have a polyclonal serum specific for TMBr-1 and TMBr-3. It is being used to study the location of brain tropomyosins in the developing rat brain and their distribution in other species. We are also searching for a cell line that expresses the brain isoforms. These cells will be essential for understanding the function of the brain tropomyosins and the mechanism by which their expression is regulated.

α-TM Diversity: Microinjection of Fluorescently Labeled Tropomyosins

M. Pittenger, L. Goodwin, D.M. Helfman

The function of the various tropomyosin isoforms in cells is, with perhaps one exception, not understood. In skeletal and cardiac muscle, tropomyosin, in association with the troponin complex, is thought to control the interaction between actin and myosin filaments in response to fluxes of cellular calcium levels. However, in the rat alone, there are 12 isoforms of tropomyosin expressed from three genes that are expressed in a tissue-specific manner. The α-TM gene itself encodes nine tropomyosin proteins (Fig. 2). To begin to understand the importance of α-TM isoform diversity and the role that these proteins play in non-muscle cells, we have utilized the cDNA clones generated in our laboratory to produce highly purified quantities of each isoform in a bacterial expression system.

In using the pET-8C expression system (made available to us by Bill Studier of Brookhaven National Laboratory), the translational start site of the cDNA of interest is placed adjacent to a T7 polymerase transcriptional initiation site. The T7 polymerase is coded on another plasmid present in the bacteria, and its expression is chemically inducible by IPTG. The induced protein may represent up to 20% of bacterial protein. The tropomyosin isoforms are purified by classical methods from the lysed bacteria, with yields of 5–30 mg of protein per liter of culture. We have analyzed these purified proteins and find them to be 95–99% homogeneous, with the majority of the contaminants being fragments of tropomyosin polypeptides and therefore difficult to remove. These are probably not degradation products but are more likely due to inappropriate translation initiation. With the aid of the Protein Chemistry core facility, we have sequenced the amino terminus of several of the purified tropomyosins and found them to be correct. Moreover, certain tropomyosins isolated from eukaryotic cells have been shown to lack the amino-terminal methionine coded by the mRNA. We find that this bacterial expression system correctly removes this methionine from isoforms where it is not normally found, while leaving it on at least one isoform that retains this methionine in vivo. The signals for this type of processing are not known but probably reside in the first coding exon.

With these tropomyosins in hand, we have begun to ask questions about the functional role of these proteins in living cells using microinjection and fluorescent analog cytochemistry. We have labeled the purified proteins with FITC, an amine-reactive compound, which usually modifies lysine residues under our conditions. The FITC-Tm was purified away from excess unreactive dye and analyzed for dye/protein ratio. We have found that a dye/protein ratio of near one is necessary to visualize localized FITC-Tm in fibroblast cells. Ratios of about 1.5 may prove optimal, but higher dye/protein ratios can cause protein denaturation and have been difficult to attain experimentally. These fluorescent derivatives were microinjected into tissue culture cells and allowed to mix with the endogenous pool. The cells were then fixed and viewed with the fluorescent microscope. So far, we have found that both the 248- and 284-amino-acid tropomyosins are found in association with actin filaments. We have not seen a preference for any isoform at the leading or trailing edge of a moving cell. The isoforms do not appear to enter the nucleus. In preliminary experiments, we have synchronized cells and tried to follow injected FITC-Tm5A as the cell progresses through the cell cycle. We have seen a speckled pattern around the nucleus of cells that appear to be entering mitosis. Many more such experiments are planned using this and other FITC-Tms. We also will study the reorganization of tropomyosin isoforms in cells migrating into the wound created in a cell monolayer. Experiments are also planned to microinject transformed rat cells to see if the isoforms maintain the same localization as in normal cells. Transformed cells have al-

tered Tm expression, and it will be of interest to note how these cells respond to such injections.

Functional Significance of β-TM Isoform Diversity

A. Kistler, M. Pittenger, K. Galactionov, D.M. Helfman

The β-TM gene generates two isoforms of tropomyosins through alternative mRNA splicing (Fig. 1). Both isoforms contain amino acids encoded by the common exons 1 through 5, 8, and 9. In skeletal muscle cells, exons 7 and 10 are utilized to produce skeletal muscle β-TM, whereas in smooth muscle and nonmuscle cells exons 6 and 11 are used, generating the TM-1 isoform. The mRNA for the skeletal muscle β-TM is expressed in skeletal muscle but is undetectable in nonmuscle and smooth muscle cells. In contrast, mRNA for TM-1 is found in fibroblasts and smooth muscle cells but not in skeletal muscle cells. No mRNAs that contain exons 6 + 10 or 7 + 11 have been detected in any tissue or cell type examined. These mutually exclusive exons encode differences in the amino acid sequences in two short regions of the tropomyosin molecule. Exons 6 and 7 encode an internal region in the protein sequence (amino acids 189–213), and exons 10 and 11 encode the carboxyl terminus (amino acids 258–284). The functional significance of these differences in the two tropomyosin isoforms is not understood. Similarly, why the tissue-specific splicing prohibits incorporation of exons 6 and 10 or 7 and 11 in the same isoform is unclear.

Using our cDNA clones and the bacterial expression system described above, we have produced and purified two naturally occurring β-TM isoforms. In addition, we have also produced two chimeric isoforms (TM610 and TM711) by swapping the carboxy-terminal exons (10 and 11) from the cDNA clones of each of the naturally occurring β-TM isoforms. The actin-binding capabilities of these proteins will be assayed in vitro and compared to tropomyosin biochemically purified from skeletal muscle tissue. We are currently labeling these purified proteins with various fluorescent probes. The labeled proteins will be introduced into tissue culture cells by microinjection. By so doing, we hope to determine whether the differences in each of the isoforms and chimeras result in differential localization or incorporation into microfilament structures during various cellular events, such as mitosis, spreading, and motility.

Why Do Transformed Cells Alter Tropomyosin Expression?

M. Pittenger, D.M. Helfman

There are dramatic alterations in tropomyosin isoform expression in cells transformed by such diverse agents as DNA and RNA tumor viruses, UV irradiation, and chemical carcinogens. The underlying reasons for these alterations are not understood. Morphological alterations are the most obvious and immediate characteristics of transformed cells in culture. It is not known whether these gross abnormalities are directly related to transformation (e.g., by interfering with cell-cell communication and/or signal transduction pathways) or if they occur only as an indirect consequence. We are interested in asking if morphological alterations are directly related to changes in tropomyosin expression. This also requires an understanding of the normal regulation of tropomyosin expression.

We have used a pulse-chase experiment and high-resolution gel electrophoresis and quantitative analysis to look at the half-life of tropomyosin proteins in normal rat embryo fibroblasts. We find that all tropomyosins have long half lives, greater than 20 hours. We will analyze some transformed rat cell lines to see if alterations in half-life of particular isoforms can account for the alteration in their abundance. To analyze tropomyosin expression through the cell cycle, we have synchronized populations of cells by thymidine block and mitotic shake off. The newly synthesized proteins in each population were pulse-labeled and resolved by two-dimensional gel electrophoresis. Only a modest change was detectable in the newly synthesized tropomyosins. This alone would not account for the changes seen in transformed cells. However, it will be interesting to see if there is any change in the degradation rate of isoforms through the cell cycle.

To address the question of whether morphological alterations are directly related to changes in tropomyosin expression, we have plated normal rat fibroblasts on a substrate that has been treated with polyHEMA to prevent cell attachment. This treatment can be varied to allow cells to spread to different extents. New synthesis of tropomyosins has then been analyzed by two-dimensional gel electrophoresis. We find that cells that cannot attach and spread properly show synthetic patterns similar to those of the transformed cells. Further experiments of this type are planned.

Transcriptional Control of Tropomyosin Gene Expression

J. Kazzaz, D.M. Helfman

The rat β-TM gene expresses two distinct isoforms via an alternative splicing mechanism (Fig. 1). Although the gene is expressed in muscle (skeletal, cardiac, and smooth) and nonmuscle cells, a single transcription initiation site is used in the various cell types that express the gene. We now wish to determine if the same *cis*-acting elements are used in muscle (skeletal, cardiac, and smooth) and nonmuscle cells (e.g., fibroblasts), and if the same or different *trans*-acting factors are involved in transcriptional control of the gene in different cell types. Sequence analysis of the 5′ end of the β-TM gene has already revealed a number of potentially important elements involved in transcriptional control in skeletal muscle cells. The gene contains three copies of a skeletal-muscle-specific enhancer element located approximately 304, 352, and 398 bp upstream of the transcriptional start site. The consensus sequence of this 14-nucleotide-long element (C/GNG/AG/ACAC/GC/GTGC/TC/TNC/G) has been found in a number of genes expressed in skeletal muscle, including the muscle creatine kinase, δ-subunit of the acetylcholine receptor, myosin light chain 1/3, desmin, and vimentin (Buskin and Hauschka, *Mol. Cell. Biol. 9:* 2627 [1989]). This element is believed to be the site of action of the myogenic regulatory factors myoD and myogenin. Since these myogenic regulatory factors are expressed in skeletal muscle but not in cardiac and smooth muscles nor nonmuscle cells, it is likely that expression of the β-TM gene in cells other than skeletal muscle will require a different set of transcriptional regulatory proteins. Work is in progress to determine the *cis*-acting elements required for expression in muscle versus nonmuscle cells and to identify the cellular factors that interact with these elements.

Cell Cycle Regulatory Proteins and Growth Control in Mammalian Cells

K. Galactionov, D.M. Helfman

It is assumed that somatic cells predominantly possess a control point located in the G_1 phase of the cell cycle, where they can be committed to the normal cell cycle or remain in a quiescent state. This point has been named "start" in yeasts and restriction point (R) in mammalian tissue culture cells. In yeasts, the function of one of the key proteins involved in cell cycle control, p34 *cdc2* (*cdc28* in *Saccharomyces cerevisae*), is dependent on other genes that regulate its function independently at G_1-S and G_2-M transition. The function of p34 *cdc2* at the G_2-M transition was better understood after the discovery of cyclins and their role in activation of p34 *cdc2* protein kinase activity (as a catalytic subunit of maturation promoting factor, which is probably the same entity as growth-dependent histone H1 kinase). On the other hand, earlier experiments on tissue culture cells suggest that some unstable protein(s) is required to pass the restriction point. Taking into account that cyclin homologs were identified which affect *start* function of *S. cerevisae*, it is possible to question the role of cyclins, their homologs, or other *cdc2*-associated proteins in G_0-G_1 transition in mammalian cells. One can also speculate on the involvement of p34 *cdc2* in the control of differentiation in mammalian cells.

We are using the mouse myogenic cell line BC3H1 as a model to study the possible role of p34 *cdc2* in the regulation of differentiation. This cell line responds to decreases of serum levels in the media by cessation of proliferation and, 24–48 hours later, induction of muscle-specific isoforms of actin, myosin, tropomyosin, etc. Because of the inability of this cell line to form myotubes, the process of differentiation can be reversed by simply changing the serum concentration of the media. In this case, the process of dedifferentiation was monitored by the loss of the muscle markers and activation of the normal cell cycle. To study changes in the p34 *cdc2* phosphorylation and associated proteins during cell cycle activation from the quiescent state, we chose Swiss-3T3 cells because the time course of the cell cycle following serum stimulation has been previously studied in detail.

Proteins associated with p34 *cdc2* during different stages of differentiation and dedifferentiation of BC3H1 cells and cell cycle activation of the 3T3 cells were detected by immunoprecipitation with G6 antibody (kindly provided by G. Draetta and D. Beach, Genetics Section) directed against a peptide, representing the carboxyl terminus of human *cdc2*. Work from David Beach's laboratory has shown that this antiserum precipitates, in addition to p34 *cdc2*, proteins with molecular masses of 62 and 60 kD from HeLa cells (Draetta and Beach, *Cell 54:* 17 [1988]). The 62-kD protein was shown to be the human cyclin B gene product, whereas the 60-kD protein rep-

resents a protein associated with the E1A gene product in adenovirus-transformed human cells (Giordano et al., *Cell 58:* 981 [1989]). Our data show that in BC3H1 cells, p34 *cdc2* forms stable complexes with the 47-, 55-, 56-, and 65- and 67-kD proteins that are different from p62 (cyclin B), which is precipitated as well. Some of these proteins associate with p34 *cdc2* mostly at G_1 (p56) and others at the G_2-M transition (47 and 62 kD). Isolation of complexes containing p56 and p34 *cdc2* are under way.

In the 3T3 cells, we found p34 *cdc2* associated with a previously unidentified protein with a molecular mass of approximately 56 kD (p56) in correlation with passing the R point. This event precedes phosphorylation of the p34 *cdc2* at the particular site(s) of the G_1/S border. p56 is phosphorylated at the same time point as p34 *cdc2*, but it is not the substrate of the p34 *cdc2* at this stage of the cell cycle. S-phase-specific "isoform(s)" of p34 *cdc2* was shown to contain phosphotyrosine. No detectable protein kinase activity toward histone H1 was found in the complexes of p56 and p34 *cdc2*, compared with the high activity of the complexes between p34 *cdc2* and p62/cyclin B in the same cells. We are now in the process of searching for possible G_1/S substrates for the complexes between p34 *cdc2* and p56, specific for the G_1-S transition.

Our data indicate that different proteins may associate with p34 *cdc2* during differentiation of the myogenic cells, compared to 3T3 cells entering a quiescent phase of the cell cycle. We plan to isolate these newly identified proteins associated with p34 *cdc2* in the myogenic cell line BC3H1. The isolated proteins will be used for preparation of antibodies and for peptide sequence analysis, which could subsequently lead us to cloning of the genes for these proteins.

PUBLICATIONS

Helfman, D.M. and W.M. Ricci. 1989. Branch point selection in alternative splicing of tropomyosin pre-mRNAs. *Nucleic Acids Res.* **17:** 5633–5650.

Helfman, D.M., J. Lees-Miller, L. Goodwin, S. Erster, and L.A. Finn. 1989. Tropomyosin gene structure, expression, and regulation. In *Cytoskeletal proteins in tumor diagnosis. Current communications in molecular biology* (ed. M. Osborn and K. Weber), pp. 85–89. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

In Press, Submitted, and In Preparation

Galactionov, K. and D.M. Helfman. 1990. Cell growth and changes in subunit composition and activity of the *cdc2* protein kinase. (In preparation.)

Goodwin, L.O., J.P. Lees-Miller, S.B. Cheley, and D.M. Helfman. 1989. Four rat fibroblast tropomyosin isoforms are expressed from the rat alpha-tropomyosin gene via alternative RNA splicing and use of two promoters. (Submitted.)

Helfman, D.M., R.F. Roscigno, G.J. Mulligan, L.A. Finn, and K.S. Weber. 1990. Identification of two distinct intron elements involved in alternative splicing of β-tropomyosin pre-mRNA. *Genes Dev.* **4:** 98–110.

Lees-Miller, J.P., A. Yan, and D.M. Helfman. 1990. Structure and complete nucleotide sequence of the gene encoding rat fibroblast tropomyosin 4. *J. Mol. Biol.* (in press).

Lees-Miller, J.P., L.O. Goodwin, and D.M. Helfman. 1990. Three novel brain tropomyosin isoforms are expressed from the rat α-tropomyosin gene through the use of alternative promoters and alternative RNA processing. *Mol. Cell. Biol.* (in press).

Novy, R.E., J.L. Lin, C.S. Lin, D.M. Helfman, and J.J.L. Lin. 1990. Expression of smooth muscle and nonmuscle tropomyosins in *Escherichia coli* and characterization of bacterially produced tropomyosins. *J. Biol. Chem.* (Submitted.)

QUEST PROTEIN DATABASE CENTER

J.I. Garrels
B.R. Franza

C. Chang, H. Sacco, P. Myers, J. Kos, M. Hannaford, G. Mak, A. Rudman, C. Blanchford, S. Fang, T. Husain, J. LaMarca, A. Husain

During the past year, the QUEST Center has been focused on the construction of two major databases, one for mouse embryos and one for yeast cells. Both of these are patterned after the rat REF52 database, built by Jim Garrels and Robert Franza, which was reported last year. At the same time, the QUEST Center has continued to support many smaller projects of two-dimensional gel analysis, ranging from human cells to bacterial cells to plants.

The mission of the QUEST Center is not only to build databases derived from computer analysis of two-dimensional gels, but also to continue to improve and disseminate the technology that makes such analysis possible. Because the QUEST facility

is now operating smoothly from gel electrophoresis to database analysis, Jim Garrels was able to spend much of 1989 on a sabbatical leave. From October 1988 through September 1989, he has worked 2 days per week with the Millipore company of Bedford, Massachusetts, helping to explore improved methods of two-dimensional gel electrophoresis. During this period, he has also set up a home-office in the Boston area to continue explorations of future two-dimensional gel software.

The year 1989 was also the renewal year for the NIH Research Resources Grant that has funded QUEST for the past 5 years. The new directions for the technological and scientific goals of QUEST have been approved at an increased funding level. In the renewal, a full-time Computer Resource Manager was requested to manage the software upgrades and the interaction of users with the software. Furthermore, the renewal application included a new program for QUEST, a protein identification section to be headed by a full-time protein chemist. Both were approved. The new Computer Resource Manager, Gerald Latter, will start in January, 1990, and a protein chemist at the senior staff level will be hired during 1990.

Our staff in the Grace Building has not changed during 1989. Cecile Chang, the Operations Supervisor, continues to interact with service and collaborative users to coordinate data processing and use of the workstations. Jim Kos has maintained the hardware and software, adding utility programs as needed. Phyllis Myers tirelessly performs the service of starting the gel matches and carefully checking the results.

In the two-dimensional gel laboratory in the McClintock Building, Ann Rudman departed in August, and Heidi Sacco began maternity leave in September. During July, Heidi recruited and trained two new laboratory technicians, Christy Blanchford and Shuling Fang, and they have run the gel laboratory during Heidi's absence. When Heidi returns part-time in 1990, she will continue to supervise the gel laboratory, but will take on responsibilities in a new core facility for two-dimensional gel scanning.

Yeast Database

J.I. Garrels, C. Chang, P. Myers [in collaboration with C. McLaughlin, University of California, Irvine, and J. Warner, Albert Einstein College of Medicine]

The yeast database has grown in the past year through the analysis of several major experiments described below. Each of these experiments has been analyzed on three different two-dimensional gel types (pH 3.5–10, 10% slab; pH 4–8, 10% slab; and nonequilibrium, 15% slab), and routinely 2000–3000 proteins per sample are scored. These gels are sensitive enough to detect many proteins that represent less than 0.001% of the total protein synthesis, although many other proteins of such low intensity cannot yet be resolved from the larger spots on the gel. The proteins of lowest detectable intensity represent about 100–300 molecules per cell.

INTRONS IN YEAST

Our experiment to study proteins encoded by genes with introns has been further analyzed. The mutant *rna2* fails to remove introns at the nonpermissive temperature, and, after the decay of normal mRNAs, proteins encoded by genes with introns fail to be synthesized. Our studies show that for *Saccharomyces cerevisiae* grown in balanced growth on glucose, only 2% of the detected proteins are coded from genes with introns. Interestingly, most of these are ribosomal proteins, which were previously identified on gels of purified ribosomes.

KINETICS OF LABELING AND MODIFICATION

A study of the kinetics of labeling, processing, and turnover has been put into the database. The vast majority of yeast proteins appear to be labeled with the same relative intensity in a 30-second pulse, a 60-minute pulse, and a 5-minute pulse, followed by a 20-minute chase. (The time to complete a polypeptide chain in yeast is about 30 seconds.) Thus, for most yeast proteins, the posttranslational modifications, if any, are rapid.

Most proteins are stable as judged from the 20-minute chase (carried out under balanced growth conditions). Protein spots that represent posttranslationally modified forms should appear with increasing intensity as pulse times increase from 2 to 60 minutes (unless the modification occurs quickly on nascent chains). There are 50 such proteins. Those that appear with lower intensity for longer pulses either are precursors to a modified form or are unstable. In this class, 31 proteins have been found.

N-ACETYLATION OF YEAST PROTEINS

One of the most common protein modifications is acetylation of the amino-terminal amino acid. Three

groups following entirely different strategies have isolated mutants that affect this process and define two genes that appear to code for subunits of the transacetylase. Whiteway and Szostak isolated *ARD1* (*Cell 43:* 483 [1985]) on the basis that it was involved in the switch between the mitotic cell cycle and alternate developmental pathways. The *NAT1* gene was isolated by Grunstein, Sternglanz, and co-workers, who screened a library of temperature-sensitive mutants to detect a mutant defective in histone acetyltransferase activity. The mutant turned out to be defective in the acetyltransferase activity for a wider range of proteins (Mullen et al., *EMBO J. 8:* 2067 [1989]), and the fact that it was functionally related to *ARD1* was noted. Smith and co-workers took the direct approach of purifying the *N*-acetyltransferase, sequencing peptides, and preparing oligonucleotide probes (Lee et al., *J. Biol. Chem. 264:* 12339 [1989]). Both Sternglanz's and Smith's laboratories conducted a preliminary survey of the two-dimensional gel electrophoresis pattern in the mutants, the former using the QUEST laboratory and the latter using Protein Databases Inc. Both groups noted that a number of proteins changed position in the mutant.

We have undertaken a detailed analysis of protein synthesis in the *ard1* and *nat1* mutants because of our intrinsic interest in protein modification and because of the possible relationship between these mutants and a switch between the mitotic cell cycle and alternative developmental pathways. The comparison of protein patterns from normal and the acetylation mutants was challenging because so many of the major proteins are shifted in position. Proteins that are *N*-acetylated in the wild type lose one negative charge in the mutant and migrate to a more basic position on the isoelectric focusing gel. Figure 1 gives an example of the wild-type, mutant, and mixed patterns, revealing charge shifts for a large number of spots.

In a pH 4–8 gel, 152 of the 1325 scored proteins are normally *N*-acetylated by the *NAT1,ARD1* enzyme. Proteins that are clearly nonacetylated number 534. In addition, 639 low-intensity proteins do not appear to be acetylated, but cannot be unam-

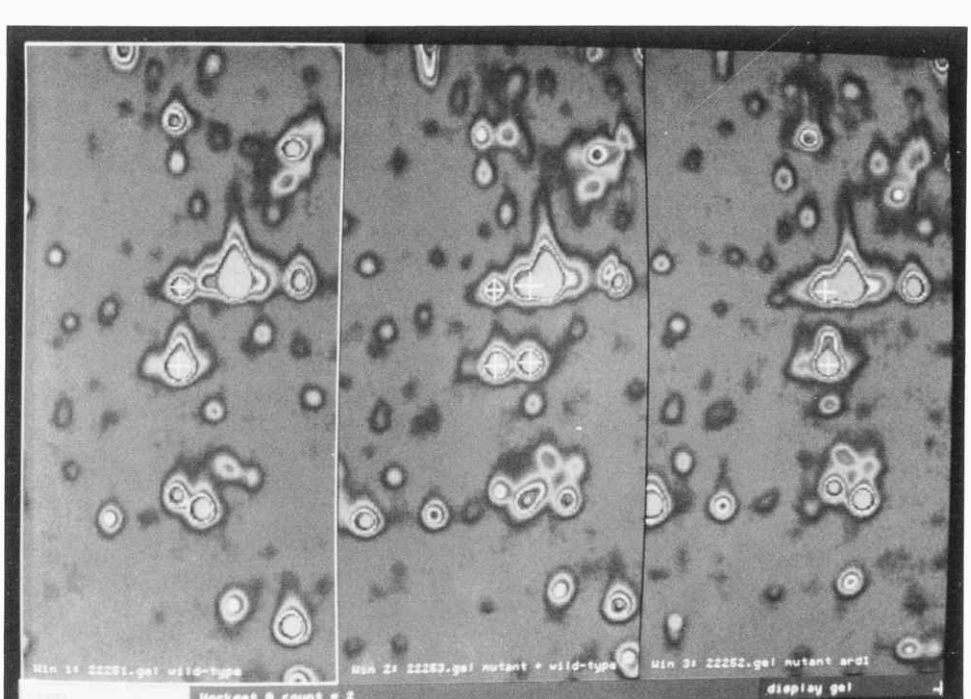

FIGURE 1 Comparison of the wild-type strain and the *N*-acetylation mutant (*ard1*) isolated by Szostak et al. The cells were labeled with [^{35}S]methionine for 15 min at log phase. In mutant *ard1*, a total of 152 proteins (about 50% of the total protein mass) were shifted toward the basic side of the two-dimensional gel (pH 4–8, 10% polyacrylamide), compared with the wild-type strain (see text). The middle graph is a mixture of wild type and mutant; + indicates a group of four spots that correspond to the spots marked either in the wild-type or mutant cells.

biguously scored. The 152 acetylated proteins represent about 50% of the total protein mass in this gel type. Thus, *N*-acetylation is heavily biased toward the major proteins. All of the *N*-acetylated proteins are stable, and they all are labeled in the shortest pulse labelings. In all 152 cases, the mutant and the wild-type proteins have the same relative abundance. Therefore, for cells in balanced growth in glucose, the lack of the *N*-acetyl group does not lead to instability that can be detected in a 15-minute pulse.

Mouse Embryo Database

J.I. Garrels, C. Chang [in collaboration with K. Latham and D. Solter, Wistar Institute]

The mouse embryo database is being developed by Drs. Davor Solter and Keith Latham as a tool to supplement many studies ongoing in Dr. Solter's laboratory. As a baseline study of the normal embryo, the database will be enormously valuable for interpretation of more-specialized experiments involving genetic manipulation of embryos. The mouse embryo database should be useful to many researchers, and like the yeast database, it is being developed with the needs of the larger community in mind.

Carefully staged mouse embryos have been labeled at 3-hour intervals, from fertilization through the four-cell stage, which is a 57-hour time span. Typically, 20–30 embryos are required per time point. Further labelings have been done to extend this study through the blastocyst stage, but only samples through the four-cell stage have been analyzed.

It is known that by the two-cell stage, much of the maternal mRNA has been degraded and new proteins coded by embryonic mRNA are being made. The timing of this switchover and its impact on the types and amounts of proteins being synthesized have not been previously studied in detail. We find that during any 3-hour interval of the late zygote/early two-cell stages, more changes occur in the pattern of protein synthesis than in the entire transition of REF52 cells from proliferation to quiescence. In contrast, cells labeled 3 hours apart in the four-cell stage appear to be as similar as duplicate cultures of REF52 cells.

To explore this period of rapid change further, we followed the fate of each of 748 proteins that can be accurately measured during the two-cell stage. Of these, 169 (23%) disappear during the two-cell stage and are probably coded from maternal mRNA. Another set of 174 proteins (23%) appear for the first time during the two-cell stage and are probably coded by newly activated embryonic genes. Most of the remaining proteins show increases or decreases of more than twofold between the beginning and the end of the two-cell stage. These may represent proteins coded by both maternal and embryonic mRNAs, but for which the switch affects the rate of synthesis. Only 78 proteins (10%) remain constant (within a factor of 2) during the entire two-cell period. In contrast, 92% of the proteins are synthesized at a constant rate throughout the four-cell stage. Figure 2 shows a portion of the gels from the two-cell stage (four of the eight gels) with the most constant proteins highlighted. The massive changes in the surrounding spots are apparent.

We plan to extend the mouse embryo database to the blastocyst stage and to supplement it with many more identified proteins.

Signal Transduction/Nuclear Regulatory Protein Database

B.R. Franza

Efforts to identify several cellular proteins involved in the processes of signal transduction and/or the control of mammalian mRNA transcription have been fruitful. Details of each of these studies are presented in Cellular *trans*-Activators of Gene Expression, in this section. We have identified the following proteins in the QUEST two-dimensional protein pattern: the large subunits of RNA polymerase II as well as certain coimmunoprecipitating proteins; mammalian cyclin B (p62); an important complex between p60 and the "MARCKS" proteins (a known substrate of protein kinase C); Fos B, a Fos-related gene product; and p13, another *cdc2*-associated protein. The QUEST two-dimensional gel system made possible the recognition that proteins other than cyclin B and p13 were associated with *cdc2* and that a major substrate of protein kinase C was associated with an E1A-associated cellular protein. These observations have prompted much experimentation at Cold Spring Harbor Laboratory during 1989.

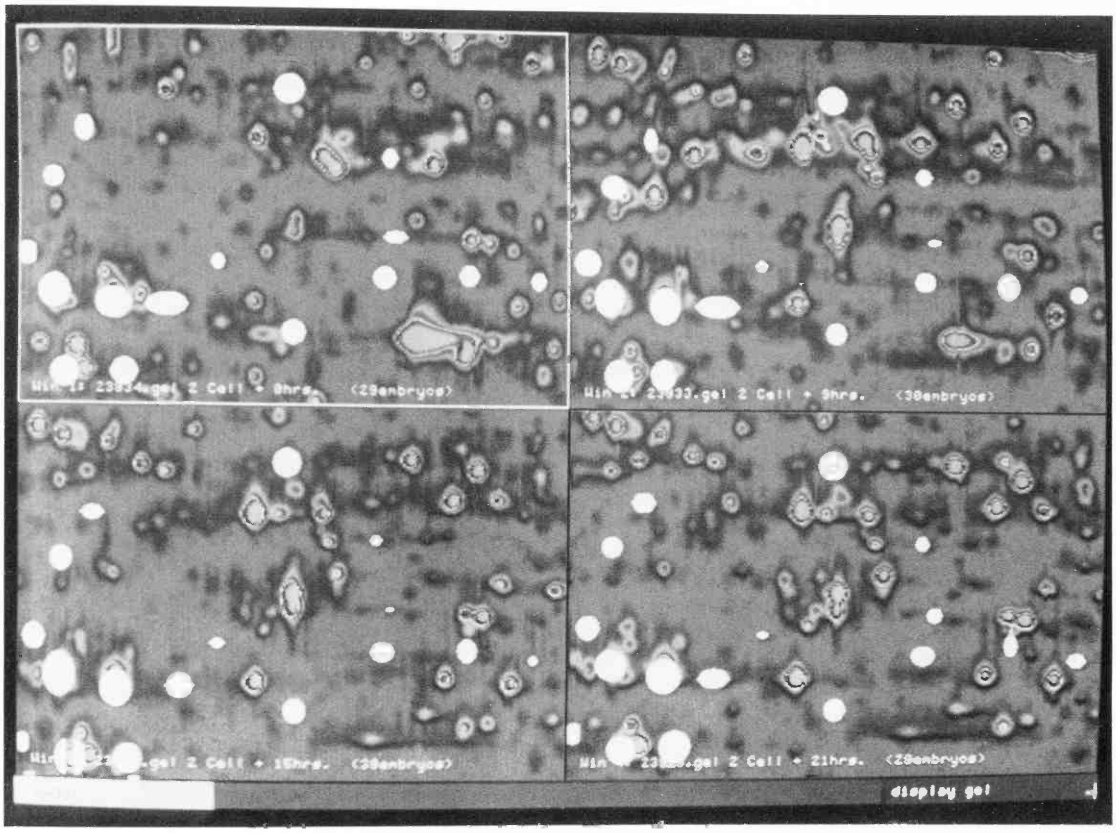

FIGURE 2 Changes of protein synthesis during the two-cell stage of mouse embryos. Gel images are shown from four time points of the two-cell stage, with constant proteins highlighted. The dramatic changes of protein synthesis are apparent, reflecting the reprogramming of protein synthesis as embryos switch from the use of maternal mRNA to newly synthesized embryonic mRNA.

Service Projects

C. Chang, P. Myers, J. Kos, J. Garrels

The QUEST center performs numerous small service and collaborative projects. Some of the key projects for 1989 are described below.

S. Shaw and Y. Shimizu (National Institutes of Health) have used the QUEST system to investigate naive and resting T cells, separated on the basis of their CD45 isoforms. Differences in the proteins patterns were found, and these proteins are being characterized to find additional regulators in the T-cell signal transduction pathway.

C. Kumar (Schering Corp.) has used the QUEST system to study normal and transformed human HOS cells, using an isoform of myosin-light-chain 2 (MLC2) as a marker. So far, he finds that MLC2 expression is repressed by *ras*, *met*, *src*, and TPA, but not by feline leukemia virus. He plans to expand these studies into an entire database for HOS cells.

J. Higginbotham (Pioneer Hi-Bred and CSHL) has used the QUEST system to study more than 1900 proteins from inbred maize lines. More than 200 substantial differences among strains were found, and these were checked to be sure that environmental factors, such as time of imbibition, were not responsible. These studies will be useful in better understanding the relationship of the inbred genotypes.

T. Holt and C. Thompson (Pasteur Institute) have used QUEST to identify and study proteins involved in the synthesis of the antibiotic bialaphos. More than 20 proteins were identified, using regulatory mutants that fail to induce the pathway, and the expression of these proteins was studied in cells pulse-labeled from early log-phase growth to late stationary phase.

New Directions in QUEST Technology

J.I. Garrels [in consultation with G. Latter]

The year 1989 was the last year of our initial 5-year Resource Grant from the NIH Biomedical Research Technology Program. Therefore, much of the past year has been spent in planning the next phase of the QUEST center. We have explored new avenues of hardware and software technology, we have tested ideas and developed preliminary data, and we have presented and defended our ideas during the review process. Throughout this period, Jerry Latter served as a consultant to QUEST. The review was successful, and Jerry has been hired to join QUEST as the Computer Resource Manager in January 1990.

WORKSTATIONS

In reequipping the QUEST center, we have decided to continue with the powerful Sun workstations for the demanding task of processing hundreds of gel images to build the master databases. We have also decided to use the Macintosh computer more for statistical analysis of the data and as a vehicle for database dissemination (see below). New Sun Sparcstation 4/330 and Sparcstation 1 workstations were ordered by the end of the year. In addition, we are thankful to Sun Microsystems for the gift of one Sun 4/330 and one Sun 4/60 Sparcstation as part of their academic grant program.

STANDARDIZED USER INTERFACE

Standardized graphics is now an essential component of modern software. X Windows give a library of powerful subroutines for almost any type of graphics or windowing operation, and programs that use X can run on virtually any workstation. Conversion of the QUEST software to X was begun by Jim Garrels using a Sun 4/260 workstation. Displays of gel images are now almost instantaneous. Previously, QUEST users could view only images from one matchset at a time; now any number of matchsets can be displayed simultaneously in overlapping windows. It is even possible to control multiple screens from the same program if desired.

One of the major responsibilities of Jerry Latter, as the new Computer Resource Manager, will be the development of a standardized user interface. Such interfaces represent standard specifications and software toolkits that allow implementation of menus and control windows with a common "look and feel." Jerry has researched these, and he is currently leaning toward the OpenWindows interface standard and the XView toolkit developed by Sun. The implementation of a standardized user interface will greatly improve our ability to train users of the QUEST system, both at CSHL and elsewhere, when we begin to disseminate the QUEST software.

New Directions in QUEST Databases

J.I. Garrels [in consultation with G. Latter]

We have also explored and tested new directions for the QUEST databases. These databases, which are the end result that proves the worth of all our technology, will be enhanced (1) by making them available to scientists everywhere who use personal computers, (2) by making them compatible with commercial programs for statistical analysis and data presentation, (3) by connecting them to other biological databases, (4) by enlarging databases through core research in key database areas, and (5) by enhancing them with many more protein identifications.

DATABASE DISSEMINATION ON THE MACINTOSH

Jerry Latter has worked during 1989 to build a prototype database interface for the Macintosh. Using the Hypercard utility in the Macintosh, Jerry has been able to present entire gel images that can be scrolled and zoomed, and he has been able to connect the spots as "buttons" that display annotations, spot names, spot graphs, etc., when "pushed" by a mouse click. Our aim is to format the spot quantitation data, as well as the spot names and annotations, from each database in a format compatible with Hypercard, and to disseminate these data with all the relevant gel images on CD-ROM disks. Such a database would give users everywhere complete access to the QUEST databases and would allow them to check important results against the original images. One of the hardest tasks in developing the QUEST system is writing the statistical analysis routines and plotting routines to cover every type of analysis that a user might want. Fortunately, a wide variety of commercial packages for the Macintosh already provide for statistical analysis, for spreadsheet analysis, and for creation of presentation quality slides and figures. Jim Garrels has explored many of these packages during the past year, and he has

set up the interface to move data easily between the QUEST program and the Macintosh.

THE NEW FOCUS FOR CORE DATABASES

New databases to be built by the QUEST staff are (1) a mammalian cell proliferation database, (2) a nuclear regulatory protein database, and (3) a yeast protein database. The first is an outgrowth of our REF52 database, but it has been broadened to include studies of interest from any mammalian cell culture system that provides information about the regulation of cell growth. The second database area is centered on the work of Robert Franza (see his section of this report). The third database area, yeast cells, continues to be led by Calvin McLaughlin and Jonathan Warner. In each of these areas, a database can be built under the direction of one or two scientists, who know both the particular field and the fine points of using the QUEST system to build databases.

We expect our work in each of these database areas to be supplemented by a full-time protein chemist, to be hired during 1990, who will specialize in protein identifications. The next generation of technology should make database construction severalfold easier and faster, and with a full-time effort at protein identification, we expect that QUEST in the 1990s will truly become a database resource.

PUBLICATIONS

Chang, C., J. Sacco, G. Mak, W. Welch, L. Mizzen, G. Morris, M.B. Mathews, A. Giordano, E. Harlow, D. Beach, G. Draetta, B.R. Franza, Jr., and J.I. Garrels. 1989. Building databases with the QUEST system: The HeLa database. In *Electrophoresis forum '89* (ed. B.J. Radola), pp. 47-51. Technical University of Munich, Munich.

Cohen, D.R., P.C.P. Ferreira, R. Gentz, B.R. Franza, Jr., and T. Curran. 1989. The product of a Fos-related gene Fra-1 binds cooperatively to the AP-1 site with Jun: Transcription factor AP-1 is comprised of multiple protein complexes. *Genes Dev.* **3:** 173–184.

Garrels, J.I. 1989. The QUEST system for quantitative analysis of two-dimensional gels. *J. Biol. Chem.* **264:** 5269–5282.

Garrels, J.I. and B.R. Franza, Jr. 1989. The REF52 protein database: Methods of database construction and analysis using the QUEST system, and characterizations of protein patterns for proliferating and quiescent REF52 cell cultures. *J. Biol. Chem.* **264:** 5283–5298.

Garrels, J.I. and B.R. Franza, Jr. 1989. Transformation-sensitive and growth-related changes of protein synthesis in REF52 cells. *J. Biol. Chem.* **264:** 5299–5312.

Giordano, A., P. Whyte, E. Harlow, B.R. Franza, D. Beach, and G. Draetta. 1989. A 60-kD *cdc-2*-associated polypeptide complexes with the E1A proteins in adenovirus-infected cells. *Cell* **58:** 981–990.

Kumar, C.C., S. Mohan, C. Chang, and J.I. Garrels. 1989. Regulation of smooth-muscle-specific myosin light chain-2 isoform by oncogenes. In *Cytoskeletal proteins in tumor diagnosis* (ed. M. Osborn and K. Weber), pp. 91–97. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Kumar, C.C., S.R. Mohan, C. Chang, and J.I. Garrels. 1989. Regulation of smooth muscle specific myosin light chain-2 isoform gene expression by oncogenes. *J. Cell. Biochem.* (suppl.) **4:** 194.

Lambert, M.E., Z.A. Ronai, I.B. Weinstein, and J.I. Garrels. 1989. Enhancement of major histocompatibility class I protein synthesis by DNA damage in cultured human fibroblasts and keratinocytes. *Mol. Cell. Biol.* **9:** 847–850.

Mizzen, L., C. Chang, J.I. Garrels, and W.J. Welch. 1989. Identification, characterization, and purification of two mammalian stress proteins present in mitochondria: Grp75, a member of the Hsp70 family, and Hsp58, a homolog of the bacterial groEL protein. *J. Biol. Chem.* **264:** 20664–20675.

In Press, Submitted, and In Preparation

Franza, B.R. 1990. Complex protein interactions at the HIV-1 direct repeat enhancer structure: A paradigm for database analysis of protein-nucleic acid interactions. *UCLA Symp. Mol. Cell. Biol. New Ser.* **119:** (In press.)

Holt, T., A. Raibaud, C. Laurent-Winter, C. Chang, P. Myers, J.I. Garrels, T. Murakami, J. Davies, and C. Thompson. 1990. The regulation of antibiotic biosynthesis in *Streptomyces hygroscopicus*. Microbial metabolites as sources for new drugs. *J. Cell Biol.* (suppl.) **14A:** 94.

Holt, T., A. Raibaud, C. Laurent, C. Chang, P. Myers, J.I. Garrels, T. Murakami, E. Takano, J. Davies, and C. Thompson. 1990. Global changes in gene expression related to antibiotic biosynthesis in *Streptomyces hygroscopicus*. Molecular biology of *Streptomyces*. *J. Cell Biol.* (suppl.) **14A:** 117.

Kumar, C.C., S.R. Mohan, C. Chang, and J.I. Garrels. 1990. Human smooth muscle myosin light chain-2 isoform is repressed in transformed cells. (In preparation.)

Latham, K.E., C. Chang, J.I. Garrels, and D. Solter. 1990. Analysis of preimplantion mouse development: Construction of a high resolution two-dimensional protein database. (In preparation.)

McLaughlin, C., J. Warner, and J.I. Garrels. 1990. The heat shock response in yeast. (In preparation.)

Warner, J., C. McLaughlin, and J.I. Garrels. 1990. Identification of proteins in yeast encoded by genes with introns. (In preparation.)

Warner, J., C. McLaughlin, and J.I. Garrels. 1990. *N*-acetylation of yeast proteins. (In preparation.)

GENETICS

Genetic techniques continue to be widely used at Cold Spring Harbor Laboratory, in particular by those who work with the ascomycete fungi, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. The Plant Genetics Group continues to use both maize and increasingly the model plant, *Arabidopsis*, for studies in molecular genetics. *Arabidopsis* is a pond weed that has a very short life cycle and is highly tractable to genetic methods. Whereas yeast has been viewed in recent years as the new *E. coli*, with the advantage that it is a eukaryote, *Arabidopsis* is becoming the yeast of the plant world. This is a simple system upon which genetic methods can readily be applied. The work of the Genetics Group, in yeast, plant, and animal cells, is described in this section.

EUKARYOTIC CELL CYCLE CONTROL

D. Beach J. Bischoff, T. Connolly, G. Cottarel, S. Davey, S. Dembski, U. Deuschle, B. Ducommun, J. LaMonica, K. Lundgren, T. Matsumoto, L. Molz

Our research has focused for the past five years on the regulation of the eukaryotic cell cycle. Initially, we studied the fission yeast exclusively, because of the appealing genetic accessibility of this organism. However, the broad conservation of the molecular elements that regulate the cell cycle, apparently in all eukaryotic cell types, allows us now to take an integrated approach that initially involves identification of regulators by the technique of yeast genetics and subsequently elaborating their role in both yeast and mammalian cells.

During the last year, the members of the "start-up" Beach laboratory largely moved on to other positions. Giulio Draetta assumed a group leader position at EMBL, Heidelberg, and Maureen McLeod became an assistant professor at Downstate Medical School, Brooklyn. Robert Booher and Leonardo Brizuela each obtained PhD degrees (SUNY Stony Brook) and moved on to postdoctoral positions at the University of California at San Francisco and Merck Sharpe and Dohme. We are joined by several new postdoctoral fellows, including Jim Bischoff, Uli Deuschle, Karen Lundgren, Tim Connolly, Giullaume Cottarel, Tomohiro Matsumoto, and Scott Davey.

Fission Yeast Cell Cycle

T. Connolly, G. Cottarel, U. Deuschle, B. Ducommun, K. Lundgren, T. Matsumoto, L. Molz, D. Beach

We have pursued the study of the *cdc2*$^+$ protein kinase and continued to identify genetic elements with which it interacts. *cdc2*$^+$ plays a central role in cell cycle regulation and is required both for the initiation of DNA replication and for entry into mitosis. Previously, we found that the products of both *suc1*$^+$ and *cdc13*$^+$ act as subunits of the protein kinase. *cdc13*$^+$ is of particular interest because it is required only at mitosis. The interaction between *cdc2*$^+$ and *cdc13*$^+$ thus defines a "G_2-specific" function of *cdc2*$^+$. Nucleotide sequencing of *cdc13*$^+$ revealed homology with a class of proteins known as mitotic cyclins. In fission yeast, we showed that the *cdc13*$^+$ gene product behaves as a cyclin. It accumulates progressively during interphase and is abruptly degraded at each mitosis (Fig. 1).

cdc2 and the *cdc13*$^+$-encoded cyclin progressively form a complex during interphase, but the enzyme is not catalytically activated until the onset of mitosis. Activation requires the function of the *cdc25*$^+$

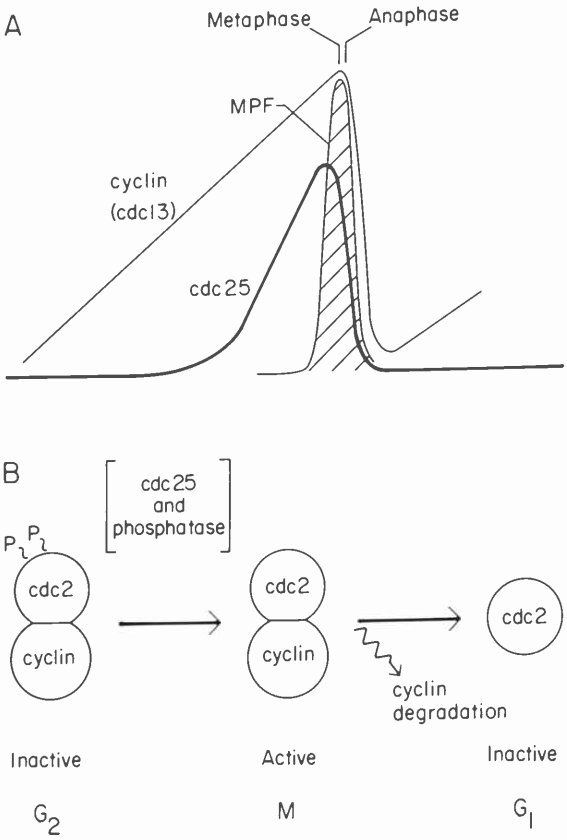

FIGURE 1 (*A*) Representation of the cell cycle oscillation of cyclin and the *cdc25* product, in comparison with the time of activation of the *cdc2*-cyclin complex (M-phase promoting factor, MPF). (*B*) Molecular representation of the *cdc2*-cyclin complex at the G_2/M transition.

gene product. Antibodies were raised against the *cdc25*$^+$ product, and immunoblotting revealed that the protein varied in abundance during the cell cycle. Thus, both the *cdc13*$^+$ and *cdc25*$^+$ products display cell cycle oscillation, but unlike the cyclin, *cdc25* is a dose-dependent activator of mitosis (Fig. 1A).

Although the accumulation of the cyclin and *cdc25* proteins is gradual, the *cdc2*/cyclin complex is activated very abruptly at the G_2/M transition. In higher eukaryotes, we found that at this stage of the cell cycle, the *cdc2* subunit of the protein kinase becomes dephosphorylated on tyrosine and threonine residues. This also occurs in fission yeast, in particular in a *cdc25*^{ts} strain after release from the restrictive temperature (Fig. 2). The *cdc25*$^+$ protein is probably not itself a phosphatase but may act to stimulate the relevant phosphatase (Fig. 1B). Direct evidence that dephosphorylation of *cdc2* contributes to activation of the enzyme has been obtained by treating *cdc2*/cyclin from either starfish or frog oocytes with potato acid phosphatase. The enzyme dephosphorylates *cdc2* and stimulates its histone kinase activity (in collaboration with L. Meijer, Roscoff, France, and C. Jessus, CNRS, Paris).

Cyclin/*cdc2* Localization at the Mitotic Centrosomes

B. Ducommun, G. Draetta, R. Booher, L. Brizuela, D. Beach

In a study of the localization of *cdc2* in mammalian cells (in collaboration with K. Riabowol, Cold Spring Harbor Laboratory, and D. Vandre, Southern Methodist University, Texas), we found that *cdc2* is predominantly a nuclear protein in interphase cells. Furthermore, microinjection of anti-*cdc2* antibodies caused cells to fail to enter mitosis. This provides the first direct evidence that *cdc2* is required for cell cycle progression in mammalian cells. During mitosis, *cdc2* becomes widely distributed throughout the cell as the nuclear membrane dissolves. At this time, extensive staining of the mitotic centrosomes becomes apparent. This suggests that *cdc2* is likely to be involved in nucleation of the mitotic spindle.

In a further study in fission yeast (in collaboration with C. Alpha and J. Hyams, UC, London), we found that both *cdc2* and its associated cyclin were localized in the spindle pole body, which is the yeast counterpart of the mitotic centrosome. The spindle-pole-body-associated cyclin showed an interesting property. Whereas the bulk of the cyclin is degraded relatively early in mitosis, the pole-body-localized protein is delayed in degradation. Thus, during spindle elongation, cyclin is observed exclusively in the pole bodies (Fig. 3).

cdc2 and Mammalian Oncoproteins

J. Bischoff, G. Draetta, L. Brizuela, D. Beach

During the course of studies of the *cdc2* protein kinase in mammalian cells, we have engaged in a series of collaborations with other laboratories at Cold Spring Harbor to investigate interconnections between *cdc2* and oncogene products.

We found that SV40 large T antigen is a particularly good substrate of *cdc2* (in collaboration with D. McVey, I. Mohr, D. Marshak, and Y. Gluzman). We took advantage of T synthesized in bacteria and were able to show that *cdc2* phosphorylates residue

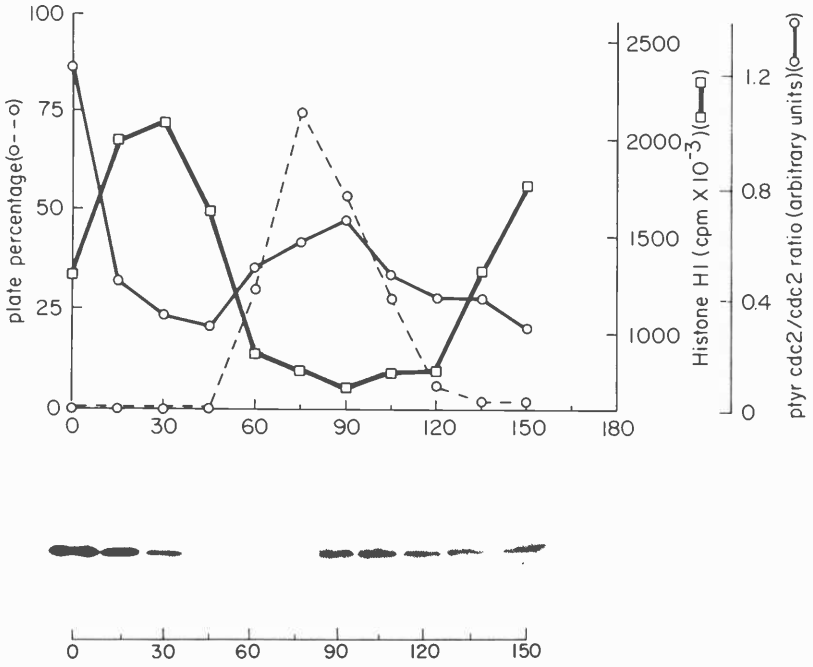

FIGURE 2 (*Top*) Activity of *cdc2*-cyclin (□) and level of phosphotyrosine in *cdc2* (heavy circles) in a *cdc25* mutant of fission yeast after release from a restrictive temperature. (*Bottom*) Immunoblot showing the phosphotyrosine content of *cdc2*.

Thr-124. This site is known to be phosphorylated in vivo and is essential for replication of SV40. Furthermore, protein that is mutant at this site is unable to support virus replication in cell-free lysates. In addition, bacterially derived T, unlike that purified from mammalian cells, cannot support DNA replication. We found that this deficit can be directly overcome by phosphorylation at Thr-124 by *cdc2*. This provides the first evidence that *cdc2* can act to control DNA replication in a higher eukaryote.

In a further study (in collaboration with A. Giordiano, R. Franza, and E. Harlow), we found that a 60-kD polypeptide, previously identified as an E1A-associated protein in adenovirus-infected cells, acts as a *cdc2* subunit in uninfected cells. p60 is not the mammalian cyclin B (*cdc13* homolog), and the *cdc2*-p60 complex shows properties different from those of cyclin B-*cdc2*. In particular, *cdc2*-p60 is most active during interphase, whereas cyclin B-*cdc2* is an M-phase enzyme. Although the respective roles of

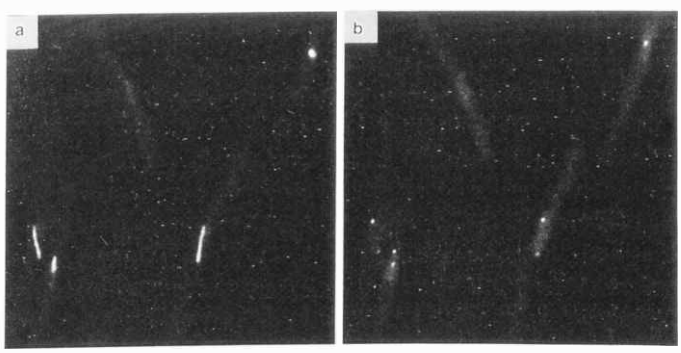

FIGURE 3 Double staining of fission yeast with anti-tubulin (*a*) and anti-cyclin (*b*). Each mitotic spindle can be seen to be spanned by regions of anti-cyclin staining. These are the spindle pole bodies.

these two complexes are not yet clear, these observations emphasize that *cdc2* is not a single protein kinase, but rather the catalytic subunit of a family of cell cycle regulatory protein kinases.

PUBLICATIONS

Booher, R. and D. Beach. 1989. Involvement of a type-1 phosphatase encoded by *bws1*$^+$ in fission yeast mitotic control. *Cell* **57:** 1009–1016.

Booher, R., C. Alpha, J. Hyams, and D. Beach. 1989. The fission yeast *cdc2/suc1/cdc13* protein kinase: Regulation of catalytic activity and nuclear localization. *Cell* **58:** 485–497.

Brizuela, L., G. Draetta, and D. Beach. 1989. Activation of the *cdc2* as a histone H1 kinase is associated with complex formation with the p62 subunit. *Proc. Natl. Acad. Sci.* **86:** 4362–4366.

Draetta, G. and D. Beach. 1989. The mammalian *cdc2* protein kinase: Mechanisms of regulation during the cell cycle. *J. Cell Sci.* (suppl.) **12:** 21–27.

Draetta, G., F. Luca, J. Westendorf, L. Brizuela, J. Ruderman, and D. Beach. 1989. *cdc2* protein kinase is complexed with both cyclin A and B: Evidence for proteolytic inactivation of MPF. *Cell* **56:** 829–838.

Giordano, A., P. Whyte, E. Harlow, R. Franza, D. Beach, and G. Draetta. 1989. A 60-kD *cdc2*-associated polypeptide complexes with the E1A proteins in adenovirus-infected cells. *Cell* **58:** 981–990.

McVey, D., L. Brizuela, I. Mohr, D. Marshak, Y. Gluzman, and D. Beach. 1989. Phosphorylation of large T antigen by *cdc2* stimulates SV40 DNA replication. *Nature* **341:** 503–507.

Meijer, L., D. Arion, L. Goldsteyn, T. Pines, L. Brizuela, T. Hunt, and D. Beach. 1989. Cyclin is a component of the sea urchin M-phase specific histone H1 kinase: Activation is associated with cyclin phosphorylation. *EMBO J.* **8:** 2275–2282.

Molz, L., R. Booher, P. Young, and D. Beach. 1989. *cdc2*$^+$ and the control of mitosis: Six interacting *mcs* elements. *Genetics* **122:** 773–782.

Morla, A., G. Draetta, D. Beach, and J. Wang. 1989. Reversible tyrosine phosphorylation of *cdc2* in the cell cycle: Dephosphorylation accompanies entry into mitosis. *Cell* **58:** 193–203.

Riabowol, K., G. Draetta, L. Brizuela, D. Vandre, and D. Beach. 1989. The *cdc2* kinase is a nuclear protein that is essential for mitosis in mammalian cells. *Cell* **57:** 393–401.

In Press, Submitted, and In Preparation

Alfa, C., B. Ducommun, D. Beach, and J. Hyams. 1990. Two step cyclin degradation in fission yeast: A subpopulation of cyclin associated with the mitotic spindle poles shows delayed anaphase destruction. (Submitted.)

Bischoff, J., G. Draetta, P. Freidman, D. Marshak, C. Prives, and D. Beach. 1990. Human p53 is a *cdc2* substrate: Phosphorylation in interphase by p60-*cdc2* and M-phase by cyclin B-*cdc2*. (Submitted.)

Ducommun, B. and D. Beach. 1990. A versatile microtiter assay for the universal *cdc2* cell cycle regulator. *Anal. Biochem.* (in press).

Ducommun, B., G. Draetta, P. Young, and D. Beach. 1990. Fission yeast *cdc25* is a cell-cycle-regulated protein. *Biochem. Biophys. Res. Commun.* (in press).

Ducommun, B., Y. Tollon, M. Gares, D. Beach, and M. Wright. 1990. Cell cycle regulation of *cdc2* in *Physarum polycephalum*. (Submitted.)

Jessus, C., B. Ducommun, and D. Beach. 1990. Direct activation of *cdc2* with phosphatase: Identification of $p13^{suc1}$-sensitive and insensitive steps. (Submitted.)

Meijer, L., P. Pondaven, and D. Beach. 1990. Activation of M-phase-specific histone H1 kinase by modification of the phosphorylation if its $p34^{cdc2}$ and cyclin components. *Genes Dev.* **4:** 9–17.

PRE-mRNA SPLICING AND snRNA SYNTHESIS IN *SCHIZOSACCHAROMYCES POMBE*

D. Frendewey D. Kim M. Gillespie
J. Potashkin I. Barta
A. Serrano

We pursue two related lines of research. Our primary interest is the splicing of mRNA precursors (pre-mRNAs). We have also recently begun to investigate small nuclear RNA (snRNA) synthesis. We are studying both of these processes in the fission yeast *Schizosaccharomyces pombe*. These two areas of research are related, in general, by the fact that the snRNAs known as U1, U2, U4, U5, and U6 are required for pre-mRNA splicing and are components of the spliceosome, the large ribonucleoprotein structure in which pre-mRNA splicing occurs. In fission yeast, this relationship exhibits an additional peculiar twist: The gene for the U6 RNA of *S. pombe* is interrupted by an intron whose structure resembles that of a pre-mRNA intron. Therefore, in fission yeast, it appears that U6 RNA, in addition to being an essential component of the pre-mRNA splicing machinery, might itself be produced from a precursor that is a *substrate* of the spliceosome.

Our initial efforts to examine these phenomena

have been genetic. To identify genes that are required for pre-mRNA splicing or snRNA synthesis, we have isolated temperature-sensitive (*ts*$^-$) mutants that are defective in these processes. Analysis of the mutants has generated the three main projects that are currently under investigation in our laboratory.

U6 RNA Synthesis

J. Potashkin, I. Barta, M. Gillespie, D. Frendewey

In our 1987 and 1988 Annual Reports, we described the isolation and initial characterization of three *ts*$^-$ pre-mRNA splicing mutants: *prp1*, *prp2*, and *prp3*. Indicative of the complete (*prp1* and *prp2*) or partial (*prp3*) block in pre-mRNA splicing, these mutants accumulate unspliced pre-mRNAs at the nonpermissive temperature (37°C). As part of our initial analysis of the *prp*$^-$ mutants, we observed that after a short period at 37°C, each had a reduced U6 RNA content compared to the wild type. The levels of the other snRNAs were normal. At the time, we had no explanation for these results. However, the discovery of an intron in the *S. pombe* U6 gene (Tani and Ohshima, *Nature 337:* 87 [1989]) suggested that the reduction in U6 RNA in the *prp*$^-$ mutants might result from inefficient splicing of the U6 precursor (U6 pre-RNA).

In 1989, we confirmed the lower U6 RNA concentration in the *prp*$^-$ mutants by a more sensitive assay and went on to show that the splicing of the U6 pre-RNA was indeed impaired in *prp1*, *prp2*, and *prp3*. The severity of the U6 splicing defects exhibited by the three mutants paralleled that seen for pre-mRNA, suggesting that the U6 pre-RNA might be spliced by the same mechanism and require some of the same gene products as pre-mRNAs. We would like to investigate these questions further in the future.

The presence of a pre-mRNA-type intron in the *S. pombe* U6 gene raises a number of interesting questions with regard to splicing and snRNA synthesis. The *S. pombe* U6 gene is the only example of an interrupted U6 or other snRNA gene. How did this intron arise and why is it maintained? The U6 RNA from all sources so far investigated is transcribed by RNA polymerase III, and this is also expected to be true for *S. pombe*. Yet the intron in the *S. pombe* U6 pre-RNA is the type found only in RNA polymerase II transcripts (e.g., pre-mRNAs). Is the U6 pre-RNA spliced by the pre-mRNA splicing machinery or does it use its own unique mechanism? It has been proposed (Brow and Guthrie, *Nature 337:* 14 [1989]) that the *S. pombe* U6 intron was inserted as the result of a mishap during the splicing of a pre-mRNA. This hypothesis predicts that the position of the intron reflects a functional domain in the U6 RNA.

To begin to address these questions, we initiated a search for intron-containing U6 genes in other *Schizosaccharomyces* strains and several more distantly related species of yeast. The primary goal of this project was to investigate the uniqueness of the *S. pombe* U6 intron. In addition, if introns were found in the other strains, their sequences might reveal conserved features peculiar to U6 introns. Our approach was to use the polymerase chain reaction (PCR) to amplify a highly conserved portion of the U6 genes from the various yeast strains. The intron resides in this region of the *S. pombe* U6 gene. The sizes of the PCR products would be the first indications of the presence or absence of introns. Subsequent sequencing would define the position and structure of any introns that were found.

The results of the amplifications showed that the U6 genes in all of the *Schizosaccharomyces* strains were interrupted by introns of approximately the same size as that found in *S. pombe*. The U6 genes from the other yeast species did not contain introns within the fragment that was amplified. The PCR products from the *Schizosaccharomyces* strains were cloned and sequenced. The sequences of the introns are shown in Figure 1. All the introns were found in precisely the same position and were approximately identical in size (50–52 bp). As expected, the positions displaying the greatest sequence conservation were at the 5′ and 3′ splice sites and the branchpoint consensus. However, an unexpected region of homology was found immediately upstream of the presumptive branchpoint. When 11 bases from the corresponding region of the *S. pombe* intron were compared with all available *S. pombe* sequences in the databases, a surprising similarity was found to the B-box promoter elements of the 7SL RNA and several tRNAs (starred bases in Fig. 1). The B box is the 3′ half of the bipartite internal promoter required for transcription of 7SL RNA and tRNA by RNA polymerase III.

This result was surprising by comparison with vertebrate U6 genes, whose transcription by RNA polymerase III does not require internal sequences. Similarity to known promoter elements is, of course, not proof of function. However, the hypothesis that

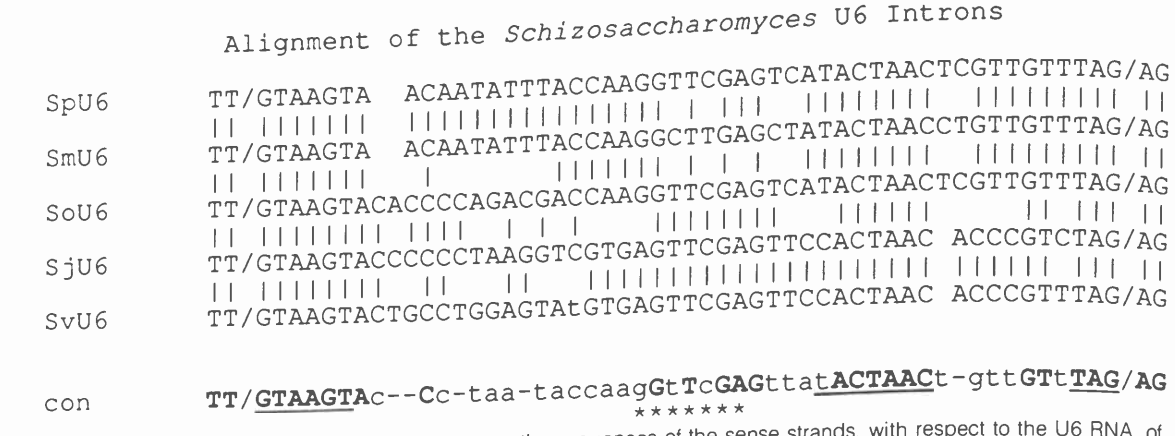

FIGURE 1 U6 gene intron sequences. Shown are the sequences of the sense strands, with respect to the U6 RNA, of the introns and two bases in the flanking exons for the U6 genes of five *Schizosaccharomyces* strains. The strains are indicated by abbreviations to the left of the sequences: (Sp) *S. pombe*; (Sm) *S. malidevorans*; (So) *S. octosporus*; (Sj) *S. japonicus* var. *japonicus*; (Sv) *S. japonicus* var. *versatilis*. The splice junctions are identified by slashes. Gaps were introduced to accommodate the best alignment of all the sequences. Identities between any two adjacent sequences are denoted by vertical bars. A consensus sequence (con) is given below the aligned sequences, with bold uppercase letters designating positions of absolute conservation. The 5′ and 3′ splice site and branchpoint consensus sequences are underlined, and the bases that match the B-box consensus are starred.

the conserved internal sequence in the *Schizosaccharomyces* U6 genes is a B-box promoter element makes several predictions. First, we would expect to find a corresponding A box in the fission yeast U6 genes. The first exon of the *S. pombe* U6 gene contains a stretch of 12 bases that is very similar in sequence and position, relative to the transcription initiation site, to those of the A box in the *S. pombe* 7SL RNA gene. Second, deletion of the intron from the *S. pombe* U6 gene should drastically reduce or abolish transcription of U6 RNA. Thus, the intron may be necessary for expression of U6 RNA, which may explain why this unusual gene structure has been maintained in the fission yeast. Third, the conserved sequence in the *S. pombe* U6 intron should bind factors that recognize the B box, and therefore the *S. pombe* U6 gene should compete for the transcription of tRNA genes in in vitro assays. We have initiated experiments to test these predictions and to define the sequences required for U6 RNA transcription in *S. pombe*.

Pre-mRNA Splicing Mutants

J. Potashkin, D. Kim, D. Frendewey

The isolation of the *prp1*, *prp2*, and *prp3* mutants identified three genes that are required for pre-mRNA splicing. During the past year, we initiated a project to clone the *prp1*$^+$ and *prp2*$^+$ genes. Since the *prp*$^-$ mutants are *ts*$^-$, our cloning strategy was to transform *prp1* and *prp2* with an *S. pombe* genomic library and select transformants that can grow at 37°C (*ts*$^+$). If the *ts*$^+$ phenotype is dependent on the plasmid, then the transformant is likely to contain the *prp1*$^+$ or *prp2*$^+$ genes or a gene that can suppress the *prp*$^-$ mutation.

We have obtained *ts*$^+$ transformants for both *prp1* and *prp2*. Plasmid has been isolated from these transformants and cloned in *Escherichia coli*. We are now testing the plasmids from the bacterial clones to see if they can cure the *ts*$^-$ phenotype in *prp1* and *prp2*, thus confirming that we have cloned either the *prp1*$^+$ and *prp2*$^+$ genes or *prp*$^-$ suppressors.

In addition to our attempts to clone one or more *prp*$^+$ genes, we have recently expanded our bank of *ts*$^-$ mutants and have initiated a screen for new *prp*$^-$ mutants. In our previous screen, we used a Northern assay to identify mutants that accumulate pre-mRNA at the nonpermissive temperature. Our new search will take advantage of our observation that unspliced U6 pre-RNA levels are elevated in the *prp*$^-$ mutants. We will again use a Northern assay, but this time we will be looking for mutants that accumulate U6 pre-RNA at 37°C. This screening method is more sensitive (because of the abundance of U6 RNA compared to mRNA), faster, and easier. Any mutants identified will also be checked for pre-mRNA splicing defects. An important question in *S. pombe* splicing is whether the U6 pre-RNA is spliced by the same mechanism as pre-mRNAs or

whether it requires its own specific factors. The U6 screen might allow us to answer this question by identifying mutations that affect the splicing of the U6 pre-RNA but not pre-mRNA.

snm1: An snRNA Maintenance Mutant

J. Potashkin, A. Serrano, D. Frendewey

In the 1988 Annual Report, we described the characterization of an unusual mutant, which at that time we called *prp4*. This ts^- mutant maintains reduced steady-state levels of U1, U2, U4, U5, and U6 snRNAs and K RNA, the RNA subunit of the tRNA processing enzyme RNase P, compared to the wild type. In addition, the mutant produces aberrantly large U2, U4, and U6 transcripts at the nonpermissive temperature. We observe a slight accumulation of pre-mRNA and a significant reduction in pre-tRNA processing in the mutant at 37°C. Because these RNA processing defects are probably secondary effects of the reduction in snRNA content, we have renamed the mutant *snm1* to indicate a defect in *sn*RNA *m*aintenance.

The initial analysis of the *snm1* mutant phenotype has been completed in the past year. This included experiments showing that the aberrantly large U2 and U4 transcripts are extended at their 3′ ends, as we had earlier expected. We also established that the larger U6 RNA that accumulates in *snm1* is not extended at its 3′ end but is the unspliced U6 pre-RNA. We have yet to determine if the reduction in U6 RNA in *snm1* is solely the result of a splicing defect or might be caused by a lower transcription efficiency or instability. We are continuing our efforts to better define the primary defect in *snm1*; i.e., does the *snm1* mutation manifest its effect at the level of transcription, processing, or stability of the snRNAs?

We have also begun a project to clone the $snm1^+$ gene by the same strategy described above for the $prp1^+$ and $prp2^+$ genes. As with *prp1* and *prp2*, we have obtained ts^+ transformants of *snm1*, isolated plasmid from the transformants, and cloned it in *E. coli*. We are now testing the bacterial clones for their ability to convert *snm1* to ts^+ upon transformation. Since *snm1* is the first mutant isolated that affects snRNA metabolism, we are confident that its analysis and the characterization of the $snm1^+$ gene will enhance our understanding of the synthesis and maintenance of an important class of RNAs.

PUBLICATIONS

Potashkin, J. and D. Frendewey. 1989. Splicing of the U6 RNA precursor is impaired in fission yeast pre-mRNA splicing mutants. *Nucleic Acids Res.* **17:** 7821–7831.

Potashkin, J., R. Li, and D. Frendewey. 1989. Pre-mRNA splicing mutants of *Schizosaccharomyces pombe*. *EMBO J.* **8:** 551–559.

In Press, Submitted, and In Preparation

Potashkin, J. and D. Frendewey. 1990. A mutation in a single gene of *Schizosaccharomyces pombe* affects the expression of several snRNAs and causes defects in RNA processing. *EMBO J.* (in press).

PLANT GENETICS

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Robertson's Mutator

V. Sundaresan, J. Brown, J. Colasanti, A. Doseff, Z.-Y. Zhao

Our main area of investigation has been the maize transposable element system called Robertson's *Mutator* (*Mu*). This system is characterized by an exceptionally high mutation rate due to the transposition of a family of elements called the *Mu* transposons, of which a 1.4-kb element called *Mu1* is the best-characterized member. At present, little is known about the protein factors that catalyze *Mu* transposition or the genes encoding them. Mutator

lines of corn carry many copies of actively transposing *Mu* elements, but they can switch to an inactive state in which the *Mu* elements are present but not transposing. Our studies have focused on the mechanism and regulation of transposition of the *Mu* elements. A second area of interest has been the regulation of plant growth. In this context, we are beginning to isolate genes for plant growth hormones, as well as genes that regulate cell division in plants.

Protein-binding Sites on the *Mu* Terminal Inverted Repeat

Z.-Y. Zhao, V. Sundaresan

To identify factors that regulate *Mu* transposition activity, we have been characterizing protein-binding sites on the terminus of the *Mu1* element. Three such sites have been identified in mobility-shift assays using labeled *Mu1* probes and nuclear protein from *Mu*-active and *Mu*-inactive plants (see Fig. 1). The binding activity for sites I and III is detected in nuclear protein from both *Mu*-active and *Mu*-inactive lines, whereas the binding activity for site II is found only in the *Mu*-active line. Thus, the protein that binds to site II is a candidate for a *Mu* transposase. The DNA sequences for sites I and II have been determined more precisely by DNase I footprinting and confirmed by mobility-shift experiments using synthetic oligonucleotides. The oligonucleotide CGGGAACGGTAA (site I) binds to protein from both *Mu*-active and *Mu*-inactive plants, whereas the oligonucleotide CGGCGTCT (site II) binds only to protein from *Mu*-active plants. Furthermore, we determined that site I is in fact a duplication of a hexamer sequence CGGG/TAA, which exhibits the same binding activity and is the unit binding site. The identification of these binding sites is particularly interesting in view of the discovery that there is an outward promoter 16 bp from the end of *Mu1* (R. Martienssen, see below). When the *Mu* system becomes inactive, it results in transcription of downstream sequences from this promoter, and suppression of mutant phenotypes can occur. When *Mu* is active, there is no detectable transcription from this promoter. Currently, our model is that a protein binding to site I is a positive regulator of transcription from this promoter. When *Mu* is active, the protein binding to site II (the putative transposase) interferes with this activation of the *Mu1* promoter by the site I protein (the two sites are only 5 bp apart). We have initiated transformation experiments to verify this model by using the particle gun system (Klein et al., *Nature 327:* 70 [1987]).

Changes in State of a *Mu1* Insertion at the *bronze1* Locus

A. Doseff, V. Sundaresan

McClintock described a "change in state" of a mutable locus as a change at the locus that alters the pattern of expression of the gene in a heritable manner (*Carnegie Inst. of Wash. Yearbook 66:* 20 [1968]). Examples of such changes in state have been extensive in both the *Spm* and *Ac-Ds* transposon systems. We have now found examples of changes in state in the *Mu* system in our studies of a *Mu1* insertion at the *bronze1* (*bz1*) locus. The allele that we are studying, *bzMum9*, was isolated by D. Robertson (Iowa State University) and is characterized by a pattern of small purple spots on a bronze background. Southern blotting and DNA sequence analysis show that a *Mu1* element is inserted into the gene at the intron-exon junction (J. Colasanti, Weiss, and V.

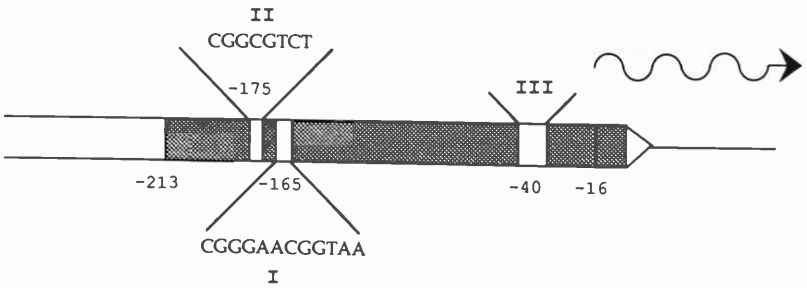

FIGURE 1 Location of the protein-binding sites I, II, and III, and the transcript initiation site on the 213-bp terminal inverted repeat of *Mu1*.

Sundaresan, unpubl.; see Fig. 2). Three heritable changes in state of *bzMum9* (*cs1*, *cs2*, and *cs3*) have been isolated, and their phenotypes and molecular structure are summarized in Figure 2.

1. *cs1* consists of an extremely high density of spots so that the kernel appears solidly colored until examined closely. We have found that this change in state carries a new 1.7-kb insertion at the upstream end of the *Mu1* element. The nature of this new insertion is as yet unknown, but it does not appear to be a *Mu* element. At present, we have no model to account for the 10–100-fold increase in excision frequency that is apparently due to this insertion.
2. *cs2* is a pseudorevertant, i.e., the kernel is a solid purple color, but the *Mu1* element is still at the locus. Interestingly, detailed Southern analysis reveals a 0.65-kb deletion of the downstream segment of *Mu1*. We propose that this deletion has created a new splice site downstream from the normal splice site, and the new splice site allows the *Mu1* element to be spliced out of the mRNA, resulting in *bz* expression. We will verify this model by analyzing the RNA from *cs2*.
3. *cs3* exhibits a pattern in which the spots are very dense on the crown of the kernel, and sparse otherwise (an "*Rnavajo*"-type pattern). So far, we have not found any change in the DNA of *cs3* by Southern blotting, but our experiments would not detect very small insertions or deletions.

Coordinate Regulation of Activity of Different *Mu* Elements

J. Brown, V. Sundaresan

The *Mu* transposon system can switch from an active state to an inactive state during propagation of *Mu* stocks, and this switch has been correlated with hypermethylation of the *Mu1* elements (Chandler and Walbot, *Proc. Natl. Acad. Sci. 83:* 1761 [1986]; Bennetzen, *Mol. Gen. Genet. 208:* 45 [1987]). We have questioned whether the switch to the inactive state affects all the *Mu* elements at the same time or whether there is a progression of inactivation. For this purpose, we utilized the *bzMum9* allele described earlier, along with a second *Mu*-induced mutation at the *waxy* locus, *wxMum5*. The kernel phenotype of *wxMum5* is that of small sectors of normal starch on a waxy background. This mutation has been found to be due to the insertion of a *Mu8* element into an exon of the *wx* gene. *Mu8* is a member of the *Mu* family of transposons and has the same termini as *Mu1*, but nonhomologous internal sequences. We crossed these two alleles together and then outcrossed them to recessive testers; the

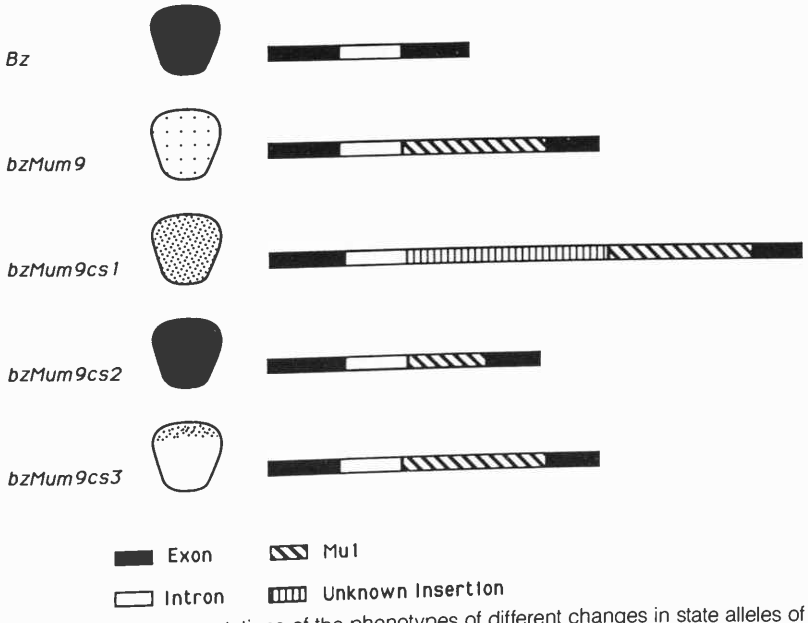

FIGURE 2 Representations of the phenotypes of different changes in state alleles of *bzMum9* and the corresponding DNA rearrangements.

bzMum9 wxMum5 recombinants are being scored for loss of mutability in the kernel. In the crosses examined so far, there is an excellent correlation between loss of mutability at *bz* and loss of mutability at *wx*, suggesting that the switch from active to inactive *Mu* occurs simultaneously for the different *Mu* elements.

Synthesis of Cytosine Methylated DNAs In Vitro

J. Colasanti, V. Sundaresan

For our studies of the effects of transposon activity on DNA methylation, it was necessary to devise a simple method to synthesize cytosine-methylated DNA. We have found that this can be done by polymerase chain reaction (PCR) in which 5-methyl-dCTP is substituted for dCTP. The reaction conditions are much the same as those for regular PCR, except that the extension time had to be increased to 5 minutes for a 1-kb fragment as the enzyme operates less efficiently with the methylated nucleotide, and the yields decrease significantly for amplification of larger fragments. Using this method, we could demonstrate that cytosine-methylated DNA is completely resistant to the restriction enzyme *Hin*fI at the concentrations normally used to cut genomic DNA, i.e., 10–100-fold enzyme excess. This would account for the observed resistance to *Hin*fI digestion of the *Mu1* elements in inactive *Mu* lines (Chandler and Walbot, *Proc. Natl. Acad. Sci. 83:* 1761 [1986]). We find that *Hin*fI at 15,000-fold excess will completely digest cytosine-methylated DNA, but use of such a vast excess of enzyme is impractical in most genomic digests.

A Suppressible Allele of the *d1* Locus of Maize

J. Colasanti, V. Sundaresan

Maize plants that are homozygous for the *dwarf 1* (*d1*) mutation are 10–40% the height of wild-type siblings, have broad dark green leaves, and undergo aberrant floral development in which vestiges of the male flowers appear in the female flower. Biochemical studies have shown that these pleiotropic effects are the result of a deficiency of the active form of the growth regulator gibberellic acid, GA_1. The conversion of the inactive form of gibberellic acid (GA_{20}) to the active form is catalyzed by an enzyme encoded by the *d1* gene.

We have been attempting to isolate the *d1* gene by transposon tagging, using the transposable element *Mu*. Initially, there were two dwarf mutants derived from the self of a Mutator line crossed with a non-Mutator line in a screen by Michael Freeling (University of California, Berkeley). One of the mutants exhibited the expected ratio of dwarf to normal plant for a recessive allele. After several outcrosses, however, restriction-fragment-length polymorphism (RFLP) analysis with *Mu*-specific probes showed that no particular element segregated with the dwarf phenotype. The other *dwarf* mutant segregated at non-Mendelian ratios upon selfing, with mutant plants appearing less frequently with each subsequent generation, until it finally disappeared.

Recent evidence with another *Mu*-induced mutation provided a precedent for a form of allelic suppression, governed by the *Mu* element, that may explain the observed frequencies and loss of the mutation. In the case of the *hcf106* mutation, the activity of this gene is correlated with the state of activity of the *Mu* element inserted within the gene (R. Martienssen, see below). When *Mu* is active, the *hcf106* gene expresses the mutant phenotype, and when *Mu* is inactive, the *hcf106* lesion is suppressed and the plants appear to be wild type. To determine if a similar phenomenon was occurring with the disappearing *dwarf* line, DNA from later generations was analyzed, and the *Mu1* elements were found to be methylated, suggesting that *Mu* activity was lost (Chandler and Walbot, *Proc. Natl. Acad. Sci. 83:* 1761 [1986]; Bennetzen, *Mol. Gen. Genet. 200:* 45 [1987]). Plants from this inactive line were crossed to active *Mu* plants (*Mu*-active plants crossed with *Mu*-inactive plants give *Mu*-active progeny), and the resulting progeny were selfed to uncover recessive alleles. One line segregated *dwarf* plants at a ratio of 3:1, suggesting that the mutation has been rescued by restoring *Mu* activity.

Because the expression of the mutation depends on the state of *Mu* activity, it is likely that the *dwarf* mutation is indeed caused by insertion of the *Mu* element. We are now outcrossing the rescued *dwarf* plants to follow the cosegregation of the *Mu* elements with the mutant phenotype and to ultimately clone the mutant gene.

Cloning of Plant Cell-division Genes

J. Colasanti, V. Sundaresan

There are several important differences in the regulation of growth and differentiation between plants and animals. In plants, for example, the state of terminal differentiation is not as clearly defined, and in several plant species, an entire new plant can be regenerated from a fully differentiated somatic cell. Furthermore, plant cells in tissue culture can be induced to form shoots, roots, or both, merely by varying the balance of certain plant growth hormones (auxins and cytokinins). It is therefore of interest to study such basic processes as regulation of cell division, which have received much attention in animal systems but have not yet been studied in plants. To this end, we have begun to clone the plant homologs of the cell-division-cycle genes. The first such gene we have cloned is the gene encoding $p34^{cdc2}$, a key regulatory protein of the cell cycle in both animals and yeasts. Using oligonucleotides to highly conserved regions of this gene, we were able to isolate a partial cDNA clone from maize, and a partial genomic clone from *Arabidopsis* (Fig. 3). There is one copy of the *cdc2* gene in the *Arabidopsis* genome by hybridization, and possibly up to five copies in the maize genome, although some of these copies might be pseudogenes. In maize, we have detected a 1.4-kb mRNA for *cdc2* that is highly abundant in the apical meristem, where the most cell division occurs, and in low levels in differentiated leaf. We are currently attempting to isolate a full-length cDNA clone for maize *cdc2*. Several new cDNA clones have been recently isolated by screening a maize seedling cDNA library (a gift from A. Barkan, University of California, Berkeley), and these are being characterized.

Maize	TNETIVLKKIRLEQEDESVPSTAIREISLLKE
Arabidopsis	.....A.....G.....
Human	.GQVVAM.....S.E.G.....
S. pombe	SGRIVAM.....D.S.G.....

FIGURE 3 Comparison of partial amino acid sequence data derived from the cloned maize and *Arabidopsis cdc2* homologs with that of human and *S. pombe cdc2*, showing a highly conserved domain. The dots represent identity with the maize sequence.

Role of the *hcf106* Gene Product in Thylakoid Biogenesis

R. Martienssen, A. Jahrsdoerfer [in collaboration with A. Barkan, University of California, Berkeley]

The nonphotosynthetic mutant of maize, *hcf106*, contains a recessive nuclear mutation that prevents the normal assembly of photosynthetic electron transport complexes and causes aberrant thylakoid membrane organization. Figure 4 shows electron micrographs of mature chloroplasts from wild-type and mutant mesophyll cells. Mutant thylakoids have lost the lateral heterogeneity characteristic of wild-type plastids, comprising alternating stromal (unstacked) and granal (stacked) lamellae. Instead, they frequently form circular structures such as the one shown in Figure 4. Furthermore, photosystems I and II, and the cytochrome f/b_6 complex, fail to accumulate to more than 5–10% of wild-type levels, whereas the ATPase and light harvesting complexes are unaffected. Consequent reductions in chloroplast pigment levels result in pale-green leaves. These emit an increased fluorescence when illuminated with ultraviolet light (thus, the designation *hcf* for *h*igh *c*hlorophyll *f*luorescence), as they are unable to channel harvested light energy into photosynthetic electron transport. Homozygous mutant seedlings die approximately 3 weeks after germination, when seed reserves are exhausted. Heterozygous plants survive to maturity, enabling propagation of the mutation.

hcf106 arose in a Robertson's *mutator* line and showed signs of somatic instability (namely, dark green sectors), suggesting that it was caused by the insertion of a *Mu* transposable element. Heterozygous plants were outcrossed in successive generations to the inbred line B73, resulting in segregation of the 20–50 *Mu1* elements found in these lines. A 3.7-kb *Sst*I fragment homologous to *Mu1* was found by Southern analysis in every mutant seedling derived from these outcrosses. This fragment was cloned into a phage vector and was subsequently identified as part of the *hcf106* gene (Martienssen et al. 1989). A 1.2-kb transcript that hybridized to the cloned locus was identified on Northern blots. This transcript was regulated by *mutator* activity, suggesting that it encoded the *hcf106* gene product (see below).

Thylakoid biogenesis in maize leaves occurs in a well-defined developmental sequence. Proplastids, which are found in younger, dividing cells at the base of the leaf, have semicrystalline prolamellar bodies and a poorly developed thylakoid membrane system.

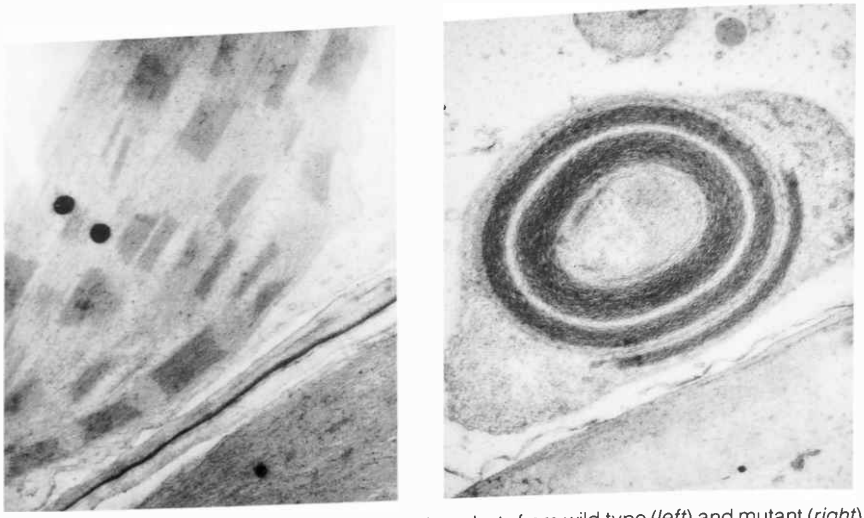

FIGURE 4 Electron micrographs of mature chloroplasts from wild-type (*left*) and mutant (*right*) mesophyll cells from 10-day-old seedling leaves. Magnification, 12,000×.

More mature plastids are found in older, elongating cells from successive leaf sections from leaf base to leaf tip. These contain thylakoids that become progressively more elaborate, while the prolamellar bodies rapidly disappear. Northern analysis has shown that the *hcf106* transcript is found primarily in the base of the leaf, consistent with a role in the early stages of thylakoid assembly and organization. Lower amounts (three- to fivefold) are found at the tip of the leaf, and still lower (but detectable) levels are found in the root. Thylakoid elaboration can also be induced in etioplasts of dark-grown plants by exposure to the light. A three- to fivefold induction of mRNA was observed following a 24-hour dark-light transition, reflecting this morphological change.

The RACE protocol for the rapid amplification of cDNA ends was used to isolate several full-length cDNA clones, and these are being sequenced. cDNA clones made by conventional methods were also obtained from A. Barkan (University of California, Berkeley), and these are also being sequenced in case of polymerase chain reaction (PCR) error. These clones include a second, distantly related sequence that appears to be the product of a low-stringency cognate gene of unknown function.

Somatic Excision of *Mu*

R. Martienssen, A. Jahrsdoerfer

The *Mu1* element at the *hcf106* locus is located in the 5′-untranslated region, close to the initiation of transcription (see below). The first 5 bp of the 9-bp target site duplication (ATCTCCAAC) are the same as the last 5 bp of *Mu1* (ATCTC). DNA from two *hcf106* revertant sectors was cloned from sectored leaves following PCR amplification using primers flanking the site of *Mu1* insertion. The sequence of the two revertant alleles and the sequence of the wild-type allele from the inbred line B73 are compared to the mutant sequence in Figure 5. The first excision precisely removed a single copy of the target-site duplication. This was not a contaminating wild-type product, since a 6-bp duplication (to the left of the target site in Fig. 5) was present in the mutant and revertant alleles, but absent from B73. The second excision left 5 bp of one repeat at the locus. This is the region of the *Mu1* terminus homologous to the target site. Thus, naively, both products might have been generated by recombination between the target site and either its duplication or the homologous sequence at the end of *Mu1*. Further analysis of other sectored leaves will be required to determine if this is a general pattern of *Mu* excision.

Suppression of *Mu*-induced Mutations

R. Martienssen [in collaboration with A. Barkan, University of California, Berkeley]

The *hcf106* mutation is unusual in being *Mu*-suppressible: When *Mu* activity is lost, homozygotes adopt a wild-type phenotype. The loss of *Mu* activ-

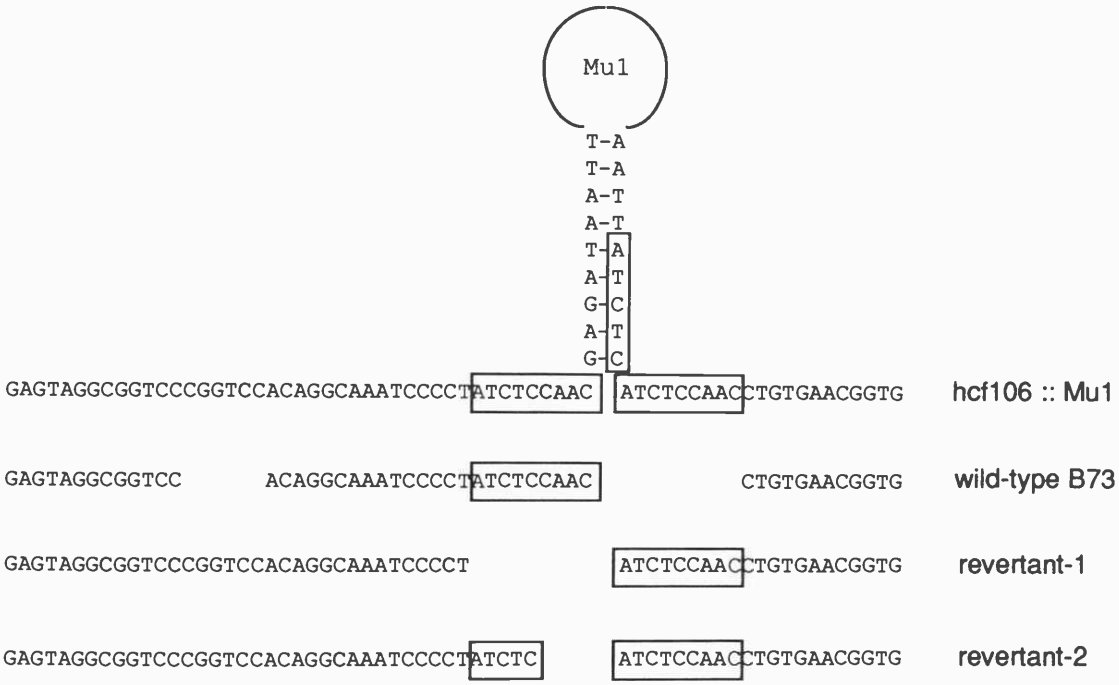

FIGURE 5 Nucleotide sequence of mutant, wild-type (from the inbred line B73), and revertant alleles of *hcf106*. The target site duplication is boxed. Only the first 9 bp of the 200-bp terminal inverted repeats are shown. *Mu1* is 1350 bp long.

ity is reversible: When *Mu*-off plants are crossed to *Mu*-on plants, most of the progeny regain *Mu* activity, but when *Mu*-off plants are selfed, no such "reactivation" occurs. Suppression of the *hcf106* mutant phenotype is also reversible. When suppressed *hcf106* homozygotes (phenotypically wild type) were crossed to *Mu*-on heterozygotes, the mutant phenotype segregated 1:1 in the resulting progeny. In contrast, when suppressed plants were selfed, no mutant progeny were observed.

Mu-off plants typically have methylated *Mu1* elements, whereas *Mu*-on plants have elements that are unmethylated at the *Hin*fI sites in their terminal inverted repeats. Consistent with this observation, all of the mutant *hcf106* seedlings examined had unmethylated elements, whereas 95% of the suppressed plants had methylated elements. An exceptional suppressed plant had unmethylated elements (including the one at the *hcf106* locus), but appeared to be *Mu*-off by other criteria (i.e., failure to detect new *Mu1* transpositions in the progeny). Suppression of the mutant phenotype in the progeny of this exceptional plant was still reversible, as shown by reactivation crosses. Suppression in this case may be due to the segregation of a *trans*-acting factor (such as transposase). Alternatively, suppression may have occurred prior to the onset of DNA methylation.

Suppression of the mutant phenotype was accompanied by accumulation of mRNA transcripts from the *hcf106* locus, despite the insertion of a *Mu1* element into the untranslated leader. In contrast, mutant seedlings had no detectable transcript (Martienssen et al. 1989). The 5′ end of the suppressed transcript was mapped by S1 and primer extension and was shown to be heterogeneous, with the longest transcript initiating at a site 15–16 bp inside the *Mu1* element (Fig. 6) (A. Barkan and R. Martienssen, in prep.). This suggests that loss of *Mu* activity results in activation (or derepression) of a promoter in the *Mu1* terminal inverted repeat. This may be associated with the binding of *trans*-acting factors to this region (Z.-Y. Zhao and V. Sundaresan, pers. comm.).

In 95% of suppressed plants, suppression of the mutant phenotype was associated with DNA methylation of *Mu1* elements. In these plants, DNA flanking the *Mu1* element at the *hcf106* locus was also affected by loss of *Mu* activity (Fig. 6). *Pvu*II and *Pst*I sites in the *hcf106* promoter were unmethylated in the wild-type and mutant alleles, but methylated in the suppressed allele. A *Pvu*I site downstream from *Mu* was partially methylated in suppressed plants as well. This may reflect a requirement for promoter competition in the mutant phenotype. When the

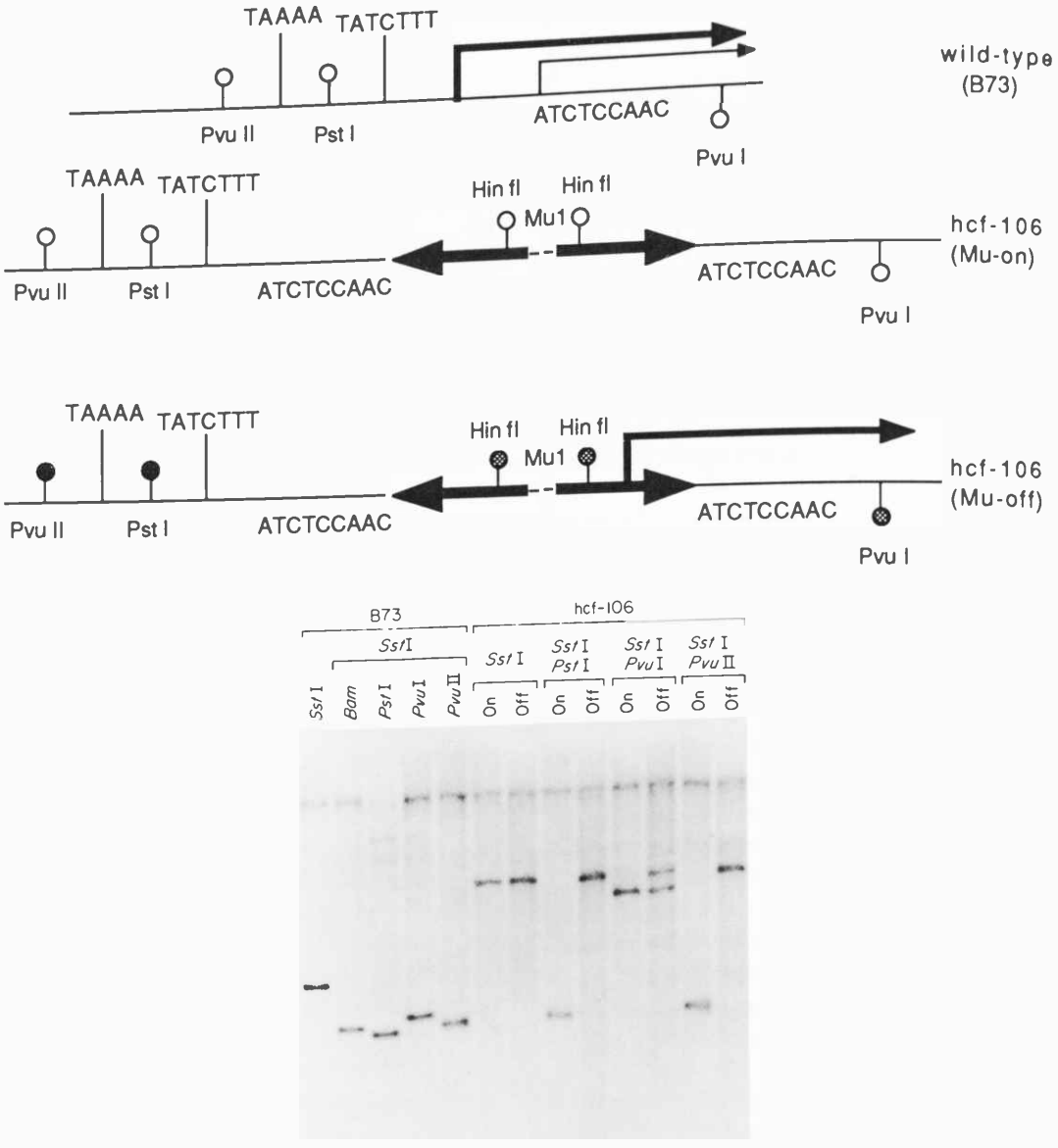

FIGURE 6 Promoter region of the *hcf106* gene from mutant, wild-type, and suppressed alleles. Arrows indicate transcription start sites. (●) Modified restriction sites; (O) unmodified restriction sites; (*crosshatched circles*) partially modified restriction sites. The Southern blot shows DNA from wild-type (B73), homozygous mutant (On), and homozygous suppressed (Off) seedlings digested with restriction enzymes and hybridized to a probe from the upstream flanking region of the *hcf106* gene. The faintly hybridizing upper band is a reduced stringency homolog unlinked to *hcf106*.

wild-type promoter is methylated, and inactive, the *Mu1* promoter becomes active, so restoring transcript levels. Transient expression experiments using the particle gun will be used to test this model.

In some families, mutant plants were observed that had large, phenotypically wild-type sectors. DNA isolated from these sectors contained modified *Mu1* elements, including the one at the *hcf106* locus (Martienssen et al. 1990). The pattern of stripes suggested that clonal switching events associated

with DNA modification had occurred in cells near the tip of the apical meristem, so that more and larger sectors were observed in successive leaves until, in the upper leaves, the entire plant was phenotypically wild type. This resulted in a gradient of transposon inactivity (and methylation) up the plant and may account for reciprocal effects in the transmission of *Mu* activity (Brown and Sundaresan, *CSHL Annual Report*, p.172 [1988]). This is because the male and female flowers are formed from different parts of the shoot apical meristem, and so have different proportions of active and inactive cells.

Molecular and Genetic Analysis of the Maize *P* Locus

T. Peterson, S. Allan, P. Athma, E. Grotewold

The primary goal of our work is to understand how a gene in a higher eukaryote is expressed in particular organs during development. Our model is the maize *P* gene, which is required for the production of red flavonoid pigments in certain parts of the plant. These pigments are nonvital, and thus mutants with altered amounts and distributions of pigment can be isolated and studied readily. Since the expression of *P* can be visualized directly without the need for a biochemical assay, subtle changes in the intensity and pattern of pigmentation are readily recognized.

We are using molecular, genetic, and biochemical methods to determine how *P* is regulated during plant development. Our first aim is to delineate the coding and regulatory regions of *P*. As reported in last year's Annual Report, we have cloned 34 kb of DNA from the *P* locus and defined a region of 7 kb that is transcribed to produce several polyadenylated RNAs (Lechelt et al. 1989). Flanking the transcribed region are two 5.8-kb direct repeats. Finally, a 250-bp sequence present within both direct repeats is also repeated at a site outside the repeats.

Faced with the complex structure of *P*, we required a method to define the *P* gene coding and regulatory sequences. We decided to implement a major program toward saturating the *P* locus with insertions of the transposable element *Ac*. Our starting allele, *P-ovov*, carries an insertion of *Ac* within the transcribed region; from this allele, we selected derivatives with altered *P* expression. To date, we have isolated and studied nearly 100 mutant derivatives of *P-ovov*. Structural analyses revealed that the mutant derivatives carry deletions (P. Athma and T. Peterson, in prep.), intragenic transpositions, and other rearrangements. In the most informative cases, *Ac* has transposed from its original site in the *P-ovov* allele to a new site. In most cases, the new site of *Ac* insertion lies within the 7-kb transcribed region. However, in a significant number of cases, *Ac* has inserted at sites located 5–7 kb upstream of the putative transcription start site. These insertions 5′ of the transcribed region may identify regulatory elements, although at present, we cannot rule out the possibility of upstream coding sequences. We have also identified four cases in which *Ac* has inserted into either one or the other of the flanking 5.8-kb direct repeats; however, in all four cases, the phenotypic expression of *P* was normal. We conclude that intact flanking direct repeats are not essential for *P* expression.

We have continued to refine our knowledge of the molecular structure of the *P* locus. In the past year, we determined the nucleotide sequence of an additional 5.3 kb of genomic DNA, which, together with 3.3 kb previously sequenced, provides 8.6 kb of completed sequence. This region covers the 7-kb transcribed region completely. The genomic sequence will be compared to the cDNA clone sequence to determine the exon/intron boundaries, to search for regulatory sequences in the 5′ region, and to design oligonucleotide primers for polymerase chain reactions (PCR) to determine the structures of mutant *P* alleles. A number of cDNA clones from *P* have been isolated, but unfortunately, these have all been too short to be useful. Since the average insert size in our cDNA libraries is quite large, we suspect that a region of strong secondary structure in the *P* mRNA is inhibiting the progress of the reverse transcriptase, resulting in short clones. To circumvent this problem, we are currently mapping the 5′ end of the *P* RNA, and with this information and the genomic sequence, we will design oligonucleotide primers for PCR amplification of *P* transcripts.

Last year, we reported that the *P-ovov* allele, which produces orange variegated pericarp and cob, resulted from a short-range transposition of *Ac* to a new site in the *P* locus. Biochemical analyses showed that the orange pigment specified by *P-ovov* derives not from synthesis of a new pigment, but rather from a reduced level of the pigments specified by *P-rr*. *P-ovov* carries an insertion of the 4.5-kb transposable element *Ac* within the transcribed region; Northern analysis indicates that *P-ovov* produces two transcripts that are the same size as

those in the wild-type allele and that the *Ac* sequences must be removed from the mRNA by splicing (Peterson 1990).

In the last year, we have obtained an important clue as to the function of the *P* gene. We examined the expression of RNA from two unlinked structural genes that encode two enzymes in the pigment biosynthetic pathway. RNAs from both genes could be readily detected in plants expressing a functional *P* gene; however, no RNA from either gene could be detected in plants homozygous for a deletion of *P*. Thus, our current hypothesis is that *P* regulates the expression of RNA from the structural genes in the pigment biosynthetic pathway.

In the coming year, we intend to utilize the particle gun transformation method to study the expression of *P* in tissue explants. In this technique, tissues can be transformed by bombardment with DNA-coated particles and examined for expression of the introduced gene. Our initial aim will be to perform a "complementation test" by introducing cloned segments of *P* into *p* pericarps. If a positive result is obtained, we will then introduce deleted and modified constructs to localize the 5′ elements required for pericarp-specific expression.

We thank Christopher Brown, Patrick Nolan, and Michael Persans for their assistance in various aspects of this work.

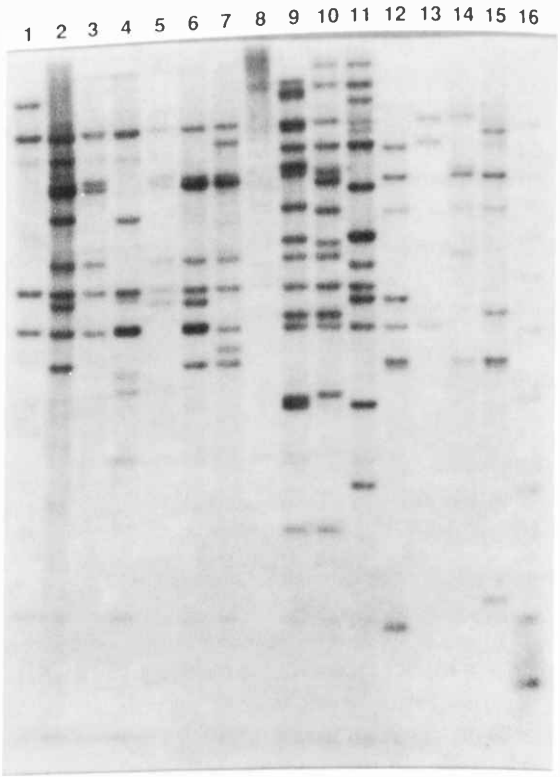

FIGURE 7 Cosegregation of a 3.2-kb *SstI* fragment with the *hm656* allele. (Lanes *1–11*) DNA from plants that inherited the mutant allele; (lanes *12–15*) DNA from siblings that did not inherit the mutant allele. The Southern blot was probed with the *Mu1* internal region.

Molecular Tagging of a Disease Resistance Gene

G. Johal, S.P. Briggs

Our focus is on efforts to describe the genes and proteins that control disease resistance in plants. The primary host molecules that determine disease resistance in animals were recognized long ago as antibodies. The study of antibodies and the genes that encode them has provided a detailed understanding of host-pathogen interactions in animals. No antibodies or functionally equivalent molecules have been found in plants. Clearly, plants and animals have evolved different mechanisms for halting infections. Although it is obvious from genetic analysis that plants do have genes for resistance to pathogens, no such genes have been isolated. The isolation of plant resistance genes would not only open up an important new field for investigation, but also permit resistance genes to be manipulated in vitro, possibly resulting in more effective forms of resistance.

We are using genetic methods rather than biochemical methods to isolate plant genes for resistance. Biochemical methods are not useful because nothing is known about the products of such genes. Our genetic approach utilizes transposable elements to mutate resistance genes; transposition into a gene disrupts both its DNA sequence and its function. Such events are detected as rare susceptible plants within a population of resistant plants. We have isolated many mutants of this type. Two of our mutants appear to result from the insertion of a well-known transposable element, *Mu1*, into a corn gene for resistance. Standard methods can now be used to clone the *Mu1* element, along with some of the flanking DNA that it inserted into. The flanking DNA is part of the resistance gene, and it can be used to clone the rest of the gene. This report describes how the two *Mu1* insertions were identified.

Earlier reports have described how our mutants were isolated. Once we had obtained a collection of

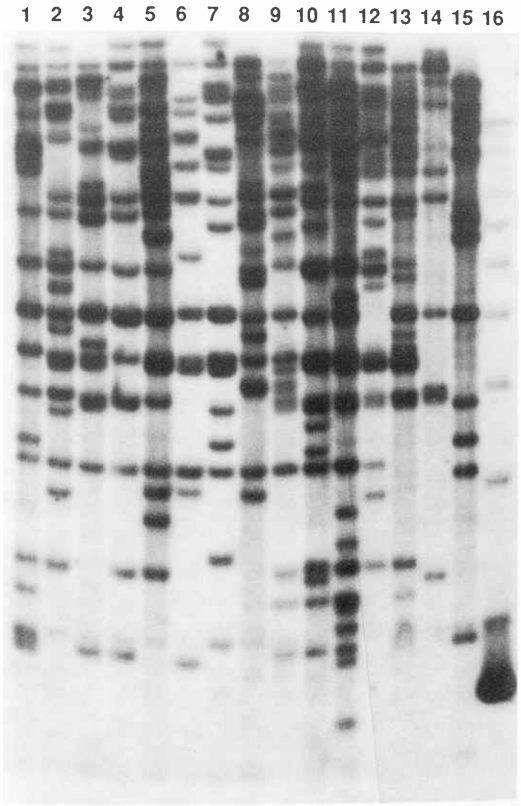

FIGURE 8 Cosegregation of a 5.8-kb *Sst*I fragment with the *hm1062* allele. (Lanes *1–12*) DNA from plants that inherited the mutant allele; (lanes *13–15*) DNA from siblings that did not inherit the mutant allele. The Southern blot was probed with the *Mu1* internal region.

mutants, the challenge was to identify which ones, if any, were caused by the *Mu1* element. The test we used was simple. Progeny were separated into two classes: those that inherited the mutant allele and those that did not. To separate the progeny into the two classes, we needed an unambiguous test to identify the mutant, or *Hm**, allele from the standard recessive, *hm*, allele. Both alleles produce the same phenotype, a fully susceptible plant. We obtained a set of restriction-fragment-length polymorphism (RFLP) probes from David Grant at Pioneer Hi-Bred International, Inc. These probes had already been localized to the region of chromosome 1 where *Hm* was known to be. By hybridizing each probe to DNA from members of a family in which *Hm* was segregating, we were able to place *Hm* on the RFLP genetic map. The family members were from the cross *K61/Pr1* × *K61*, where *K61* is a homozygous *hm* inbred and *Pr1* is a homozygous *Hm* inbred. Polymorphisms were detected with *Sac*I and *Hin*dIII. We scored *Hm* by inoculating each plant with the fungal pathogen, *Helminthosporium carbonum* race 1, isolate SB111. Probes PIO200644 and PIO200044 were found to map 5 cM proximal and distal to *Hm*, respectively. These probes were used to identify each *Hm** allele and to determine which family members inherited *Hm**.

We then examined the same progeny for the presence of the *Mu1* element. For a probe, we used an internal *Ava*I-*Bst*NI fragment of *Mu1* (pAB/5 obtained from Loverine Taylor), which does not hybridize to the highly repetitive terminal inverted repeats. Nevertheless, the probe detects 10–50 fragments in DNA from *Mutator* plants, when an enzyme that cuts outside of the element is used. To simplify the restriction pattern, we crossed our mutants with non-*Mutator* inbreds to inactive the *Mutator* system (so that no new fragments are created) and to reduce the number of fragments by segregation. This strategy permitted us to observe each of several fragments segregating in the *Hm** families. In two cases, we found that a particular size DNA fragment that contained *Mu1* was only present in progeny which inherited a mutant (*Hm**) allele. This could be explained by an insertion of *Mu1* into the resistance gene (*Hm*), so that *Mu1* and the mutant allele (*Hm**) are always inherited together. The *Hm** alleles designated *hm1*-656 and *hm1*-1062 are associated with a 3.2-kb and 5.8-kb *Sac*I fragment, respectively (see Figs. 7 and 8).

PUBLICATIONS

Colasanti, J., A.J.S. Klar, and V. Sundaresan. 1989. *R* locus imprinting is tissue-specific and not positional. *Maize Genet. Newslett.* **63:** 53.

Lechelt, C., T. Peterson, A. Laird, J. Chen, S. Dellaporta, E. Dennis, P. Starlinger, and W.J. Peacock. 1989. Isolation and molecular analysis of the maize *P* locus. *Mol. Gen. Genet.* **219:** 225–234.

Martienssen R.A., A. Barkan, M. Freeling, and W.C. Taylor. 1989. Molecular cloning of a maize gene involved in photosynthetic membrane organisation that is regulated by Robertson's *Mutator*. *EMBO J.* **8:** 1633–1639

Martienssen R.A., A. Barkan, W.C. Taylor, and M. Freeling. 1989. A *Mu*-suppressible *hcf* mutant, and non-reciprocal transmission of *Mu* activity. *Maize Newslett.* **63:** 37.

Sundaresan, V. 1989. Dosage of *Mu*. *Maize Genet. Newslett.* **63:** 54.

In Press, Submitted, and In Preparation

Athma, P. and T. Peterson. 1990. *Ac* transpositions reflected as twin sectors on *P-ovov* ears. *Maize Genet. Newslett.* (in press).

Athma, P. and T. Peterson. 1990. Deletions at the maize *P* locus are promoted by the transposable element *Ac*. (In preparation.)

Brown, J. and V. Sundaresan. 1990. Intragenic recombination at the maize *A1* locus. (Submitted.)
Colasanti, J. and V. Sundaresan. 1990. Synthesis of cytosine methylated DNAs *in vitro*. (Submitted.)
Colasanti, J.M. Freeling, and V. Sundaresan. 1990. A new *Mu*-induced suppressible mutation. (Submitted.)
Grotewold, E. and T. Peterson. 1990. Isolation of cDNA clones homologous to the *P*-gene flanking regions. *Maize Genet. Newslett.* (in press).
Johal, G. and S.P. Briggs. 1990. Placement of *hml* on the Pioneer RFLP map. *Maize Genet. Newslett.* (in press).
Johal, G. and S.P. Briggs 1990 *Mul* associated *hml* alleles *Maize Genet. Newslett.* (in press)
Peterson, T. 1990. Intragenic transposition of *Ac* generates a new allele of the maize *P* gene. (Submitted.)
Martienssen R.A., A. Barkan, W.C. Taylor, and M. Freeling. 1990. Somatically heritable switches in the DNA modification of *Mu* transposable elements monitored with a suppressible mutant in maize. *Genes Dev.* (in press).

G_1 CYCLINS OF *SACCHAROMYCES CEREVISIAE*: FIRST TICK OF A TWIN CLOCK?

B. Futcher B. Elliott, K. Erickson, I. Fitch, R. Nash, G. Tokiwa, M. Tyers

Our main interest has been the *WHI1* gene of *Saccharomyces cerevisiae*, which appears to be a rate-limiting regulator of the cell cycle. Sequencing showed that the Whi1 protein is a cyclin homolog. This was surprising, because Whi1 regulates the G_1/S transition, whereas other known cyclins regulate G_2/M. This suggested that there might be two classes of cyclins, G_1 cyclins and mitotic cyclins, that operate at different points in the cell cycle. That is, we imagine that a control mechanism containing the Cdc28 protein kinase and G_1 cyclins controls the G_1/S transition, and a different but homologous mechanism containing Cdc28 and mitotic cyclins controls the G_2/M transition. In some species, different Cdc28/Cdc2 homologs might specialize for either G_1/S or G_2/M. We call this the "Twin Clock" hypothesis, and we are attempting to prove it by identifying "mitotic" cyclins in *S. cerevisiae* and "G_1" cyclins in other species.

Whi1 is not the only cyclin homolog working in the G_1 phase. Steve Reed and co-workers have found two other cyclin homologs in *S. cerevisiae* called Cln1 and Cln2. The simultaneous deletion of all three of these cyclin homologs is lethal and prevents cells from leaving the G_1 phase (Richardson et al., *Cell 6:* 1127 [1989]). Because of the sequence homology and functional overlap between *WHI1* and *CLN1* and *CLN2*, the *WHI1* gene has been renamed *CLN3*.

Immunological and Biochemical Characterization of the Cln3 Protein

M. Tyers, G. Tokiwa, K. Erickson, B. Futcher

Since Cln3 bears considerable homology with the cyclins, it is expected to have some biochemical properties in common with them. Other cyclins oscillate in abundance during the cell cycle, reaching a peak in mitosis and being degraded at metaphase/anaphase. These cyclins are found in a complex with their cognate Cdc2/Cdc28 kinase homolog and serve to activate the kinase. The Cln3-1 mutant protein is a dominant, hyperactive protein that pushes cells through the G_1/S transition prematurely. At the molecular level, the *CLN3-1* mutant allele has a stop codon two thirds of the way through the open reading frame. This removes the last third of the protein. This missing third contains PEST regions (Pro-, Glu-, Ser-, and Thr-rich regions), which are thought to be signals for proteolysis. A hypothesis for the phenotype of *CLN3-1* mutants is that the wild-type protein is short-lived because of its PEST signals but that the mutant protein is long-lived because of the absence of PEST signals. This would increase the relative abundance of the mutant protein and so make it appear to be hyperactive.

To see whether Cln3 and Cln3-1 behaved as predicted, we needed a good antibody against Cln3.

None of the simple approaches we tried produced antibodies of sufficient sensitivity to detect Cln3 in yeast. An approach that did work was based on the epitope-tagging method developed by L. Field, R. Lerner, M. Wigler, and co-workers. A nine-amino-acid epitope was tandemly triplicated and then used to tag the Cln3 gene at its amino terminus, its carboxyl terminus, or at an internal site. The tagged Cln3 genes were transcribed from the inducible yeast GAL1 promoter, which increased steady-state mRNA levels about 200-fold. This overexpressed, triply tagged protein could be detected in yeast extracts by immunoprecipitation and by Western blotting by a high-affinity monoclonal antibody. The tagged proteins seemed to have wild-type function. Triplication of the epitope was essential—Cln3 with a single tag was not detectable in yeast. Calculation of Cln3 abundance suggested that in a wild-type strain, the normal protein is present at only a few copies per cell, which explains our inability to detect it.

This system has only been working for a short time, and so our results are still preliminary. We have used glucose repression of the GAL promoter to measure the half-life of Cln3 and Cln3-1. Cln3 is very unstable, with a half-life of only a few minutes, but Cln3-1 is stable with a half-life of more than 1 hour. Thus, the carboxyl terminus of Cln3 does seem to cause rapid turnover, and the dominant, hyperactivation of Cln3-1 does seem to be caused by the lack of proteolysis. This bodes well for the PEST hypothesis. As yet, we have seen no evidence that Cln3 abundance varies in the cell cycle, nor have we seen any evidence of an effect of α factor on the protein. Both Cln3 and Cln3-1 are phosphoproteins in vivo. When Cln3 or Cln3-1 is immunoprecipitated, a protein kinase activity is coprecipitated that phosphorylates Cln3 and Cln3-1 in vitro. It is not yet clear if this protein kinase is Cdc28.

These results do not necessarily reflect the normal behavior of the protein. The proteins we have studied are tagged and overexpressed. We are working on ways of controlling against or eliminating potential artifacts.

Cloning of a Mitotic Cyclin from *S. cerevisiae*

I. Fitch, B. Futcher

A prediction of the Twin Clock Hypothesis is that *S. cerevisiae* should have at least one mitotic cyclin as well as its three G_1 cyclins. A pair of degenerate polymerase chain reaction (PCR) primers was made corresponding to two highly conserved regions of type-B mitotic cyclins. These were used to amplify *S. cerevisiae* genomic DNA. A fragment of the appropriate size was obtained. The corresponding gene was cloned and has been partially sequenced. It is approximately 60% identical to the Cdc13 protein of *Schizosaccharomyces pombe*, a known type-B mitotic cyclin. The alignment is shown in Figure 1; the fragment is referred to as CLN10.

We think that this fragment may come from a gene for an *S. cerevisiae* B-type mitotic cyclin. Genetic experiments will determine if loss of function of this gene leads to a G_2 or mitotic arrest.

The *whi3* Mutation

R. Nash, B. Futcher

Like *WHI1-1*, *whi3* is a mutation that causes cells to divide at aberrantly small sizes. We originally ob-

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CLN10        LQLVGTSCLFIASKYEEIYSPSIKHFAYETDGACSVEDIKEGERFI

cdc13        LQLVGIAALFIASKYEEVMCPSVQNFVYMADGGYDEEEILQAERYI


             LEKLDFQISFANPMNFLRRISKADDYDIQSRTLAKFLMEIS   CLN10

             LRVLEFNLAYPNPMNFLRRISKADFYDIQTRTVAKYLVEIG   cdc13
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FIGURE 1 Alignment of the Cdc13 protein and the CLN10 fragment.

tained the *whi3* mutation by transposon mutagenesis using the Ty transposon. Genetic crosses showed that the inserted Ty element was inseparable from *whi3* (although this does not absolutely prove that the Ty element caused the mutation). We have cloned the inserted Ty element and flanking genomic DNA on both sides. Some of the flanking DNA has been subcloned and used as a probe to clone the wild-type (uninterrupted) DNA from the same locus. This DNA is unusual in that it undergoes recombination and rearrangement in *E. coli;* partly because of this, it is not yet clear whether the cloned DNA contains the intact *WHI3* gene.

Relationship between Heat-shock Resistance and Stationary Phase

B. Elliott, B. Futcher

Yeasts that have exhausted their growth medium and have stopped growing or dividing are said to be in stationary phase. Such stationary phase cells characteristically have a 1C DNA content, have stores of glycogen and trehalose, survive very well upon storage, and are resistant to heat shocks and other environmental stresses. Stationary phase has been compared and equated with the G_0 state of mammalian cells.

For historical reasons, it has been assumed that only G_1 phase cells are capable of taking on stationary phase characteristics, and this has led to the idea that resistance to stress is dependent on being in a special, G_1-like cell cycle phase. We have tested this by measuring the extent to which cells can survive heat shock under different conditions. We find that fast-growing cells are very sensitive to heat shock, nongrowing, stationary phase cells are very resistant to heat shock, and cells growing at various intermediate rates have a corresponding intermediate degree of heat-shock resistance. Within these populations growing at different rates, we have examined individual cells at known places in the cell cycle. We find that position in the cell cycle makes little or no difference to the heat-shock resistance of each cell. Rather, heat-shock resistance seems to be correlated mainly with growth rate. Therefore, it now seems unlikely that the resistance to stress of stationary phase cells has anything to do with any special cell cycle compartment. Rather, stationary phase is probably just arrest in G_1 phase, and not a good model for the G_0 phase of mammalian cells.

PUBLICATIONS

Futcher, A.B. 1989. The copy number control system of the 2 μm circle plasmid of *S. cerevisiae*. In *Genetic engineering, principles and methods* (ed. J.K. Setlow), pp. 33–48. Plenum Press, New York.

In Press, Submitted, and In Preparation

Futcher, A.B. Yeast cell cycle control. *Current Opinion in Cell Biology*. (in press).

TRANSCRIPTION AND CELL CYCLE REGULATION IN YEAST

K.T. Arndt A. Sutton
K. Tice

In almost all aspects of basic cellular processes, the yeast *Saccharomyces cerevisiae* has been found to be very similar to higher eukaryotic cells. For this reason, and the ease of manipulation and the powerful genetic approaches available with yeast, we are using yeast in our laboratory to study transcription regulation and cell cycle control.

Transcriptional Regulation of the *HIS4* Gene

K. Tice

Our ultimate goal is to understand completely the regulation of the yeast *HIS4* gene and use *HIS4* regu-

lation as a model for the transcriptional regulation of other systems. The *HIS4* gene is under complex transcriptional control. Two independent systems activate *HIS4* transcription: basal control and general amino acid control. In general amino acid control, starvation for any one or more amino acids causes an increase in the levels of the GCN4 protein. The GCN4 protein binds to the sequence TGACTC, repeated five times in the *HIS4* promoter, to activate *HIS4* transcription. GCN4 binds one of these *HIS4* elements, repeat sequence C, much more tightly than the others, and most (about 80%) of the GCN4-dependent activation of *HIS4* transcription is due to repeat sequence C. We are using defective GCN4 derivatives to obtain mutations in the general transcription machinery (see below).

The basal level control activates *HIS4* transcription in the absence of amino acid starvation. In addition, either phosphate or adenine starvation will cause a further increase in the already high basal levels of *HIS4* transcription. Activation of the basal level transcription of *HIS4* requires two *trans*-acting proteins, encoded by the *BAS1* and *BAS2* genes. Mutations in *BAS2* cause a phosphate requirement (*BAS2* is the same gene as *PHO2*), and mutations in either *BAS1* or *BAS2* cause an adenine requirement. Thus, in yeast, the phosphate, purine, and histidine pathways are coregulated. Analysis of the metabolites in these pathways shows the biological rational for the coregulation.

DNA sequence analysis indicates that BAS1 contains an amino-terminal region similar to that of Myb proteins and that BAS2 contains a centrally located homeo box. The Myb domain is the DNA-binding domain for BAS1, and the homeo box region is the DNA-binding domain for BAS2. The Myb motif was first identified in the v-*myb* gene of avian myeloblastosis virus. Cellular homologs of v-*myb* were then found in all vertebrates. In addition, Myb-type proteins have been found in *Drosophila* and corn (C1). The Myb motif contains three repeats of a sequence, whose most striking feature is three regularly spaced tryptophans. Within each repeat, the first and second tryptophans are separated by 18 or 19 amino acids, and the second and third tryptophans are separated by 18 amino acids. The third tryptophan of a given repeat is separated by 12 amino acids from the first tryptophan of the next repeat.

The regions of the *HIS4* promoter to which BAS1 and BAS2 binds were determined by DNase I footprinting using proteins prepared from overproducing strains of *Escherichia coli*. The relative positions of binding of BAS1, BAS2, and GCN4 are shown in Figure 1. The combined BAS1 + BAS2-binding region corresponds almost exactly to the *cis*-acting regions of the promoter that were determined to be required for basal level transcription of *HIS4*. Interestingly, at high BAS1 concentrations, BAS1 binds to the GCN4-binding site. We have also determined that (1) the amino-terminal fragment of BAS1 containing the Myb motif has full DNA-binding activity, (2) BAS1 and BAS2 bind adjacently on the *HIS4* promoter, (3) even though the BAS1- and BAS2-binding sites overlap by eight to ten bases, they can bind simultaneously to the *HIS4* promoter, (4) the En protein (encoded by the homeo box *engrailed* gene, which is the homeo box gene to which *BAS2* is most closely related) binds almost identically to the *HIS4* promoter as does the BAS2 (En and BAS2 protect the same DNA sequences from DNase I and bind at about the same affinity), and (5) BAS1 and BAS2, from yeast or *E. coli*, show no evidence of cooperative binding to the *HIS4* promoter.

Basal level transcription of the *HIS4* gene requires both BAS1 and BAS2. Either protein alone, even when overexpressed, can only poorly activate *HIS4* transcription (at 1/100th the level of when both BAS1 and BAS2 are present). Since BAS1 and BAS2 do not bind cooperatively to the *HIS4* promoter, the nature of the requirement for both BAS1 and BAS2 for basal level *HIS4* transcription remains to be determined. In addition to activation of *HIS4* transcription, BAS2 is required to activate the transcription of the secreted acid phosphatases (the major

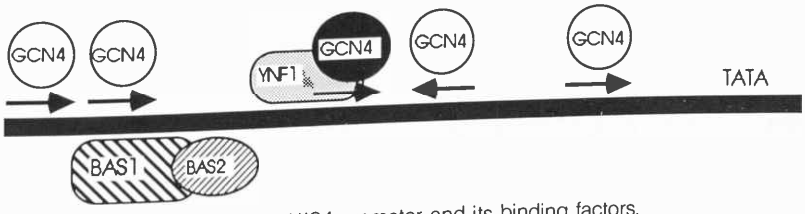

FIGURE 1 *HIS4* promoter and its binding factors.

secreted acid phosphatase is encoded by the *PHO5* gene). However, BAS1 has no role in the activation of the transcription of the secreted acid phosphatases. In the next year, we would like to determine if BAS2 requires a second DNA-binding protein (to take the place of BAS1) for activation of the secreted acid phosphatases.

The function of BAS1 and BAS2 in yeast may have relevance to their homologs in higher systems. Like BAS2, the En protein of *Drosophila* cannot activate transcription by itself. The question remaining is whether En functions as a repressor (by competing for the binding of other homeo box proteins that are able to activate transcription by themselves) or whether En is similar to BAS2 in that a second DNA-binding protein is required for activation of transcription. The avian v-Myb protein can transform only myeloid cell lineages in vitro or in vivo. One explanation of this result is that vertebrate Myb proteins, like BAS1, require a second DNA-binding protein for activation of transcription of genes that lead to the transformed phenotype. The BAS1 DNA-binding site is similar to that defined for v-Myb protein in vitro. In the near future, we would like to determine if a Myb/En system can functionally substitute for the BAS1/BAS2 system for activation of *HIS4* transcription.

BAS1, BAS2, and GCN4 are the three activation proteins for *HIS4* transcription. In strains deleted for all three proteins, *HIS4* transcription is extremely low and the strains are His^-. However, as measured by gel-shift assays using total yeast extracts, these three activator proteins represent only a very small fraction of the binding activity to the *HIS4* promoter. The major binding activity is due to a protein we have termed YNF1 that binds to the *HIS4* promoter as shown in Figure 1. In the absence of GCN4, BAS1, and BAS2, YNF1 is not able to activate *HIS4* transcription. In addition, the binding sites for YNF1 and GCN4 overlap; GCN4 binds much tighter to the middle repeat element than to the other four repeat elements, and most GCN4-dependent transcription occurs through the middle repeat sequence. In vitro DNA-binding studies show that either GCN4 or YNF1 can bind to their respective sites. They cannot bind simultaneously. This result argues that YNF1 might be a repressor of GCN4-dependent *HIS4* transcription. However, using *HIS4* promoters that contain point mutations in the YNF1-binding site that completely eliminate YNF1 binding in vitro (which are completely outside the GCN4-binding site), we have found that the presence or absence of YNF1 binding has almost *no* effect on the steady-state levels of GCN4-dependent transcription.

We have very recently determined that YNF1 is the same protein as RAP1. RAP1 (which is probably the same as GRF1-TUF) is a very abundant DNA-binding protein in yeast that binds to silencers, telomeres, and the promoters of many of the genes encoding ribosomal proteins and glycolytic enzymes. In addition, most of the RAP1 protein is found associated with the nuclear matrix or scaffold. The binding of RAP1 to the *HIS4* promoter is somewhat unexpected, since it was believed that RAP1 *by itself* is a transcriptional activation protein. In the context of the *HIS4* promoter, RAP1 *cannot* activate transcription by itself, since a strain lacking GCN4, BAS1, and BAS2 has extremely low levels of *HIS4* transcription. Future experiments will be directed to show if RAP1 is required for the rapid kinetics of GCN4-dependent induction of *HIS4* transcription, such as occurs when general control is induced by amino acid starvation. We also want to determine if RAP binding to the *HIS4* promoter correlates with association of *HIS4* with the nuclear matrix. These experiments will allow us to determine if association with the nuclear matrix is required for rapid kinetics of induction. It may be that genes that are not associated with the nuclear matrix are in repressed chromatin structures and cannot be induced until that region of DNA undergoes DNA replication, which then makes the promoter free to associate with activator proteins. We already know that nucleosomes are phased on the *HIS4* promoter and that the nucleosomes are excluded from the RAP1-binding site.

A Genetic Selection for General Transcription Factors

A. Sutton

When the levels of *HIS4* transcription are quantitated by assaying the levels of β-galactosidase from a *HIS4-lacZ* fusion, a wild-type strain gives about 400 units of activity, whereas a strain containing deletions of the *GCN4*, *BAS1*, and *BAS2* genes gives less than one unit of activity. As a result, a strain deleted for the three activators of *HIS4* transcription is His^-. We have reverted this strain to His^+ with the assumption that mutations in genes encoding general transcriptional factors could result in in-

creased *HIS4* transcription. This reversion analysis has identified four suppressor genes that permit *HIS4* transcription in the absence of GCN4, BAS1, and BAS2. We have termed these suppressors *sit* genes for *s*uppressors of *i*nitiation of *t*ranscription. These suppressor genes encode factors that affect the transcription of many diverse genes.

Two of the suppressors, *SIT1* and *SIT2*, are encoded by *RPB1* and *RPB2*, the genes for the two largest subunits of RNA polymerase II. All strains containing suppressor mutations in *RPB1* and *RPB2* have reduced transcription of the *INO1* gene and an inositol requirement.

Mutations in *SIT3* or high-copy-number *SIT3* increase *HIS4* transcription in the absence of GCN4, BAS1, and BAS2. *SIT3* is the only suppressor that suppresses when the wild-type gene is present in high copy number. The increase in *HIS4* transcription by high-copy-number *SIT3* or by *sit3* alleles is largely independent of the *HIS4* TATA sequence. We sequenced the *SIT3* gene and found that it is identical to *GCR1*, a gene previously identified as being required for high-level transcription of almost all glycolytic enzymes (whose combined mRNAs constitute over 50% of the mRNA in a yeast cell). Presently, we are not sure exactly how SIT3 functions. Our current model is that SIT3 is a protein that if altered (*sit3* mutants) or if overexpressed (high-copy-number *SIT3*) can recognize transcriptional activation proteins at almost any promoter to stimulate TATA-independent transcription.

The SIT4 protein is over 50% identical to the catalytic subunit of bovine type-2A protein phosphatase. In the haploid, *sit3* in any pairwise combination with *sit1*, *sit2*, or *sit4* is viable, consistent with the view that SIT3 encodes a factor that functions as an accessory role to the RNA polymerase II holoenzyme. In contrast, all pairwise combinations between *sit1*, *sit2*, and *sit4* are inviable. The inviability of *sit1 sit2* double mutants is easily understood as an interaction between altered versions of the largest subunits of RNA polymerase II that leads to a more severe phenotype than either of the single mutants. One interpretation of the gene interactions between *sit4* and the *sit1* and *sit2* RNA polymerase II mutations is that *SIT4* encodes a factor that interacts with RNA polymerase II. This model needs to be tested directly by Western and immunoprecipitation analyses to determine the phosphorylation state of RPB1 in strains containing wild-type *SIT4*, *sit4* mutations, and overexpression of SIT4.

Progression from G_1 to S Phase Requires the SIT4 Protein Phosphatase

A. Sutton

Regulation of the cell cycle, most importantly the decision of whether or not to initiate a new cycle, is a major determinant of cell proliferation. For the yeast *Saccharomyces*, cells that are nutritionally starved (such as by limiting an essential nutrient in the growth medium) or cells in a saturated culture arrest in G_1. Cells that have arrested at this point in G_1 are in a physiological state distinct from G_1 cells in actively growing cultures. In general, cells initiate a new cycle only when they will be able to complete the entire cycle.

Much of what is known about regulation of the cell cycle comes from strains containing conditional mutations that arrest in G_1 at the nonpermissive temperature. Strains containing *cdc19*, *cdc25*, or *cdc35* (adenylate cyclase) arrest in G_1 at what seems to be close to the nutritional arrest point (unbudded uninucleate G_1 cells with no spindle pole satellites). Conditional mutations in the *CDC28* gene, which encodes a protein kinase, cause arrest in G_1 as unbudded uninucleate G_1 cells containing spindle poles with a satellite structure. This G_1 arrest point in *cdc28* mutants has been operationally defined as START. Supposedly, if a normal cell passes START, it is committed to completion of the cycle.

As stated above, we originally identified the *SIT4* gene as a suppressor of a *HIS4* transcriptional defect. All *sit4* strains are temperature sensitive for growth. We have found that at the nonpermissive temperature, *sit4* strains (and mutations in *sit4* created solely with a temperature criterion) have a cell-cycle-arrest phenotype and arrest as large nonbudded uninuclear G_1 cells. Reciprocal shift experiments show that SIT4 is required *only* for progression from G_1 into S phase. In the first experiment, a *sit4-102* strain was arrested in late G_1 by α-factor. The cells were released from α-factor arrest by washing away the α-factor and shifted to the nonpermissive temperature at various times after release. If the released cells were shifted to the nonpermissive temperature at any time *before* they began to initiate a bud (which takes 30–40 min), they did not initiate a cell cycle but arrested as large unbudded uninucleate cells with a 1N DNA content. In contrast, if the released cells were shifted to the nonpermissive tem-

perature at any time *after* they initiated a bud, the cells continued with their current cell cycle (e.g., the bud continued to grow) and arrested as large unbudded uninucleate cells in late G_1. In the second experiment, unsynchronized *sit4-102* cells were first arrested at the nonpermissive temperature as large unbudded cells. When α-factor was added and the cells shifted to the permissive temperature, the cells initiated budding and completed a cell cycle *before* arresting in late G_1 by α-factor. In a third set of experiments, *sit4-102* cells in the G_1 stage of the cell cycle were isolated by centrifugal elutriation. The synchronized cells were grown until they formed a very small bud, at which time both α-factor was added and the cells were shifted to the nonpermissive temperature (for *sit4-102*). The cells continued with their current cell cycle and arrested exclusively at the α-factor arrest point (as "schmoos"). These experiments, and the proper controls, show that the execution point for the essential function of SIT4 is in very late G_1 (and *only* in late G_1) and that this essential function of SIT4 is required for progression into S phase.

The localization of the SIT4 protein was determined by indirect immunofluorescence using antibodies directed against the amino terminus of SIT4. These experiments show that SIT4 is localized primarily to the cytoplasm and mostly excluded from the nucleus. SIT4 coimmunoprecipitates with two larger proteins that might be regulatory subunits. Although SIT4 is not phosphorylated, the two larger SIT4-associated proteins are phosphorylated. We are currently investigating if the association of these two proteins with SIT4 or the phosphorylation state of these two proteins varies in the cell cycle. The steady-state levels of SIT4 do not vary in the cell cycle.

We are also investigating the role of SIT4 in progression from G_1 into S phase using two different genetic approaches. Hopefully, these approaches will identify substrates of SIT4 or regulators of SIT4 activity. The first approach we have taken is to identify wild-type genes that, in high copy number, suppress the slow-growth defect of *sit4* His^+ suppressor strains. Using this approach, we have obtained four different genes. None of these genes rescue the temperature-sensitive defect of *sit4* strains. One of these genes is another protein phosphatase that we call *PPH2*. DNA sequence analysis of the *PPH2* gene shows that it is 86% identical to type-2A protein phosphatases and that the similarity extends to the very carboxyl terminus. In contrast, SIT4 has no similarity to the carboxyl terminus of type-2A or type-1 protein phosphatases. Since overexpression of *PPH2* partially rescues only a subset of the defects of a *sit4* strain, we believe that SIT4 represents a class of phosphatase distinct from the canonical type 1 and type 2A.

A second gene (which we call *SIS1*) that, in high copy number, suppresses the slow-growth defect of *sit4* strains encodes a predicted protein that is 28% identical overall (for an overlap of 330 amino acids) to bacterial DnaJ proteins. The SIS1 protein has a predicted molecular mass of 37.6 kD and is essential for viability. Immunoprecipitation experiments show that SIS1 is phosphorylated and coimmunoprecipitates with a protein (p40) of 40 kD that is not phosphorylated. We are currently investigating if SIS1 is a substrate of SIT4 and if the association of SIS1 with p40 varies in the cell cycle. A third gene, called *SIS2*, that, in high copy number, suppresses the slow-growth defect of *SIT4* strains encodes a predicted protein of 53 kD that is not similar to any protein in the current databases. We are investigating the function of this protein in relation to the role of SIT4 in G_1/S progression.

Our second genetic approach to SIT4 function is to obtain second-site suppressors of the temperature-sensitive arrest phenotype of *sit4-102* strains. This analysis has identified three different complementation groups, which we call *pts1* through *pts3*. Interestingly, about half of the alleles of both *pts1* and *pts2* cause complete failure to germinate. In addition, all *pts1* strains are sensitive to nutrient limitation. Hopefully, the *PTS* genes have identified important G_1 control proteins that relate to SIT4 function.

PUBLICATIONS

Arndt, K.T., C. Styles, and G.R. Fink. 1989. A suppressor of a *HIS4* transcriptional defect encodes a protein with homology to the catalytic subunit of protein phosphatases. *Cell* **56:** 527–537.

Tice-Baldwin, K., G.R. Fink, and K.T. Arndt. 1989. BAS1 has a Myb motif and activates *HIS4* transcription only in combination with BAS2. *Science* **246:** 931–935.

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

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SMALL NUCLEAR RNA GENES

The human U2 and U6 small nuclear RNA (snRNA) genes constitute a fascinating model to examine the links between formation of a transcription complex at a specific promoter and the ability of this transcription complex to elongate and recognize (or ignore) transcription termination signals. In addition, they also provide a so far unique system to study the mechanisms that govern RNA polymerase selection by transcriptional promoters.

The U2 snRNA gene is transcribed by RNA polymerase II. The promoter contains a proximal element located around position −50 and functionally equivalent to a TATA box, in that it is essential for efficient transcription and localizes the start site of transcription. It also contains an enhancer located around position −200 and consisting of an Sp1-binding site adjacent to an octamer motif. Termination of transcription just downstream from the region corresponding to the mature 3′ end of the RNA is directed by the 3′ box, a short sequence conserved among RNA polymerase II snRNA genes. This termination signal is peculiar in that it is recognized only by transcription complexes derived from snRNA promoters and not by transcription complexes derived from mRNA promoters. Reciprocally, the RNA polymerase II snRNA promoters are peculiar in that the transcription complexes they direct are not capable of transcribing through long DNA templates, but they will terminate at sequences even only remotely related to the 3′ box.

The U6 snRNA gene belongs to a growing family of RNA polymerase III genes that include the 7SK and probably the RNase MRP RNA genes. In these genes, the elements essential for initiation of transcription are not contained within the coding sequences, but rather lie within the 5′-flanking sequences. Curiously, two of these promoter elements are similar in their sequence and spatial arrangement to the U2 promoter elements. Thus, the U6 promoter contains an enhancer region that is characterized, like the U2 enhancer, by the presence of an octamer motif. It also contains a proximal element that matches in 13 out of 17 positions the human U2 proximal element. The third element of the U6 promoter is not found in the U2 promoter and consists of an A/T-rich region located −18 to −26 nucleotides upstream of the start site of transcription and reminiscent of the TATA box of mRNA promoters. Its function is, however, quite different. In the human U6 promoter, the A/T box constitutes a dominant element that defines the promoter as a U6 promoter. Mutation of the A/T box converts the U6 promoter into a predominantly RNA polymerase II promoter, whereas insertion of the A/T box into the U2 promoter converts it into an RNA polymerase III promoter. We are interested in characterizing in detail the *cis*-acting elements involved in transcription by RNA polymerase II and III and in identifying the *trans*-acting factors involved in transcription of the U2 and U6 genes.

HUMAN IMMUNODEFICIENCY VIRUS TYPE 1

The human immunodeficiency virus type 1 (HIV-1) contains, in addition to the genes normally found in retroviruses, a set of genes encoding regulatory proteins. One of these genes encodes the *tat* protein, a potent *trans*-activator of viral gene expression. *tat* acts through a sequence called TAR, located downstream from the transcriptional start site. TAR could thus represent a DNA element, an RNA element, or both. Indeed, the mechanism of *tat trans*-activation is the subject of considerable controversy, and *tat* effects at the levels of translation, initiation and elongation of transcription, and other ill-defined pathways have all been described in the literature. We are particularly intrigued by the proposal that TAR may be a termination site and *tat* an antiterminator; in this model, *tat* would increase gene expression by allowing the RNA polymerases to bypass a transcriptional block located close to the initiation site of transcription. In support of this model, short transcripts whose 3′ ends map just downstream from TAR can be detected in steady-state RNA from cells expressing the HIV-1 long terminal repeat (LTR). However,

in contradiction to this model, deletion of TAR does not result in increase of expression from the HIV LTR. Thus, TAR does not seem to contain a negative element such as a premature termination site. This paradox could be resolved if the HIV-1 promoter directed the formation of a transcription complex with not much processivity, comparable in this respect to the transcription complex formed on the U2 promoter. Such a transcription complex might stop at a premature termination site such as TAR if TAR were present, and might stop randomly if the premature termination site were deleted. As an approach to understand the mechanism of *tat trans*-activation, we would like to determine how the short transcripts are generated and what the role of HIV promoter elements is in *tat trans*-activation.

cis-Acting Elements and Factors Involved in Transcription of the Human U6 snRNA Gene

S. Lobo, P. Reinagel, J. Lister, L. Johal,
S. Ifill, N. Hernandez

ROLE OF THE REGION AROUND THE U2 CAP SITE IN RNA POLYMERASE SELECTION

We have continued our analysis of the *cis*-acting elements involved in transcription by RNA polymerase II and III in the U2 and U6 promoters. We had shown before that insertion of the U6 A/T box into the U2 promoter extending from position −247 to −7, i.e., lacking the U2 cap site, converts it into an RNA polymerase III promoter (see Fig. 1A, constructs pU2/-247/RA.2 and pU2/TA). This observation suggests that the U2 and U6 promoters bind a common set of transcription factors and that RNA polymerase specificity is determined by the absence or presence of the factor(s) binding to the A/T box. However, in the *Xenopus* U6 gene, transcription by RNA polymerase III requires, in addition to the A/T box, the sequences surrounding the start site of transcription. This observation raises the possibility that in the complete U2 promoter with an A/T box inserted, the sequences surrounding the cap site just upstream of the potential RNA polymerase III start site could suppress RNA polymerase III transcription. To exclude this possibility, we modified the construct pU2/TA by site-directed mutagenesis to create pU2/TA/U2Cap (Fig. 1A). In this modified construct, the natural U2 sequence surrounding the U2 cap site was restored, so that the U2 promoter sequences now extend to position +7 downstream from the U2 cap site. Analysis of expression from this construct in vivo and in vitro showed that 50% of the total transcription was directed by RNA polymerase III, compared to 80% with the parent construct. Thus, although the U2 cap site sequences

A.

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                        PROXIMAL ELEMENT
                        ┌──────────────┐
pU2/-247/RA.2           CTCACCGCGACTTGAATGTGGATGAGAGTGGGACGGTGACGGCGGGCGCGAAGGCccctCgAgtACcTagaGt
pU2/U2Cap               ..................................................GAGCGCATCGCTTagaGt
pU2/TA                  ..............................t.t.tatat..........ccctCgAgtACcTagaGt
pU2/TA/U2Cap            ..............................t.t.tatat..........GAGCGCATCGCTTagaGt
```

B.

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                                PROXIMAL ELEMENT
                                ┌──────────────┐
pU2/-247                GGC TGGGGCTCTCACCGCGACTTGAATGTGGATGAGAGTGGGACGGTGACGGCGGGCGCGAAGGCGAGCGCATCGCT
LS-34/-43               ... ......................ccctc...g.....................................

U6/Hae/RA.2             ACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCg
LS5/Hae                 ............................tctcgagaa.....................................g

pU2/-247/RA.2           GGC TGGGGCTCTCACCGCGACTTGAATGTGGATGAGAGTGGGACGGTGACGGCGGGCGCGAAGGCccctCgAgtACcTa
pU2/TA                  ... ....................................tTtAtatat..........ccctCg.gtA.c.a
pU2/TA/(LS-34/-43)      ... ......................ccctc...g.......tTtAtatat..........ccctcg.gta.c.a
```

FIGURE 1 (*A*) 5′-flanking sequence in different U2 constructs. The nucleotides that differ from the wild-type U2 sequence are represented by boldface characters. Underscores indicate the different start sites of transcription; uppercase underscored characters represent RNA polymerase II start sites, and lowercase underscored characters represent RNA polymerase III start sites. The proximal element homology is indicated. (*B*) 5′-flanking sequences in different U2 and U6 constructs. Symbols are the same as in *A*.

coinfluence the relative levels of RNA polymerase II and III transcription, the U6 A/T box is nevertheless the dominant element.

CHARACTERIZATION OF THE U6 A/T BOX

The U6 A/T box has the sequence TTTATATAT. This sequence is nearly perfectly conserved in the human 7SK gene (TTTATATAG), but quite divergent in the mouse RNase MRP RNA gene (ATAAATTAG). To characterize the sequence flexibility in this region for RNA polymerase III transcription, we replaced the U6 A/T box with TATA boxes from different mRNA promoters. Analysis of expression of the different constructs showed that a large number of different sequences can replace the A/T box without suppressing RNA polymerase III transcription. The minimal requirement seems to be a run of at least six A and T residues. We are in the process of refining this analysis and determining whether sequences flanking the A/T box also play a role. A detailed knowledge of the sequence requirements in this region for RNA polymerase III transcription will be useful to discriminate between factors binding specifically to the U6 A/T box and therefore probably involved in U6 transcription and factors binding nonspecifically to A/T-rich regions.

CHARACTERIZATION OF THE REGION IMMEDIATELY 3′ OF THE PROXIMAL ELEMENT

We also examined in some detail the region immediately downstream from the proximal element homology in the U2 and U6 promoters. We showed before that in the U2 promoter, a linker-scanning mutation that modifies this region and overlaps with the proximal element homology itself only by 1 bp (Fig. 1B, mutant LS-34/-43) is as detrimental to transcription as mutations that modify the central part of the proximal element homology. This suggested that the region immediately downstream from the proximal element contains some element required for RNA polymerase II transcription, although this region is not conserved in different RNA polymerase II snRNA promoters. In the human U6 promoter, a clustered point mutation that modifies essentially the same region (LS5, see Fig. 1B) has little effect on RNA polymerase III transcription. However, when combined with a mutation that debilitates the A/T box and switches the U6 promoter to a predominantly RNA polymerase II promoter, the LS5 mutation has a marked deleterious effect, again suggesting that this region is required for RNA polymerase II transcription. However, because the above mutations are different and were introduced in the context of different promoters, the above data cannot be rigorously interpreted.

To determine whether the region downstream from the proximal element represents an element required mainly for RNA polymerase II transcription, we introduced the LS-34/-43 mutation in the context of the pU2/TA promoter that directs both RNA polymerase II and RNA polymerase III transcription, thus creating the pU2/TA/(LS-34/-43) construct (Fig. 1). Analysis of expression from this construct showed that introduction of the LS-34/-43 mutation suppressed RNA polymerase II transcription but had little effect on RNA polymerase III transcription. Thus, at least one element in the U2 and U6 promoters appears to be required by RNA polymerase II but not by RNA polymerase III. The U2 and U6 transcription complexes thus differ by at least one factor in addition to the A/T box binding factor, and the absence of this factor in nuclear HeLa cell extracts might explain the apparent paradox of active RNA polymerase III but inactive RNA polymerase II transcription from snRNA promoters in vitro.

Why does the U6 gene contain a polymerase II promoter element? This element may represent a remnant from when the snRNA promoters may have been transcribed by the same RNA polymerase. Alternatively, there may be slightly divergent U6 gene copies in the human genome that are transcribed by RNA polymerase II. Little is known about the genomic organization and the copy number of the true U6 genes in the human genome, although the total number of U6 loci including pseudogenes has been estimated to be 200. Finally, the U6 gene we have analyzed may itself be transcribed by RNA polymerase II under circumstances not reproduced in our assay.

We are now interested in identifying and eventually purifying transcription factors involved in U2 and U6 transcription, and one of the intriguing questions is how the 3′ box signals termination of transcription by RNA polymerase II. To determine whether the factors recognize the 3′ box at the DNA level, we performed competition experiments in vivo, in which an excess of 3′ box sequences was transfected together with a reporter gene containing a 3′ box followed by a polyadenylation site. Even the highest concentration of 3′ box sequences did not result in an increase of polyadenylated RNA rela-

tive to RNA ending at the 3′ box from the reporter construct. This suggests either that the amount of 3′ box binding factor in the nucleus is very large, or that the 3′ box does not bind a factor at the DNA level, or that the U2 transcription complex is locked in a stable structure such that individual factors cannot readily exchange between different binding sites. However, similar experiments in which an excess of U6 proximal element sequences was cotransfected with the U2 gene or an excess of U2 proximal elements was cotransfected with the U6 gene resulted in a decrease of U2 and U6 transcription, respectively, suggesting that the U2 and U6 transcription complexes are not completely stable and demonstrating that the same factors can bind to the U2 and U6 proximal elements in vivo. To identify the factor(s) that interacts with the U6 proximal element, we have more recently begun to fractionate the U6 in vitro transcription system. This approach should also allow the identification of the factor(s) interacting with the A/T box.

Mechanism of *trans*-Activation of the HIV-1 Promoter by the Viral *trans*-Activator *tat*

R. Ratnasabapathy, M. Sheldon, S. Ifill, L. Johal, N. Hernandez

To study the mechanism of *tat trans*-activation, we are following two approaches. In the first one, different constructs are transfected into T cells or HeLa cells, and *tat trans*-activation and formation of short transcripts are monitored by RNase T1 protection. In the second, formation of short transcripts is studied in an in vitro transcription system.

IN VIVO ASSAYS

Figure 2 shows the hybrid gene construct used in the transfection experiments and from which all other constructs are derived. It consists of HIV-1 sequences from −147 to +80, i.e., containing the HIV promoter and the entire TAR sequence. The HIV sequences are followed by a fragment from adenovirus 2 (Ad2) containing the L3 polyadenylation site, and this ensures that transcripts that read through the potential premature termination sites in TAR will have discrete polyadenylated 3′ ends and will therefore be stable. This artificial transcription unit is followed by two fragments: (1) a fragment carrying the T3 promoter, so that riboprobes can be generated from each construct without additional cloning steps, and (2) a fragment (not shown in the figure) carrying the SV40 origin of replication, so that the plasmid can be replicated in vivo by inclusion of an SV40 T-antigen expression vector in the transfections. 5′ to the HIV-1 promoter are two tandem polyadenylation sites (not shown in the figure) derived from SV40. The presence of the tandem SV40 polyadenylation sites reduces considerably the amount of stable RNA extending through the HIV-1 promoter in transient transfections, presumably because RNA derived from cryptic promoters within the vector becomes polyadenylated at these sites.

This construct was transfected into Jurkat cells with or without a second plasmid directing expression of the *tat* protein, and the transcripts generated were characterized by RNase T1 protection mapping. We detect bands whose sizes correspond to short transcripts ending downstream from the TAR region as well as transcripts extending to the Ad2 L3 polyadenylation site. Control experiments have confirmed that (1) these transcripts correspond to correctly initiated RNA, (2) the bands are not the results of artifactual cleavage of the hybrids by RNase T1, and (3) the long transcripts correspond to polyadenylated RNA, whereas the short transcripts are not polyadenylated. In the presence of *tat*, the amount of polyadenylated RNA increases, whereas the amount of short transcripts decreases, both in T cells and in HeLa cells. This observation is similar to the results reported by M. Peterlin and colleagues for experiments in COS cells and is consistent with *tat* acting as an antiterminator of transcription.

We have generated a whole series of constructs in which the HIV-1 promoter was replaced by different mRNA promoters or by the U2 and U6 promoters. Surprisingly, all of these hybrid constructs

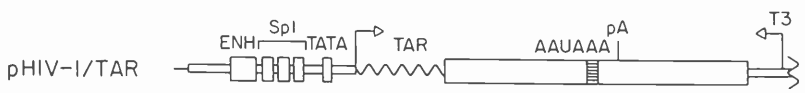

FIGURE 2 Hybrid gene in HIV-1 construct. The HIV-1 promoter elements, the TAR region, the L3 polyadenylation site, and the T3 promoter are indicated.

still generated short transcripts and were *trans*-activated by *tat*. The only exception was the U6 promoter, which when placed upstream of TAR generated short transcripts but was not *trans*-activated by *tat*. Thus, the HIV-1 promoter does not contain any element required for short transcript formation or for *tat trans*-activation. We have now generated more constructs with substitutions in the TAR region, so as to define which regions of TAR are required for short transcript formation.

TRANSCRIPTION IN VITRO

We have prepared nuclear extracts from HeLa cells and from Jurkat cells. These extracts direct efficient transcription initiation from the HIV-1 promoter. Initiation is accurate, and transcription is directed by RNA polymerase II. Interestingly, a large amount of short transcripts is generated in vitro, which are identical in size to the short transcripts observed in vivo. The amounts of short transcripts vary in different extracts, but they are always detectable. Preliminary experiments suggest that these short transcripts are not the results of a processing reaction uncoupled from transcription, but rather are generated by termination of transcription or by a processing reaction coupled to transcription. Thus, in time courses, the short transcripts appear at the same time as long transcripts. Moreover, in experiments in which transcription is allowed to proceed for 1 hour, then blocked by addition of α-amanitin, and incubation is continued for various periods of time, the amounts of short transcripts do not increase during the incubation period. We have yet to find a mutant that does not generate short transcripts in vivo nor in vitro to convince ourselves of the specificity of the in vitro reaction. If it does accurately reflect in vivo events, we will determine by competition or depletion experiments whether specific factors are involved in premature termination of transcription in the TAR region.

PUBLICATIONS

Lobo, S. and N. Hernandez. 1989. A seven base pair mutation converts a human RNA polymerase II snRNA promoter into a RNA polymerase III promoter. *Cell* **58:** 55–67.

In Press, Submitted, and In Preparation

Lobo, S., S. Ifill, and N. Hernandez. 1990. *cis*-Acting elements in the human U2 and U6 promoters involved in RNA polymerase II and RNA polymerase III transcription. (Submitted.)

Ratnasabapathy, R., M. Sheldon, L. Johal, and N. Hernandez. 1990. TAR is an inducer of short transcripts for both mRNA and snRNA promoters. (In preparation.)

STRUCTURE AND COMPUTATION

This section includes five laboratories interested in the detailed structural properties of proteins and computational biology. It includes the laboratories of Jim Pflugrath and John Anderson, who are interested in macromolecular crystallography, and Rich Roberts, whose group has had a long-standing interest in restriction enzymes and their associated methylases. During the last year, the program lost one of its founding members, Dr. Mark Zoller, who has joined Genentech Corporation in San Francisco to pursue his interests in site-directed mutagenesis. Fortunately, Dr. Jeff Kuret, a former postdoctoral fellow of Dr. Zoller, was persuaded to stay on and has accepted the position of Staff Investigator. His laboratory studies protein kinases, and Dr. Kuret has established a close collaboration with Dr. Pflugrath. The program was also fortunate in enticing Dr. Tom Marr to leave Los Alamos National Laboratory, where he has been responsible for designing the new GENBANK database. Dr. Marr has accepted the position of Senior Staff Investigator at Cold Spring Harbor Laboratory. His extensive background in biology and computer science will considerably enhance computational biology at the Laboratory.

NUCLEIC ACID CHEMISTRY

R.J. Roberts

A. Dubey
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S. Klimasauskas
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Studies of the *Msp*I Methylase

A. Dubey

The *Msp*I restriction-modification system recognizes the sequence 5′-CCGG-3′. The complete system has been previously cloned and sequenced (Lin et al., *Nucleic Acids Res. 17:* 3001 [1989]). A subfragment, containing the methylase gene, was cloned next to a fragment containing the inducible pTac promoter, and this derivative overproduces the methylase to high levels. Dr. B. Mollet in this laboratory had previously attempted the purification of the *Msp*I methylase directly from this strain and found that it was possible to purify small amounts of the methylase quite readily; however, most of the enzyme was in the form of large aggregates of unknown specific activity. This was caused by overexpression of the *Msp*I methylase in *Escherichia coli* strain RR1, which results in the formation of inclusion bodies. We have now examined the formation of these inclusion bodies and discovered that they are temperature-sensitive and are not produced at temperatures below 30°C. At normal growth temperatures of 37°C, about 50% of the total *Msp*I methylase produced forms biologically inactive aggregates. At 30°C, greater than 99% of the total *Msp*I methylase is soluble. We are continuing to fine-tune the overexpression system to produce higher yields of soluble and enzymatically active *Msp*I methylase.

In the recombinant strain overexpressing the *Msp*I methylase, a shorter protein with an apparent molecular mass of 40 kD has been identified in addition to the 51-kD species. Amino-terminal sequence analysis of these two proteins shows that the 40-kD protein is a truncated form of the *Msp*I methylase,

missing exactly 40 amino acids from the amino terminus. The new amino terminus is located at the second methionine within the open reading frame that encodes the protein. This suggests that the two forms are produced by alternative sites of translation initiation. However, the missing amino acids from the amino terminus are not sufficient to account for the differences in apparent molecular mass, and we are continuing to analyze the lower-molecular-mass form. We are presently trying to identify the *Msp*I methylase from the original *Moraxella* strain so as to discover which of the two forms of the methylase represents the bona fide protein. We are also trying to separate the two forms of the enzyme so as to characterize them. For these studies, we have prepared polyclonal antibodies against the *Msp*I methylase to help identify the protein in cells that are not overexpressing it.

A new scheme for the purification of the *Msp*I methylase is presently being devised using phosphocellulose and hydroxyapatite chromatography. Considerable purification has been achieved, although at least one further step will be necessary before homogeneous protein is available for kinetic studies and crystallization.

The Architecture of Cytosine Methylases

J. Meyertons, S. Klimasauskas

The primary goal of this work is to identify the protein domains of the *Hha*I (recognition sequence: 5′-G^{me}CGC-3′) and *Hpa*II (recognition sequence: 5′-C^{me}CGG-3′) methylases that form part of their respective restriction-modification systems. These enzymes are responsible for the sequence-specific recognition of DNA, although nothing is known about the detailed mechanisms by which this is achieved. A plasmid was constructed with the two methylase genes in tandem and arranged in the same orientation relative to transcription. *Hha*I and *Hpa*II methylase gene fusions were made by cleaving the plasmid between the methylase genes and digesting the plasmids with BAL-31 nuclease. This resulted in hybrid methylases that had the amino-terminal region of the *Hha*I methylase fused to the carboxy-terminal region of the *Hpa*II methylase, and vice versa.

An alternative scheme to generate hybrid methylases is also being pursued. The polymerase chain reaction (PCR) is being used to construct exact deletions from the plasmid containing both methylase genes such that the amino terminus of one methylase becomes joined to the corresponding carboxyl terminus of the second methylase. These constructions take advantage of the conserved sequence motifs that are present within the cytosine methylases so that the boundaries in the hybrids are defined by the sequence motifs. Thus, in each case, the overall architecture of the methylase is maintained.

To detect the hybrid methylases, an assay was devised that relied upon the *Mcr* system of *E. coli*. The *Mcr* system is a sequence-specific, modification-dependent restriction system that cleaves DNA at 5-methylcytosine residues. The two *Mcr* systems known are identified as A and B. The *Mcr*A system restricts DNA methylated by the *Hpa*II methylase, whereas both *Mcr*A and *Mcr*B systems restrict DNA methylated by the *Hha*I methylase. Methylated plasmids transformed into an *E. coli* strain with an active *Mcr* system are cleaved and destroyed. To permit detection of active hybrid methylases, *E. coli* strains with temperature-sensitive mutations in the *Mcr* genes were constructed. Three *E. coli* strains with temperature-sensitive *Mcr* mutations were identified. The *Mcr* temperature-sensitive mutations in *E. coli* will be further characterized, because these strains could potentially have value in studying both prokaryotic and eukaryotic methylases.

Isolation of Mutants of Integration Host Factor

D. Roberts

Integration host factor (IHF) is a site-specific DNA-binding protein in *E. coli* that has been shown to play a role both in regulation of gene expression and in site-specific recombination (for review, see Friedman, *Cell 55:* 545 [1988]). It is a heterodimer composed of two homologous subunits, IHF-α and IHF-β, each about 100 amino acids long. IHF binds DNA by contacts in the minor groove (Craig and Nash, *Cell 39:* 707 [1984]; Yang and Nash, *Cell 57:* 869 [1989]). Binding of IHF to DNA causes the DNA to bend (Prentki et al., *EMBO J. 6:* 2479 [1987]; Stenzel et al., *Cell 49:* 709 [1987]; Robertson and Nash, *J. Biol. Chem. 263:* 3554 [1988]). We are interested in learning more about the structure and function of IHF, and as a first step in studying this, we have isolated many new mutants of IHF.

IHF mutants were isolated with the genes on plasmids. Both genes have been cloned (Miller, *Cold Spring Harbor Symp. Quant. Biol. 49:* 691 [1984]; Flamm and Weisberg, *J. Mol. Biol. 183:* 117 [1985]). We subcloned small fragments containing the genes (350–400 bp) and inserted them downstream from the *lac* promoter. These plasmids were mutagenized by growth through the mutator strain *mut*D and screened for mutants using two different screens. First, mutants were selected as survivors of heat induction of a Mu lysogen. IHF is required for growth and replication of the bacteriophage Mu (Ross et al., *J. Bact. 187:* 905 [1985]). A lysogen of a Mu phage with a temperature-sensitive repressor was made IHF$^-$, transformed with the mutagenized plasmids, and plated at 42°C. Only transformants with defective IHF genes on the plasmids should survive. The second screen was to look for increased β-galactosidase activity from a *lacZ* fusion to a promoter known to be repressed by IHF. The promoter used was the Tn*10* transposase promoter.

We isolated 80 mutants of IHF, 50 mutants of IHF-α, and 30 mutants of IHF-β. These mutants were tested for IHF function in five different genetic assays, involving five different IHF-binding sites. About two thirds of the mutants were defective in all assays, and sequence analysis showed that these were either single-base insertions, single-base deletions, stop codons, or introduction of a proline in an α-helical region. The remaining mutants were all missense mutations. Since IHF is homologous to the bacterial histone-like protein HU, and the crystal structure of HU has been determined (Tanaka et al., *Nature 310:* 376 [1984]; White et al., *Proteins 5:* 281 [1989]), the effects of the missense mutations can be interpreted with reference to the structure of HU. Most of these changes were in amino acids that are conserved between IHF and HU in the region likely to interact directly with DNA. The strongest mutant was nonfunctional in all assays and was a glycine-to-glutamic acid change in the DNA-binding region. The effect of this mutation can be easily explained by the inability of the protein to interact with DNA due to the presence of a larger and charged amino acid residue. The rest of the mutants appeared to be defective in some assays but functional in others and included changes of phenylalanine to serine, arginine to cysteine, arginine to histidine, and proline to serine. Although these changes could weaken the protein:DNA interaction, it is not clear why the mutant proteins function in some assays but not in others. These mutants will be investigated further for DNA binding and bending to determine whether specific DNA contacts are affected.

Prediction of Protein Function from Sequence

G. Otto

We are continuing our work on the comparative analysis of sequences with the aim of predicting protein function. The general strategy is to compare a test sequence of unknown function with sets of sequences sharing known functions. Significant similarities between the test sequence and sequences of one of these sets imply that the test sequence shares the function of that set. One focus of this work is on the methods of measuring sequence similarity. We define similarity as a probability that an optimal alignment of two sequences is due just to random matching of unrelated sequences. This definition leads to a useful array of techniques for (1) comparing sets of sequences, (2) deriving a sequence pattern or motif from a set of sequences, and (3) searching for matches to a given pattern in a set of sequences.

An important goal of this work is to develop a database of sequence patterns that are predictive of specific protein functions. This will be useful as a screen to analyze newly determined sequences for possible functions. As the Human Genome Initiative begins to produce large volumes of sequence, it will be essential that methods are available to analyze those sequences automatically. A predictive motif database would be extremely useful in this regard. We have designed a prototype version of this database and are presently implementing it. In addition to exploring ways to find new motifs for entry into the database, we are tackling the difficult problem of making sure that it is completely up to date. With each new release of the sequence databases, it is necessary to ensure that the formal description of each motif is still predictive.

Advanced Computer Tools for the Analysis of Biological Sequences

J. Posfai, K. Zachmann, R.J. Roberts

FUNCTIONAL MOTIFS IN PROTEIN SEQUENCES

The sizes of sequence data libraries have increased dramatically over the past few years. In many cases,

sequences of functionally related proteins are now available from different organisms. The successful global comparisons of such related sequences often reveal the presence of conserved elements that represent regions of the proteins that are important for function and/or structure. This in turn can allow the definition of amino acid sequence motifs that are diagnostic of function or structure. A database of such diagnostic motifs would be of great importance in assigning function to new protein sequences. This database cannot conveniently be built and maintained by manual methods, since these would be too time consuming given the ever-increasing number of known sequences. Therefore, we have been developing algorithms and computer tools that enable such motifs to be generated semiautomatically.

The first step is to identify groups of functionally related sequences from the databases. This is achieved by use of the key words that appear in the annotation of database entries. For instance, these key words in the PIR database are treated as descriptors of knowledge about protein functions (e.g., binding a substrate and catalyzing a reaction), and proteins that share key words are considered to be functionally related. Such sequences are retrieved from the database, and a filtering program eliminates any duplicate sequences. This is necessary because the databases frequently contain identical duplicates. At the next step, the pairwise sequence similarity scores are calculated by the RDF method (Lipman and Pearson, *Science 227:* 1435 [1985]). A program that provides a convenient visual representation of similarity helps in the selection of the globally related sequences. This program shows the sequences selected and their interrelationships as dots and edges in a graphic display. The investigator manipulates these symbols by a high-level interface (buttons, slides, scrollbars, pop-up menus, mouse). Exceptionally high or exceptionally low scores of selected subsets of the sequences can be displayed. The valency of each point, defined as the number of sequences with exceptional scores connected to it, is also displayed. The order in which the sequences are put on the screen is determined by an algorithm, which results in globally similar sequences appearing as closely packed dots with many internal connections. The final decision in selecting the sequences for global alignment from all sequences on the screen is left to the operator, since human pattern recognition is still superior to that of the best computer algorithms. Additional tools for alignment, motif detection and testing, incorporation into the database, and update are either in prototype form or being designed.

DETECTION OF ERRORS IN DNA SEQUENCES

The DNA sequence libraries have been estimated to contain one error in every 10,000 bases. It would be extremely useful to be able to detect the locations of sequence errors before sequences were placed in databases. We have designed an algorithm that would help to detect insertion/deletion errors located in coding regions. Since these errors shift the reading frame of translation, any sequence similarity of the gene product of the correct DNA sequence with other protein sequences will be shifted from one frame of translation to another frame near the location of the error. A comprehensive error checking of a new DNA sequence by this principle requires a program that screens the protein sequence library for local similarities with any of the six translation frames of the DNA sequence. The other reading frames must be checked for continued similarity around the starts and ends of any similarities found. Such adjoining similarities could indicate possible sequence errors and would suggest that the primary data be checked carefully for inconsistencies.

Because of the sizes of sequence databases, the simple program outlined is extremely demanding in computational resources. To get results in reasonable times, instead of straightforward algorithms, we have applied a special tool of computer science, called a "finite state machine" at two critical stages of the checking process. This imaginary machine is created as a series of pointers, tables of states, and vectors of outputs when the program starts. At a cost of memory usage and computational time at the start of the program, considerable speed is gained at every cycle of the repeated steps when processing large amounts of data. One machine performs the translation from DNA sequence to amino acid sequence. This way we can actually build the translated sequences more quickly than we would get them from pretranslated sequences stored in disk files. A second machine is used to report seven out of ten (or similar) hits between two matched segments by performing a single comparison between two amino acids instead of ten comparisons.

Using this approach, we detected two errors in a particular DNA sequence in the EMBL database in which we were interested. Careful examination of the

literature that appeared after the initial determination of the sequence revealed that in a subsequent publication, the original authors had found and corrected the errors, thus verifying the validity of our approach. As our knowledge of sequences becomes more and more complete for every new sequence, the chances of finding similar ones in the databases increases and so does the potential of our method. The basic idea of searching for interrupted similarities in a protein or DNA sequence can be generalized as a technique for error detection and may also find application in the detection of consecutive introns.

Restriction Endonucleases

L. Hamablet, S. Klein, D. Macelis, J. Martling, J. Meyertons, S. Miceli, R.J. Roberts

The collection of restriction endonucleases continues to grow, and more than 1300 enzymes have now been characterized; 157 different specificities are known. During the last year, 28 new enzymes have been isolated and characterized as part of a collaborative program with I. Schildkraut and D. Comb (New England BioLabs). Among these are six valuable new specificities. Of special interest is an enzyme that we have found from a *Frankia* species that recognizes the octanucleotide sequence GGCCGGCC. This enzyme has been characterized in detail; unfortunately, it is present in extremely small quantities, and the *Frankia* strain from which it is isolated grows extremely slowly. The enzyme will be extremely valuable for genome mapping studies, since it cleaves DNA such as the human genome very rarely. Within known human DNA sequences, the frequency of *Fse*I sites is very similar to that of *Not*I sites.

In collaboration with New England BioLabs, we have begun experiments to clone the gene for *Fse*I. Initially, we have been exploring a new general strategy with which to clone cytosine methylase genes, taking advantage of the sequence motifs that we have found in cytosine methylases to design PCR primers. Initial tests of this approach in a strain of *Staphylococcus aureus*, which produces an isoschizomer of *Sau*3AI, have proved quite successful. The initial experiments with DNA from the *Frankia* species gave rise to an amplified segment that had all of the characteristics of a cytosine methylase, although it remains to be determined whether this fragment that we have cloned truly contains the counterpart methylase of the *Fse*I restriction enzyme gene.

For many years, we have maintained a database of information about restriction enzymes. This database, REBASE, has now been fully implemented in a relational format. We have been using the ORACLE Database Management System for this purpose and have written a large number of report generators so that the contents of the database can be disseminated to individual investigators. Presently, we send electronically either a standard generic form that may be reformatted by the recipient or a variety of specific formats that will directly support known packages of computer programs for identification of restriction enzyme recognition sites. One of the most popular of these is a file that can be used directly by the UWGCG software package. Further software tools are being developed that help in the acquisition and management of these data. Another major project currently under way is a package of programs that will aid in the identification of new restriction enzymes based mainly on digestion patterns on known DNAs and, when necessary, preliminary mapping of a few cleavage sites. This project involves the improved implementation of existing programs, many of which were written at Cold Spring Harbor Laboratory, together with new routines to digitize the incoming data and to allow the rapid visualization of results.

PUBLICATIONS

Hamablet, L., G.C.C. Chen, A. Brown, and R.J. Roberts. 1989. *Lpn*I, from *Legionella pneumophila*, is a neoschizomer of *Hae*II. *Nucleic Acids Res.* **17:** 6417.

Lin, P.M., C.H. Lee, and R.J. Roberts. 1989. Cloning and characterization of the genes encoding the *Msp*I restriction modification system. *Nucleic Acids Res.* **17:** 3001–3011.

Posfai, J. and R.J. Roberts. 1989. Predictive motifs of cytosine methylases. *J. Cell Biochem.* (suppl.) **213**.

Pósfai, J., A.S. Bhagwat, G. Pósfai, and R.J. Roberts. 1989. Predictive motifs derived from cytosine methyltransferases. *Nucleic Acids Res.* **17:** 2421–2435.

Roberts, R.J. 1989. Restriction and modification enzymes and their recognition sequences. In *CRC practical handbook of biochemistry and molecular biology* (ed. G.D. Fasman), pp. 470–511. CRC Press, Boca Raton.

Roberts, R.J. 1989. Restriction enzymes and their isoschizomers. *Nucleic Acids Res.* **17:** r347–r387.

Roberts, R.J. 1989. Benefits of databases. *Nature* **342:** 114.

In Press, Submitted, and In Preparation

Bhagwat, A.S., B. Johnson, K. Weule, and R.J. Roberts. 1990. Primary sequence of the *Eco*RII endonuclease and properties of its fusions with β-galactosidase. *J. Biol. Chem.* **265:** 767–773.

Card, C.O., G.G. Wilson, K. Weule, J. Hasapes, A. Kiss, and R.J.

Roberts. 1990. Cloning and characterization of the *HpaII* methylase gene. *Nucleic Acids Res.* **18:** (in press).
Meyertons, J.L., S.M. Miceli, M.P. Lechevalier, and R.J. Roberts. 1990. FseI, A new type II restriction endonuclease which recognizes the octanucleotide sequence 5′ GGCCGGCC 3′ *Nucleic Acids Res.* **18:** (in press)
Roberts, R.J. 1990. Restriction enzymes and their isoschizomers *Nucleic Acids Res.* **18:** (in press)

MACROMOLECULAR CRYSTALLOGRAPHY

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A primary objective of the Macromolecular Crystallography Group is to determine the structures of a number of biologically important proteins to atomic resolution in order to better understand their functions in cell processes such as signal transduction, growth control, and development. Our studies are designed to benefit from and to complement the genetic and biochemical experiments performed by other groups at the Laboratory. Together with these biochemical data, crystallographic models of kinases, oncogene products, *trans*-acting factors, and other proteins will help us to comprehend the roles of these macromolecules in cell division, in cell transformation, and, ultimately, in afflictions such as cancer, AIDS, and Alzheimer's disease.

Our crystallographic experiments require well-formed crystals of the macromolecule being investigated. To grow crystals of a useful size (>0.3 mm on a side), one must have available several milligrams of the highly purified macromolecule. Although this does not guarantee that crystals can be produced, it is the minimal prerequisite before attempting to crystallize the molecule. To this end, a major part of our laboratory and time is devoted to producing and purifying to crystallographic homogeneity the proteins we are studying. When a suitable crystal is obtained, it is placed in an intense X-ray beam smaller than a pencil lead. The X-ray beams diffracted by the crystal are measured on our area detector. We must quantitate tens of thousands of these diffracted beams from several dozen different crystals. Fortunately, these experiments are computer controlled, with much of the software written in-house. Only after all the data are collected can we begin to construct a three-dimensional model of the macromolecule—all the atoms and bonds—with the aid of computer graphic tools.

Structural Studies of *PvuII* Endonuclease

J. Anderson, F.-C. Chuan [in collaboration with I. Schildkraut, New England BioLabs]

We have two crystal forms of the restriction endonuclease from *Proteus vulgaris*, R·*PvuII*, that diffract X-rays to 2.5 Å resolution. R·*PvuII* binds to the DNA site CAGCTG and cleaves both strands between the internal cytosine and guanine. One of the crystal forms is space group P422, with $a=b=176.2$ Å, $c=50.6$ Å. It grows as trapezoidal prisms by vapor diffusion in hanging drops over reservoirs containing 0.13 M ammonium acetate, 0.065 M sodium acetate, and 20% PEG-4000 (pH 5.5). Each 4-6-µl drop yields 1–10 crystals in several days. Crystals of the second crystal form, which appear much less frequently under the same conditions, are orthorhombic needles with unit cell dimensions of 106, 84, and 47 Å. The space group has not yet been determined, due to the scarcity of crystals large enough for diffraction experiments.

We are currently collecting native data from the P422 crystals and are preparing to make heavy atom derivatives. We are making slight modifications of the crystallization conditions in an effort to increase the frequency and size of the orthorhombic needles so that we can characterize them and collect data from them. We are also preparing to set up cocrystallizations of R·*PvuII* with oligonucleotides carrying

its recognition site in order to determine the structure of the complex and compare it to that of the free protein.

Structural and Functional Studies of Fos and Jun Proteins

C.K. Cheung, E. Chang, F.-C. Chuan, J. Anderson
[in collaboration with C. Abate and
T. Curran, Roche Institute]

The protein products of the proto-oncogenes c-*fos* and c-*jun* interact to form a DNA-binding transcription factor that is a major component of the activity of the eukaryotic transcription factor AP-1. Fos/Jun heterodimers bind to DNA at AP-1 sites (TGACTCA) in the promoters of many genes, including the human immunodeficiency virus (HIV). Once bound, they can activate or repress transcription. We intend to crystallize these proteins and determine their structures in the presence and absence of oligonucleotides carrying AP-1 sites. This will help us to understand the role that these proteins play in normal cellular regulation and in the regulation of HIV, and the role altered versions of these proteins play in oncogenesis.

OVEREXPRESSION AND PURIFICATION OF JUN

We overexpressed Jun in *Escherichia coli* using a bacteriophage T7 expression system. Bacterial cells freshly transformed with the expression plasmid and induced at 37°C with isopropyl-β-D-thiogalactoside produce about 30% of their total protein as Jun. Jun is in the insoluble fraction of the crude cell lysate, and after an initial centrifugation, the overexpressed protein is already 80–90% pure. The pellet is resuspended in 6 M urea and loaded onto a heparin-Sepharose column equilibrated in 6 M urea. About 4 mg of Jun eluting in 1 ml from the heparin column at about 100 mM NaCl in a linear salt gradient is denatured with SDS and β-mercaptoethanol (BME) and layered as a single sample onto a 1.5-mm thick 10% SDS-polyacrylamide gel. After electrophoresis, the protein is located by staining a small slice of the gel with Coomassie blue. The protein band is sliced out of the gel, diced, and electroeluted in 50 mM ammonium bicarbonate overnight in a Bio-Rad electroelution apparatus; at the same time, the SDS is removed by dialysis against a large excess of 50 mM ammonium bicarbonate. About 2 mg of Jun is recovered after electroelution. By silver-staining, it is judged to be greater than 98% pure.

STRUCTURAL DOMAINS OF JUN

After digestion of purified Jun in phosphate-buffered saline (PBS) with a variety of proteases (*Staphylococcus aureus* V8 protease, chymotrypsin, protease K, papain, trypsin, and thermolysin), relatively resistant fragments consistently appear. The fragments have apparent molecular weights of approximately 30K from SDS-PAGE. Since sites for these proteases exist throughout the protein, we interpret this to mean that the 30K polypeptides are folded in such a way that the sites are protected from cleavage. In other words, these fragments represent folded structural domains. The 30K fragments from V8 and chymotrypsin digestion were isolated by gel electroelution, and their amino-terminal sequences were determined. The V8 fragments have rather heterogeneous amino termini, but they tend to begin in the neighborhood of residues 80 and 110. The chymotryptic fragment begins at residue 99. The differences in amino termini probably reflect the availability of protease sites in the sequence. We made a preparative digestion of Jun with chymotrypsin, and purified the 30K fragment, called Jun99, by electroelution as described above for intact Jun.

GEL SHIFT EXPERIMENTS SHOW THAT BACTERIAL JUN PROTEINS ARE ACTIVE

Both Jun and Jun99 prepared as described above bind to DNA carrying an AP-1 site in initial gel-shift experiments (C. Abate). The binding of both is improved in the presence of a truncated Fos protein comprising the Fos DNA-binding domain, which forms heterodimers with Jun and Jun99.

CRYSTALLIZATION OF FOS AND JUN PROTEINS

We are currently carrying out crystallization trials with purified Jun. When sufficient quantities of purified Jun99 become available, we will attempt to crystallize it as well. We are attempting to cocrystallize Jun with the Fos DNA-binding domain. We will also try to cocrystallize the proteins with oligonucleotides carrying an AP-1 site. Once crystals are in hand, we will determine their structures using X-ray crystallography.

Structural and Functional Analyses of CRE-binding Proteins

E. Chang, T. Sellati [in collaboration with M. Gilman and D. Marshak, Cold Spring Harbor Laboratory]

Transcription of many cellular and viral genes is induced by cAMP. This often involves the binding of protein factor(s) to a conserved sequence called the cAMP-responsive element (CRE; TGACGTCA) in the promoter region of these genes. Many CRE-binding (CREB) proteins have been cloned and isolated by a number of research groups. However, the mechanism by which these proteins stimulate transcription remains unknown. We are studying the CREB protein with the hope of learning how this protein regulates gene expression in response to cAMP. To facilitate our studies, we have overexpressed two CREB proteins, CREB2 and CREB6, in *E. coli*. CREB2 and CREB6 are very similar to each other, except that CREB6 contains 14 additional amino acid residues in its amino-terminal region. These CREB proteins are produced in the soluble fraction of *E. coli* extracts and can be easily purified to 90% homogeneity.

CREB2 has been further characterized. Several lines of evidence emerging from these studies suggest that the *E. coli*-produced CREB2 protein is functionally active and therefore has a conformation similar to its counterpart from mammalian sources: (1) The *E. coli*-produced CREB protein retains its sequence-specific binding activity. Results from gel-shift experiments indicate that CREB2 binds to a DNA fragment containing the −65 CRE sequence from mouse c-*fos* promoter but fails to bind to a similar fragment carrying mutations in the CRE. (2) The DNase I footprint generated by *E. coli*-made CREB2 protein on the c-*fos* CRE is similar to that determined by other groups for CREB protein isolated from mammalian cells. (3) The *E. coli*-made CREB2 protein stimulates transcription in vitro from a c-*fos* promoter containing the wild-type −65 CRE sequence but not the same promoter carrying a mutant CRE. (4) The *E. coli*-made CREB protein, like the CREB proteins produced by mammalian cells, can be phosphorylated by protein kinases.

We are currently studying several aspects of CREB structure and function. Since proteins produced in bacteria are largely unmodified, the recombinant CREB proteins will be very useful for studying the effects of phosphorylation on CREB structure and function. We will phosphorylate the *E. coli*-made CREB proteins with protein kinase(s) and determine whether the modification affects their DNA-binding and transcriptional activities. Limited proteolytic digestion of the *E. coli*-produced CREB2 protein with *S. aureus* V8 protease generates a 30-kD protease-resistant fragment. We will determine if phosphorylation will change the protease digestion pattern. In addition to the full-length CREB protein, we have overexpressed several truncated CREB proteins in *E. coli*. We are now characterizing these proteins with respect to their ability to bind to the CRE and to stimulate CRE-dependent transcription. These experiments initiate our efforts to identify important structural and functional domains of CREB.

Finally, we are further purifying the *E. coli*-made full-length and truncated CREB proteins. Once this is accomplished, we will try to crystallize the proteins and determine their three-dimensional structures in the presence and absence of the cognate DNA substrate. Results from such studies will help to reveal the structural basis for substrate specificity. Furthermore, these results, together with the results of similar studies of Jun (see above), will help to explain how the similarities and differences between CREB and Jun affect their ability to recognize and bind to similar DNA sequences.

Yeast cAMP-dependent Protein Kinase

J.W. Pflugrath, T. Malone, D. Sterner [in collaboration with J. Kuret, Cold Spring Harbor Laboratory]

Protein kinases are key regulatory molecules that modulate many cellular processes including cell growth, differentiation, and proliferation. Nearly 100 protein kinases have been identified thus far, each of which is capable of integrating input signals and coordinating physiological responses by phosphorylating a specific range of substrate proteins. A detailed three-dimensional structure would expand our knowledge of this class of important enzymes to the molecular level. Toward this end, we have initiated a crystallographic study of the cAMP-dependent protein kinase from *Saccharomyces cerevisiae*, also known as TPK1.

From extremely pure preparations of genetically engineered TPK1 (see J. Kuret's section), we grew kinase crystals in the hexagonal space group $P6_122$ (or its enantiomorph) with unit cell lengths of 61 Å

by 61 Å by 320 Å. Density measurements of these crystals indicate the presence of 12 protein molecules in the unit cell or one per asymmetric unit. We measured diffraction intensities on our Enraf-Nonius FAST area detector with monochromatized X-rays generated by a GX-21 rotating anode operated at 45 kV, 95 mA. All the data collection experiments were controlled by the software package MADNES, developed in part by us. The reproducibility of the data as determined by R_{sym} ranged from 4% to 8%. To solve the crystallographic phase problem, we have soaked numerous crystals in a stabilizing buffer containing different heavy atom compounds. Although many such treatments cracked or dissolved the crystals, we collected diffraction intensities on the intact crystals to find any that had incorporated the heavy atom. Isomorphous difference Patterson maps calculated with data from two separate crystals treated with a gold compound and a mercury compound showed significant substitution. These should allow us to calculate an electron density map in the coming months. Further heavy atom screening will continue. Finally, data from several native crystals were collected at the National Synchrotron Light Source beamline X12-C with the generous assistance of R. Sweet. We will continue to use this national resource.

Area Detector Software

J. W. Pflugrath [in collaboration with the EEC Cooperative Workshop on Position-Sensitive Detectors]

Although we continue to develop and improve the device-independent area detector software system MADNES, the year ended with the software in a mature state. This system is used by our group and dozens of other crystallography laboratories worldwide to collect and process X-ray diffraction data from several kinds of area detectors. Our group coordinates the maintenance and debugging of MADNES. This is done through a digest distributed worldwide to interested colleagues via a computer network. In the past year, MADNES was adapted for use on two new kinds of detectors, including a new experimental CCD-based detector of M. Strauss and E. Westbrook of Argonne National Laboratory for use at synchrotrons. As more scientists use the program, it has become clear that a new intuitive user interface is required. We have begun to address this need.

PROTEIN KINASE STRUCTURE AND FUNCTION

J. Kuret A. O'Connor
K. Kopecek

Protein kinases are enzymes that catalyze the transfer of the γ-phosphoryl group of ATP to nucleophilic amino acids of protein substrates, thereby modulating the substrates' biochemical function. They are employed by cells for regulatory purposes because of the fine control and high signal amplification achievable by reversible protein phosphorylation; evidence of their importance in cell biology can be found throughout this volume. Our research combines the techniques of X-ray crystallography and molecular genetics to study these enzymes in detail.

Yeast cAMP-dependent Protein Kinase

J. Kuret, A. O'Connor [in collaboration with J.W. Pflugrath, Cold Spring Harbor Laboratory]

Our first aim is to elucidate the structural basis of protein kinase activity, a goal that is crucial for understanding how these enzymes modulate a limited subset of proteins. For years, this question has been approached by chemical, enzymological, and genetic

methods by many investigators, with each new technique surpassing its predecessor in elegance. Yet, in the end, the problem can be solved only with a high-resolution three-dimensional structure of a protein kinase. Therefore, we initiated a crystallographic study of the yeast cAMP-dependent protein kinase (cAMPdPK) catalytic subunit (TPK1). We chose to study this enzyme because (1) its gene has been cloned (by Mike Wigler's Laboratory, see Molecular Genetics of Eukaryotic Cells Section), (2) mutant proteins with altered catalytic properties can be obtained using genetic screens in yeast, (3) it can be overexpressed in a soluble form in yeast, and (4) it retains the well-characterized structural and kinetic features of the mammalian enzyme. Thus, the yeast system allows us to study the structure and function of a protein kinase using diverse techniques.

The first step in our structural approach to kinase function was to simplify the primary structure of TPK1 as much as possible while retaining its enzymatic properties. We reasoned that by reducing main-chain flexibility and microheterogeneity, the resolution obtainable from X-ray crystallography could be maximized. Moreover, by minimizing the mass of TPK1, we could employ a wide range of heavy atom compounds for isomorphous replacement and make nuclear magnetic resonance (NMR) analysis of its active site practical. A similar strategy proved valuable in the successful study of c-Ha-*ras* p21 tertiary structure.

Examination of the primary structure of TPK1 reveals an amino terminus extending far beyond the beginning of the canonical protein kinase domain and bearing little similarity to any known kinase structure, including TPK2 or TPK3 (two isozymes of TPK1). Many *Saccharomyces cerevisiae* genes encode proteins far larger than their mammalian homologs, including *BCY1* (the cAMPdPK regulatory subunit), without conferring any obvious functional advantage on them. This strange organization of the *S. cerevisiae* genome may result from reverse transcription of yeast mRNA to cDNA, which, after insertion into chromosomes by recombination, creates genes deficient in introns and extended at their 5′ ends. Regardless of their origin, nonessential sequences can complicate structural studies. In the case of TPK1, the amino-terminal 83 amino acids contain five of the eight most flexible regions in the protein, as predicted by the algorithm of Karplus and Schulz. Moreover, this same region contains the most hydrophilic region of the protein, as predicted by the method of Hopp and Woods, which is consistent with it being highly solvated and flexible.

Considering that the structural features potentially detrimental to crystallography were located in the amino-terminal 83 amino acid residues of TPK1, that the sequence similarity among TPK1, TPK2, and TPK3 begins 14 residues from the first glycine of the putative nucleotide binding loop, and that all protein kinases contain at least ten amino acids amino-terminal to this glycine, we decided to eliminate residues 1–80 of TPK1 and replace them with the synthetic amino terminus Met-Pro-. We chose Met-Pro- as the amino terminus for truncated TPK1 because this sequence is a good substrate for the yeast methionyl aminopeptidase and is a poor substrate for known posttranslational modifications. Thus, the engineered sequence (termed TPK1Δ) eliminates nearly 20% of the mass of TPK1, reduces main-chain flexibility, retains homology with TPK2 and TPK3 at the amino terminus, and cannot be modified post-translationally.

We overexpressed TPK1Δ in yeast, developed a novel purification procedure that yielded enzyme of very high purity, and characterized its biochemical properties. As summarized in Table 1, TPK1Δ is very similar to its parent in substrate affinity, maximum velocity, and sensitivity to the protein kinase inhibitor analog PKI_{5-24}. We conclude that TPK1Δ retains all the structural features necessary for protein kinase activity and that determination of its three-dimensional structure will help us to localize the amino acid residues responsible for catalysis and substrate recognition.

With highly purified and concentrated enzyme in hand, we searched for suitable TPK1Δ crystallization conditions using the hanging-drop vapor diffu-

TABLE 1 Properties of Catalytic Subunits: Yeast TPK1 and TPK1Δ

Kinase	TPK1	TPK1Δ
Physical properties		
quarternary structure	monomer	monomer
amino acid residues	396	318
molecular mass	46,000	37,300
amino-terminal blocking group	?	none
Kinetic properties		
V_{max} (μmole/mg/min)	13.2	11.1
K_m Kemptide (μM)	101	83
K_m MgATP (μM)	33	45
K_i PKI_{5-24} (nM)	280	125
casein phosphorylation	no	no

sion technique combined with an incomplete factorial search strategy. Remarkably, after consuming just 1.5 mg of protein, we discovered hexagonal crystals growing over a period of 2–4 days at 4°C. After careful refinement of precipitant concentrations, we were able to grow crystals of the same morphology at room temperature as well (Fig. 1). These conditions have consistently yielded crystals from several separate preparations of the protein. Our best crystals are obtained at this temperature by seeding, are hexagonal dipyramids, and have the dimensions 0.5 × 0.5 × 1.5 mm. The close of the year found Jim Pflugrath employing the data collection software package MADNES to record the crystal's diffraction pattern and screening for suitable heavy atom reagents required to solve the structure by the method of isomorphous replacement.

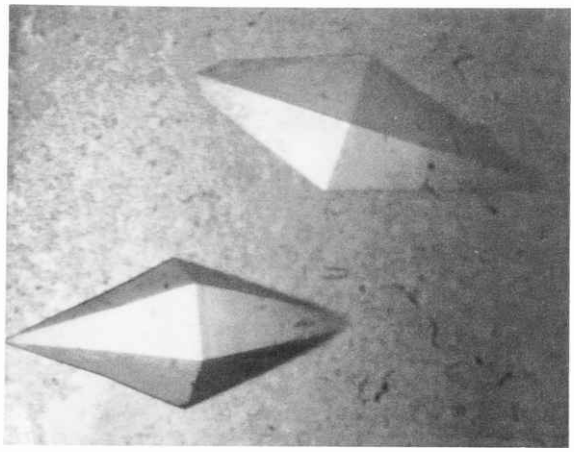


FIGURE 1 Crystals of TPK1Δ.

Yeast Casein Kinase 1

J. Kuret, A. O'Connor

We are also using *S. cerevisiae* to study casein kinase 1, a ubiquitous protein kinase in eukaryotes. Our principal goals are to purify the enzyme from yeast, to determine its primary structure by sequencing its gene, and to elucidate its biological function by a combination of genetic and biochemical approaches. During the past year, we successfully purified casein kinase 1 from *S. cerevisiae* to near homogeneity using classical purification procedures. The enzyme is a monomer of 54,000 daltons in solution, possesses a blocked amino terminus, is inhibited by heparin ($IC_{50} = 80$ ng/ml), has an isoelectric point of 9.0, and has a turnover number of approximately 4.3 when assayed with casein. Although these physical and kinetic features are very similar to those of mammalian casein kinase 1, definitive proof must be provided at the level of amino acid sequence. We are in the process of isolating the cDNA encoding casein kinase 1 from a yeast cDNA expression library prepared in bacteriophage λ.

In press, Submitted, and In Preparation

Kuret, J. and F. Murad. 1990. Adenohypophyseal hormones and related substances. In *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 8th edition (ed. Gilman et al.). Macmillan, New York. (In press.)

Kuret, J. and A. O'Conner. 1990. Purification and physical properties of casein kinase-1 from *Saccharomyces cerevisiae*. (In preparation.)

Kuret, J. and J. Pflugrath. 1990. Crystals of the cAMP-dependent protein kinase from *Saccharomyces cerevisiae* diffract X-rays to 2.7 Å resolution. (In preparation.)

Murad, F. and J. Kuret. 1990. Estrogens and progestins. In *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 8th edition (ed. Gilman et al.). Macmillan, New York. (In press.)

NEUROSCIENCE

Neuroscience at Cold Spring Harbor Laboratory has focused on the role of growth factors in the differentiation and aging of neural cells. We have been bolstered this year by the dramatic finding that the neurite extension factor, a form of the protein S100β, is elevated in certain cells surrounding the amyloid plaques in Alzheimer's disease. We are also pursuing the role of this factor in Down's syndrome, a developmental neurological disease. As we look forward to the new Neuroscience Center at Cold Spring Harbor, our studies continue to probe the fundamentals of growth and differentiation of neurons, particularly as it pertains to human neurological diseases.

NEURONAL GROWTH AND DIFFERENTIATION

D.R. Marshak Y.S. Bae, N. Chester, J.C. Figueiredo, S. Pesce, A. Vongs

During the last 4 years, the work in our laboratory related to neuroscience has centered on the outgrowth of processes, or neurites, from embryonic neurons in cell culture. This system serves as a model for the development of neurons in vivo, by allowing us to isolate and characterize a neurite extension factor. We have characterized the structure and function of the factors and have developed reagents to permit analysis of brain tissue taken from patients with several neurological diseases on autopsy. The past year has revealed some spectacular findings related to the neurite extension factor protein, S100β, and neurological diseases.

Role of S100β in Alzheimer's Disease

D.R. Marshak, S. Pesce [in collaboration with W.S.T. Griffin, University of Arkansas]

A significant segment of the population over 65 years of age is afflicted with Alzheimer's disease (AD), a neurodegenerative process that results in memory loss and progressive dementia. The hallmarks of AD neuropathology are prevalent in temporal lobe structures, such as the hippocampal formation, that have been associated with learning and memory. These neuropathological symptoms include an abnormal number of neurons that contain neurofibrillary tangles, extracellular plaques with β-amyloid cores, and reactive astrocytes. A puzzling feature of plaque pathology is the presence of enlarged neurites encircling the β-amyloid deposits. The presence of soluble neurotrophic factors could give rise to inappropriate growth of neurites. In support of this idea, we have shown that the levels of a neurotrophic factor, the protein S100β, and its encoding mRNA are elevated in AD, but not control, brain temporal lobe. Moreover, AD temporal lobe extracts had more S100β-specific neurotrophic activity than similar extracts from age-matched controls. We have suggested that elevated levels of S100β contribute to the cellular neuropathology in AD.

Levels of S100β in Down's Syndrome

D.R. Marshak, S. Pesce [in collaboration with P. Whitaker, SUNY Stony Brook, and S. Kish, Toronto]

Down's syndrome (DS) is a developmental disorder arising from extra copies of all or part of human chromosome 21, by either trisomy, partial duplication, or translocation. In addition to mental retarda-

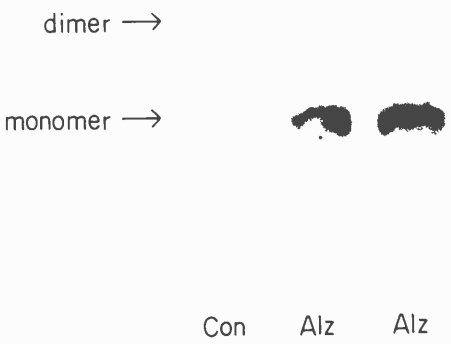


FIGURE 1 Autoradiograph of protein immunoblots of AD and control samples of temporal lobe. Samples extracted in hypotonic buffer containing nonionic detergent were subjected to polyacrylamide gel electrophoresis, transferred to nitrocellulose filters, and incubated with S100β antibodies followed by radioiodinated goat anti-rabbit immunoglobulin.

tion, DS patients that live to middle age invariably develop a degenerative neurological disorder that is indistinguishable from AD. This is further supported by the findings that genes related to AD reside on chromosome 21, although distinct from the DS regions. Because the gene for S100β is located on chromosome 21 near the telomere of the long arm, we tested samples of DS autopsy brain for S100β levels. It appears that immunoreactive levels of S100β are elevated in DS early in life, reaching fivefold over normal levels at 3 months and decreasing to normal levels by 9 months. This correlation holds for both frontal and parietal cortex in 21 normal and DS samples. We are trying to obtain autopsy tissue from DS patients of various ages, including middle age, elderly, and fetal, to attempt to identify the critical time of S100β elevation in human brain development.

Molecular Cloning and Expression

D.R. Marshak, S. Pesce, J. Figueiredo, N. Chester

Isolation of a cDNA clone for S100β was done for several reasons. First, cloning of the cDNA in an Okayama-Berg vector allowed transfection in COS cells and expression of the protein in mammalian cells. Second, subcloning the coding region into a commercial vector (pBS⁺; Stratagene) allowed production of a cRNA probe for slot-blot and Northern blot analyses of RNA from cells and tissues. Third, the clone provides a vector for constructing transgenic mice that overexpress the protein as models of neurological disease. Oligonucleotides corresponding to the 5′ and 3′ ends of the coding region were synthesized and used to screen a rat brain cDNA library made in pcD2, a mammalian expression vector that utilizes the SV40 early promoter. Of 63 clones that were positive with the 3′ probe, 15 clones that were also positive for the 5′ probe were selected. Restriction enzyme maps and DNA sequence analysis permitted the selection of a full-length clone with a total insert of 1.6 kb. Transfection of COS cells followed by labeling with [^{35}S]methionine and immunoprecipitation yielded a full-length protein product comigrating on polyacrylamide gels with native S100β. Extracts of the transfected cells showed neurite outgrowth activity, whereas extracts of mock-transfected or untransfected cells showed no neurite extension factor activity.

The coding region of the cDNA clone was isolated as a 916-bp *Bam*HI fragment and subcloned into pBS⁺. Restriction maps of this construct indicated that the insert was oriented with the 5′ end of the complementary strand under control of the T3 promoter. Synthesis of RNA probes using the T3 polymerase resulted in positive slot blots and Northern blots of brain RNA. Finally, transgenic mice are being constructed to provide an animal model for

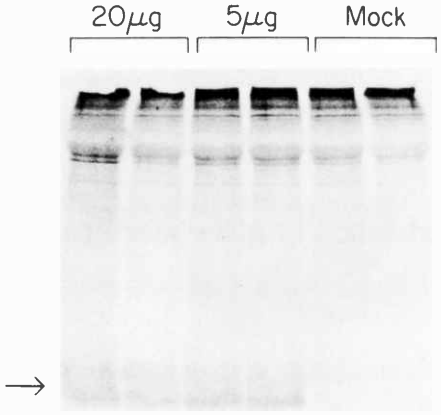


FIGURE 2 Immunoprecipitation of S100β expressed in COS I cells. Cells were transfected with 5 μg or 20 μg plasmid (as indicated) or with salmon sperm DNA (Mock). The cells were labeled and extracted as described in the text. The extracts were subjected to polyacrylamide gel electrophoresis and autoradiography. Arrow shows the position of migration of S100β.

Down's syndrome. We are also investigating trisomy ten mice (with C. Epstein, UCSF) that have an extra copy of the S100β gene and severe neurological defects. The cDNA probe has also been used in collaboration with K. Gardiner of the Eleanor Roosevelt Institute in Denver to confirm the localization of the gene and continue fine-structure mapping of chromosome 21. Present experiments revolve around the construction of expression plasmids that utilize tissue-specific promoters for specific populations of cells in the brain.

Role of S100β In Vivo

D.R. Marshak, S. Pesce [in collaboration with E. Azmitia, New York University, and P. Whitaker, SUNY Stony Brook]

We have begun to examine the role of S100β in vivo using the rat model system. The findings of Drs. Whitaker and Azmitia indicate that serotonergic neurons of the brain stem that innervate the hippocampus appear to respond to S100β as a neurotrophic factor. Combined with our findings of increased S100β in temporal lobe areas including the hippocampus, this suggests that S100β may play a key role in the normal development of contacts between serotonergic neurons of the Raphe nuclei and neurons of the hippocampus. Abnormal levels of S100β either during development (as in Down's syndrome) or in aging (as in Alzheimer's disease) may lead to errors or lesions in these connections. We have demonstrated that serotonergic agonists can specifically release S100β from glial cells that surround the neurons of the hippocampus, indicating that the neurotransmitter compound itself might regulate the release of the neurotrophic factor in the developing brain. Further studies indicate that chemical lesion of the connections between the brain stem and the hippocampus results in up-regulation of serotonin

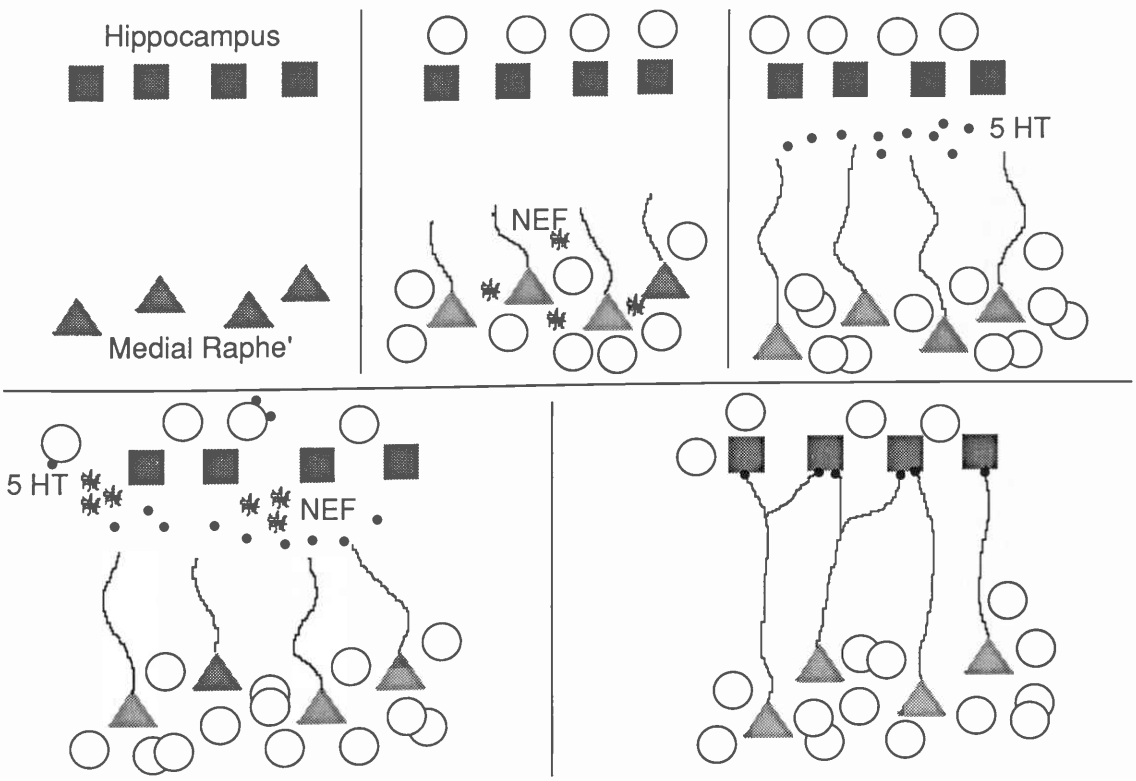


FIGURE 3 Working model of neurite factor (NEF) action in the developing nervous system. In each panel, the triangles represent developing serotonergic neurons of the Raphe nuclei, the squares represent target neurons in the hippocampus, and circles represent astroglial cells. When astrocytes of the Raphe proliferate, they release NEF (*), which induces neurite outgrowth from the serotonergic neurons. These cells grow axons that innervate the hippocampus and release serotonin (5-HT). The serotonin induces NEF from hippocampal glia, which in turn promotes continued outgrowth of axonal processes. The final result is a mature serotonergic innervation of the hippocampus.

receptors and sensitivity to S100β release by serotonergic agonists. Injection of radiolabeled S100β into the hippocampus indicates that much of the S100β released is associated with hippocampal neurons. Further studies are in progress to map the pathways and pharmacology of S100β action in vivo, which may reveal some of the characteristics of S100β function in normal brains and in diseased states.

Structural Analysis of Neurite Extension Factor

D.R. Marshak [in collaboration with J. Pflugrath, Cold Spring Harbor Laboratory]

Previously, sequence analysis and gel electrophoresis indicated that neurite extension factor (NEF) consisted of a disulfide form of the protein S100β. Subsequent analysis of various preparations of NEF suggested that the active species was a disulfide dimer of two identical subunits. In further experiments, monomer and dimer species have been identified on immunoblots of brain extracts of humans and in animal models, including rat, chicken, and cow, indicating that the dimer is present in vivo. Separation of monomer and dimer species has been accomplished by high-performance electrophoresis chromatography on polyacrylamide gels and by HPLC gel-filtration chromatography in the presence of acetonitrile at either pH 6.0 phosphate buffer or 0.1% (w/v) trifluoroacetic acid. In all cases, the dimeric form appears to be the active species. However, concentration of the protein under acidic conditions gives rise to polymers, which can be reduced by treatment with 2-mercaptoethanol. Analysis of the protein by plasma desorption mass spectrometry gave a subunit molecular weight of 10,553, consistent with an acetylated amino terminus, and no other covalent modifications. Therefore, the present evidence points to a disulfide dimer as the active species.

Further analyses of the three-dimensional structure of the S100β monomer and dimer are being accomplished by solving the X-ray crystal structure. In collaboration with J. Pflugrath at Cold Spring Harbor Laboratory, we are attempting to grow crystals of S100β suitable for X-ray analysis. We have also succeeded in synthesizing the protein chemically using solid-phase methods on an automated instrument. The protein has 91 amino acids and took approximately 4 weeks to synthesize after several initial failures. Purification and characterization of the product are now under way. We would like to compare the three-dimensional structures of the native, synthetic, and dimerized forms of S100β.

Regulation of Brain Protein Kinases

Y.S. Bae, D.R. Marshak

The action of growth factors in the nervous system has been implicated in the onset of symptoms of neurological disease, such as Alzheimer's disease. We have begun to study the regulation of the signal transduction systems that are stimulated by growth factors in the brain. In particular, we have purified casein kinase II (CK-II) from bovine brain to examine the stimulation and repression of the activity. Brain CK-II appears to have an endogenous inhibitor as well as compounds that can activate the enzyme. Our current research focuses on the isolation and characterization of molecules that regulate this kinase. CK-II is important to transcriptional control, and it is possible that alterations in CK-II levels or activity are associated with degeneration of neurons.

TABLE 1 Mass Spectrometric Analysis of Bovine Pancreatic Thread Protein (PTP)

Protein	Observed M/Z	Calculated M/Z	Difference (%)
Unmodified intact PTP			
$[M+H]^+$	15,036.11	15,035.96	0.001
$[M+2H]^{2+}$	7,519.99	7,518.49	0.020
$[M+3H]^{3+}$	5,013.30	5,012.66	0.013
Unmodified A chain			
$[M+H]^+$	11,073.13	11,073.39	0.002
$[M+2H]^{2+}$	5,537.04	5,537.20	0.003
Unmodified B chain			
$[M+H]^+$	3,973.07	3,969.63	0.087
Pyridylethylated A chain			
$[M+H]^+$	11,390.35	11,388.81	0.014
$[M+2H]^{2+}$	5,694.72	5,694.91	0.003
Pyridylethylated B chain			
$[M+H]^+$	4,286.40	4,285.05	0.032
$[M+2H]^{2+}$	2,144.03	2,143.03	0.047

Structural Analysis of Pancreatic Thread Protein

D.R. Marshak [in collaboration with J. Crabb, W. Alton Jones Cell Science Center, and J. Gross, Massachusetts General Hospital]

The pancreatic thread protein (PTP) is a macromolecule that forms helical structures that can be identified under electron microscopy and is a component of insoluble occlusions found in the pancreas. Interestingly, monoclonal antibodies against the protein identify the same protein in brain lesions found in Alzheimer's disease. The protein was isolated from bovine pancreas and was found to consist of two disulfide-bonded chains. Plasma desorption mass spectrometry allowed us to measure the molecular weight of the intact protein, as well as the separate A and B chains. Upon reduction and alkylation of the protein with 4-vinylpyridine, each chain was found to have three cysteinyl residues by measuring the increase in mass due to the chemical modification. Further analysis of proteolytic fragments of the unreduced protein allowed us to assign the complete disulfide bonding pattern. This is another example of the utility of mass spectrometry in conjunction with classic methods of protein chemistry in the analysis of proteins involved in neurological disease.

PUBLICATIONS

Jaiswal, R.K., D.R. Marshak, and R.K. Sharma. 1989. Rat adrenocortical and human platelet α_2-adrenergic receptors are biochemically indistinguishable. *Mol. Cell. Biochem.* **86:** 41–53.

In Press, Submitted, and In Preparation

Cai, L., W.R. Harris, D.R. Marshak, J. Gross, and J.W. Crabb. 1990. Structural analysis of bovine pancreatic thread protein. (Submitted.)

Marshak, D.R. 1990. S100β as a neurotrophic factor. *Prog. Brain Res.* (in press).

Marshak, D.R., S.A. Pesce, L.C. Stanley, and W.S.T. Griffin. 1990. Increased S100β neurotrophic activity in Alzheimer's disease temporal lobe. *Nature* (Submitted.)

Sugrue, M.M., J.S. Brugge, D.R. Marshak, P. Greengard, and E.L. Gustafson. 1990. Immunocytochemical localization of the neuron-specific form of the c-*src* gene product, pp60$^{c\text{-}src(+)}$ in rat brain. *J. Neurosci.* (in press).

CSH LABORATORY JUNIOR FELLOWS

In 1986, Cold Spring Harbor Laboratory began a new 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 one fellow each year to work independently at the Laboratory for a period of up to 3 years on projects of their choice. Each fellow is provided with a salary, research support, and technical assistance so that they can accomplish their goals free from other distractions. The interaction among research groups at the Laboratory and the program of courses and meetings on diverse topics in molecular biology contribute to a research environment that is ideal for innovative science by these fellows.

The first Cold Spring Harbor Fellow (1987) was Adrian Krainer, a former graduate student with Tom Maniatis at Harvard University. The 1988 Fellow was Carol Greider from Elizabeth Blackburn's laboratory at the University of California, Berkeley. The 1989 Fellowship was awarded to Eric Richards from Fred Ausubel's laboratory at the Massachusetts General Hospital. Dr. Richards joined the Laboratory during the summer of 1989 and is studying the molecular biology of the centromeres and telomeres of *Arabidopsis*.

C.W. Greider
E.J. Richards

Telomerase: The Long and the Short of It

C.W. Greider, L.A. Harrington, S. Kaplan

TELOMERASE BIOCHEMISTRY AND REACTION MECHANISM

Telomerase is an RNA-containing enzyme that synthesizes repeats of the telomeric sequence d(TTGGGG)$_n$ onto the ends of chromosomes. This enzyme may be involved with both normal telomere replication and healing of broken chromosomes. Without a mechanism to elongate telomeres, one would predict that chromosomes might shorten from their ends due to the inability of DNA polymerases to replicate completely both strands at the end of a DNA molecule. Thus, telomeres, in conjunction with telomerase, serve to replicate and protect chromosome ends. We are interested in both the biochemistry of telomerase and the biology of telomere replication in vivo.

The telomerase enzyme is a unique kind of DNA polymerase; it contains an essential RNA component that provides a template for the d(TTGGGG)$_n$ repeats which the enzyme synthesizes. Last year, we cloned and sequenced the *Tetrahymena* telomerase RNA component and showed that it contained the sequence 5′-CAACCCCAA-3′. The presence of one and a half repeats of the sequence CCCCAA suggested that d(TTGGGG)$_n$ sequence primers could be stably bound, elongated, translocated, and then further elongated in a processive manner as shown in Figure 1A. However, an alternative is that telomerase may add a single repeat, dissociate from the primer, and then rebind for a further round of d(TTGGGG) addition (Fig. 1B). To distinguish between the processive and distributive mechanisms, we carried out primer challenge experiments. Telomerase was reacted in the presence of excess primer under standard reaction conditions (1 μM [^{32}P]-dGTP, 100 μM dTTP, 1 mM Mg^{++}, 10 mM Tris at pH 8.0) for 2 minutes, and the reaction was then diluted 30-fold into two different reaction mixtures. The first mixture contained unlabeled dGTP, 100 μM dTTP,

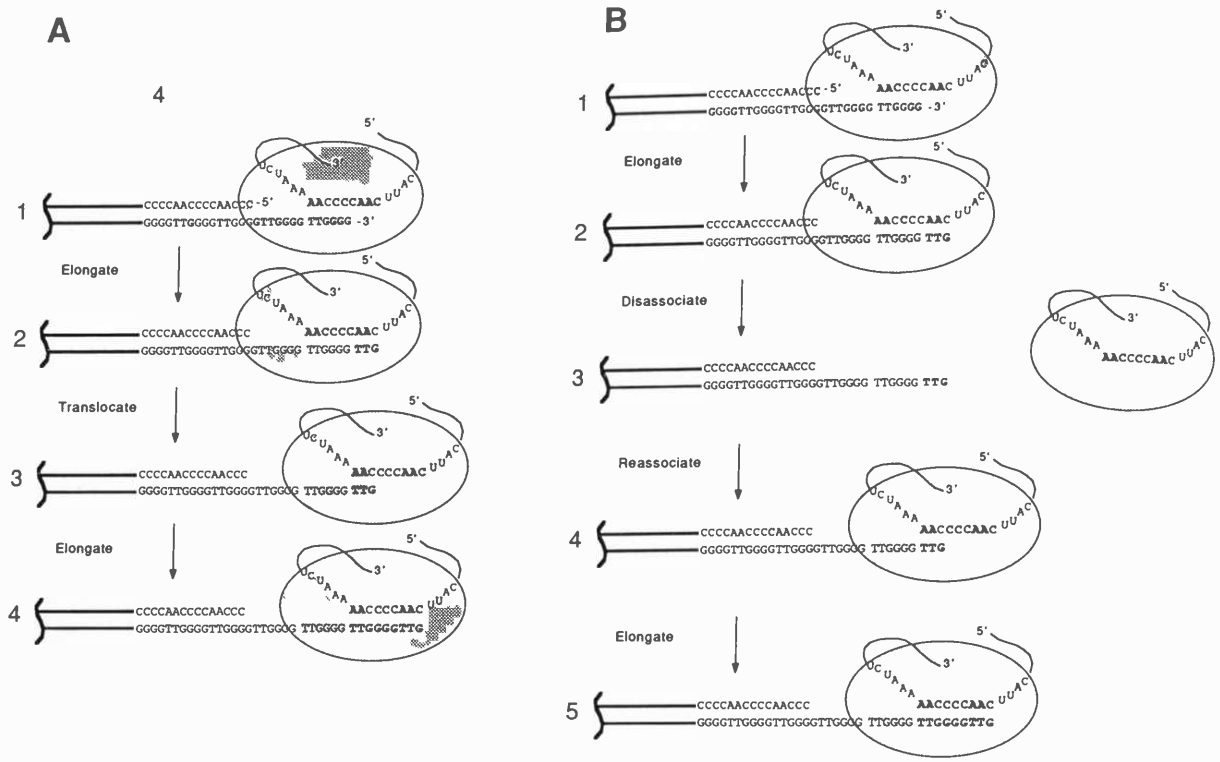


FIGURE 1 (*A*) Processive model for elongation of telomeres by telomerase. The *Tetrahymena* telomere is shown containing an overhang on the d(TTGGGG)$_n$ strand. After recognition of the d(TTGGGG)$_n$ strand by telomerase, the 3′-most nucleotides are hybridized to the CAACCCCAA sequence in the RNA (*1*). The sequence TTG is then added one nucleotide at a time (*2*). Translocation then repositions the 3′ end of the TTGGGG strand such that the 3′-most TTG nucleotides are hybridized to the RNA component of telomerase (*3*). Elongation occurs again, copying the template sequence to complete the TTGGGGTTG sequence (*4*). (*B*) Distributive model for elongation of telomeres by telomerase. This model is similar to that described in *A*, except that instead of the translocation step, the enzyme dissociates from the telomere (*3*) and must reassociate (*4*) for a further round of d(TTGGGG)$_n$ addition.

and a large excess of primer d(TTGGGG)$_4$ oligonucleotide. The second mixture contained unlabeled dGTP, 100 μM dTTP, and no primer oligonucleotide. The reactions were then allowed to proceed for 3, 4, or 10 minutes (Fig. 2). The labeled products increased in size, even after dilution into excess primer oligonucleotide, suggesting that the telomerase reaction is processive.

Another interpretation for the results seen in Figure 2 is that the telomerase preferentially elongates long products over short ones. To test this, we mixed d(TTGGGG)$_8$ with d(GGGGTT)$_3$ and showed that even at a molar ratio of 1:0.3, the shorter oligonucleotide was the preferred substrate. Kinetic analysis confirmed these results. d(TTGGGG)$_8$ has a K_m of 22 nM, and d(TTGGGG)$_4$ has a K_m of 2 nM. From these experiments, we conclude that d(TTGGGG)$_n$ repeat synthesis by telomerase is processive.

The telomerase reaction produces a characteristic 6-bp ladder of products (see Fig. 2). Careful examination of the pattern of these products showed that there is a strong pause after the addition of the second dT and the first dG in the sequence d(TTGGGG). The pause at the first dG residue is consistent with the model proposed in Figure 1A, in which elongation proceeds to the end of the template and then a translocation step occurs after the addition of the first dG residue. These data suggest that the entire CAACCCCAA template is used by telomerase.

We are actively pursuing purification of the telomerase enzyme with the aim of identifying the (as yet still elusive) protein components and of obtaining very pure enzyme for further biochemical dissection of the enzyme mechanism. The experiments described above were done using enzyme purified over two columns, with a purification of about 40–60-fold. Since it's initial identification, telomer-

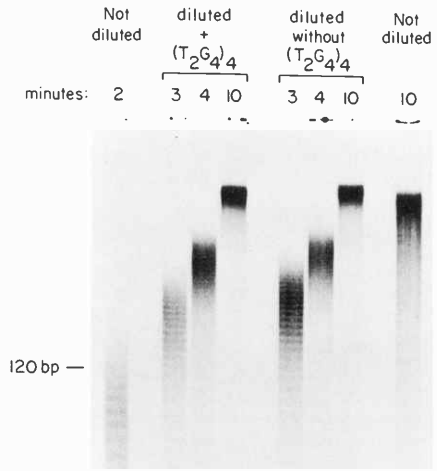


FIGURE 2 The telomerase reaction is processive. Telomerase was reacted for 2 min in the presence of 1 μM [^{32}P]dGTP, 100 μM dTTP, and 3000 nM d(TTGGGG)$_4$; 30 μl of this reaction was diluted into 900 μl of either 1 μm cold dGTP, 100 μM dTTP, 2700 nM d(TTGGGG)$_4$ (lanes labeled diluted + (T_2G_4)$_4$) or 1 μm cold dGTP, and 100 μM dTTP (lanes labeled diluted without (T_2G_4)$_4$). The reactions were then stopped after 3, 4, or 10 min total reaction time and run on a sequencing gel. As controls, the undiluted reaction was also stopped at either 2 or 10 min total reaction time.

ase has proven to be extremely labile, making purification over more than two columns very difficult. We are taking both conventional and affinity approaches to purification and hope to soon have highly purified enzyme for structural and functional analysis.

TETRAHYMENA TELOMERASE RNA EXPRESSION IN YEAST

In addition to telomerase biochemistry, we are pursuing several aspects of telomere biology in vivo. One approach is to express the *Tetrahymena* telomerase RNA component in the yeast *Saccharomyces cerevisiae*. Identification of the RNA component of a yeast telomerase through direct hybridization has not been possible. This is not unexpected since the sequence of the telomerase RNA from the ciliate *Euplotes crassus* does not cross-hybridize with *Tetrahymena* RNA, although the RNA can fold into a structure similar to that of the *Tetrahymena* RNA (D. Shippen-Lentz and E.H. Blackburn, pers. comm.). To express the *Tetrahymena* RNA in yeast, the gene was cloned directly behind the bacteriophage T7 promoter. This construct allows the bacteriophage T7 polymerase to produce a transcript identical in sequence to the RNA found in *Tetrahymena*. This T7 construct was transformed into a yeast strain that contains the T7 RNA polymerase gene cloned behind the yeast GAL10 promoter (a gift from Rolf Sternglanz). When these yeast are grown on galactose, the T7 RNA polymerase is induced and the telomerase RNA is transcribed. The telomerase RNA is stable in yeast as it can be visualized by Northern analysis of total yeast RNA.

We are examining the chromosomal telomeres in the yeast cells expressing the telomerase RNA. If the *Tetrahymena* RNA competes with the putative yeast telomerase RNA, telomeres might become shorter. If the *Tetrahymena* RNA substitutes for the yeast RNA, *Tetrahymena* telomeric d(TTGGGG)$_n$ repeats might be incorporated into the yeast telomeres. These possibilities are being examined by Southern blots and by cloning and sequencing yeast telomeres.

TELOMERES SHORTEN DURING MAMMALIAN AGING

As a second approach to in vivo telomere biology, we have recently begun a collaboration with Drs. B. Futcher and C. Harley to examine the behavior of human telomeres with aging. This study is rooted in the observations of Hayflick (*Exp. Cell Res. 25:* 585 [1961]) that primary (i.e., nontransformed) fibroblasts can go through only a limited number of doublings in vitro. Fibroblasts taken from a young donor can go through about 90 doublings, whereas those from an old donor go through many fewer doublings before senescence. Similar observations were made for other cell types and other species. The reason for the limit on the number of cell divisions is unknown.

In 1973, before the structure of telomeres was known, Olovnikov proposed that chromosome shortening due to incomplete telomere replication might limit the number of divisions a cell can go through (*J. Theor. Biol. 41:* 181 [1973]). Recently, Cooke and Smith (*Cold Spring Harbor Symp. Quant. Biol. 51:* 213 [1986]) observed that human sperm telomeres are

about 15 kb long, whereas telomeres from other somatic tissues are only about 10 kb long. This observation, in conjunction with the proposal by Olovnikov, suggested that human telomeres are elongated only in the germ line and that these telomeres are then gradually shortened during vegetative growth. Loss of telomeres may be a direct cause of cellular senescence.

To examine this theory, we passaged primary fibroblasts from different aged donors and examined telomere length after increasing the number of doublings in vitro. We found that mean telomere length decreases by about 30 bp per generation, that the distribution of telomere lengths becomes broader, and that the total hybridization signal to the telomeric probes decreased as cells aged (Figs. 3 and 4). We also found that telomere lengths at early passage correlated with the age of the donor; older donors had shorter telomeres. These observations are consistent with sequences being lost from the chromosome ends with each round of replication and telomere lengthening occurring in the germ line.

To follow up the observations on telomere length and aging, we plan to look at the lengths of telomeres in a number of different tissues from individuals of different ages, as well as from individuals who have specific aging diseases, such as progeria and Werner's Syndrome. Since mouse telomeres have the same sequence as humans, we can further test the correlation of aging with telomere length in animal models. In addition, the recent identification of telomerase activity in HeLa cells allows us to look directly at whether telomerase activity is restricted to the germ line in mammalian cells. Thus, we hope to directly test whether telomere shortening is a cause or an effect of aging in mammals.

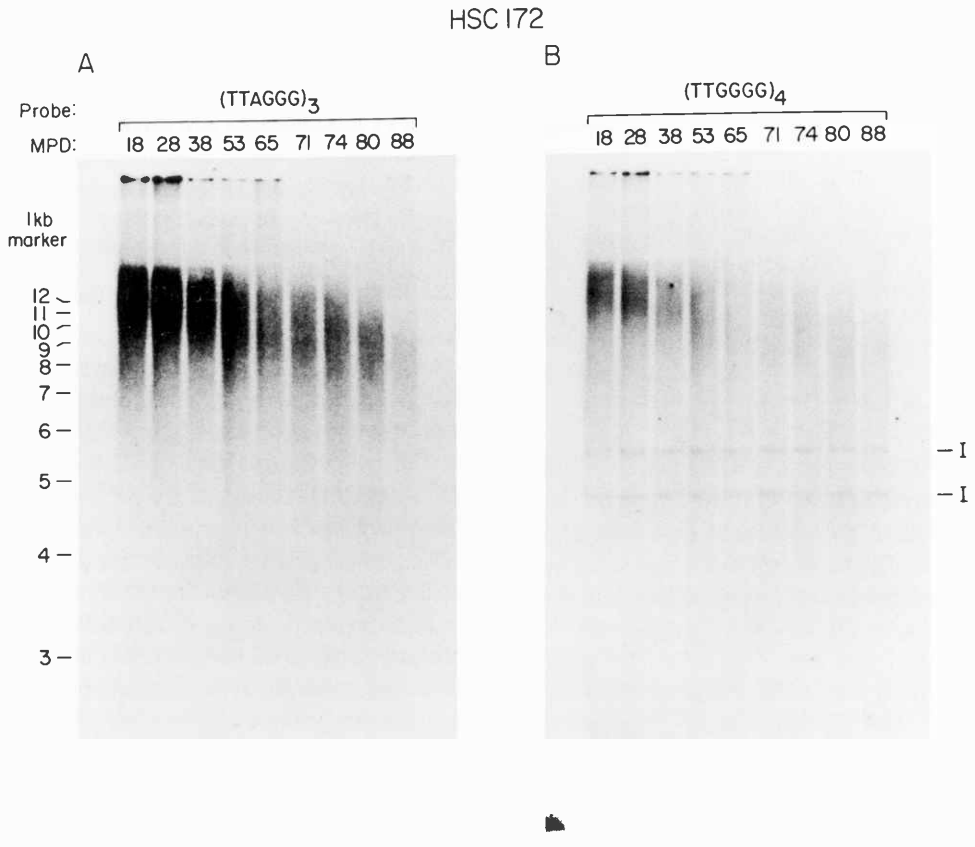


FIGURE 3 Primary fetal lung fibroblasts (HSC172) were passaged until senescence occurred (88 Mean Population Doublings, "MPD 88"). The DNA from these cells was digested with *MspI* and *RsaI*, run on a 0.7% agarose gel, and blotted to Nytran. (*A*) The blot was probed with the human telomeric probe $d(TTAGGG)_3$. (*B*) As a control, the same blot was stripped and reprobed at low stringency with $d(TTGGGG)_4$ to identify internal repetitive DNA bands (indicated by an I).

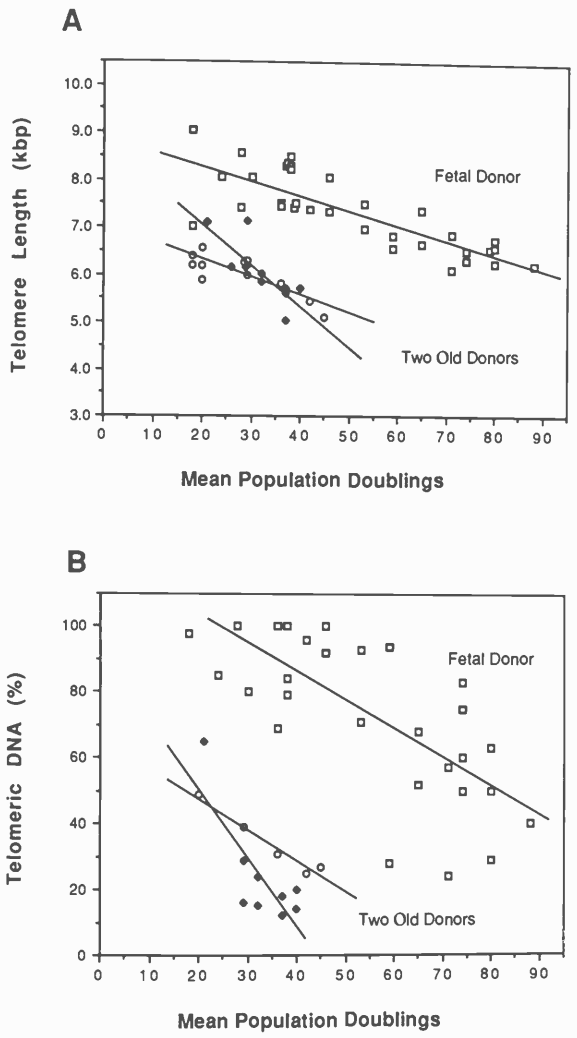


FIGURE 4 Mean telomere length (*A*) and total telomeric DNA (*B*) were determined by scanning Southern blots similar to those shown in Fig. 3. Mean telomere length is plotted as a function of in vitro age for three cell lines. HSC172 (□) is a fetal fibroblast cell line, and F001 (△) and F002 (○) are two primary fibroblast lines established from old individuals (70 and 90 years).

Characterization of Centromeric DNA from *Arabidopsis thaliana*

E.J. Richards, David Fessell

Centromeres are the chromosomal regions responsible for the proper partitioning of the chromosomes during mitosis and meiosis. One component of the centromere is the kinetochore, a complex nucleoprotein structure that attaches to the spindle microtubules and drives chromosome movement. In addition, control of sister-chromatid separation is exerted at the centromere, resulting in sister-chromatid separation at anaphase in mitosis and meiosis II and sister-chromatid nondisjunction at meiotic anaphase I.

I am interested in identifying and characterizing the DNA sequences required for centromere function as a first step toward understanding how centromeres work. At present, functional centromeric DNA sequences have been identified in only two systems, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. The picture that emerges from centromere studies in the yeasts is rather confusing; the budding yeast centromeres are quite small (120 bp) and do not resemble the large (50-100 kb) arrays of repetitive DNAs that make up *S. pombe* centromere regions. Although it is generally thought that the *S. pombe* centromeres may provide a simple model for the larger, more structurally complex centromeres of higher eukaryotes, I have decided to begin characterizing a higher eukaryotic centromere directly.

I have chosen to work on the flowering plant, *Arabidopsis thaliana*, for several reasons. The streamlined genome of this plant, with only 70 Mbp/n and a low repeated DNA content, facilitates chromosome walking and physical mapping efforts. In addition, *A. thaliana* is a good genetic system, and this attribute has been exploited to construct restriction-fragment-length polymorphism (RFLP) maps, providing molecular landmarks sprinkled throughout the genome. The availability of viable aneuploid mutants and strains carrying chromosomal rearrangements is particularly useful for molecular chromosome studies.

CENTROMERE 1: CHROMOSOMAL WALKING AND PHYSICAL MAPPING

I am presently trying to characterize and isolate an *A. thaliana* centromere using a combination of physical mapping and chromosome walking techniques. The approach is similar to that first used to isolate yeast centromeres: begin with molecular markers that flank a centromere and walk across the gap. The centromere of *A. thaliana* chromosome 1 is the best target, since it is the most precisely mapped, and several genetic and RFLP markers are closely linked to this centromere. Included among these RFLP markers is an AT-rich moderately repetitive element I previously identified as residing adjacent to an array of highly reiterated telomere-similar repeats. The challenge now is to put together a fine-structure genetic and physical map of the region to delimit the centromere. Toward this end, I am setting up crosses

using aneuploid strains in order to isolate chromosomes with crossover between centromere 1 and neighboring morphological and RFLP markers. The results of these experiments will establish a more precise genetic map position for centromere 1 and order flanking markers. These markers can then be used to construct large-scale restriction maps of the region, providing a location of the centromere in physical terms.

While the mapping project is under way, I have begun a chromosomal walk in the region. Using the centromere-1-linked moderately repeated element as a low-copy-number probe, I have isolated several YAC clones from a library constructed in C. Sommerville's laboratory (Michigan State University). The clones are currently being analyzed to determine if they are derived from centromere 1.

USING TELOCENTRIC CHROMOSOMES TO ISOLATE CENTROMERIC SEQUENCES

Several *A. thaliana* aneuploid strains have been isolated that contain telocentric chromosome derivatives, in addition to the normal diploid complement of ten metacentric chromosomes (i.e., telotrisomic mutants). The telocentric chromosomes were recovered after fracture through the centromere and consequently should contain a new, "healed" telomere close to the centromere. Using telomeric probes, it should be possible to identify and isolate the healed telomeres with large regions of flanking sequence stepping into the centromere. In essence, this is another version of the chromosome walk just described, except in this case, the starting point is a telomere, rather than flanking RFLP marker.

Because of the potentially large size of the centromere and the uncertain molecular distance between the healed telomeres and their "adjacent" centromeres, a YAC cloning system is being used to isolate large inserts. I have recently shown that cloned *A. thaliana* telomeres can serve as telomeres in *S. cerevisiae*, demonstrating the feasibility of isolating large telomeric restriction fragments directly by selection for telomere function in yeast.

PUBLICATIONS

Greider, C.W. and E.H. Blackburn. 1989. A telomeric sequence in the RNA of *Tetrahymena* telomerase required for telomere repeat synthesis. *Nature* **337:** 331–337.

In Press, Submitted, and In Preparation

Harley, C.B., A.B. Futcher, and C.W. Greider. 1990. Telomeres shorten during aging of human fibroblasts. (Submitted.)

Richards, E.E., W.D.B. Loos, J. Giraudat, H.M. Goodman, and F.M. Ausubel. 1990. The centromere region of *Arabidopsis thaliana* chromosome 1 contains telomere-similar sequences. (In preparation.)

COLD SPRING HARBOR MEETINGS

54th COLD SPRING HARBOR SYMPOSIUM ON QUANTITATIVE BIOLOGY

Immunological Recognition

May 31–June 7, 1989

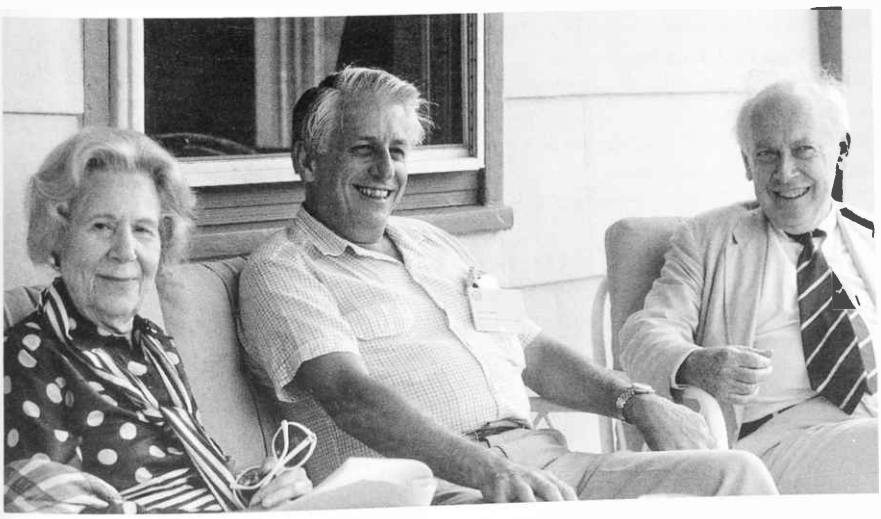
ARRANGED BY

James D. Watson, Cold Spring Harbor Laboratory
John R. Inglis, Cold Spring Harbor Laboratory

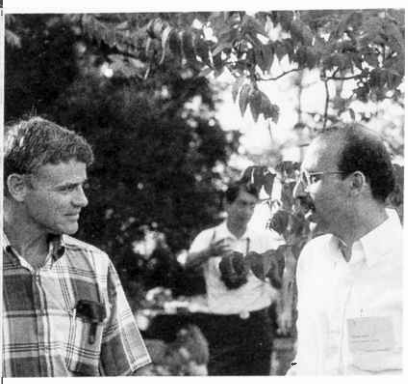
446 participants

The stark necessity of healthy immune function has been thrust into public consciousness in the 1980s by the tragedy of AIDS. The consequences of immune function gone awry can also be seen in such treatable but still incurable disorders as rheumatoid arthritis, multiple sclerosis, diabetes, and the myriad forms of allergy. To understand immune responses, and thus their malfunction, requires knowledge of the genetics of lymphocytes, the complexity of the signals they deliver and respond to at the cell surface, and the extraordinary assortment of responses they make when stimulated.

Research in the 1980s cut through to the molecular basis of these events. Antigen receptor molecules from T cells were identified and found to be encoded in a unique set of rearranging gene segments, separate from but evolutionarily related to the immunoglobulin genes. The analysis of other cell-surface molecules revealed diversity among T cells that correlated with differences in function. Crucially, it was observed that the products of MHC loci could bind peptide fragments of antigen and deliver them to the surface of antigen-presenting cells. A structural basis for T-cell antigen recognition became apparent when the solution of the crystal structure of the class I histocompatibility molecule HLA-A2 showed



L. Annenberg Hazen, G. Nossal, J.D. Watson



L. Hood, G. Osman

P. Kourilsky
J. Maryanski

F. Melchers, W. Kerr

that it had a groove in which both peptide and the variable T-cell receptor might bind. X-ray crystallography also clarified the interaction between an antibody and its ligand, with the solution of the structure of an immune complex.

By 1989, therefore, the time was clearly ripe for a Cold Spring Harbor Symposium focusing on the molecular, cellular, and structural aspects of immunological recognition. The theme of the meeting centered on the interactions among MHC molecules, peptides, and antigen receptors that trigger T-cell activation, but sessions were devoted to the shaping of T-cell and B-cell repertoires by these events and their consequences for lymphocyte activation, tolerance, and autoimmune responses.

The final program included 114 speakers and 446 participants. Every available seat in Grace Auditorium was occupied throughout the week, and the aisles were filled. The meeting opened with splendid presentations by Leroy Hood, Philippa Marrack, Don Wiley, Emil Unanue, and Hugh McDevitt, whose talks highlighted the themes of the coming week. Then came 14 sessions of great intensity and an elegant and wide-ranging summary by Jonathan Howard.

The meeting was supported in part by the National Cancer Institute and National Institute of Allergy and Infectious Diseases; the National Science Foundation; the U.S. Department of Energy, and the Lucille P. Markey Charitable Trust.

PROGRAM

Opening Remarks
J. D. Watson

T-cell Development
Chairman: S. Tonegawa, *Massachusetts Institute of Technology*

B-cell Development
Chairman: T. Honjo, *Kyoto University*

Interactions between T-cell Receptors, Antigen, and MHC Molecules. I
Chairman: B. Benacerraf, *Dana-Farber Cancer Institute*

Self-recognition by B Cells and Antibodies
Chairman: F. Melchers, *Basel Institute for Immunology*

Lymphocyte Activation. I
Chairman: R. Klausner, *National Institutes of Health*

Recognition by Antibodies
Chairman: D. Davies, *National Institutes of Health*

Antigen Processing
Chairman: H. Eisen, *Massachusetts Institute of Technology*

Histocompatibility Antigens
Chairman: W. Bodmer, *Imperial Cancer Research Fund Laboratories*

Tolerance in T Cells
Chairman: J. Miller, *Walter and Eliza Hall Institute of Medical Research*

Interactions between T-cell Receptors, Antigen, and MHC Molecules. II
Chairman: R. Zinkernagel, *University of Zurich*

Lymphocyte Activation. II
Chairman: N. Mitchison, *University College*

Selection of T-cell Receptors
Chairman: P. Kourilsky, *Institut Pasteur*

Interactions between T-cell Receptors, Antigen, and MHC Molecules. III
Chairman: M. Gefter, *Massachusetts Institute of Technology*
Summary: J. Howard, *AFRC, Institute of Animal Physiology*

MEETINGS

Genome Mapping and Sequencing

April 26–April 30, 1989

ARRANGED BY

Charles Cantor, Columbia University College of Physicians & Surgeons
Maynard Olson, Washington University School of Medicine
Richard Roberts, Cold Spring Harbor Laboratory

252 participants

This year's meeting on Genome Mapping and Sequencing was the second in what is likely to be a continuing series. The meeting emphasized current techniques in use for genome mapping and sequencing and highlighted some of the systems in which great progress is being made. The importance of small technical breakthroughs was exemplified by studies of telomeres. In 1988, a single presentation, included at the last minute, described the cloning of a stretch of DNA that hybridizes to all human telomeres. At this year's meeting, a whole session needed to be devoted to telomeres. Among the many ongoing mapping endeavors, several groups reported good progress in the hunt for specific human disease genes, including the much sought after cystic fibrosis gene. On the technical front, spectacular progress was announced in the use of confocal microscopy to map genes directly onto chromosomes. The final session of the meeting was devoted to computation and served to illustrate the important role that computer science will play within genome research.

PROGRAM

Genome Organization
Chairman: V. McKusick, *Johns Hopkins University*

Telomeres
Chairman: C. Cantor, *Columbia University College of Physicians & Surgeons*

Polymorphism
Chairman: W. Bodmer, *ICRF, London*

Mapping Techniques. I
Chairman: M. Olson, *Washington University School of Medicine*

Mapping Techniques, II
Chairman: S. Brenner, *MRC Laboratory, Cambridge*

Mapping Experience
Chairman: D. Cohen, *CEPH, Paris*

Sequencing
Chairman: E. Soeda, *Riken Institute*

Computation
Chairman: D. Lipman, *National Library of Medicine*

C. Cantor

P. Pearson, D. Lipman

M. Olson, G. Cahill

Regulation of Liver Gene Expression

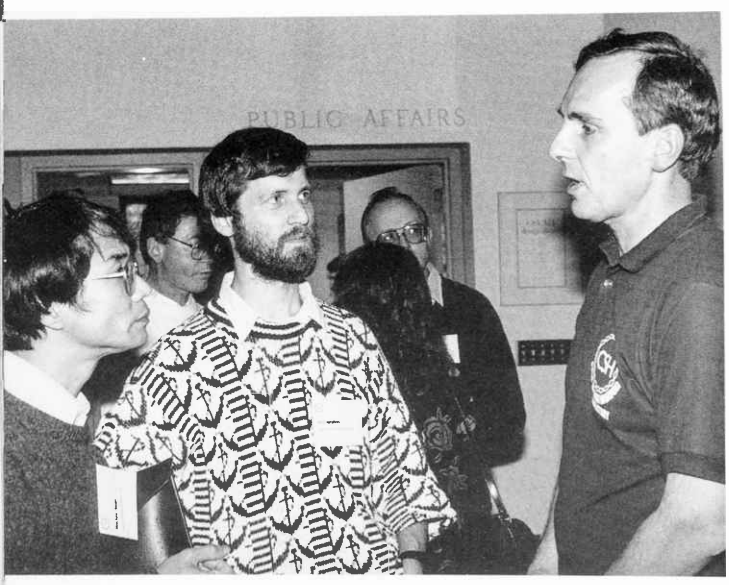
May 7–May 11, 1989

ARRANGED BY

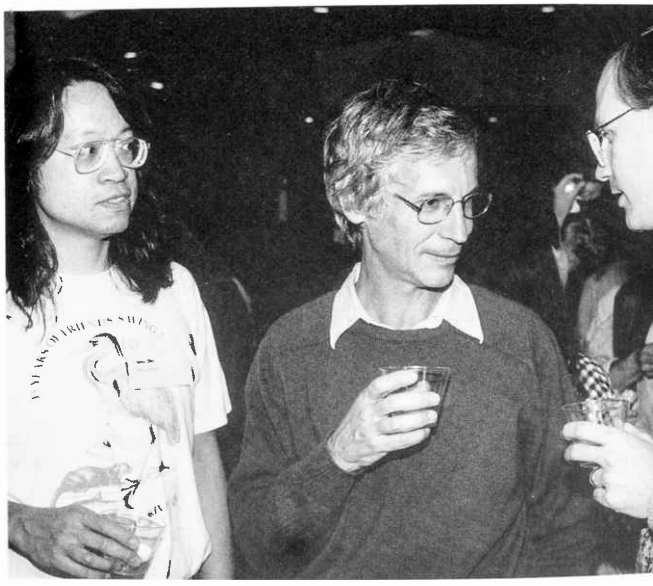
Gerald Crabtree, Stanford University
George Fey, Research Institute of Scripps Clinic
Shirley Tilghman, Princeton University

265 participants

The second meeting of this series was attended by more than 250 participants, including speakers presenting exemplary work from other areas of gene regulation, such as *Drosophila* and yeast. The focus of the meeting was on mechanisms of gene regulation. Highlights included the characterization and cloning of hepatocyte nuclear factor 1 (HNF1), a key factor determining hepatocyte-specific transcription of albumin, fibrinogen, and other liver marker genes. With the description of the cloning of CBP and DBP and the purification of HNF3 and HNF4, other factors determining liver-specific transcription, significant progress was reported toward the solution of a key problem in this field: How can interactions among factors that each may not be restricted to one cell type generate cell-type-specific transcription? Novel types of control elements in the far-upstream region of the albumin gene were discovered that allowed the expression of this gene in hepatic cells only when the cells were cultured in special media and on a collagen matrix. This result demonstrates the existence of control mechanisms that signal the contact of cells with the extracellular matrix to the nucleus and activate the expression of specific genes. Along with bone marrow cells, the liver has emerged as the target of choice for the correction of gene defects. Several examples were presented for phenotypic corrections of defects in genes that are primarily expressed in the liver. These included correction of the



M. Hattori, S. Humphries, G. Fey



H. Choy, M. Yaniv, R. DuBois

spf mutation by expression of a correct human gene in transgenic mutant mice and the correction of a hereditary deficiency of LDL receptors in Watanabe rabbits. In this case, the correct wild-type gene was introduced with retroviral vectors into cultured Watanabe rabbit hepatocytes. Attempts to reintroduce corrected cells into animals were described, including injection into the portal vein and homing in the liver, as well as growth of hepatic cells on cytodex beads and reimplantation of these grafts at extrahepatic sites. Important progress was described in improving long-term acceptance of liver cell allografts in rats.

Recent work was presented on myo D, a master gene capable of converting fibroblasts to muscle cells, and on the search for corresponding genes determining the hepatic phenotype by somatic cell genetics. Another highlight of the meeting was the announcement of the purification and cloning of HGF, a novel hepatocyte growth factor. Important progress was reported in the identification of characteristic markers for hepatic precursor cells, mature hepatocytes, and biliary epithelial cells. With the help of these markers, it was possible to follow the differentiation from embryonal progenitor cells in culture to both hepatocytes and biliary epithelial cells, and to study the influence of growth- and differentiation-promoting agents on this process.

The liver is increasingly appreciated as an excellent experimental system for the study of gene regulation. It offers the logistic feasibility of analyzing both the transcriptional machinery at the biochemical level and a number of unique biological processes involving gene regulation, such as regeneration and hepatocellular carcinogenesis. The participants thanked the host laboratory and its meetings staff for their excellent work and expressed their hopes that this series of meetings will be continued at Cold Spring Harbor.

G. Crabtree

S. Woo

G. Schutz

PROGRAM

Transcription. I
Chairman: G. Crabtree, *Stanford University*

Hormonal Modulation of Gene Expression
Chairman: G. Schutz, *German Cancer Research Center*

Transcription. II
Chairman: M. Yaniv, *Institut Pasteur*

Regulation of Liver Acute-Phase Genes
Chairmen: H. Baumann, *Roswell Park Memorial Institute*
G. Fey, *Scripps Clinic and Research Foundation*

Developmental Regulation
Chairman: S. Tilghman, *Princeton University*

Somatic Cell Genetics
Chairman: K. Fournier, *Fred Hutchinson Cancer Research Institute*

Liver Genes and Disease
Chairman: S.L.C. Woo, *Baylor College of Medicine*

Growth Regulation and Regeneration of Liver Cells
Chairman: B. Knowles, *The Wistar Institute*

Requirement for Hepatic Differentiation in Cultured Cells
Chairman: H. Isom, *Pennsylvania State University College of Medicine*

C. elegans

May 10–May 14, 1989

ARRANGED BY

Scott Emmons, Albert Einstein College of Medicine
Jonathan Hodgkin, MRC Laboratory of Molecular Biology
Judith Kimble, University of Wisconsin
James McGhee, University of Calgary

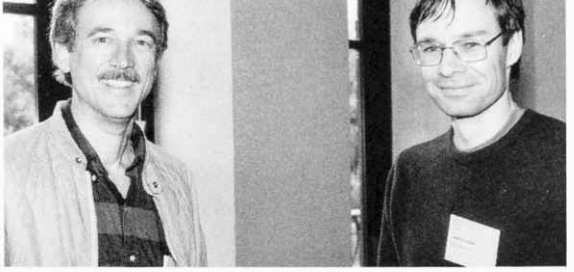
385 participants

Attendance at this year's *C. elegans* meeting was almost 30% higher than two years ago, reflecting the continuing growth and excitement in the field. Much research on *C. elegans* has focused on a description of the organism. This information sets the stage for genetic and molecular analyses of many important problems in biology.

Analysis of genes identified by mutations continues to be a central approach of *C. elegans* research. Most of the genes governing the sex of the animal have been identified, and molecular studies of their products are well under way. A likely candidate for the X-chromosome-specific sequence whose dosage determines sex has been identified. Genes have been described that are required for specification of fates of early embryonic blastomeres, determination of position and polarity within the body, control of programmed cell death, development of the vulva in the hermaphrodite and copulatory structures in the male, timing of events during development, and many other functions. From analysis of cloned genes, it has been found that laminin plays a central role in determination of position and direction in the body. Other developmental control genes have similarity to growth factors and homeobox genes of higher organisms. Powerful

Wine and cheese

P. Meneely, P. Mains



S. Emmons, J. Hodgkin

transformation methods are now available for analysis of gene function. Sequences have been identified that direct expression of the myosin-heavy-chain gene to specific muscle tissues. Future work will address the question of how expression of key genes is regulated in the genetic program whose outcome is the nematode worm.

PROGRAM

Sex Determination and Dosage Compensation
Chairman: V. Ambros, *Harvard University*

Cell Biology
Chairman: J. Thomas, *University of Washington*

Developmental Pathways and Control Genes
Chairman: A. Fire, *Carnegie Institution of Washington*

Cell Fate and Differentiation
Chairman: P. Meneely, *Fred Hutchinson Cancer Research Center*

Nervous System
Chairman: D. Moerman, *University of British Columbia*

Genome Organization and Gene Expression
Chairman: J. Shaw, *University of Minnesota*

Embryogenesis and Positional Information
Chairman: E. Schierenberg, *University of Cologne*

RNA Processing

May 17–May 21, 1989

ARRANGED BY

Michael Green, Harvard University
Anita Hopper, Hershey Medical Center, Pennsylvania State University
Norman Pace, Indiana University

448 participants

RNA processing encompasses the cellular mechanisms that convert primary transcripts into functional RNAs and degrade RNAs not destined for further use by the cell. The large attendance at the 1989 RNA Processing meeting testifies to the rapid growth of the field and to the recognition that RNA processing affects many areas of cellular function. The five-day meeting included eight sessions of short

presentations, three poster sessions, and a concluding session of four review lectures of broad interest. Diverse topics were discussed, ranging in detail from the atom to the organism level.

A recurring theme of the meeting was the analysis of the molecular structure involved in RNA processing. For example, local RNA structure determines catalytic activities and substrate sites; long-range folding of large RNAs aligns splicing sites and can determine developmentally controlled, alternative splicing pathways. RNA processing often requires the assembly of macromolecular complexes, commonly composed of stable RNAs and discrete proteins. Increasing success was reported in dissecting the constituents of such complexes, for instance those involved in splicing or polyadenylation, and in reconstituting them in vitro. The involvement of RNA processing in regulatory mechanisms in several systems was discussed. Sex determination in *Drosophila*, for instance, is dependent in part on alternative mRNA processing.

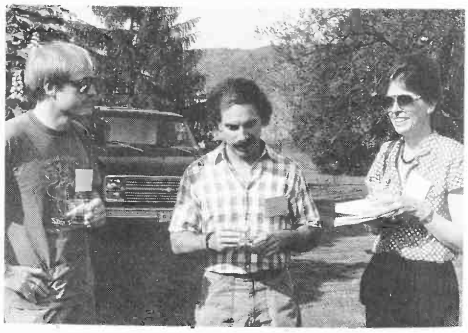
The range of cellular events that rely on RNA or RNA processing continues to expand. Recently discovered examples discussed at the meeting included the service of RNA as a template for telomere formation in ciliates and probably humans, and "editing," the alteration of mRNA sequences that occurs in many organisms by modification, excision, or addition of nucleotides. Particularly,

A. Hopper, J. Abelson

M. Green, M. Zapp

D. Draper, M. Yarus

J. Dahlberg, J. Cuddihy, R. Krug



M. Culbertson, R. Spritz, J. Steitz

extensive additions to and excisions from some mRNAs in trypanosome mitochondria question the source of sequence information for the editing process. Putative templates remain unidentified, however.

The annual RNA Processing meeting is an important forum for communications of recent results and methods. The large size of the 1989 meeting, and the fact that many applicants could not be accommodated, prompted suggestions for finer focus of future meetings, for example, on splicing phenomena or catalytic RNAs. However, the consensus of participants at the 1989 RNA Processing meeting seemed to be that the eclectic coverage provided by the current format is of overriding value in this rapidly advancing field.

The meeting was supported in part by the National Science Foundation and the National Institutes of Health (National Institute of General Medical Sciences).

PROGRAM

Catalytic RNA
Chairman: J. Szostak, *Massachusetts General Hospital*

Splicing: Specificity, Regulation, and Pathways
Chairman: J. Beggs, *University of Edinburgh*

snRNPs: Formation and Function
Chairman: J. Steitz, *Yale University*

Splicing: Factors and Spliceosome Assembly
Chairman: J. Abelson, *California Institute of Technology*

3′ ENDS, hnRNPs, and Export
Chairman: T. Platt, *University of Rochester Medical Center*

Stable RNAs
Chairman: J. Dahlberg, *University of Wisconsin*

Regulation and Turnover
Chairman: B. Baker, *Stanford University*

Special Topics Reviews
Chairman: W. Keller, *Biocenter of the University of Basel*

RNA Tumor Viruses

May 24–May 28, 1989

ARRANGED BY

Hung Fan, University of California, Irvine
Eric Hunter, University of Alabama, Birmingham

398 participants

The 1989 RNA Tumor Virus meeting considered retrovirus replication, structure, and pathogenesis. In the last few years, the meeting has become an important forum for basic research in human retroviruses–this year nearly half of the presentations concerned HIV and related primate viruses, or HTLV. Some of the highlights included discussion of recent advances in retrovirus replication. In particular, progress in identifying several retrovirus receptors was described, including molecular cloning of the ecotropic murine leukemia virus receptor.

Reports on the mechanism of retroviral integration included descriptions of in vitro integration systems and mechanistic studies on the integrase protein. Considerable attention was focused on human retrovirus regulatory proteins, particularly *tat*, *rev*, and *tax*, although important information was presented, the highlight was the description of high-resolution structures of avian and human retrovirus proteases; these will be important in structure-function studies and, potentially, in the design of new anti-retrovirus drugs. Sessions on retrovirus pathogenesis included discussions on the mechanisms of virus-induced tumorigenesis and immunodeficiency. Several animal model systems for immunodeficiency were presented, including feline, murine, and human viruses.

P. Jolicoeur, R. Kettmann

J. Witkowski, B. Cullen

A. Cochrane, C. Rosen

PROGRAM

Replication—Entry through Integration
Chairmen: H. Varmus, *University of California, San Francisco*
S. Goff, *Columbia University*

Transcription and Transcriptional Control
Chairmen: N. Hopkins, *Massachusetts Institute of Technology*
C. Rosen, *Roche Institute of Molecular Biology*

Replication—RNA Splicing through Virus Assembly
Chairmen: A.M. Skalka, *Fox Chase Cancer Center*
A. Rein, *Frederick Cancer Research Facility*

Nonstructural/Regulatory Genes
Chairmen: M. Martin, *NIAID, National Institutes of Health*
B. Cullen, *Howard Hughes Medical Institute, Duke University*

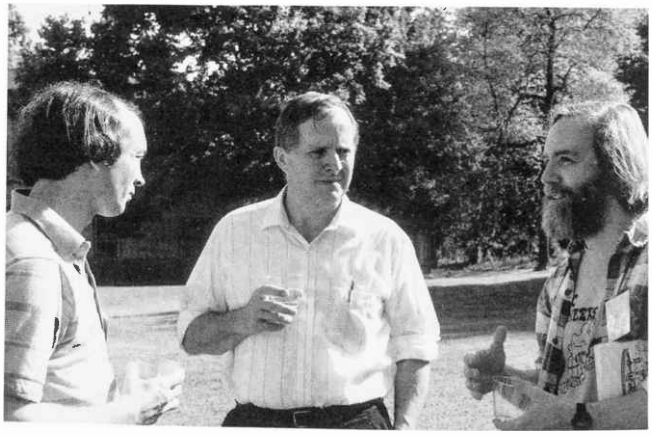
Oncogenesis
Chairmen: P. Jolicoer, *Clinical Research Institute of Montreal*
W. Hayward, *Memorial Sloan Kettering-Cancer Center*

Protein Structure and Function Endogenous Viruses and Evolution
Chairmen: V. Vogt, *Cornell University*
J. Coffin, *Tufts University School of Medicine*

Vectors and Gene Transfer
Chairmen: M. Linial, *Fred Hutchison Cancer Research Center*
S. Hughes, *NCI Frederick Cancer Research Facility*

Immunosuppression and Cytopathogenesis
Chairmen: H. Robinson, *University of Massachusetts Medical Center*
F. Lilly, *Albert Einstein College of Medicine*

J. Schultz, W. Wachsman, H. Fan



R. Craigie, D. Grandgenett, S. Goff

Yeast Cell Biology

August 15–August 20, 1989

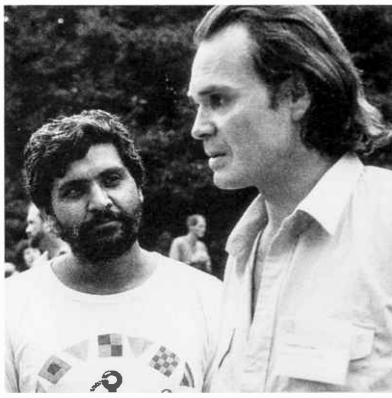
ARRANGED BY

Scott Emr, California Institute of Technology
Amar Klar, NCI-Frederick Cancer Research Facility
John Pringle, University of Michigan
Steve Reed, Scripps Clinic and Research Foundation

325 participants

Participants of the meeting on Yeast Cell Biology considered the many ways in which the experimental tractability of yeasts is being exploited in investigations of fundamental problems in eukaryotic cell biology. The utility of this approach depends in part on the degree to which structures and functions are conserved between yeasts and the larger eukaryotes, and the meeting provided further evidence that such conservation runs very deep indeed. The meeting also provided abundant illustrations of just how experimentally tractable yeasts have become. Many papers gave dramatic evidence that the traditional barriers (real and psychological) to application of conventional cell biological approaches (light microscopy, electron microscopy, and cell fractionation/in vitro reconstitution) to yeast have been overcome. At the same time, the genetic approaches for which the yeasts have long been renowned have become even more diverse and powerful as a result of conceptual and technical advances presented at the meeting. These genetic approaches are of two general types, both of which were much in evidence. In "forward genetics," potentially novel features of the cell are explored by isolating and analyzing mutants with interesting phenotypes: Molecular genetic methods (including DNA sequencing and the use of fusion proteins to elicit antibody production) are then used to explore the functions of the gene products at the molecular level. This approach has brought many exciting recent advances, such as the realization of the very wide range of functions in which GTP-binding/hydrolyzing proteins are involved, the discovery of cyclin-like molecules involved in G_1 control of the cell cycle, and the discovery of an apparently novel pathway of secretion. Equally potent is the approach of "reverse genetics," in which molecular genetic methods are used first to clone the gene encoding a putatively important protein and then to make mutations in that gene to explore the role of the protein in vivo. This approach continues to produce many surprises, such as the new result that mutants unable to acidify their vacuoles are apparently healthy. In a more general way, the practice of reverse genetics in yeast (and a few other suitable organisms) has greatly increased our appreciation of the subtlety of cellular functions and of the degree of redundancy and overlap in the functions of individual proteins.

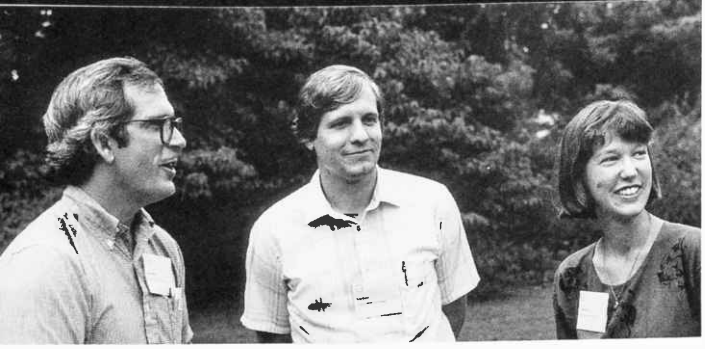
In summary, use of the powerful amalgam of genetic and nongenetic approaches that can be applied to yeast has produced exciting progress in understanding virtually all parts of the yeast cell, from the cell wall to the nucleolous and microtubule, as reported at the 1989 Yeast Cell Biology meeting. There is no reason to think that the pace of discovery will slow between now and the next such meeting in 1991 and every reason to think that the results obtained will be of wide interest to cell biologists generally.



A. Klar, V. Larionov

D. Gallwitz

J. Thorner



J. Rine, S. Emr, L. Silveria

E. Cabib, R. Trimble

PROGRAM

Nucleus and Plasma Membrane
Chairman: J. Woolford, *Carnegie Mellon University*

Cytoskeleton and Morphogenesis
Chairman: F. Solomon, *Massachusetts Institute of Technology*

Chromosome Structure, Function, and Segregation
Chairman: M. Grunstein, *University of California, Los Angeles*

Secretion and Protein Sorting. I
Chairman: R. Trimble, *New York State Department of Health*

Cell Cycle and Growth
Chairman: K. Tatchell, *North Carolina State University*

Secretion and Protein Sorting, II
Chairman: E. Jones, *Carnegie Mellon University*

Signal/Transduction/Cell Wall
Chairman: J. Thorner, *University of California, Berkeley*

Mating Type, Meiosis, and Sporulation
Chairman: B. Byers, *University of Washington*

Cell Cycle Control
Chairman: P. Fantes, *University of Edinburgh*

Secretion/Endocytosis
Chairman: S. Michaelis, *Johns Hopkins University School of Medicine*

Peroxisomes and Mitochondria
Chairman: H. Ruis, *Universitat Wien*

Molecular Genetics of Bacteria and Phages

August 22–August 27, 1989

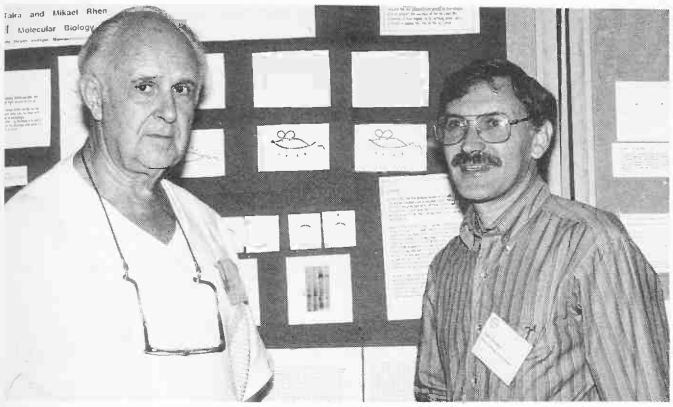
ARRANGED BY

Gary Gussin, University of Iowa
Lucia Rothman-Denes, University of Chicago
Andrew Wright, Tufts Medical School

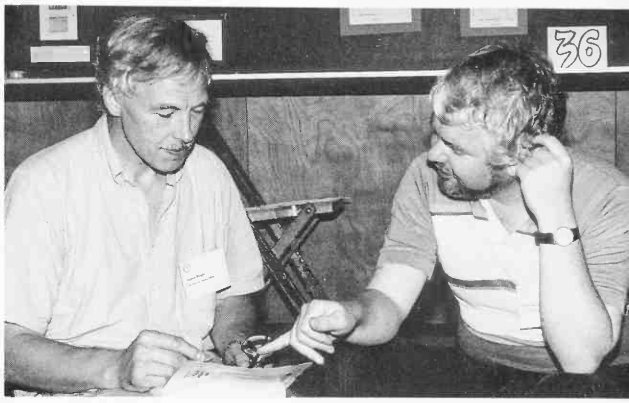
315 participants

The 1989 meeting on Molecular Genetics of Bacteria and Phages was extremely successful and attracted more than 300 people, including many young investigators and graduate students. There were 112 talks and 130 posters covering all aspects of prokaryotic molecular genetics. Prior to each session, the chairperson

gave a brief overview of the topics to be discussed; this proved a useful addition to the previous format. Topics included DNA replication site-specific recombination, transcriptional activation, antitermination of transcription, and posttranscriptional control by RNA degradation or by translational control. Mechanisms of signal transduction, membrane assembly, and protein secretion were also discussed.



W. Szybalski, H. Murialdo



A. Wright, S. Busby

G. Mosig, N. Sternberg, A. Abeles

There has been considerable progress in a number of these areas during the past year. Of particular note was the number of systems in which DNA bending or looping plays a role in regulation. Several reports supported a ubiquitous role for IHF as a regulator of gene expression, as an essential element in site-specific recombination, and as a factor required for packaging of phage DNA. In each of these cases, IHF facilitates the formation of unique DNA structures through its ability to bend DNA. The most dramatic example of DNA loop formation involved in regulation was that shown to occur in the *deo* operon of *E. coli* (Dandanell and Hammer). The *deoR* repressor, containing eight identical subunits, binds to three operator sites and forms single and double loops, the latter being consistent with its high efficiency and cooperative behavior observed in vivo. These studies all illustrate how sites at a distance interact with one another. Significant progress in the understanding of structural elements that alter stability (Emory and Belasco; Wagner et al.) and new methods of analysis (Mackie) and understanding of this complex process seems within reach.

Wine and cheese

Among the exciting results presented was the demonstration by Ebright that a DNA-binding protein, cap, could by simple chemical modification of a cysteine residue in the DNA-binding helix be converted into an endonuclease, with unaltered DNA-binding affinity and specificity. It should now be possible to design other reagents of this type that will allow cleavage of DNA at specifically selected sites. The report (Van Dyk et al.) that overproduction of the *groES* and *groEL* gene products, major heat shock proteins, suppresses a wide range of temperature-sensitive mutations in *E. coli* further strengthens the notion that these proteins play a major role in protein folding. Overall the presentations were of very high quality and the meeting stimulated intense interest and discussion.

PROGRAM

Replication/Repair
Recombination/Excision/Transposition
Chromosome Packaging
RNA Polymerase-Promoter Interactions
Transcription Termination/Antitermination
Posttranscriptional Regulation
Protein Secretion/Membrane Assembly/Signal Transduction
Transcriptional Regulation. I: Activation
Transcriptional Regulation. II
Thermal Regulation/Modern Genetics

Eukaryotic DNA Replication

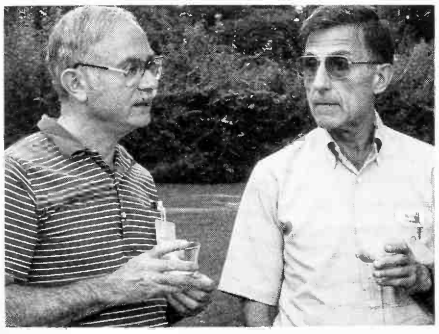
August 30–September 3, 1989

ARRANGED BY

Thomas Kelly, Johns Hopkins University School of Medicine
Bruce Stillman, Cold Spring Harbor Laboratory

290 participants

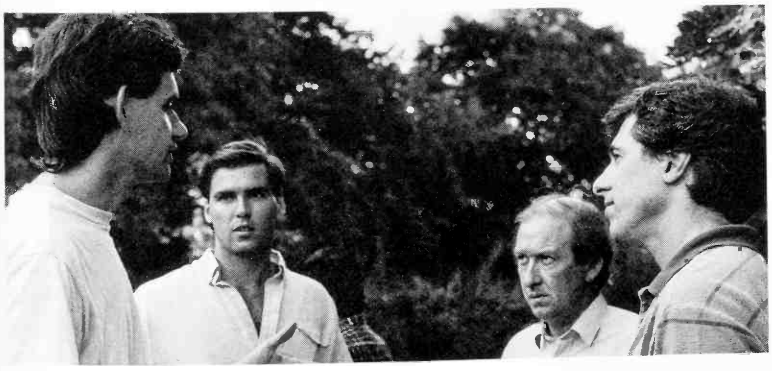
This was the second meeting on Eukaryotic DNA Replication held at Cold Spring Harbor; the first was held two years ago. The meeting focused on all aspects of the subject, including two sessions on replication of virus DNA; two sessions on the replication of cell chromosomal DNA, including yeast and mammalian cells; a session on the replication of extrachromosomal elements and selective amplification of DNA; one session on replication proteins; and one session on the control of DNA replication. In addition, two large poster sessions were held. As with the previous meeting, the 1989 meeting saw a large amount of high-quality science, indicating that the field is strong and will be for some time to come. It has also become clear that the field of DNA replication is branching out to include topics on gene regulation, cell cycle control, genome amplification and organization, and cell biology.



M. Horwitz, M. Goulian

D. Beach, M. Fairman

C. Prives, T. Kelly



B. Stillman, M. McAlear, R. Laskey, N. Heintz

E. Johnson, S. Berberich

PROGRAM

Replication Proteins
Chairman: P. Burgers, *Washington University School of Medicine*

Virus DNA Replication. I
Chairman: C. Prives, *Columbia University*

Cell Chromosome Replication. I
Chairman: J. Huberman, *Roswell Park Memorial Institute*

Virus DNA Replication. II
Chairman: P. van der Vliet, *University of Utrecht*

Extrachromosomal Replication
Chairman: G. Wahl, *The Salk Institute*

Control of Cell Cycle and S Phase
Chairman: D. Beach, *Cold Spring Laboratory*

Cell Chromosome Replication. II
Chairman: R. Laskey, *University of Cambridge*

Regulation of Eukaryotic mRNA Transcription

September 6–September 10, 1989

ARRANGED BY

Winship Herr, Cold Spring Harbor Laboratory
Robert Tijan, University of California, Berkeley
Keith Yamamoto, University of California, San Francisco

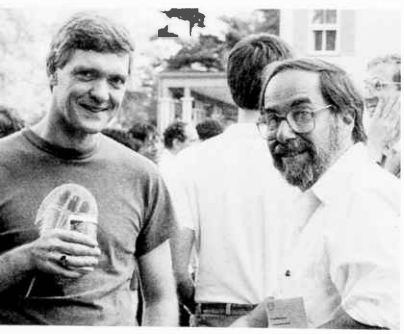
427 participants

The 1989 Cancer Cells meeting, Regulation of Eukaryotic mRNA Transcription, was attended by an international group of over 400 scientists sharing overlapping interests in regulation of transcription in eukaryotic cells. The reports presented by the participants spanned a broad spectrum of topics, including the biochemistry of general transcription factors, structural studies, yeast genetics, and *Drosophila* development. In the first session, several groups reported the molecular cloning of

P. Rigby, J. Alwine, T. Kadesch

R. Treisman, K. Yamamoto, P. Sigler

P. Sharp



J. Witkowski, D. Baltimore

the gene encoding the yeast TATA box binding factor TFIID, a major breakthrough in understanding the structure and function of basic initiation factors. The sequence of this gene revealed that it had also been isolated genetically, because mutations in this gene affect the selection of transcription initiation sites in yeast. Another basic transcription factor, the RNA-polymerase-II-associated protein complex RAP 30/74, was shown to process DNA helicase activity, suggesting it may be involved in establishing an open transcription initiation complex. Many studies described the dissection of sequence-specific DNA-binding transcription factors revealing in increasing detail the many structural motifs involved in both DNA binding and transcriptional activation. An unexpected finding revealed that a chromosomal translocation associated with a class of leukemias results in the fusion of a transcription factor expressed in lymphoid cells to a novel homeodomain, thus probably conferring a new DNA-binding specificity. One session focused on the influence of chromatin and DNA topology on regulation of transcription, with one report describing how sequence-specific factors are already bound to the promoter in vivo before transcription of the gene is activated; this indicates that other levels of regulation (e.g., protein modification) are involved. In the final session, reports on the analysis of homeodomain genes in *Drosophila* revealed how regulation of transcription factor activity can have dramatic effects on development. The meeting closed with a striking presentation in which individual

H. Weintraub, W. Driever

transcription complexes were revealed within the nuclei of stained *Drosophila* embryos. The success of the meeting was indicated by the large number of scientists standing in the aisles or sitting on the stairs of Grace Auditorium during the sessions.

The meeting was funded by the National Science Foundation and National Institutes of Health.

PROGRAM

RNA Polymerase II and Basal Initiation Factors
Chairman: A.L. Greenleaf, *Duke University Medical Center*

Initiation Complexes and Post-Initiation Events
Chairman: C. Gross, *University of Wisconsin*

Tissue Selective Enhancer and Promoter Factors
Chairman: R. Roeder, *Rockefeller University*

Mechanisms of Transcription Regulation: Activation Domains and Protein/Protein Interactions
Chairman: M. Ptashne, *Harvard University*

Regulation of Transcription Factor Activity
Chairman: D. Baltimore, *Whitehead Institute and Massachusetts Institute of Technology*

Structure and Function of Sequence-Specific DNA Binding Proteins
Chairman: P.B. Sigler, *Yale University*

The Influence of Template Topology, Nuclear Matrices, and Chromatin Structure on Transcription
Chairman: J. Wang, *Harvard University*

Transcription of Developmentally Regulated Genes
Chairman: M. Levine, *Columbia University*

Translational Control

September 13–September 17, 1989

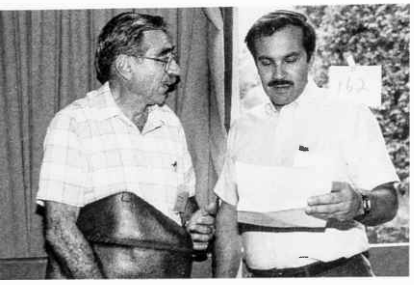
ARRANGED BY

Michael Mathews, Cold Spring Harbor Laboratory
Nahum Sonenberg, McGill University

313 participants

The first Translational Control meeting was held at Cold Spring Harbor two years ago. That meeting engendered great enthusiasm among its participants, many of whom felt that it signaled a renaissance of research in protein synthesis. Judging from the increased attendance, the wealth of new information and the high caliber

B. Thimmappaya, M. Mathews, B. Hayes



R. Bablanian, W. Merrick

N. Gupta, A. Roy, U. Maitra

M. Grunberg-Manago, B. Safer

of the presentations made during this year's meeting, these expectations have been translated into fact. Molecular genetic approaches have allowed great strides to be made in defining the mechanism of translation initiation in yeast and in higher eukaryotes. Major contributions to this knowledge have stemmed from the cloning of the genes for many of the eukaryotic initiation factors. Advances have been made in defining the *cis*-acting elements within prokaryotic and eukaryotic mRNAs that function in translational control, and it has become clear that secondary and tertiary structure elements are key components in translational control mechanisms operating during the initiation and elongation phases and in ribosome frame shifting. Regulatory factors that either suppress or enhance translation have been characterized, and it is anticipated that many more such *trans*-acting factors will be identified in the next few years. The control of translation during development and growth has attracted considerable attention: As more genes involved in the regulation of development, growth, and differentiation are cloned, it becomes increasingly apparent that many of them are translationally controlled.

PROGRAM

mRNA 5′ Untranslated Region
Chairman: H. Voorma, *University of Utrecht*

Initiation
Chairman: W. Merrick, *Case Western Reserve University*

Elongation and Termination
Chairman: A. Spirin, *Academy of Science of the USSR*

Prokaryotes
Chairman: M. Grunberg-Manago, *Foundation Edmond de Rothschild*

Phosphorylation of Initiation Factors
Chairman: R. Thatch, *Washington University*

Stability, Poly (A), and Other Tales
Chairman: D. Cleveland, *Johns Hopkins Medical School*

Growth, Development, and Heat Shock
Chairman: R. Jackson, *University of Cambridge*

Initiation Factor Genes and Their Regulation
Chairman: A. Wahba, *University of Mississippi Medical Center*

L. Slobin, A. Spirin

N. Sonenberg, A. Moustakas

Modern Approaches to New Vaccines Including Prevention of AIDS

September 20–September 24, 1989

ARRANGED BY

Fred Brown, Wellcome Biotechnology Ltd.
Robert Chanock, NIAID, National Institutes of Health
Harold S. Ginsberg, Columbia University
Richard Lerner, Research Institute of Scripps Clinic

276 participants

The seventh annual meeting on Modern Approaches to New Vaccines offered an opportunity for molecular biologists, microbiologists, and clinical investigators to interact and broaden their perspective of immunoprophylaxis. Biomedical scientists concerned with prevention of viral, bacterial, or parasitic diseases met to exchange the latest information on (1) the molecular and immunological properties of important human and animal pathogens; (2) the application of this knowledge to the development of new vaccine strategies, and (3) the evaluation of the resulting candidate vaccines for safety and efficacy in the laboratory or in clinical trials.

Among the many exciting advances described were the induction of antibodies that catalyze peptide cleavage at a specific site, the development of a combinatorial system for cloning and expressing the antibody repertoire of an animal in *E. coli*, the demonstration of the role of HIV *gag*-specific cytotoxic T cells in resistance to AIDS, and the first experimental evidence that immunization with an inactivated simian immunodeficiency virus (SIV) induces effective resistance in monkeys to subsequent SIV challenge. Equally notable were the first descriptions of the sequence and genome organization of the feline immunodeficiency virus (FIV), which now appears to be a promising experimental surrogate for human AIDS. Evidence was also presented indicating that HIV-2 evolved in West Africa following infection of humans by an SIV indigenous to sooty mangabey monkeys (SIV_{smm}). Finally, the feasibility of using synthetic peptides as immunogens received a significant boost from a study demonstrating that reactivity of a synthetic peptide with a monoclonal antibody directed against it could be increased several orders of magnitude by substitution of appropriate amino acids at certain sites in the linear sequence.

The meeting was supported in part by NIAID and the Rockefeller Foundation.

F. Brown, E. Norrby

J. Treanor, B. Murphy

R. Lerner, H. Ginsberg

F. Schödel, M. Popescu

PROGRAM

Immunology, Bacteriology and Parasitology
Chairman: R. Lerner, *Research Institute of Scripps Clinic*

AIDS and Bacteriology
Chairman: M.B.A. Oldstone, *Research Institute of Scripps Clinic*

Virology. I
Chairman: B. Murphy, *NIAID, National Institutes of Health*

Virology. II
Chairman: R. Chanock, *NIAID, National Institutes of Health*

AIDS and Immunology
Chairman: E. Norrby, *Karolinska Institutet*

R. Chanock

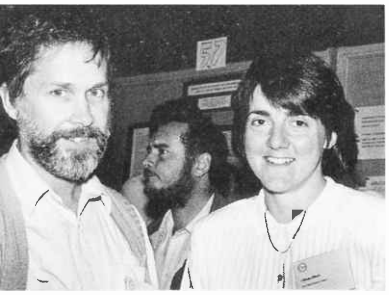
AIDS. I
Chairman: P.R. Johnson, *Georgetown University*
AIDS. II
Chairman: H. Ginsberg, *Columbia University College of Physicians & Surgeons*
Virology and Parasitology
Chairman: E. Wimmer, *State University of New York, Stony Brook*
Virology, Parasitology, and Bacteriology
Chairman: F. Brown, *Wellcome Biotechnology Ltd.*
Summary: P.R. Johnson, *Georgetown University*

Hepatitis B Viruses

September 25–September 28, 1989

R. Burke, H. Schaller

M. Lai, J. Monjardino



W. Mason, A. Jilbert

ARRANGED BY

Patricia Marion, Stanford University Medical School
Heinz Schaller, University of Heidelberg

268 participants

At the meeting on the Molecular Biology of Hepatitis B Viruses, new information was presented on the replication, transcriptional regulation, and gene product synthesis of hepatitis B virus (HBV) and on the host response to this virus family, including the viral association with hepatocellular carcinoma. The development of more efficient systems to replicate the various hepadnaviruses via transfection of viral DNA into cultured cell lines has resulted in significant progress in the genetic analysis of all aspects of virus replication. Viral sequences important in encapsidation of the viral RNA pregenome, in initiation of both minus- and plus-strand DNA synthesis, and in circularization of viral DNA have been located. Regions of the *X* gene essential for reverse transcriptase activity and RNase H activity were described, as well as regions of the structural proteins that are important in membrane insertion or in assembly and eventual secretion.

In other sessions, speakers described new studies of the cellular factors interacting with specific sequences of the viral enhancer and presented recent findings on the *trans*-activating functions of the *X* gene product. In the area of pathology and immunology of the HBV family, T-cell response to envelope antigens and the *X* gene product were detected, although response to nucleocapsid antigens appeared to be stronger. In a session on the role of hepadnaviruses in liver cancer, factors contributing to the development and growth of liver malignancy were described, along with an aberrant oncogene structure or expression associated with tumors in one of the animal models, but not in HBV infection, and a high rate of specific modification of a single chromosome in human tumors. These studies support the conclusion that chronic HBV infection may lead to hepatocellular carcinoma by more than one route.

Recent studies of the replication of hepatitis delta virus (HDV), dependent on HBV for secretion and subsequent infection, were presented. Specific interactions between the HDV-encoded protein and the RNA were described, along with the possible roles of two variants of the HDV protein created by RNA editing.

PROGRAM

Replication. I
Chairman: J. Summers, *University of New Mexico School of Medicine*
Replication. II
Chairman: H. Schaller, *University of Heidelberg*
Hepatocellular Carcinoma
Chairman: K. Koike, *Cancer Institute, Tokyo*
Hepatitis Delta Virus
Chairman: M. Lai, *University of Southern California School of Medicine*
Structural Proteins. I
Chairman: P. Marion, *Stanford University Medical School*
Structural Proteins. II
Chairman: W. Gerlich, *Georg August University, Gottingen*
Transcription
Chairman: W. Mason, *Fox Chase Cancer Center*
X Gene
Chairman: W.S. Robinson, *Stanford University*
Pathology/Immunology
Chairman: D. Ganem, *University of California, San Francisco*

P. Marion, R. Cheung, J. Cullen

K. Koike

W. Robinson

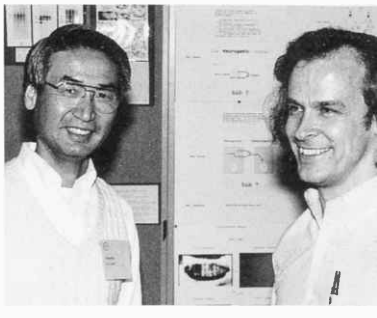
Molecular Neurobiology of *Drosophila*

October 4–October 8, 1969

ARRANGED BY

William Pak, Purdue University
Donald Ready, Purdue University

179 participants



W. Pak, D. Ready

Approximately 175 researchers representing essentially all *Drosophila* neurobiology laboratories gathered for the third biennial meeting. For this meeting, 153 abstracts were contributed and three poster sessions were held, in addition to the seven slide sessions. Lively discussions ranged from the molecular structure of ion channels to mutational analysis of learning.

Considerable progress was reported on the neurogenic genes that regulate CNS development. Biochemical analysis of one neurogenic gene product, *Notch*, has borne out the predictions of protein structure and topology deduced from the gene sequence and has revealed subtleties of cellular localization. Growing evidence suggests that *Notch* may have a role in cell-cell adhesion.

A growing family of genes that direct neural patterning have been recognized as *myc*-related transcription factors. A combinatorial network of interactions among these gene products was the focus of much discussion. Several papers described patterns of gene activity in the developing nervous system as revealed by enhancer traps. A notable early success of this method is the identification of a new gene of the steroid receptor superfamily that is required both in the early embryonic nervous system and in the later development of the compound eye. G proteins and phospholipase C second-messenger cascades figured prominently

D. Gailey, J. Hall


J. Palka, J. Posakony


J. Campos-Ortega, J. Hall


M. Seeger, I. Hariharan, G. Rubin

in discussions of phototransduction. Evidence was presented suggesting that sensory receptor-specific phosphoprotein phosphatase plays a role in both visual and olfactory reception.

Different forms of the *Shaker* potassium channel gene products are expressed differentially in the CNS. The functional significance of these variant transcripts is beginning to be sorted out by patch-clamp analysis of *Xenopus* oocytes expressing combinations of *Shaker* transcripts. Functional domains of the *Shaker* proteins are being elucidated by measurements made on channels altered by site-directed mutagenesis.

Discussions of genes important to behaviors were highlighted by the report that the *per* gene transcript cycles in synchrony with the circadian rhythm. Antibodies to the *per* protein showed staining in neurons, photoreceptors, and glial cells in the fly head, and the intensity of staining varied with the circadian rhythm.

PROGRAM

Neurogenesis
Chairman: J. Campos-Ortega, *Universitat zu Koln*

Nervous System Development: myc-Related Proteins and Others
Chairman: J. Posakony, *University of California, San Diego*

Sensory System Development
Chairman: G.M. Rubin, *University of California, Berkeley*

Sensory Transduction
Chairman: C. Zuker, *University of California, San Diego*

Early Neuronal Differentiation and Eye Development
Chairman: S. Benzer, *California Insitute of Technology*

Ion Channels
Chairman: R. Aldrich, *Stanford University School of Medicine*

Behavior
Chairman: J. Hall, *Brandeis University*


BANBURY
CENTER

BANBURY CENTER DIRECTOR'S REPORT

The Banbury Center continues to be used throughout almost the entire year, with the exception of an all-too-brief respite during the winter! Seventeen meetings were held here, in addition to four advanced lecture courses on neurobiology and one on mapping human diseases. More than 500 people participated at these meetings. The majority came from the United States, but many were visitors from Europe and as far afield as Japan and Australia. Bea Toliver and Ellie Sidorenko in the Center's office and Katya Davey at Robertson House did a wonderful job looking after all aspects of the meetings. These meetings seem to cover an increasingly diverse range of topics, a continuing testimony to the power of molecular biology and genetics.

Topics in Basic Research

Although it is increasingly difficult to maintain a distinction between "basic" and "applied" research, in 1989, there were two meetings that perhaps qualify as basic research. One meeting was called **Molecular Clocks of Evolution**. A considerable amount of data has been produced using differences in DNA between different species to measure the relatedness of species. Some of the data obtained using DNA as a molecular clock have required radical revision of previously accepted taxonomic relationships, and the purpose of our meeting was to examine whether the assumptions underlying the use of molecular clocks are justified. It was intriguing to see data with time scales measured in millions of years rather than the minutes or hours of conventional molecular biological data!

Evolution was also the underlying theme of the meeting **Molecular Genetics of Early *Drosophila* and Mouse Development**. Homology between related genes of

Banbury Meeting House, rear view

different species is a very powerful tool for studying gene function, as the research on *Drosophila* homeo box genes and their mouse equivalents has shown. In addition, new advances such as homologous recombination offer the possibility of directly manipulating the genomes of organisms like the mouse, which were previously restricted to classic breeding experiments. This meeting brought together the world's leading scientists working on mouse and *Drosophila* development with a view to determining whether there are common themes to studies of the molecular development of these two very different organisms. As *Science* reported (*244*: 652–654 [1989]), the two groups did find common ground, and we hope that the meeting has stimulated continuing interactions in this field.

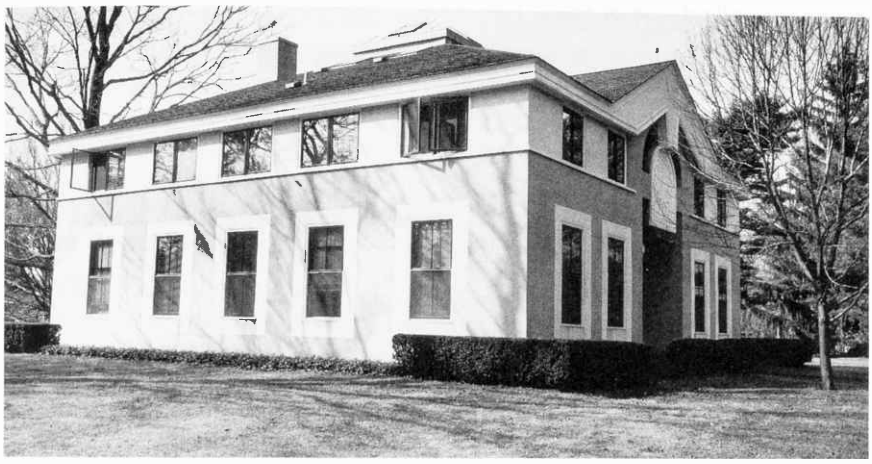
Molecular Genetics and Human Inherited Diseases

Extraordinary advances continue to be made in the exploitation of recombinant DNA techniques for studying human inherited diseases, as shown by four meetings held here this year. One of the great hopes of molecular genetics is that it will provide an understanding of the genetics of human cancer, a promise that comes closer to fulfillment each year. Some of the most important recent advances in cancer research concern anti-oncogenes or tumor suppressor genes and their interactions with oncogenes. This was the topic of the Banbury meeting, **Recessive Oncogenes and Tumor Suppression**, held in March. It would be invidious to pick out individual contributions, for the whole meeting was remarkably stimulating and encouraging. The field seems to be at one of those rare and exciting moments in research when a series of apparently unconnected observations and findings are about to be unified.

The gene for Duchenne muscular dystrophy (DMD) was the first gene responsible for a human inherited disease to be cloned without any prior knowledge of the protein involved. Research on DMD has moved rapidly from analysis of DNA to studies of the function of the protein, named dystrophin, in muscle, brain, and other tissues. **Dystrophin**, a very exciting meeting in March, drew together research workers from all over the world to discuss, and in some cases to attempt to reconcile, the latest results. It is a remarkable comment on the rapid progress made possible by recombinant DNA techniques that a session could be devoted to discussing therapeutic strategies for affected boys.

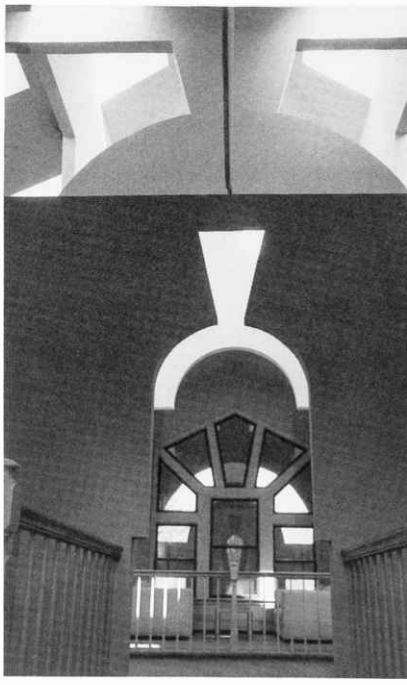
A major question is whether the strategies used to clone the DMD and cystic fibrosis genes are applicable to other disorders, and whether other, more rapid and efficient methods can be devised. The meeting, **Molecular Cytogenetics: Bridging the Resolution Gap**, examined new strategies for handling and analyzing DNA intermediate in size between the DNAs studied by cytogenetics and by cloning techniques. It seems that although one can optimize techniques and strategies, a considerable element of luck is involved in finding a sought-after gene in kilobases of DNA.

Although cloning is laborious and time-consuming, the successes with the DMD and cystic fibrosis genes demonstrate that it is possible to clone the genes for human diseases caused by single genes. Most common disorders, however, while showing strong familial tendencies, do not have a simple pattern of inheritance and probably result from interactions between the environment and one or more of a number of genes. Alcoholism is a classic example of such a complex disease, and the **Molecular Genetics and Biology of Alcoholism** meeting reviewed the data on the inheritance of alcoholism and attempted to evaluate future strategies. As in the meeting held last year on schizophrenia, very few firm



Sammis Hall, guest house

conclusions could be reached, except that there is a genetic component in alcoholism. It seems unlikely that a real effort can begin on cloning the genes involved in alcoholism without many more studies of families, a project about to begin under the auspices of the National Institute on Alcohol Abuse and Alcoholism.



Neurobiology of Vision

For 2 weeks in the middle of the summer, the Conference Center comes to resemble mission control as it is inundated by computers used for the Computational Neuroscience course. Advantage was taken of their presence to hold a Banbury Center meeting on **Computational Models of Visual Processing.** The meeting covered such topics as spatial sampling, object recognition, and scene analysis and involved hands-on computer modeling as well as more usual presentations and discussions. There is no doubt that this kind of integrated workshop/lecture format contributed enormously to the success of the meeting.

HIV and AIDS

Two meetings on HIV and AIDS dealt with quite different aspects of the problem. The November meeting, **Control of HIV Gene Expression**, was a follow-up to a similar meeting held in February of 1988. There were some concerns that the gap between the two meetings was too long, but in the event it seemed just right. Research has continued to demonstrate the complexities of the HIV genome and of the interactions between the regulatory systems of the virus and the host cell. It is still proving to be a highly controversial field despite (or because of) the intense research activities. It was a particular pleasure to welcome Harold Varmus, newly anointed Nobel Laureate and longtime associate of Banbury Center, to the meeting.

The **Immunological Aspects of AIDS** meeting provided an opportunity for researchers to determine what progress has been made in this field and what topics require special attention. The primary target of HIV is the immune system, and it might be expected that by now there would be a clear understanding of how the virus produces an immunodeficient state. Such an understanding may be a prerequisite for designing efficient and safe vaccines. However, the difficulties of working with complex cell-culture systems or with animals make progress in this vital area of research frustratingly slow.

Practical Applications of Basic Research

One strategy proposed for dealing with viral infections is to target viral proteins that are of critical importance to the life cycle of the virus. The viral proteinases that cleave polyprotein precursors into individual viral proteins are one group of such proteins. A very exciting meeting, **Viral Proteinases as Targets for Chemotherapy**, examined what has been done in this research area. This was one of the largest meetings of 1989, a testimony to the potential importance of this approach for controlling viral infections, including AIDS.

The **Applications of Basic Research in Mammalian Development** meeting exemplified the Banbury Center's objective of bringing together diverse groups of people. The topics at this meeting ranged from sex determination, through cryopreservation of embryos and gene transfer in domestic animals, to preimplantation diagnosis of human diseases!

Environmental Hazards

The **Mutation Induction and Heritability in Mammalian Germ Cells** meeting dealt with such important topics as How do mutations arise in germ-line cells? Can these mutations be repaired? How can they be detected? In particular, participants reviewed the ways in which new technical developments such as polymerase chain reaction can be used to get estimates of germ cell mutation rates.

Sloan Foundation Workshops for Congressional Staff and Science Journalists

The workshop for Congressional staff dealt not with a research topic, but with an issue of public policy, the question of scientific misconduct. The meeting was entitled **The Ethos of Scientific Research** because it was intended to examine the set of beliefs held by scientists that maintains the integrity of the way in which research is done. There have been many other meetings on this topic, but we believe that ours was unique. The participants included those directly involved with the issue in Washington and a group of distinguished scientists. For the first time, these two groups had the opportunity to interact directly with each other in an informal setting. Whatever the outcome in terms of legislation, there is no doubt that the two groups—scientists and staff—left the meeting with a clear appreciation, if not understanding, of the intense feelings that this issue generates on both sides.

Applications of the New Molecular Genetics was the theme of the science journalists' workshop in May. The meeting covered topics that were united in being amenable to new analysis because of new experimental techniques like homologous recombination for gene targeting in mice. For the first time, we made use of the facilities of the DNA Learning Center to give the journalists an opportunity to experience a little of what they write about! This was a great success, and similar laboratory experiments will be featured in the new series of workshops supported by the Sloan Foundation.

H. Wendt and B. Dovey at Baring Brothers Meeting

The Baring Brothers-CSHL Meeting

For the second year, the London merchant bankers Baring Brothers and Cold Spring Harbor Laboratory hosted a meeting for senior executives of companies with strong links to biotechnology. As in previous years, the topic of the meeting was chosen to involve fascinating research that has potential applications in biotechnology. Stanley Cohen introduced **Growth Factors in Development**, a

meeting intended to introduce participants to the research being done on growth factors and morphogens and to point to the therapeutic potential of these biologically active substances. Once again, the practical experience in the laboratory at the DNA Learning Center was a tremendous success. On this occasion, DNA samples were analyzed for the sickle cell anemia mutation using polymerase chain reaction, restriction enzyme digest, and gel electrophoresis.

Other Meetings

The Center has been made available on occasion to other groups. The Deans of the Associated Medical Schools of New York, the Board of Trustees of Huntington Hospital, and Cold Spring Harbor School District held meetings here. In view of the Center's increasing interest in science policy and human genetics, we were particularly pleased to be able to host a joint meeting of the National Institutes of Health and the Department of Energy human genome initiatives at Banbury.

Funding

The Corporate Sponsor Program provided the funding for five of the Banbury Center meetings in 1989 and, in so doing, provided the bedrock on which the Center's meeting program is built. The five meetings were Applications of Basic Research in Mammalian Development; Viral Proteinases as Targets for Chemotherapy; Recessive Oncogenes; Molecular Cytogenetics; and The Molecular Genetics of Early *Drosophila* and Mouse Development. The importance of the contributions from the Laboratory's Corporate Sponsors cannot be overemphasized, especially in a period of declining Federal support for meetings.

Corporations and private foundations seem to be taking on this role increasingly. The Alfred P. Sloan Foundation has already undertaken to support the Congressional Staff and Science Journalist's Workshops for a further 3 years,

NIH-DOE Human Genome Meeting

beginning in 1990. The Sloan Foundation also helped to underwrite the costs of the **Molecular Clocks of Evolution** meeting as part of the Foundation's continuing interest in promoting research on molecular evolution. The Muscular Dystrophy Association funded the meeting on **Dystrophin**, and the Christopher D. Smithers Foundation gave very generously to support the meeting on the **Molecular Genetics and Biology of Alcoholism**. The book of this meeting will reach a wider audience than is usually the case because of the generosity of an anonymous donor who has underwritten the production costs of the book. Given the Center's interest in human molecular genetics, I hope to persuade other disease-specific foundations to consider holding workshops here.

Partial support for three meetings was obtained from Federal sources. The Environmental Protection Agency underwrote the costs of the **Mutation Induction and Heritability in Mammalian Germ Cells** meeting, and the National Institute for Allergy and Infectious Diseases supported the meetings on HIV and AIDS.

Banbury Center Publications

Two books published in 1989 deserve special mention. *Polymerase Chain Reaction*, part of the *Current Communications in Molecular Biology* series, was based on the meeting sponsored by Perkin-Elmer Cetus, and it seems set to become a best seller as applications of PCR increase day by day. The second important publication was *Banbury Report 32: DNA Technology and Forensic Science*. This could not have come at a more opportune moment as controversy continues unabated about the standards to be applied in this real-life application of molecular genetics. The papers and especially the discussions in this book should make a very valuable contribution to this debate.

Banbury Center Publicity

Publicity for Banbury Center meetings can be a two-edged sword. On the one hand, it provides a means of informing people about what goes on at our meetings, but, on the other hand, it may cause administrative difficulties given the

Robertson House provides housing and dining accommodations at Banbury Center

restricted numbers that can attend meetings here. On balance, the advantages are likely to outweigh the disadvantages, and journalists and writers attended some meetings in 1989 either for background or to write reports. The report in *Science* on The Molecular Genetics of Early *Drosophila* and Mouse Development meeting was an excellent example of how we can achieve a wider audience for the information presented at our meetings. The DNA Technology and Forensic Science meeting also received wide publicity, as did the Congressional Workshop, The Ethos of Scientific Research, although not all of the latter was favorable.

Looking Forward to 1990

The Center will continue to hold meetings on topics in five main areas: (1) basic research and technical developments in molecular biology and genetics; (2) advances in the applications of molecular genetics to human inherited disorders, especially complex disorders like alcoholism; (3) environmental issues; (4) the increasing impact of molecular biology and genetics on the public; and (5) issues of scientific policy that are of special importance to the research scientist, e.g., animal experimentation and granting mechanisms. These latter topics will become increasingly important as public oversight of what scientists do continues to increase. We have to lead, and not simply respond, in matters where there is legitimate public interest in our activities. The Center will continue to play a unique role in furthering all aspects of research in molecular biology and encouraging thoughtful and thought-provoking examination of its relationships with society.

Jan A. Witkowski

Publications

Ballantyne, J., G. Sensabaugh, and J.A. Witkowski, eds. 1989. *DNA Technology and Forensic Science. Banbury Rep.* **32**.

McCabe, E.R.B., J. Towbin, J. Chamberlain, L. Baumbach, J.A. Witkowski, G.J.B. van Ommen, M. Koenig, L.M. Kunkel, and W.K. Seltzer. 1989. cDNA for the Duchenne muscular dystrophy locus demonstrates a previously undetectable deletion in a patient with dystrophic myopathy, glycerol kinase deficiency and congenital adrenal hypoplasia. *J. Clin. Invest.* **83:** 95–99.

Ward, P.A., J.F. Hejtmancik, J.A. Witkowski, L. Baumbach, S. Gunnel, J. Speer, P. Hawley, U. Tantravahi, C.T. Caskey, and S. Latt. 1989. Prenatal diagnosis of Duchenne muscular dystrophy: Prospective linkage analysis and retrospective dystrophin cDNA analysis. *Am. J. Hum. Genet.* **44:** 270–281.

Witkowski, J.A. 1989. Dystrophin-related muscular dystrophies. *J. Child Neurol.* **4:** 251–271.

Witkowski, J.A. 1989. *The NEB Transcript* **2:** 1–7.

Witkowski, J.A. 1989 [correspondence]. Reprint requests and *Current Contents. Nature* **337:** 684.

Witkowski, J.A. 1989 [book review]. *Is Science Necessary?* by Max Perutz. *Trends Genet.* **5:** 387.

Witkowski, J.A. 1989 [book review] *Science as a Process* by David Hull. *Trends Biochem. Sci.* **14:** 420–421.

Witkowski, J.A. 1990. *Milestones in the development of DNA technology.* American Chemical Society. (In press.)

Witkowski, J.A. 1990. Julian Huxley in the laboratory: Embracing inquisitiveness and widespread curiosity. In *Julian Huxley, Biologist and Statesman of Science* (ed. A. Van Helden and K. Waters). Cambridge, Cambridge University Press. (In press.)

MEETINGS

Sloan Foundation Congressional Workshop on the Ethos of Scientific Research

January 26 - January 28

ARRANGED BY

J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory, New York

SESSION 1

P. Woolf, Princeton University, New Jersey: Some characteristics of publicly reported cases.
D. Rennie, Journal of the American Medical Association, Mill Valley, California: The editor, the journal, and scientific misconduct.
B. Mishkin, Hogan & Hartson, Washington, D.C.: Need for institutional policies and procedures.

SESSION 2

D. Zuckerman, House Subcommittee on Human Resources & Intergovernmental Relations, Washington, D.C.: Role of Congress in helping prevent and investigate scientific fraud.
B. Chafin, House Subcommittee on Oversight & Investigations, Washington, D.C.: Collapse of self-regulation in the absence of oversight.
W. Stewart, National Institutes of Health, Bethesda, Maryland: Keeping Congress out of science.

D. Zuckerman, D. Nathans

SESSION 3

H. Wortis, Tufts University, Boston, Massachusetts: Science investigation.
D. Korn, Stanford University Medical School, California: Institutional policy and process.
J. Newburgh, National Institutes of Health, Bethesda, Maryland: Response and role of NIH.
C. Scheman, Association of American Universities, Washington, D.C.: Issues relating to the development of institutional policies.

G. Booth, N. Zinder, H. Varmus

M. Mathews, M. Ptashne, R. Axel

Recessive Oncogenes and Tumor Suppression

March 29 - April 1

ARRANGED BY

W. Cavanee, Ludwig Institute for Cancer Research, Montreal, Canada
N. Hastie, Western General Hospital, Edinburgh, Scotland
E.J. Stanbridge, University of California, Irvine

SESSION 1: Somatic Cell Genetics

Chairperson: E.J. Stanbridge, University of California, Irvine

H. Harris, University of Oxford, England: Role of differentiation in the suppression of malignancy.

I. Herskowitz, University of California, San Francisco: Response and resistance to negative growth factors in yeast: Function and control of synthesis of peptide receptors.

J.C. Barrett, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Tumor suppressor genes as negative regulators of cell growth.

C. Harris, National Cancer Institute, Bethesda, Maryland: Tumor suppression studies of human lung cancer.

N. Bouck, Northwestern University, Chicago, Illinois: Suppressor control of an inhibitor of angiogenesis.

H. Zarbl, Massachusetts Institute of Technology, Cambridge: Genetic analysis of transformation effector and suppressor genes.

B.E. Weissman, University of North Carolina, Chapel Hill: Tumor suppressor genes in pediatric cancers.

SESSION 2: Deletions and Cancer

Chairperson: N. Hastie, Western General Hospital, Edinburgh, Scotland

W.K. Cavenee, Ludwig Institute for Cancer Research, Montreal, Canada: Loss of genetic information in cancer predisposition and progression.

R.L. White, Howard Hughes Medical Institute, Salt Lake City, Utah: High-resolution mapping of inherited tumor genes.

B. Vogelstein, John Hopkins Oncology Center, Baltimore, Maryland: Genetic alterations in colorectal tumors.

N. Hastie, Western General Hospital, Edinburgh, Scotland: Wilms' tumor locus at 11p13.

B.A.J. Ponder, Institute of Cancer Research, Surrey, England: Genetics of multiple endocrine neoplasia type 2.

SESSION 3: Retinoblastoma

Chairperson: W.K. Cavenee, Ludwig Institute for Cancer Research, Montreal, Canada

M.F. Hansen, M.D. Anderson Cancer Center, University of Texas, Houston: Molecular genetics of familial mixed cancer.

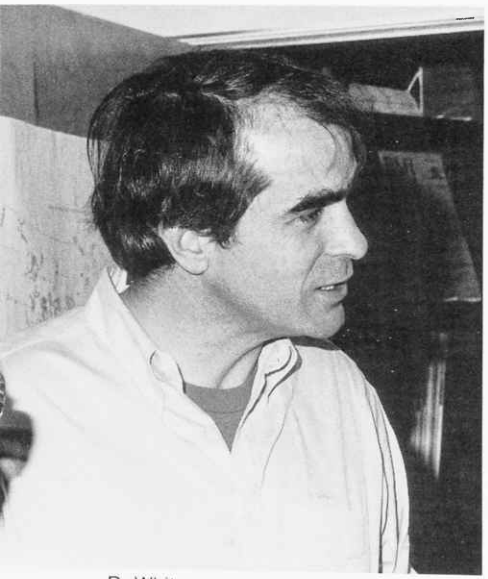
W.-H. Lee, University of California at San Diego, La Jolla: Molecular basis of tumor suppression by the retinoblastoma gene.

B. Phillips, Hospital for Sick Children, Toronto, Canada: Characterization of mutations in the retinoblastoma gene.

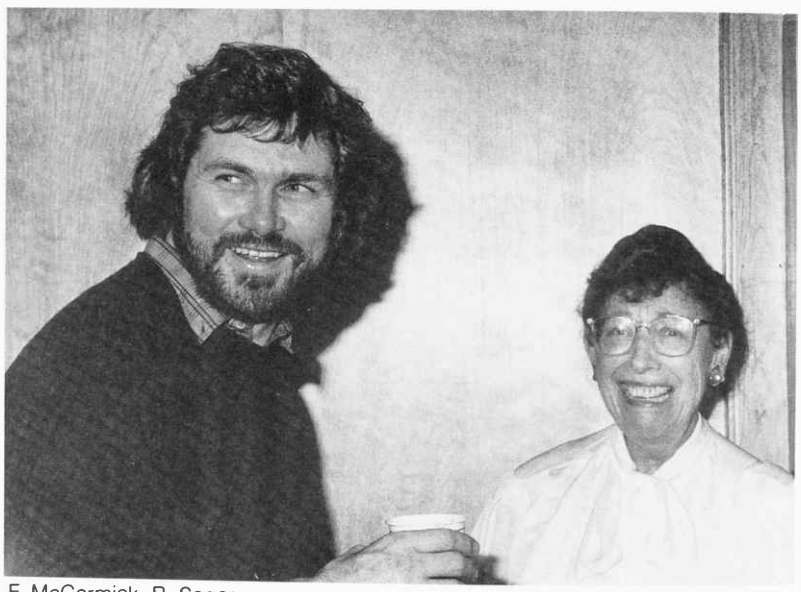
W. Benedict, The Woodlands, Texas: The retinoblastoma gene and its product.

D. Livingston, Dana-Farber Cancer Institute, Cambridge, Massachusetts: SV40 T antigen-RB interactions.

R.A. Weinberg, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: The retinoblastoma gene.



R. White



F. McCormick, R. Sager

SESSION 4: Other Candidate Tumor Suppressor Genes

Chairperson: R.A. Weinberg, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts

B.M. Mechler, Johannes Gutenberg University, Mainz, Federal Republic of Germany: Molecular basis for tumor suppression in *Drosophila*.

M. Noda, The Institute of Physical and Chemical Research, Ibaraki, Japan: Biological activities of the transformation suppressor gene Krev-1 and its mutants.

R. Schaefer, University of Zurich, Switzerland: Molecular and functional analysis of tumor suppressor genes by transfection.

L.A. Liotta, National Cancer Institute, Bethesda, Maryland: Metastasis suppressor gene.

R. Sager, Dana-Farber Cancer Institute, Cambridge, Massachusetts: The *gro* gene as putative cytokine and tumor suppressor.

SESSION 5: Strategies for Cloning Tumor Suppressor Genes

Chairperson: R.L. White, Howard Hughes Medical Institute, Salt Lake City, Utah

J.D. Minna, National Cancer Institute, Bethesda, Maryland: Chromosomal deletion in lung cancer.

E. Stanbridge, University of California, Irvine: Strategies for cloning human tumor suppressor genes.

L. Strong, M.D. Anderson Cancer Center, University of Texas, Houston: Molecular genetics of the Wilms' tumor locus.

A. Balmain, Beatson Institute for Cancer Research, Glasgow, Scotland: Chromosomal changes during tumor progression.

SESSION 6: Oncogene-Tumor Suppressor Gene Interactions

Chairperson: M. Ptashne, Harvard University, Boston, Massachusetts

E. Harlow, Cold Spring Harbor Laboratory, New York: Protein complexes between p105-RB and DNA tumor-virus-transforming proteins.

A.J. Levine, Lewis Thomas Laboratory, Princeton University, New Jersey: The p53 proto-oncogene can suppress E1A plus *ras*-mediated transformation.

F. McCormick, Cetus Corporation, Emeryville, California: Role of GAP in *ras* transformation.

M.H. Wigler, Cold Spring Harbor Laboratory, New York: Signal processing along the *ras* pathway.

S. Aaronson, National Cancer Institute, Bethesda, Maryland: Growth-factor-activated pathways in the neoplastic process.

J. Massague, University of Massachusetts Medical School, Worcester: Control of growth and phenotype by TGF-β.

Dystrophin

April 2 - April 5

ARRANGED BY

L.M. Kunkel, The Children's Hospital, Boston, Massachusetts
R. Worton, Hospital for Sick Children, Toronto, Canada

SESSION 1: The Human Dystrophin Gene: Putting Together a Physical Map

Chairperson: L.M. Kunkel, The Children's Hospital, Boston, Massachusetts

G.-J.B. van Ommen, University of Leiden, The Netherlands: Review of physical map.

K.E. Davies, John Radcliffe Hospital, Oxford, England: Deletions in the central and 3′-end of the dystrophin gene: Genotype and phenotype.

U. Francke, Yale University, New Haven, Connecticut: Characterization of dystrophin deletions by cDNA studies and long-range mapping.

R.J. Bartlett, Duke Unviersity Medical Center, Durham, North Carolina; A. Burghes, Ohio State University, Columbus; J.S. Chamberlain, Baylor College of Medicine, Houston, Texas; M. Koenig, The Children's Hospital, Boston, Massachusetts, and A. Speer, Akademie der Wissenschaften der German Democratic Republic: Filling out the map.

SESSION 2: Transcriptional Regulation and Expression

Chairperson: R. Worton, Hospital for Sick Children, Toronto, Canada

J.-C. Kaplan, Institut National de la Sante et de la Recherche Medicale, Paris, France: Transcripts of the dystrophin gene.

U. Nudel, The Weizmann Institute of Science, Rehovot, Israel: Expression and promoter studies in rodent myogenic cells.

L. Rowland

A. Engel, E. Bonilla

H. Klamut, Hospital for Sick Children, Toronto, Canada: Human gene promoter and expression in myogenic cells.
H. Blau, Stanford University, California: Cell biology of dystrophin.
R. Brown, Massachusetts General Hospital, Boston: Studies of DMD gene expression in vitro.

SESSION 3: Dystrophin Structure and Function

Chairperson: E. Lazarides, California Institute of Technology, Pasadena

E. Bonilla, Columbia University College of Physicians & Surgeons, New York, New York: Localization of dystrophin in specialized regions of the muscle fiber.
E. Zibrzycka-Gaarn, Hospital for Sick Children, Toronto, Canada: Site-specific antibodies to dystrophin and localization to membrane vesicles.
K. Arahata, National Institute of Neuroscience, Tokyo, Japan: Immunohistochemical study of dystrophin in DMD and BMD.
M. Cullen, Newcastle General Hospital, England: Ultrastructural localization of dystrophin in human muscle by gold labeling.
S. Watkins, Dana-Farber Cancer Institute, Cambridge, Massachusetts: Light and immunoelectron microscopic localization of dystrophin.
G.-J.B. van Ommen, University of Leiden, The Netherlands: Dystrophin localization in embryonic, fetal, and adult mouse.
K. Campbell, University of Iowa, Iowa City: Purification of dystrophin from rabbit muscle membranes.
T.J. Byers, Harvard University, Cambridge, Massachusetts: Relationship of dystrophin to α- and β-spectrins.
M. Koenig, The Children's Hospital, Boston, Massachusetts: Dystrophin structure and possible function.

SESSION 4: Cytoskeletal Proteins: Structure and Function

Chairperson: A.G. Engel, Mayo Clinic, Rochester, Minnesota

V. Bennett, Duke University Medical Center, Durham, North Carolina: Proteins of the spectrin-based membrane skeleton.
E. Lazarides, California Institute of Technology, Pasadena: Control of assembly of the membrane skeleton.
D. Critchley, University of Leicester, England: Cytoskeletal proteins linking actin to the membrane in cell matrix junctions.
S. Georgatos, Rockefeller University, New York: Nuclear lamina: Organization principles and anchorage functions.
S. Craig, Johns Hopkins University Medical School, Baltimore, Maryland: Muscle costameres: Transmembrane linkage between extracellular matrix, sarcolemma, and myofiber bundles.

SESSION 5: Mutational Spectrum and Clinical Correlates

Chairperson: K. Davies, John Radcliffe Hospital, Oxford, England

K. Fischbeck, University of Pennsylvania Hospital, Philadelphia: Clinical spectrum of defects in the dystrophin gene.
A. Burghes, Ohio State University, Columbus: Correlation of genotype and phenotype at the 5′ end of the gene.
R. Worton, Hospital for Sick Children, Toronto, Canada: Deletions and duplications of exons in DMD and BMD: More on the frame-shift idea.
M. Koenig, The Children's Hospital, Boston, Massachusetts: The frame-shift hypothesis: Correlation of mutation type with phenotype.
J.S. Chamberlain, Baylor College of Medicine, Houston, Texas: PCR technology for rapid deletion analysis.
M. Zatz, Universidade de Sao Paulo, Brazil: DNA and dystrophin studies in Brazilian families.
E. Hoffman, The Children's Hospital, Boston, Massachusetts: Dystrophin quality and quantity in DMD and BMD.

SESSION 6: Animal Models and Disease

Chairperson: M. Cullen, Newcastle General Hospital, England, and L.M. Kunkel, The Children's Hospital, Boston, Massachusetts

G. Karpati, Montreal Neurological Institute and Hospital, Quebec, Canada: Dystrophin expression in mosaic muscle fibers of carrier *mdx* females and transplanted *mdx* males.

T.A. Partridge, Charing Cross & Westminister Medical School, London, England: Relationship between morphology and content of introduced dystrophin in muscle of the *mdx* mouse.

J.L. Mandel, Institute of Biological Chemistry, Strasbourg, France: Dystrophin gene and protein in chicken.

B.J. Cooper, Cornell University, Ithaca, New York: Expression of dystrophin in the *grmd* dog.

R. Bartlett, Duke University Medical Center, Durham, North Carolina: Studies of the golden retriever muscular dystrophy.

SESSION 7: What Does the Future Hold?

Chairperson: D. Wood, Muscular Dystrophy Association, New York, New York

T.A. Partridge, Charing Cross Hospital, London, England: Future of myoblast transplantation: The minimum requirement before consideration of clinical trials.

L.M. Kunkel, The Children's Hospital, Boston, Massachusetts: Speculation on the function of dystrophin and approaches to correction of the defect.

D. Wood, Muscular Dystrophy Association, New York, New York: Where do we go from here? What is MDA's current response to the 'big' question—how soon will we have a cure?

Viral Proteinases as Targets for Chemotherapy

April 16 - April 19

ARRANGED BY

E. Wimmer, State University of New York, Stony Brook
S. Oroszlan, NCI-Frederick Cancer Research Facility, Maryland
H.-G. Krausslich, State University of New York, Stony Brook

SESSION 1

Chairpersons: J.H. Strauss, California Institute of Technology, Pasadena, and **M.J. Schlesinger,** Washington University, St. Louis, Missouri

E. Wimmer, State University of New York, Stony Brook: Polyprotein processing in viral replication.

B.L. Semler, University of California at Irvine: Molecular genetics of picornavirus protein processing.

P. V. Pallai, Boehringer Ingelheim Pharmaceuticals, Ridgefield, Connecticut: Peptide cleavage by purified 3C.

E. Wimmer, State University of New York: Poliovirus proteinase 2A.

A.C. Palmenberg, University of Wisconsin, Madison: Cleavage specificity of the EMC 3C proteinase.

J. Wellink, Agricultural University, Wageningen, The Netherlands: Processing of the CPMV polyproteins.

SESSION 2

Chairpersons: A.C. Palmenberg, University of Wisconsin, Madison, and **B.L. Semler,** University of California at Irvine

W. Dougherty, Oregon State University, Corvallis: Molecular genetic analysis of a plant virus proteinase and its conserved cleavage site.

R.J. Fletterick, University of California, San Francisco: X-ray crystallography of trypsin variants.

J.F. Bazan, University of California, San Francisco: Viral branches of the trypsin-like proteinase family.

M.J. Schlesinger, Washington University, St. Louis, Missouri: A second sindbis virus autoproteinase: In vitro processing of the viral nonstructural polyprotein.

J. Strauss, California Institute of Technology, Pasadena: *cis*-Acting structural and nonstructural proteinases encoded by sindbis virus.

J. Weber, Centre Hospitalier Universitaire, Sherbrooke, Canada: Adenovirus proteinase: Properties of a recombinant enzyme expressed in *E. coli*.

M. Johnston, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland: AIDS drug discovery and development: Present and future.

SESSION 3

Chairpersons: K. von der Helm, University of Munich, Federal Republic of Germany, and **S. Oroszlan,** NCI-Frederick Cancer Research Facility, Maryland

S. Oroszlan, NCI-Frederick Cancer Research Facility, Maryland: Retroviral polyprotein processing: An overview.

Y. Yoshinaka, Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan: Retroviral proteinase as an aspartic proteinase.

R.B. Luftig, Louisiana State University Medical Center, New Orleans: Bacterial expression and activity of the Mo-MLV proteinase.

M. Hatanaka, Kyoto University, Japan: HTLV-I proteinase.

M. Roberts, NCI-Frederick Cancer Research Facility, Maryland: Lentiviral proteinases.

C. Debouck, Smith Kline & French Laboratories, King of Prussia, Pennsylvania: Expression and structure-function characterization of the HIV-1 proteinase and its *gag-pol* substrate.

M. Graves, Hoffmann-La Roche Inc., Nutley, New Jersey: Characterization of HIV-1 proteinase.

K. von der Helm, University of Munich, Federal Republic of Germany: Action and inhibition of the HIV proteinase.

H.-G. Krausslich, State University of New York, Stony Brook: Processing of HIV polyproteins by bacterially expressed HIV-1 proteinase.

R.I. Swanstrom, University of North Carolina, Chapel Hill: Genetic analysis of the HIV proteinase.

J.M. Louis, National Cancer Institute, NIH, Bethesda, Maryland: Studies with wild-type and mutant HIV-1 proteinase expressed in *E. coli*.

SESSION 4

Chairperson: R.J. Fletterick, University of California, San Francisco

M.N.G. James, University of Alberta, Edmonton, Canada: Crystallographic studies of AMV proteinase.

A. Wlodawer, NCI-Frederick Cancer Research Facility, Maryland: Refined structure of a proteinase from RSV.

M. Navia, Merck, Sharp & Dohme Research Laboratories, Rahway, New Jersey: Three dimensional structure of the aspartyl-proteinase from HIV-1.

S. Foundling, E.I. du Pont de Nemours & Co., Wilmington, Delware: Crystallographic analysis of AMV proteinase.

J. Erickson, Abbott Laboratories, Abbott Park, Illinois: Structural relationships between viral and eukaryotic aspartic proteinases and the implications for retroviral drug design.

R.S. DesJarlais, University of California, San Francisco: Inhibitor design from known structures.

S. Oroszlan, NCI- Frederick Cancer Research Facility, Maryland, and R.B. Luftig, Louisiana State University Medical Center, New Orleans: Synthetic nonpeptide inhibitors.

SESSION 5

Chairperson: E. Wimmer, State University of New York, Stony Brook

C.S. Craik, University of California, San Francisco: Expression and characterization of HIV-1 and HIV-2 proteinases.

S.B.H. Kent, California Institute of Technology, Pasadena: A synthetic approach to the molecular biology of retroviral proteinases: Structure-function studies of the HIV-1 and HIV-2 enzymes and their substrates.

B.M. Dunn, University of Florida, Gainesville: Kinetic studies on viral proteinases utilizing synthetic peptide substrates.

J. Leis, Case Western Reserve University, Cleveland, Ohio, and A.M. Skalka, Fox Chase Cancer Center, Philadelphia, Pennsylvania: Biochemical analysis of the ASLV proteinase.

C. Carter, State University of New York, Stony Brook: Substrate specificity determinants of the HIV-1 proteinase.

P.L. Darke, Merck, Sharp & Dohme Research Laboratories, West Point, Pennsylvania: HIV-1 proteinase: Substrates, inhibitors, and subunit interactions.

P. Strop, Institute of Organic Chemistry and Biochemistry, Prague, Czechoslovakia: Specificity and inhibition studies on MAV proteinase.

M.T. Skoog, Boehringer Ingelheim Corporation, Ridgefield, Connecticut: Substrate specificity of HIV proteinase.

B.D. Korant, E.I. du Pont de Nemours & Co., Wilmington, Delware: Potential use of proteinase inhibitors as antiviral agents.

Molecular Genetics of Early Drosophila and Mouse Development

April 20 - April 23

ARRANGED BY

M. Cappechi, University of Utah, Salt Lake City

SESSION 1

W. Bender, Harvard Medical School, Boston, Massachusetts: Segmental regulation of the bithorax complex of *Drosophila*.

W. McGinnis, Yale University, New Haven, Connecticut: A homeo domain switch changes the regulatory function of the deformed protein in *Drosophila* embryos.

M.P. Scott, University of Colorado, Boulder: Genes that control pattern formation during development.

M. Krasnow, Stanford University Medical Center, California: Transcriptional regulation by homeotic gene products in cultured *Drosophila* cells and in vitro.

SESSION 2

M. Levine, Columbia University, New York, New York: Spatial regulation of homeo box gene expression in *Drosophila*.

M. Capecchi, University of Utah, Salt Lake City: Creating mice with specific mutations by gene targeting.

A. Joyner, Mt. Sinai Hospital Research Institute, Toronto, Canada: Molecular genetic approaches to the analysis of mammalian development.

P. Gruss, Max Planck Institute of Biophysical Chemistry, Gottingen, Federal Republic of Germany: Precision mutagenesis by homologous recombination.

SESSION 3

E. Robertson, Columbia University, New York, New York: Developmental potential of embryonic stem cells.

E.F. Wagner, Research Institute of Molecular Pathology, Vienna, Austria: In situ analysis of c-*fos* expression in transgenic mice.

R. Beddington, ICRF Developmental Biology Unit, Oxford, England: Using *lacZ* as an in situ cell marker to analyze tissue lineages in the midgestation mouse embryo.

F.H. Ruddle, Yale University, New Haven, Connecticut: Mammalian Antennapedia class homeo box genes: Organization, expression, and evolution.

SESSION 4

B. Hogan, Vanderbilt University Medical School, Nashville, Tennessee: Developmental expression of the murine *vgr-1* gene: A new member of the TGF-β multigene family.
P. Gruss, Max Planck Institute of Biophysical Chemistry, Gottingen, Federal Republic of Germany: The *hox-1.1* promoter directs expression to a specific region of the embryo in transgenic mice.
G. Martin, University of California, San Francisco: New approaches to identifying genes that control early mammalian embryogenesis.
D. Duboule, European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: The organization of the murine *hox* gene family resembles that of *Drosophila* homeotic genes.
R. Krumlauf, National Institute for Medical Research, London, England: The murine and *Drosophila* homeo box clusters are derived from a common ancestor based on similarities in structure and expression.

SESSION 5

A.P. McMahon, Roche Institute of Molecular Biology, Nutley, New Jersey: *int-1* and pattern regulation.
R. Nusse, Netherlands Cancer Institute, Amsterdam: *int-1* and *int-4*, two genes active in mouse mammary tumorigenesis and in normal embryogenesis.

Sloan Foundation Journalists' Workshop on Applications of the New Molecular Genetics

May 7 - May 9

ARRANGED BY

J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory, New York

SESSION 1

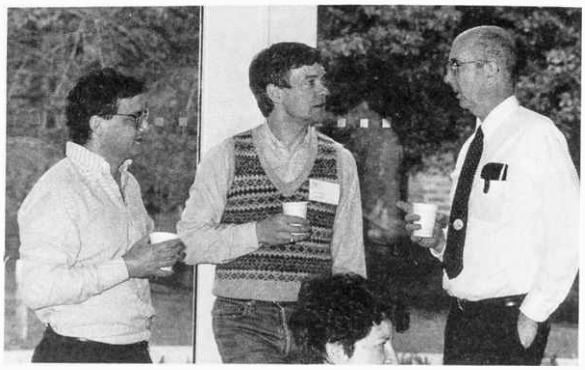
C. Cepko, Harvard University, Boston, Massachusetts: Studies of cell lineages in developing animals using retroviruses as markers.
N. I. First, University of Wisconsin, Madison: Applications of new methods of embryo manipulation in animal husbandry.
M. Capecchi, University of Utah, Salt Lake City: Homologous recombination and gene targeting.

SESSION 2

D. Micklos and M. Bloom, DNA Learning Center, Cold Spring Harbor Laboratory, New York: Laboratory experiment and Smithsonian *Search for Life* exhibit.

SESSION 3

M. Martin, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland: Transgenic mice and other animal models for the study of AIDS pathogenesis.
G.A. Evans, The Salk Institute, La Jolla, California: Cell ablation studies for developmental studies.
A.N. Rowan, Tufts University Veterinary School, North Grafton, Massachusetts: Ethical issues of the new genetics.



J. Palca, J.A. Witkowski, R. Cooke

D. Barnes, C. Joyce, M. Martin

Computational Models of Visual Processing
Workshop on Computational Neuroscience

June 26 - June 30

ARRANGED BY

J.A. Movshon and **M. Landy,** New York University, New York

SESSION 1: Sampling and the Retina

A.J. Ahumada, Jr., NASA Ames Research Center, Moffett Field, California: Learning receptor position.

L.T. Maloney, New York University, New York: Retinal sampling and early vision.

D.R. Williams, University of Rochester, New York: Photoreceptor sampling and image motion.

D.C. Hood, Columbia University, New York, New York: Models of the human rod receptor and the ERG.

SESSION 2: Central Visual Neurons

P. Lennie, University of Rochester, New York: Construction of chromatically opponent receptive fields.

M.J. Hawken, New York University, New York: Spatial organization of receptive fields in primate V1.

R. Shapley, New York University, New York: Linear and nonlinear contributions to direction selectivity of simple cells in cat area 17.

SESSION 3: Encoding

A.B. Watson, NASA Ames Research Center, Moffett Field, California: Modeling visual neurons.

D.J. Heeger, Massachusetts Institute of Technology, Cambridge: Nonlinear model of cat striate cortex.

D.J. Field, University of Cambridge, England: Redundancy of natural scenes and its relation to visual coding.

SESSION 4: Channels

D.G. Pelli, Syracuse University, New York: On the nature of the noise that limits visual detection.

J. Nachmias, University of Pennsylvania, Philadelphia: Ruminations on subthreshold summation.

H.R. Wilson, University of Chicago, Illinois: Spatial mechanisms and visual pattern discrimination.

SESSION 5: Motion and Transparency

E.H. Adelson, Massachusetts Institute of Technology, Cambridge: Analysis of orientation and motion.

N. Grzywacz, Whitaker College, Massachusetts Institute of Technology, Cambridge: Does the visual cortex really worry about the aperture problem?

D. Kersten, Brown University, Providence, Rhode Island: Transparency: Psychophysics and computation.

SESSION 6: Texture

N. Graham, Columbia University, New York, New York: Low-level visual processes in texture segregation.

C. Chubb, New York University, New York: Expanding the class of distinct textures with identical local average energy spectra.

J.R. Bergen, David Sarnoff Research Center, Princeton, New Jersey: Energy measures, gain controls, and texture perception.

SESSION 7: Three-dimensional Structure

J.P. Frisby, University of Sheffield, England: PMF89, switcher, and needles: A medley of AIVRU's stereo algorithms.

A.J. Parker, University Laboratory of Physiology, Oxford, England: Stereo, surfaces, and shape.

H. Bulthoff, Brown University, Providence, Rhode Island: Integration of depth modules.

S. Ullman, Massachusetts Institute of Technology, Cambridge: 3D object recognition.

SESSION 8: Surface Properties

M. D'Zmura, University of California, Irvine: Constraints on the recovery of surface reflectance.

D. Brainard, Stanford University, California: Predicting the illuminant's effect on color appearance.

Molecular Genetics and Biology of Alcoholism

October 10 - October 13

ARRANGED BY

H. Begleiter, State University of New York Health Science Center, Brooklyn
C.R. Cloninger, Washington University School of Medicine, St. Louis, Missouri

SESSION 1: Genetic Studies I

Chairperson: J. Sambrook, University of Texas Medical Center, Dallas

A. Chakravarti, University of Pittsburgh, Pennsylvania: Sampling design issues for segregation and linkage studies in alcoholism.

J.S. Searles, University of Pennsylvania, Philadelphia: Methodological limitations of genetic models of alcoholism: Critique of extant research and suggestions for future directions.

C.R. Cloninger, Washington University School of Medicine, St. Louis, Missouri: Clinical and genetic heterogeneity in alcoholism.

R.E. Tarter, University of Pittsburgh, Pennsylvania: Vulnerability to alcoholism: From individual differences to different individuals.

T. Reich, Jewish Hospital of St. Louis, Missouri: Familial transmission of alcoholism and related disorders.

SESSION 2: Genetic Studies II

Chairperson: P.M. Conneally, Indiana University Medical Center, Indianapolis

A.C. Heath, Washington University, St. Louis, Missouri: Contributions of twin family studies to understanding the inheritance of alcoholism.

N.G. Martin, Queensland Institute of Medical Research, Herston, Australia: Twin studies of alcohol metabolism and sensitivity.

R.J. Cadoret, University of Iowa, Iowa City: Use of the adoption paradigm to delineate the role of genes, environment, and their interaction in genesis of alcoholism.

C.C.H. Cook, Middlesex Hospital Medical School, London, England: Candidate genes and favored loci for alcoholism.

D. Goldman, National Institute on Alcohol Abuse and Alcoholism, Bethesda, Maryland: Candidate loci for alcoholism: Cloning and analysis of ethanol metabolic genes and tryptophan hydroxylase.

T.-K. Li, Indiana University School of Medicine, Indianapolis: Animal models in the study of genetics and biology of alcoholism.

SESSION 3: Putative Markers I

Chairperson: E. Gordis, National Institute on Alcohol Abuse and Alcoholism, Rockville, Maryland

H. Begleiter, State University of New York Health Science Center, Brooklyn: Neurophysiologic markers in sons of alcoholic fathers: Data and criteria.

M. Schukit, University of California, San Diego: Challenges of children of alcoholics with alcohol or diazepam.

B. Tabakoff, National Institute on Alcohol Abuse and Alcoholism, Bethesda, Maryland: Neurochemical and peripheral markers in alcoholism.

D.P. Agarwal, Institute of Human Genetics, Hamburg, Federal Republic of Germany: Human aldehyde dehydrogenases: Genetic implications for alcohol sensitivity, alcohol drinking habits, and alcoholism.

T.J. Peters, King's College School of Medicine, London, England: Alcohol-acetaldehyde flush reactions in Caucasians: Identification of biochemical defects and genetic aspects.

SESSION 4: Putative Markers II

Chairperson: R.S. Sparkes, University of California, Los Angeles, Health Science Center

E.P. Noble, Neuropsychiatric Institute, University of California, Los Angeles: Alcoholic fathers and their sons: electrophysiologic, neurophysiologic, personality and family correlates.

P. Propping, Institute of Human Genetics, University of Bonn, Federal Republic of Germany: Alcohol effect on the EEG: Are there possibilities for applications of molecular genetics?

V. Hesselbrock, University of Connecticut, Farmington: Social/behavioral factors that may attenuate/potentiate genetic effects.

E.M. Wijsman, University of Washington, Seattle: Linkage analysis of alcoholism: Problems and solutions.

J. Ott, Columbia University, New York, New York: Genetic linkage analysis under uncertain disease definition.

SESSION 5: General Discussion

Chairperson: A. Chakravarti, University of Pittsburgh, Pennsylvania

P.R. Billings, Harvard Medical School, Boston, Massachusetts: Genetics and behavioral disorders: What will be the consequences of identifying a major gene in alcoholism?

E. Gordis, National Institute on Alcohol Abuse and Alcoholism, Rockville, Maryland: National Institute on Alcohol Abuse and Alcoholism and research on the genetics of alcoholism.

Applications of Basic Research in Mammalian Development

October 15 - October 18

ARRANGED BY

R.A. Pedersen, University of California, San Francisco
A. McLaren, MRC Mammalian Development Unit, University College London, England
N.L. First, University of Wisconsin, Madison

Keynote Address

N.L. First, University of Wisconsin, Madison: Application of gamete and embryo biotechnology to animal production.

SESSION 1: Sex Determination

A. McLaren, MRC Mammalian Development Unit, University College London, England: Sex determination in mice.

P.S. Burgoyne, MRC Mammalian Development Unit, University College London, England: Mammalian Y chromosome function in early embryos and male gametogenesis.

SESSION 2: Gametogenesis

A.R. Bellve, Columbia University College of Physicians & Surgeons, New York, New York: The thecins, a novel class of proteins associated with the sperm perinuclear matrix.

C. Racowsky, Arizona State University, Tempe: Regulation of meiotic maturation in hamster oocytes.

J. Eppig, The Jackson Laboratory, Bar Harbor, Maine: Growth and development of oocytes in vitro.

SESSION 3: In Vitro Production of Embryos

P.M. Wassarman, Roche Institute of Molecular Biology, Nutley, New Jersey: Fertilization in mammals.

J.J. Parrish, University of Wisconsin, Madison: In vitro fertilization of laboratory and domestic species.

SESSION 4: Cryopreservation of Germ Cells and Embryos

W.F. Rall, American Type Culture Collection, Rockville, Maryland: New approaches to the cryopreservation of mammalian embryos by vitrification.

J. Van Blerkom, University of Colorado, Boulder: Consequences of cryopreservation in oocytes and embryos.

SESSION 5: Embryo Multiplication

R.S. Prather, University of Missouri, Columbia: Cloning by nuclear transfer in laboratory and domestic animal embryos.

V.E. Papaioannou, Tufts University School of Medicine, Boston, Massachusetts: Developmental regulation of half embryos.

SESSION 6: Early Embryo Survival

D.G. Whittingham, MRC Experimental Embryology and Teratology Unit, St. George's Hospital Medical School, London, England: Genetic evaluation of embryos.

H.J. Leese, Department of Biology, University of York, England: Assessment of embryo metabolism.

J. Butler, University of Idaho, Moscow: Metabolism during preimplantation development in farm animals.

R. Wales, School of Veterinary Studies, Murdoch University, Western Australia: Energy metabolism during development and differentiation of sheep embryos.

SESSION 7: Early Embryo Development

R.A. Pedersen, University of California, San Francisco: Origin and growth of the extraembryonic cell lineages in normal and parthenogenetic mouse embryos.

S. Heyner, Albert Einstein Medical Center, Philadelphia, Pennsylvania: The insulin family of peptides: Expression and role in development.

J.D. Biggers, Harvard Medical School, Boston, Massachusetts: Regulation of pH in early mammalian embryos.

D. Albertini, Tufts University School of Medicine: Role of cytoskeleton in early embryo development.

SESSION 8: Gene Transfer in Laboratory and Domestic Species

K.M. Ebert, Tufts University School of Veterinary Medicine, North Grafton, Massachusetts: Present status of transgenic livestock.

C. Spadafora, University of Rome, Italy: Sperm as vectors for producing transgenic mice.

J. Kopchick, Ohio University, Athens: Mutagenesis of the bovine growth hormone gene: Structure/function studies employing transgenic mice.

SESSION 9: Sex Identification in Agriculture and Human Genetics

K.L. White, Louisiana State University, Baton Rouge: Identification of the sex of preimplantation mammalian embryos.

A.H. Handyside, Royal Postgraduate Medical School, Hammersmith Hospital, London, England: Preimplantation diagnosis of human inherited disease.

A. Bellve,
N. First,
R. Pedersen

Molecular Cytogenetics: Bridging the Resolution Gap

October 30 - November 2

ARRANGED BY

F.S. Collins, University of Michigan Medical School, Ann Arbor
D.H. Ledbetter, Baylor College of Medicine, Houston, Texas
H.F. Willard, Stanford University School of Medicine, California

M. Burmeister, K. Davies

T. Hassold, H. Cooke

SESSION 1: Bridging Technologies

Chairperson: F.S. Collins, University of Michigan Medical School, Ann Arbor

M. Burmeister, University of California, San Francisco: Fine structure genetic analysis of human chromosomes by using radiation hybrid mapping.

D.C. Ward, Yale University School of Medicine, New Haven, Connecticut: High-resolution gene mapping by in situ hybridization.

B.J. Trask, Lawrence Livermore National Laboratory, California: Genomic distance estimated from the proximity of fluorescence in situ hybridization sites in interphase nuclei.

B. Horsthemke, University of Essen, Federal Republic of Germany: Microdissection and molecular analysis of human chromosome regions involved in contiguous gene syndromes.

K.E. Davies, John Radcliffe Hospital, Oxford, England: Physical mapping and microdissection in the fragile X region.

A.P. Monaco, Imperial Cancer Research Fund Laboratories, London, England: Human genome linking with cosmids and yeast artificial chromosomes.

SESSION 2: Chromosome Structure

Chairperson: H.F. Willard, Stanford University School of Medicine, California

H. Cooke, MRC Human Genetics Unit, Western General Hospital, Edinburgh, Scotland: Human telomeres.

L. Clarke, University of California, Santa Barbara: Structure-function analysis of centromeric DNA in fission yeast.

H.F. Willard, Stanford University School of Medicine, California: Molecular studies of X-inactivation.

S.D.M. Brown, St. Mary's Hospital Medical School, London, England: Long-range genetic and physical mapping of mouse chromosomes.

P. Goodfellow, Lincoln's Inn Fields, London, England: Structure of the pseudoautosomal boundary.

SESSION 3: Reverse Genetic Cloning Strategies

Chairperson: B.S. Emanuel, Children's Hospital of Philadelphia, Pennsylvania

F.S. Collins, University of Michigan Medical School, Ann Arbor: Lessons from the cystic fibrosis gene search.

B.S. Emanuel, Children's Hospital of Philadelphia, Pennsylvania: Molecular and cytogenetic studies of 22q11.

S.F. Warren, Emory University School of Medicine, Atlanta, Georgia: Isolation of human Xq28 within a somatic cell hybrid by fragile site-directed rearrangement: Application to saturational cloning and genome analysis.

SESSION 4: Contiguous Gene Syndromes

Chairperson: D.H. Ledbetter, Baylor College of Medicine, Houston, Texas

U. Francke, Howard Hughes Medical Institute, Stanford University, California: Contiguous gene syndrome in the Xp21 region.

A. Ballabio, Baylor College of Medicine, Houston, Texas: Contiguous gene syndromes in the distal short arm of the human X chromosome.

R.L. Nussbaum, University of Pennsylvania, Philadelphia: Identifying human genetic disease by map position: Application of X-linked ophthalmological disease.

G.A. Bruns, The Children's Hospital, Boston, Massachusetts: Organization of the WAGR region.

D.H. Ledbetter, Baylor College of Medicine, Houston, Texas: Molecular dissection of the Miller-Dieker lissenopholy syndrome.

SESSION 5: Origin and Mechanism of Chromosome Abnormalities

Chairperson: T. Hassold, Emory University Hospital, Atlanta, Georgia

T. Hassold, Emory University Hospital, Atlanta, Georgia: Parental origin effects: imprinting and the parental origin of chromosome abnormality.

R.D. Nicholls, University of Florida College of Medicine, Gainesville: Prader-Willi and Angelman syndromes: Are both parental contributions to chromosome 15q11q13 necessary for normal human development?

W. Reik, Institute of Animal Physiology and Genetics Research, Cambridge, England: Role of DNA methylation in mammalian genome imprinting.

A. Chakravarti, University of Pittsburgh, Pennsylvania: Analysis of nondisjunction in Down's syndrome and ovarian teratoma.

C. Sapienza, Ludwig Institute for Cancer Research, Montreal, Canada: Genetics of genome imprinting.

J.G. Hall, University of British Columbia, Vancouver, Canada: Genomic imprinting: Relevance for human diseases.

Control of HIV Gene Expression

November 7 - November 10

ARRANGED BY

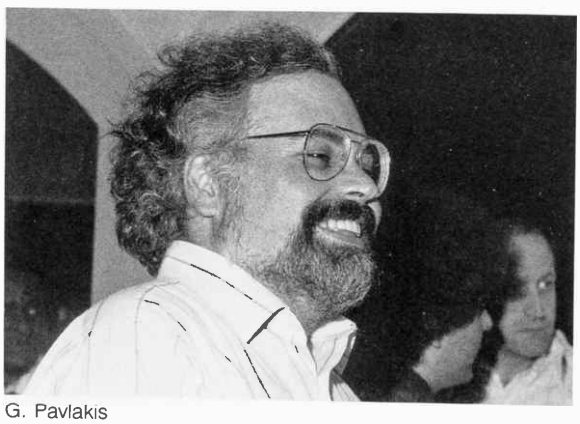
B.R. Cullen, Howard Hughes Medical Institute, Duke University Medical Center, Durham, North Carolina
R. Franza, Cold Spring Harbor Laboratory, New York
F. Wong-Staal, National Cancer Institute, Bethesda, Maryland

SESSION 1: HIV Rev Function

Chairperson: B.R. Cullen, Howard Hughes Medical Institute, Duke University Medical Center, Durham, North Carolina

C.A. Rosen, Roche Institute of Molecular Biology, Nutley, New Jersey: Regulation of HIV gene expression by *rev*.

G.N. Pavlakis, NCI-Frederick Cancer Research Facility, Maryland: Transcriptional organization and regulation of HIV-1 expression.



G. Pavlakis

J. Rusche, Repligen Corporation, Cambridge, Massachusetts: Biochemical characterization of HIV-1 *rev* interaction with the RRE.

M. Emerman, Fred Hutchinson Cancer Center, Seattle, Washington: Regulation of HIV envelope gene expression/mechanism of *rev* gene action.

M. Hatanaka, Kyoto University, Japan: Nucleolar targeting signals in HIV.

SESSION 2: HIV-1 *tat* Function

Chairperson: F. Wong-Staal, National Cancer Institute, Bethesda, Maryland

B.M. Peterlin, Howard Hughes Medical Institute, University of California, San Francisco: Activation and *trans*-activation of HIV.

A.P. Rice, Cold Spring Harbor Laboratory, New York: *tat* regulation of HIV-1 LTR-directed gene expression.

A. Frankel, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: Biochemistry, activity, and inhibition of the *tat* protein from HIV.

K.-T. Jeang, National Institutes of Health, Rockville, Maryland: Activation of the HTLV-1 and HIV-1 L7R by viral and cellular factors.

A.J. Kingsman and S.M. Kingsman, University of Oxford, England: Activation of the HIV LTR by *tat* in *Xenopus* oocytes.

N. Sonenberg, McGill University, Montreal, Canada: Function of the *tar* sequence in posttranscriptional control of HIV-1 gene expression.

M. Green, St. Louis University School of Medicine, Missouri: Functional *tat* protein domains and inhibition of viral gene.

SESSION 3: Other HIV Gene Products

Chairperson: M.A. Martin, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland

W. Haseltine, Dana-Farber Cancer Institute, Cambridge, Massachusetts: HIV-1 *vpr* gene function.

M. Rosenberg, Smith Kline & French Laboratories, King of Prussia, Pennsylvania: Role of HIV-1 proteinases.

L. Ratner, Washington University, St. Louis, Missouri: HIV regulation by *nef*.

S. Venkatesan, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, Maryland: Functional studies of HIV-1 *nef* and *rev* regulatory proteins.

SESSION 4: Cellular Factors in HIV-1 Gene Regulation

Chairperson: R. Franza,
Cold Spring Harbor Laboratory, New York

A.S. Fauci, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland: Effects of lymphokines on HIV-1 gene expression.

G.J. Nabel, Howard Hughes Medical Institute, University of Michigan Medical Center, Ann Arbor: *k*B binding proteins: Heterogeneity and mechanisms of activation.

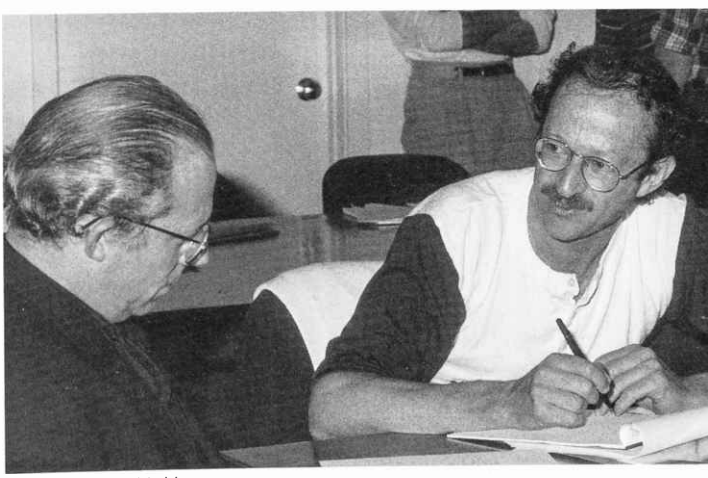
R. Gaynor, University of California, Los Angeles, School of Medicine: Transcriptional regulation of HIV.

A. Rabson, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland: Role of LTR regulatory sequences in HIV replication.

M. Feinberg, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: Mutational analysis of LTR function in HIV replication.

E. Verdin, Free University of Brussels, Belgium: Identification of an inducible intragenic enhancer in HIV-1.

I. Chen, University of California, Los Angeles, School of Medicine: HTLV gene regulation.



W. Haseltine, H. Varmus

SESSION 5: HTLV and Animal Lentiviruses

Chairperson: M. Yoshida, Cancer Institute, Tokyo, Japan

W.C. Greene, Howard Hughes Medical Institute, Duke University Medical Center, Durham, North Carolina: Mechanism of HTLV-I *rex* action.

J. Hauber, Sandoz Research Institute, Vienna, Austria: Functional analysis of the HTLV-1 *rex* gene regulation.

J.E. Clements, John Hopkins University School of Medicine, Baltimore, Maryland: Regulation of gene expression of visna virus by *tat* and cellular factors involves AP-1 recognition sequences.

D. Derse, National Cancer Institute, Frederick, Maryland: EIAV *tat* is structurally and functionally related to HIV and SIV *tat* proteins but lacks a methionine initiation codon.

A. Fauci, F. Wong-Staal

Mutation Induction and Heritability in Mammalian Germ Cells

November 12 - November 15

ARRANGED BY

J.W. Allen, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina
M.J. Moses, Duke University Medical Center, Durham, North Carolina
L.B. Russell, Oak Ridge National Laboratory, Tennessee

SESSION 1: Germ-line Properties Affecting Mutation Induction and Recovery

Chairperson: M.A. Handel, University of Tennessee, Knoxville

L.D. Russell, Southern Illinois University, Carbondale: Structural evidence for entry of substances into the seminiferous tubule.

I.B. Fritz, University of Toronto, Canada: Cell-to-cell interactions in the seminiferous tubule.

D.G. De Rooij, University of Utrecht, The Netherlands: Relation between the proliferative activity of spermatogonial stem cells and their sensitivity to irradiation and adriamycin and to translocation induction.

M.A. Handel, University of Tennessee, Knoxville: Heritable mutations in the analysis of spermatogenesis.

N.B. Hecht, Tufts University, Medford, Massachusetts: Regulation of postmeiotic genes.

G. Sega, Oak Ridge National Laboratory, Tennessee: Molecular targets, DNA breakage, DNA repair: Their roles in mutation induction in mammalian germ cells.

A. Wyrobek, Lawrence Livermore National Laboratory, California: Detection of specific locus mutation in human sperm.

SESSION 2: Aberrant Chromosome Structure/Behavior

Chairperson: M.J. Moses, Duke University Medical Center, Durham, North Carolina

I.-D. Adler, GSF-Institut fuer Saeugetiergenetik, Neuherberg, Federal Republic of Germany: Clastogenic effects of acrylamide in different germ cell stages.

M.J. Moses, Duke University Medical Center, Durham, North Carolina: The synaptonemal complex as an indicator of chromosome damage.

J.W. Allen, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina: Mutagen effects on the synaptonemal complex.

M.E. Dresser, Oklahoma Medical Research Foundation, Oklahoma City: Structure-function relationships in meiotic chromosome behavior: An experimental approach.

B.F. Brandriff, Lawrence Livermore National Laboratory, California: Human sperm cytogenetics and the one-cell zygote.

M.T. Davisson, The Jackson Laboratory, Bar Harbor, Maine: Chromosome aberrations associated mutations: Effect on mapping new mutants.

SESSION 3: Variables Affecting the Rate and Nature of Mutations

Chairperson: L.B. Russell, Oak Ridge National Laboratory, Tennessee

B.M. Cattanach, MRC Radiobiology Unit, Didcot, England: Factors affecting the recovery of mutations from mouse spermatogonial stem cells following X-irradiation.

J. Favor, GSF-Institut fuer Saeugetiergenetik, Neuherberg, Federal Republic of Germany: Mutagenic action of ENU in germ cells of the mouse as interpreted from specific locus mutation results.

S.E. Lewis, Research Triangle Institute, Research Triangle Park, North Carolina: Electrophoretically detected mosaic mutants in the mouse.

J. Peters, MRC Radiobiology Unit, Didcot, England: Comparison of electrophoretic and specific locus mutation responses and analysis of glucose phosphate isomerase mutants in the mouse.

J.D. McDonald, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Investigation of inborn errors of phenylalanine metabolism by efficient mutagenesis of the mouse germ line.

L.B. Russell, Oak Ridge National Laboratory, Tennessee: Relation of germ-cell stages to nature of induced mutations.

SESSION 4: Nonmutational Genetic Effects on Early Development

Chairperson: M.F. Lyon, MRC Radiobiology Unit, Didcot, England

D. Solter, The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania: Genomic imprinting and mammalian development.

L.M. Wiley, University of California, Davis: A chimera embryo assay reveals a decrease in embryonic cellular proliferation induced by sperm from X-irradiated mice.

W.M. Generoso, Oak Ridge National Laboratory, Tennessee: Developmental anomalies: Mutational consequences of zygote exposure.

O. Smithies, University of North Carolina, Chapel Hill: Manipulation of the mouse genome by homologous recombination.

SESSION 5: Utilization of DNA Techniques in the Detection of Germ-line Mutations

Chairperson: H. Mohrenweiser, Lawrence Livermore National Laboratory, California

H. Mohrenweiser, Lawrence Livermore National Laboratory, California: Detection of insertion, deletion, and rearrangement mutations in the genome.

B.W. Kovacs, University of Southern California School of Medicine, Los Angeles: Quantitation and characterization of human germinal mutations at hypervariable loci.

N. Arnheim, University of Southern California, Los Angeles: Analysis of DNA sequences in individual gametes.

R. Woychik, Oak Ridge National Laboratory, Tennessee: Insertional mutagenesis in transgenic mice.

SESSION 6: Genetic Risk Estimation

Chairperson: W. Russell, Oak Ridge National Laboratory, Tennessee

W. Russell, Oak Ridge National Laboratory, Tennessee: Problems and possibilities in genetic risk estimation.

V.L. Dellarco, U.S. Environmental Protection Agency, Washington, D.C.: Quantitative genetic risk assessment: Induction of heritable translocations by ethylene oxide as an example.

M.C. Cimino, U.S. Environmental Protection Agency, Washington, D.C.: Use of germ cell mutagenicity data at the U.S. Environmental Protection Agency.

J.B. Bishop, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Mammalian heritable effects research in the National Toxicology Program.

P.B. Selby, Oak Ridge National Laboratory, Tennessee: Importance of the direct method of genetic risk estimation and ways to improve it.

B.A. Bridges, MRC Cell Mutation Unit, University of Sussex, Brighton, England: Meeting summary.

Molecular Clocks of Evolution

November 28 - December 1

ARRANGED BY

D.J. Melnick, Columbia University, New York, New York
M. Goodman, Wayne State University School of Medicine, Detroit, Michigan
R.J. Britten, California Institute of Technology, Corona del Mar

SESSION 1: Paleontological Calibration of Critical Taxonomic Branch Points with Particular Reference to Molecular Data

Chairperson: P.G. Gingereich, University of Michigan, Ann Arbor

L.L. Jacobs, Southern Methodist University, Dallas, Texas: Geological dating and molecular clocks.
E.C. Olson, University of California, Los Angeles: Calibrations of ancient vertebrate origins.
J. Lake, University of California, Los Angeles: Origin of the multicellular animals.
E.S. Vrba, Yale University, New Haven, Connecticut: Using molecular and morphological evolutionary rates to study evolutionary processes in a group of mammals.
E. Mayr, Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts: General discussion.

SESSION 2: Specific Applications of Molecular Clocks

Chairperson: R.E. Pollack, Columbia University, New York, New York

T. Gojobori, National Institute of Genetics, Mishima, Japan: Molecular evolutionary clocks of viral genes.
W.-H. Li, University of Texas, Houston: Molecular clocks and mammalian phylogeny.
R. Cann, University of Hawaii, Honolulu: Human mtDNA, calibrating evolutionary rates, new technology for detecting natural selection.
V. Sarich, University of California, Berkeley: General discussion.

SESSION 3: Rate Variation within and between Genomes

Chairperson: W.M. Brown, University of Michigan, Ann Arbor

J.R. Powell, Yale University, New Haven, Connecticut: Intragenomic heterogeneity in rates of DNA evolution in *Drosophila*; a note on DNA clocks in higher primates.
R.J. Britten, California Institute of Technology, Corona del Mar: Differences in rate of evolution between regions of the *Drosophila melanogaster* genome.
T.I. Bonner, National Institute of Mental Health, Bethesda, Maryland: Evidence for unequal rates of DNA evolution in primates.
T. Ohta, National Institute of Genetics, Mishima, Japan: Nearly neutral mutations and the molecular clock.
T.H. Jukes, University of California, Berkeley: General discussion.

SESSION 4: The Effects of Selection on Rates of Molecular Evolution

Chairperson: M. Goodman, Wayne State University School of Medicine, Detroit, Michigan

W.M. Fitch, University of Southern California, Los Angeles: When do clocks go wrong? How often? How badly?
M. Goodman, Wayne State University School of Medicine, Detroit, Michigan: Darwinian evolution and the acceleration/deceleration pattern in rates of mutation and fixation.
M. Riley, University of Massachusetts, Amherst: Effects of natural selection on nucleotide polymorphism.
M. Nei, University of Texas Health Science Center, Houston: Effects of positive and negative Darwinian selection on molecular clocks: Data from the MHC and immunoglobulin families.
E. Zuckerkandl, Linus Pauling Institute of Science and Medicine, Palo Alto, California: General discussion.

E. Olson, F. Ayala, H. Carson

R. Pollack, E. Mayr

SESSION 5: Molecular Consequences of Behavior, Demography, and Biogeography

Chairperson: R.L. Honeycutt, Texas A & M University, College Station

H.L. Carson, University of Hawaii, Honolulu: Hybridization of species may confound the molecular clock.

R. DeSalle, Yale University, New Haven, Connecticut: Hawaiian *Drosophila*: Biogeography and molecular clocks.

D.J. Melnick, Columbia University, New York, New York: Rapid rates of molecular change as a consequence of behavior and biogeography.

F.J. Ayala, University of California, Irvine: General discussion.

Immunological Aspects of AIDS

December 4 - December 7

ARRANGED BY

M. Oldstone, Scripps Clinic and Research Foundation, La Jolla, California
S. Putney, Repligen Corporation, Cambridge, Massachusetts

SESSION 1: HIV Genomic Variability

Chairperson: B.H. Hahn, University of Alabama at Birmingham: Determinants of pathogenicity in naturally occurring strains of HIV-2.

S. Wain-Hobson, Institut Pasteur, Paris, France: Genetic variation of HIV-1 in vitro.

C.-Y. Ou, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia: HIV evolution and transmission in infected persons.

G. Myers, HIV Sequence Database, Los Alamos, New Mexico: HIV protein pattern analysis.

S. Putney, Repligen Corporation, Cambridge, Massachusetts: Variation of the neutralizing determinants of HIV-1.

SESSION 2: HIV-Cell Interactions

Chairperson: R.A. Weiss, Chester Beatty Laboratories, London, England: HIV neutralization and interaction with receptors.

E.G. Engelman, Stanford University School of Medicine, California: Mechanisms responsible for $CD4^+$ T-lymphocyte dysfunction and depletion in HIV-injected patients.

M.A. Martin, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland: Structural and functional relations of the HIV envelope gene.

Y. Riviere, Institut Pasteur, Paris, France: Study of the primary cellular immune response to HIV-1, using recombinant vaccinia viruses.

M. Oldstone, Scripps Clinic and Research Foundation, La Jolla, California: Anatomy of CTL vaccine.

A.S. Fauci, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland: Cytokine modulation of HIV expression.

D.E. Mosier, Medical Biology Institute, La Jolla, California: Consequences of HIV infection of hu-PBL-SCID mice.

J.M. Coffin, Tufts University School of Medicine, Boston, Massachusetts: Genetic variation in retroviruses.

SESSION 3: HIV-Immune System Interactions: Antibody

Chairperson: J.J. Skehel, National Institute for Medical Research, London, England

P.L. Nara, NCI-Frederick Cancer Research Facility, Maryland: HIV-1 neutralization: A humoral paradox.

J. Salk, The Salk Institute for Biological Studies, San Diego, California: Strategies for the control of HIV infection and/or disease using an envelope-depleted inactivated HIV immunogen.

R. De Mars, University of Wisconsin, Madison: A human mutant cell - gene transfer system for studying immune responses to HIV proteins.

D.D. Ho, University of California, Los Angeles, School of Medicine: Quantitation of HIV-1 in the blood of infected persons.

R.S. Fujinami, University of California, San Diego, La Jolla: Common determinant between HIV gp41 and human astrocytes.

SESSION 4: HIV-Immune System Interactions: CTL

Chairperson: M. Oldstone, Scripps Clinic and Research Foundation, La Jolla, California

B.D. Walker, Massachusetts General Hospital, Boston: Specificity of HIV-1-reactive CTLs.

F. Gotch, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, England: Are HIV-specific CTLs terminally differentiated in HIV-seropositive patients?

S. Koenig, National Institutes of Health, Bethesda, Maryland: Cell-mediated immunity in HIV-seropositive individuals.

J.A. Berzofsky, National Cancer Institute, NIH, Bethesda, Maryland: Viral sequence variation and MHC polymorphism in immune control of virus and viral escape.

J.L. Whitton, Research Institute of Scripps Clinic, La Jolla, California: Virus sequence requirements for induction of cytotoxic and lymphocytes and for CTL-mediated antiviral immunity.

J. Chiller, Eli Lilly and Company, Indianapolis, Indiana: General discussion.

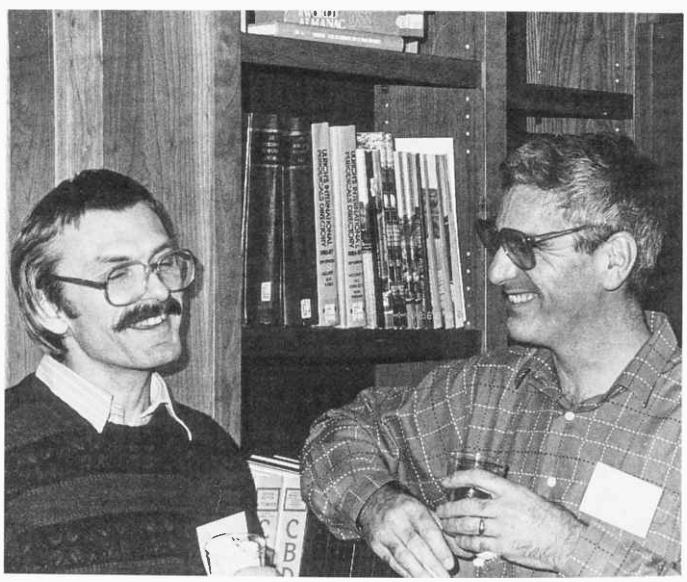
SESSION 5: Viral Pathogenesis: Lessons from Animal Models

Chairperson: O. Narayan, Johns Hopkins University School of Medicine, Baltimore, Maryland: HIV-1 neutralization: A humoral paradox.

N.L. Letvin, Harvard Medical School, Southborough, Massachusetts: The $CD8^+$ lymphocyte response in SIV-infected rhesus monkeys.

J.N. Coffin, Tufts University School of Medicine, Boston, Massachusetts: Genetic variation in retroviruses.

J. Salk, S. Putney



S. Wain-Hobson, R. Weiss


COLD SPRING HARBOR
LABORATORY PRESS

1989 PUBLICATIONS

Molecular Cloning: A Laboratory Manual (2nd edition)
J. Sambrook, E.F. Fritsch, and T. Maniatis

Molecular Biology of Signal Transduction
Symposia on Quantitative Biology 53

Drosophila: *A Laboratory Handbook and Manual*
M. Ashburner

Molecular Diagnostics of Human Cancer (*Cancer Cells 7*)
M. Furth and M. Greaves (eds.)

Vaccines 89
R. Lerner, H. Ginsberg, R. Chanock, and F. Brown (eds.)

DNA Technology and Forensic Science (Banbury Report 32)
J. Ballantyne, G. Sensabaugh, and J. Witkowski (eds.)

Current Communications in Molecular Biology Series

Polymerase Chain Reaction
H. Erlich, R. Gibbs, and H. Kazazian, Jr. (eds.)

Recessive Oncogenes and Tumor Suppression
W. Cavenee, N. Hastie, and E. Stanbridge (eds.)

Viral Proteinases as Targets for Chemotherapy
H.-G. Kräusslich, S. Oroszlan, and E. Wimmer (eds.)

Cytoskeletal Proteins in Tumor Diagnosis
M. Osborn and K. Weber (eds.)

Therapeutic Peptides and Proteins: Formulation, Delivery, and Targeting
D. Marshak and D. Liu (eds.)

Development and Application of Molecular Markers to Problems in Plant Genetics
T. Helentjaris and B. Burr (eds.)

Perspectives on the Molecular Biology and Immunology of the Pancreatic β Cell
D. Hanahan, H. McDevitt, and G. Cahill, Jr. (eds.)

Molecular Genetics of Early Drosophila *and Mouse Development*
M. Capecchi (ed.)

Journals

Genes & Development (Volume 3, numbers 1–12B)

Cancer Cells: A Monthly Review (Volume 1, numbers 1–4)

Other

CSHL Annual Report, 1988

Abstract/program books for 14 CSHL meetings

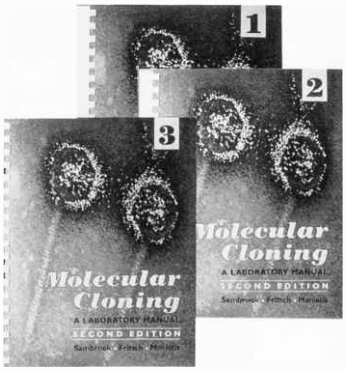
COLD SPRING HARBOR LABORATORY PRESS

This Report marks the emergence in 1989 of Cold Spring Harbor Laboratory Press as a distinct operating unit of the Laboratory. Like the Banbury and DNA Learning Centers, the Press has its own staff and operating budget. It combines the acquisition and development of new publishing projects with the work of the long-established Publications (book editorial and production) Department headed by Nancy Ford; the Fulfillment and Marketing Departments headed by Charlaine Apsel; and the new Journal Department headed by Judy Cuddihy. Thirty-three staff members are currently employed by the Press, located in the Carnegie Building, Urey Cottage, the Grace Auditorium Bookstore, and our warehouse in Cold Spring Harbor village. Because of the size and dispersed nature of the operation, communication between departments has to be good, in the interests of efficiency.

The mission of CSHL Press is threefold: to provide revenue for the Laboratory from monographs, journals, and other media; to extend the Laboratory's educational activities by publishing the proceedings of its meetings and technical manuals from its courses; and to produce Laboratory materials such as this *Annual Report* and the meetings abstract/program books.

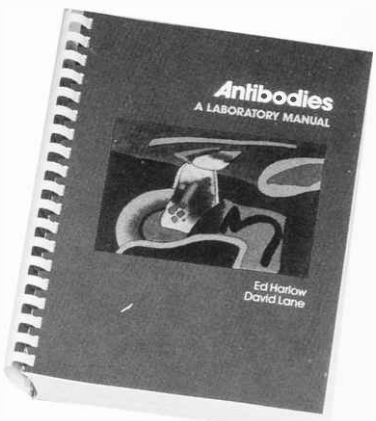
The "Bible of Molecular Biology": A New Testament

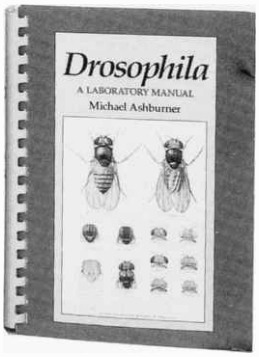
1989 proved to be an outstanding year. Among the most notable events, pride of place must go to the publication in November of the second edition of *Molecular Cloning: A Laboratory Manual*. "Eagerly awaited" has become a publisher's cliché, but it was never more appropriate than in this case. After four years of labor, Joe Sambrook, Ed Fritsch, and Tom Maniatis, with associate author, Nina Irwin, and the assistance of over 60 scientific advisors, produced a manual that is comprehensive, clear, authoritative, and as easy to use as any three-volume work can be. This enterprise would not have been possible without the extraordinary dedication of managing editor, Nancy Ford, and her assistants, Chris Nolan and Michelle Ferguson. The publication of the first edition of *Molecular Cloning,* in 1982, put recombinant DNA technology within the grasp of scientists in every biomedical discipline. It also alerted other publishers to the importance of technical manuals in molecular biology. The subsequent seven years produced many competing books, but the clamor of 25,000 advance orders for the second edition and the sale of a further 10,000 copies within three months of publication have shown us once again that a reputation for quality and reliability is a most precious asset.

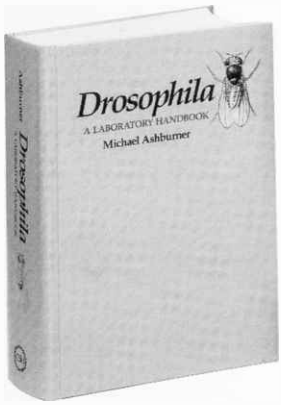


Strong Sales and Significant Influence

The success of *Molecular Cloning* did not obscure the impact made this year by another of our laboratory manuals, *Antibodies*, by Ed Harlow, a senior scientist at Cold Spring Harbor Laboratory, and David Lane of the Imperial Cancer Research Fund Laboratories, UK. Published late in 1988, the book's sales climbed rapidly past 10,000 copies in 1989 and collected a sheaf of admiring reviews. These noted, among other virtues, the exceptional clarity of the writing about immunology, a discipline notorious for the obscurity of its jargon. *Antibodies* is a unique book and has attracted international interest. A French edition is in preparation, and we have hopes of a version in Japanese.







A total of 14 books were published this year. Nine originated in meetings at the Banbury Center and two of these are particularly notable. *The Polymerase Chain Reaction*, in our rapidly published *Current Communications* series, summarizes a wide range of investigators' experiences with a technique for DNA amplification that is only three years old. Its literature is sparse, but its practical applications seem almost limitless. The book has therefore been much in demand, selling over 2000 copies. Influence, however, does not always need large sales. With more modest circulation, *Banbury Report 32*, containing contributions from lawyers as well as scientists, has contributed significantly to the debate on the use of DNA technology in forensic analysis, a subject now frequently found on the nation's news pages. The innovative meetings at Banbury under Jan Witkowski's direction suggest interesting publishing opportunities, and we continue to explore the best ways of conveying to a wide audience a sense of having been there.

In the last days of the year, we received from the printer the first copies of a magisterial work by Michael Ashburner. *Drosophila: A Laboratory Handbook and Manual* distills into two volumes, 1700 pages long, an exceptional scholar's knowledge of the tiny fruitfly from which so much knowledge of gene function has come. These beautifully designed books will be classics.

The hallmark of CSHL Press publications continues to be an obsessive concern for accuracy and quality. It is a pleasure to acknowledge the work of the book editorial and production staff, who aim for the same high standards on every project, whether it is a handsome monograph, an inexpensive paperback, the meetings abstract books (14 in 1989), or the Laboratory's *Annual Report*. Particular mention should be made of the important leadership given to the department by Annette Kirk and Dorothy Brown while Nancy Ford was wholly committed to work on *Molecular Cloning*. Lee Martin was a valuable addition to the Production staff in 1989. We also sought extra assistance from advanced technology, with the purchase of an optical scanner capable of reading manuscript pages straight into computer files for editing. Our in-house microcomputer-based typesetting system was applied for the first time to a large book, *Vaccines 89*, with satisfactory results and savings of both time and money. More books will be typeset in this way next year.

Continued Growth in Quality of *Genes & Development*

For *Genes & Development*, its third year of publication was one of continuous growth: 538 manuscripts were offered for publication, a 38% increase over the previous year, and 196 were published in 13 issues. A growing proportion of papers submitted are now judged outstanding, rather than simply worthy of publication, and as a result, the quality of issues has never been higher. This was reflected in 1989 by the frequent selection of papers published in the journal for editorial comment by other publications.

Much credit for this increase in quality lies with the editors who select the papers to be published and the referees they consult. Reviewers are the gatekeepers for any primary journal, and our thanks go to the editorial board members and several hundred referees who unstintingly gave their time and expertise to ensure that *Genes & Development* will continue to grow into a journal of the highest quality.

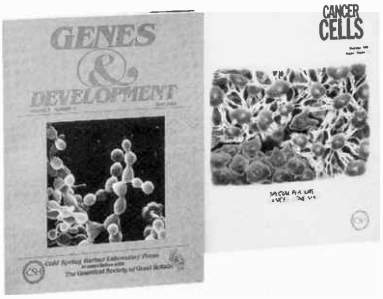
Its rising reputation was reflected in a gratifying 30% increase in subscriptions. The journal now has subscribers in 35 countries, and as the year ended, the level of new as well as renewed subscriptions promised more circulation growth in 1990.

When a new journal is launched, high costs are incurred from the outset, while

revenues accrue slowly. The investment required to reach break-even point can be daunting, and careful cost control is imperative. To help defray the journal's expenses, the decision was taken to levy a charge of $20 per page on papers published in the journal in 1990. This assistance, with an increase in the other sources of revenue, should bring to an end in 1990 the period of investment in *Genes & Development* required from the Press and its co-owner, the Genetical Society of Great Britain.

In January, Terri Grodzicker of the Laboratory's scientific staff became the journal's U.S. editor, and she was able to combine successfully the growing demands of the job with her duties as Assistant Director for Academic Affairs. Her experience and enthusiasm have been invaluable. Toward the end of the year, Grahame Bulfield, the journal's European editor, announced his intention to step down because of newly assumed responsibilities as Director of his institute. Grahame was a founding editor of *Genes & Development*, and we are grateful for all he did for the journal in its first three years. He will be succeeded in 1990 by Nicholas Hastie, a senior scientist at the MRC Human Genetics Institute in Edinburgh. Nick is highly respected as a scientist with imagination, energy, and wide-ranging interests. These are ideal qualifications in an editor, and we look forward to Nick's joining the editorial team. The journal department was also strengthened by the addition of Michele Cleary, who brought both publishing and laboratory experience to the role of editorial assistant.

A Second Journal Is Launched



The evolution of our Journal Department continued in 1989, when months of preparation resulted in the September launch of the Press's second journal, *Cancer Cells: A Monthly Review*. This subscription-based magazine of cancer research replaced the once-yearly books published in the *Cancer Cells* series. The field it covers is large and diverse, bringing together scientists of many disciplines and a variety of medical specialists. Some scientists investigate cell function at a molecular level and others pursue findings with therapy or diagnosis in mind, whereas physicians try rationally to apply the emerging tests and treatments. *Cancer Cells'* ambitious aim is to interest and educate them all.

With the help of Judy Cuddihy and the enthusiastic members of the Journal Department, the editor, Paula Kiberstis, put together four monthly issues in 1989 containing invited short reviews, features, and commentaries that were informative and occasionally provocative. These were delivered in an attractive, contemporary design, between the notable covers that have become a feature of both our journals. The subscription response to the substantial initial promotion campaign mounted by our Marketing Department was highly satisfactory. These are early days, but *Cancer Cells* has made a most promising start.

Striking Publicity and Overwhelming Orders

Our marketing activities in 1989 remained centered upon direct mail. Brochures, leaflets, and a three-times-yearly newsletter, the *Notebook*, giving advance information about our publications, were sent to many thousands of potential customers worldwide. A complete catalog was circulated in the fall, and display advertisements were placed in selected, large-circulation journals as appropriate. Comments received from the scientific community suggest that our publicity was more noticeable this year. Much of the credit belongs to the eye-catching brochures, catalogs, and advertisements designed by new staff member Jim Suddaby. With another 1989 recruit, Lisa Sweeney, Jim also produced each issue of the *Notebook* using desktop publishing techniques devised in-house.

Our marketing activities also include the sale of publications at the larger scientific conferences. Organized by Penny Sheppard, these appearances at meetings attended by as many as 15,000 delegates provide valuable contacts with current and prospective authors and reassurance that, in our core markets, we are more than a match in sales for the large commercial houses. In 1989, we attended eight American meetings, a larger number than usual, in fields as diverse as human genetics, neuroscience, and microbiology. In addition, CSHL Press exhibited for the first time at a European scientific meeting, the International Immunology Congress in West Berlin, and at the Frankfurt Book Fair, where useful discussions with a number of booksellers led to changes in sales policy that will benefit both our customers and ourselves.

Charlaine Apsel combined the direction of these marketing activities with a substantial hands-on role as head of the Fulfillment Department. The work of this department proceeded steadily for much of the year, buoyed by the strong sales of the new titles and the back list. But after publication of *Molecular Cloning* and our catalog, orders arrived in unprecedented numbers by phone, FAX, and mail, often several hundreds each day. Despite these pressures, the Customer Service staff remained efficient and good-humored. Jackie Matura was a welcome and timely addition to their ranks in mid-year.

The many thousands of advance orders for the manual were fulfilled within three weeks of its publication. The Warehouse Manager, Guy Keyes, and those who assisted him deserve congratulations, not just for this exceptional effort but for maintaining an efficient dispatch service throughout the year.

The increase in our active titles this year and the need to keep adequate copies of several best-selling titles in stock have swamped our warehouse. Rented trailers are being used as an expedient, but our need for more storage space will become critical in 1990 with the expected publication of at least 15 books. These will include the next four titles in our prestigious monograph series, the next edition of the serial compendium *Genetic Maps* in a completely revised format, and several new technical manuals. We also anticipate the start of an important new book series, *Genome Analysis,* edited by Kay Davies of Oxford University and Shirley Tilghman of Princeton University. For her invaluable help in the development of these and other new projects, I am extremely grateful to my assistant, P.J. Harlow.

What Lies Ahead?

For many science publishers, the late 1980s have not been the best of times. Anxiety about traditional products and markets has been fueled by declining library budgets, falling R&D in Western industries, and the quickening growth of electronic systems for copying, storing, and distributing information. Mergers and acquisitions are creating a limited number of commercial giants with diverse publishing interests, strong enough, it is hoped, to exploit the changes to come. CSHL Press is the antithesis of that trend, a small independent house specializing in certain areas of biology. Yet we have certain key advantages: proximity to a world-class research center, the respect earned by the excellence of our past and present publications, an experienced and professional staff close to the scientific community, and active support for prudent expansion from James Watson, Morgan Browne, and the Laboratory's Executive Committee. Our aim is to grow without compromising our reputation for quality. If that can be accomplished, there will still be a special place for CSHL Press, whatever the shape of science publishing in the decade to come.

John R. Inglis

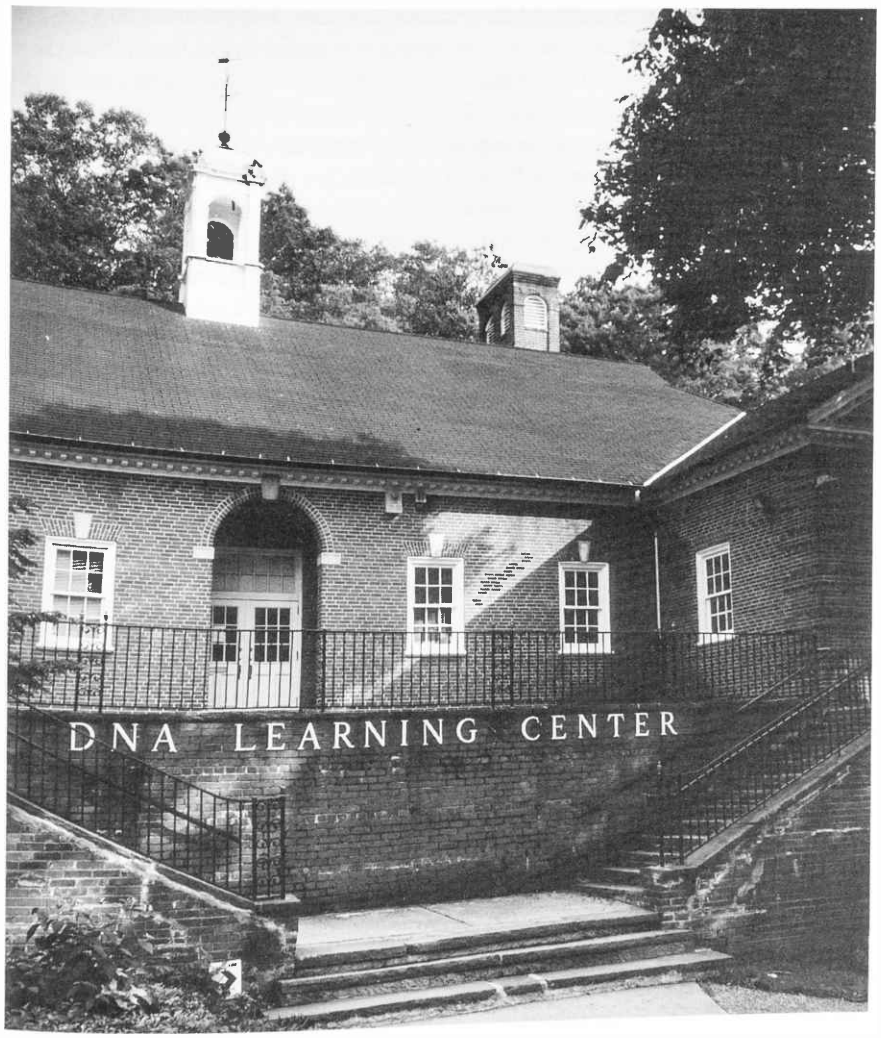

DNA
LEARNING CENTER
SMITHSONIAN
EXHIBIT
THE SEARCH FOR LIFE
COLD SPRING HARBOR LABORATORY
1890-1990
CSH
CENTENNIAL

DNA LEARNING CENTER

David A. Micklos, Director
Mark V. Bloom, Assistant Director

In 1944, the eminent physicist Erwin Schrödinger published a small book entitled *What is Life? The Physical Aspects of the Living Cell*, in which he mused on cracking the molecular "code-script" that governs the inheritance and expression of life functions. Schrödinger, whose wave equation helped describe the structure of the atom, stood in awe of the self-replicating ability of living things. He even went so far as to suggest that the essence of the mystery of life might entail new physical principles: "From all we have learnt about the structure of living matter, we must be prepared to find it working in a manner that cannot be reduced to the ordinary laws of physics."

Schrödinger's book influenced a generation of scientists to quest after the molecular "code-script" of life, including such pioneers of molecular biology as Max



Delbrück, Francis Crick, and James Watson. No new laws of physics were uncovered in cracking the genetic code. The fact that all molecules are constructed according to the same physical chemical rules made it possible for Watson and Crick to deduce the structure of the DNA molecule. With a deeper understanding of the physical principles of DNA structure and function has come the ability to treat DNA as "merely" a molecule with predictable biochemical properties.

During the last 15 years, treating DNA as an ordinary molecule has led to the extraordinary ability to dissect any of the 100,000 human genes that compose the human chromosomes—the human genome. The dissection of the molecular pathway through which hereditary information flows between DNA, RNA, and protein molecules has added rich detail to our understanding of how human life develops and changes—from fertilized egg to adulthood. It has also enabled scientists to isolate and map to their chromosomal positions the genes responsible for a number of genetic illnesses, including muscular dystrophy and cystic fibrosis.

The Human Genome Project

The inauguration of the Human Genome Project in 1988 marked the beginning of a national commitment to apply DNA technology toward understanding human health and development. Its goal is to determine the sequence of the estimated three billion bits of molecular information—the arrangement of nucleotide rungs of the DNA ladder—that constitute the entire code-script of human life.

Possession of an increasingly complete set of hereditary information will bring numerous benefits to humankind. By pushing back the threshold of early disease detection, DNA diagnosis will increase therapy options and play a positive role in personal health management. Understanding the molecular basis of disease should lead to therapies that treat the cause, rather than the symptoms, of illness. Similar techniques are now used to produce DNA fingerprints, which are gaining

Glasnost—DNA style! In December a group of high school students, visiting from Moscow, came to the Learning Center for an introduction to DNA experimentation. Assistant Director Mark Bloom helps Soviet students with their experiment.

acceptance as the most definitive evidence of identity in rape, murder, and paternity cases.

The day is not far off when a battery of DNA diagnoses will likely be part of a routine visit to the family doctor, providing an extraordinarily detailed picture of an individual's own genetic frailties. DNA fingerprints will join fingerprints and thumbprints kept on file by law enforcement agencies. In 1989, Genetic Therapy, Inc., was formed as the first company to explore methods to replace and repair defective genes.

The Social Imperative

Since the explosion of information made possible by the invention of the printing press, a democracy has needed to be a society of literates. Similarly, the explosive growth of DNA-based technology demands a society of DNA literates. It is clear that the science of DNA will increasingly generate important public policy issues. If we indeed believe in the Madisonian concept of an informed citizenry that participates in public decision making, then DNA literacy can no longer be considered an esoteric pursuit.

Unfortunately, all indicators point to the fact that DNA science is moving so fast that the gulf between technological advance and public understanding is wider than ever before in the history of science. For example, a 1986 National Science Foundation study found that 57% of Americans claim to have little or no understanding of DNA. This state of public ignorance threatens the nation's ability to make informed policy decisions about issues generated by the new biology.

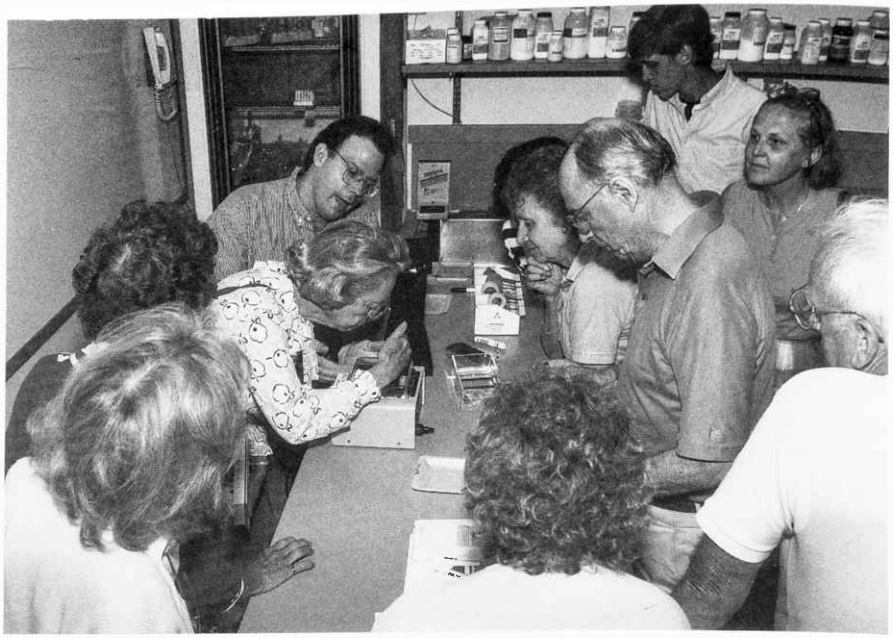
To a great extent, public education will determine whether such information realizes its compassionate potential for personal health management or is feared as a sort of science fiction fortune telling. Clearly, society cannot afford another era of abuse of genetics, such as was incurred during the Nazi quest for racial purity. Public involvement is key to the development of guidelines governing access to this genetic information to ensure protection of individual privacy rights.

As applications of DNA science leave the laboratories, trained personnel from nearly every segment of society must interface with this new technology. Young people entering the medical, agricultural, manufacturing, and even legal professions will be expected to have a basic command of DNA science. There is growing concern within the scientific community that a predicted shortage of American-born biologists during the next several decades could severely diminish our leadership in health-related research and development.

The Problems of Science Education

We are in the infancy of a scientific and social revolution of monumental proportions. Even so, the exciting prospects and problematic aspects of human genetics remain, for most young people, in the realm of science fiction. Thus, the excitement of the Human Genome Project offers an important opportunity to substantially reorganize science and health education to include new emphasis on human genetics. Failure to do so means failure to examine many of the most exciting topics science has to offer, failure to present socially and personally relevant issues, and, in the final analysis, failure to fulfill teaching's most important function—to prepare citizens capable of informed votes on policy issues.

We live in an age when young people are buffeted by all manner of distractions that keep them from pondering the biological mystery of life. Students



DNA experiments are offered to members of the Long Island Biological Association on a regular basis. Mark Bloom helps LIBA members interpret their DNA gel.

socialized to be fascinated by money, and what it can buy, have little time for physics or metaphysics. However, in working directly with DNA, the molecule of life, we may have the last decent chance to interest young people in careers in biological research.

The restructuring of biology curricula must not be aimed merely at the academically gifted, but must be geared to the abilities of all young people. In the 1990s, a basic understanding of human genetics must be considered as important as a basic understanding of hygiene and nutrition. "Retooling Biology Education for the Gene Age," a study conducted by the DNA Learning Center of 252 high school biology teachers from 10 states confirmed this opinion; they rated genetics along with ecology as the biology topics "most important in preparing students for adult life."

Our experience with over 1000 advanced high school teachers in 24 states suggests that DNA literacy is only trickling through formal channels of science communication. Curriculum setters at the state and even national levels have done little to square teaching syllabuses with the reality of modern biology. Biology curricula have evolved over the years by simply cramming in more and more facts. So, at best, human genetics still must compete with frog biology for mention in the classroom.

High school biology teachers are hamstrung by overambitious and outdated syllabuses. Survey data show that the vast majority spend most of their class time lecturing from textbooks. Yet the textbooks they teach from are typically five to ten years out of date and fail to account for the biology that appears in the news almost daily. Fewer and fewer biology students are given the opportunity for any sort of meaningful laboratory experience, let alone "advanced" experiments with DNA. It is a sad fact that biology education has changed little from the days of our grandparents. Hands-on laboratories are the exception; rote memorization is

the norm. Thus, at a time when scientists are embarking upon the most ambitious project in the history of biology, students are required to memorize terms and definitions of observational biology, a historical science of little relevance to society.

DNA Science: A First Course in Recombinant DNA Technology

Molecular biology is generally construed as the culminating experience of the biology major's academic career. We believe that lab-teaching in molecular biology should be an initiating experience—introduced in general survey courses and comprehensively instituted in advanced high school and beginning college level courses. Through its Postgraduate Training Program and laboratory manual *Molecular Cloning*, Cold Spring Harbor Laboratory has played a key role in educating research biologists in molecular biological techniques. The DNA Learning Center extends this training to high school and college teachers through the DNA Science Workshop program.

The laboratory exercises developed for the workshop will be formally published as *DNA Science: A First Course in Recombinant DNA Technology* by David Micklos and Greg Freyer. A joint publication of Cold Spring Harbor Laboratory Press and Carolina Biological Supply Company, the lab/text is the first specifically written at the advanced high school and freshman college levels. In addition to ten field-tested protocols, extensive notes give the novice teacher access to technical subtleties that are the distillation of our teaching experience and research collaborations with over 1100 educators across 24 states.

A separate text, written in a semi-journalistic style, builds upon basic biological principles presented in a general survey course. Classical genetics and DNA structure are reviewed in a historical chapter that traces the development of molecular biology. An initial technique chapter explains the theory behind methods actually used in the laboratory. The last four chapters discuss topics of current interest that illustrate the use of recombinant DNA in basic and applied research, drawing examples directly from prominent research journals, including *Cell, Science,* and *Nature*.

Starting Early

A basic introduction to human genetics should, in fact, begin in elementary and middle school. These teachers, who interface with a cross section of America's youth, constitute a major conduit through which scientific literacy can flow to society. As a content area, human genetics at once builds upon the traditional health emphasis of elementary and middle-school science and offers an entrée to pure science. Genetics emphasizes science as a problem-solving venture involving the collecting, sharing, and analyzing of data. It is also consistent with the across-curriculum approach that incorporates science, math, and social studies and emphasizes the evaluation of science and its practical applications.

Children start their lives as natural scientists. Survey data tell us that the majority of elementary school students are enthusiastic about science; however, student interest decreases dramatically through the middle and high school years. Making best use of this window of opportunity during the elementary years may have two important effects. First, it can inculcate basic tenets of scientific literacy that are essential for all children as they grow into adulthood. Second, it may light an academic spark for science that will survive into the college years.

The Challenge to Informal Science Education

Although the nation's schools are the logical place to begin building a DNA literate public, these formal channels of science education do not appear to be moving quickly to integrate human genetics into curricula at the precollege and even beginning college levels. This challenges agents of informal science education to move quickly to fill the void in public education and play an active role in preparing teachers to integrate human genetics at all levels. In this way, museums and science centers can help bootstrap biology teaching into the gene age.

In 1985, Cold Spring Harbor Laboratory instituted enrichment activities for precollege teachers and students under the title of the DNA Literacy Program. Key was the development and testing of entry-level experiments in molecular genetics. Although these laboratories were once viewed as somewhat esoteric, there is now growing conviction that they are essential to a general biology education. This sentiment has been legitimized by the Educational Testing Service, which recommended the teaching of DNA manipulation laboratories in the 1989–1990 Advanced Placement (AP) Biology syllabus. These laboratories will become compulsory for AP students in 1993–1994. Similar laboratories are included in the 1989 edition of a widely used text published by the Biological Science Curriculum Study.

As national curriculum setters, the Educational Testing Service and Biological Science Curriculum Study have, in effect, mandated DNA laboratory teaching at the high school level. Our experience in 24 states and Canada indicates that even the elite cadre of AP and honors teachers do not have the hands-on experience needed to introduce these laboratories with confidence. Thus, science centers can play a critical role in updating teachers on molecular genetic techniques, with which most have had no formal training. However, training activities are likely to affect only the upper echelon of most motivated teachers. Science centers must therefore take responsibility for the students of less-motivated teachers, who do little laboratory teaching of any sort.

The DNA Learning Center initiated a laboratory field trip program in spring of 1988, following completion of the *Bio2000* Laboratory. The program was an immediate success; every laboratory space has been continuously booked since that time, with a standing waiting list of 30 schools. Two laboratories are currently offered:

Bacterial Transformation: This experiment illustrates the direct link between an organism's genetic complement (genotype) and its observable characteristics (phenotype). Students introduce a new gene into the bacterium *Escherichia coli*, giving it the ability to grow in the presence of the antibiotic ampicillin. Teachers take culture plates back to their schools for incubation and discussion of results.

DNA Restriction Analysis: This experiment demonstrates that DNA can be precisely manipulated and that it behaves as predicted by the structure discovered by Watson and Crick in 1953. Students use restriction enzymes to cut purified DNA, and the resulting fragments are separated according to size using gel electrophoresis. Students take home Polaroid snapshots of their results.

Our experience with rural schools in Alabama and public schools in New York City indicates that DNA laboratories need not be confined to gifted high school students. Laboratories are perhaps even more important to the nongifted student, for whom involvement of several senses increases chances for internalization of the biological concepts. These students may possess greater manual dexterity, and achieve better results, than their academically gifted peers. Success with

laboratory manipulation may provide a handle with which the nongifted student can pull a theoretical concept into his or her realm of experience.

In spring 1989, we conducted a learning experiment that supports our contention that there is no intrinsic reason why young people should not be given the opportunity to try their hands at DNA manipulation labs. Eighteen gifted fifth and sixth graders from local school districts were invited into the *Bio2000* Laboratory for a Saturday laboratory program, "Fun with DNA." During two introductory sessions, the youngsters observed and categorized *Drosophila*

DNA Learning Center Director David Micklos looks over experiments performed by elementary school students.

mutations, analyzed inheritance of kernel characteristics in corn, used classmates' trait data for a ministudy on population genetics, constructed models of DNA molecules, and learned to handle sophisticated micropipets. In the final session, the students successfully performed the DNA restriction analysis described above.

We found that the students' grasp of concepts was comparable to, or better than, that of many of the high school students we have taught. Working with these eager and inquisitive young scientists was at once invigorating and saddening. Invigorating because it showed us the full measure of childhood thirst for understanding of the natural world. Saddening, because we can only wonder in how precious few of these young people the spark of science will be kept alive through the remainder of their precollege schooling.

In an era when fewer teachers have the time or equipment to offer meaningful laboratory experiences, the laboratory field trip program is a model for a cost-effective means to provide pooled laboratory resources to a local region. Since its opening, the *Bio2000* Laboratory has served 2000 students (105 classes) from March to December 1988 and 3800 students (183 classes) in all of 1989. A DNA teaching lab like ours can be equipped for $10,000–20,000, and a field trip program can be operated at a cost of $30,000–50,000 per year (exclusive of utilities and facility overhead). By making routine the performance of several laboratory experiences, museums, regional science centers, vocational technology centers, and "magnet" schools can at once take up the slack in laboratory teaching and help to train teachers for independent instruction.

Vector DNA Science Workshops

The silver *Vector* vans that crisscross the country during the summer to give in-service training to high school and college instructors have become the identifying emblem of the DNA Literacy Program. Through summer 1990, the *Vector* staff will have instructed nearly 1400 educators at 69 workshops across 28 states and Canada. Allied programs in North Carolina and California will have independently instructed an additional 621 teachers at 27 workshops. Recognizing the educational value of this workshop experience, the State University of New York at Stony Brook agreed to offer a credit option to *DNA Science Workshop* participants nationwide. Teachers who complete both a workshop and follow-up are eligible for three graduate credits from the Continuing Education Department. A workshop has been held at the Stony Brook campus each summer since 1987, sponsored by the University's Center for Biotechnology.

Our experience over the past four years has strengthened our conviction that the *DNA Science Workshop* is equally valuable to college teaching faculty who have little or no practical experience in molecular genetic analysis. Our first workshop geared specifically to college teachers was held at Bethany College in West Virginia in June 1989. This workshop was supported by a grant from the National Science Foundation to Bethany College and was attended by faculty members from a consortium of eight small colleges from West Virginia, Ohio, and Pennsylvania. Positive feedback from this workshop reinforced our belief that the information needs of college instructors are not far different from those of the high-caliber AP teachers we have regularly encountered. We envision the Bethany workshop as a model for a nationwide series of workshops patterned after our successful high school program.

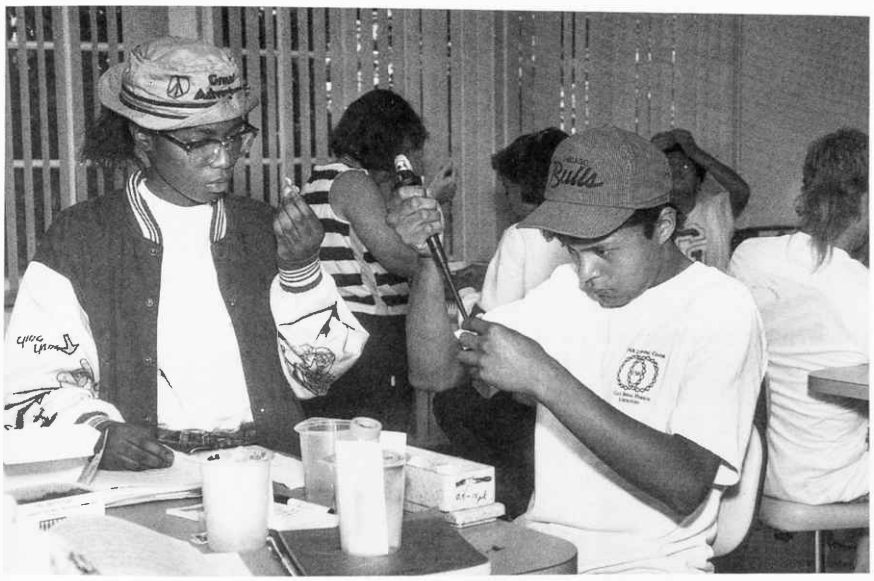
Colleges and universities provide infrastructures conducive to implementing experiments introduced during the *DNA Science Workshop*. The entire course can serve as the core of a sophomore-level molecular biology course, or individual experiments can be integrated at various levels into the biology curricula, including courses on general biology, cell biology, microbiology, genetics, and biochemistry. Costs to equip and supply a DNA teaching laboratory are well within the means of most college biology departments.

Educational Collaborations

The Curriculum Study has grown to include 24 Long Island school districts, which receive numerous benefits, including lectures by scientists, reduced admission fees to Learning Center programs, teacher in-service workshops, and equipment purchase options. Curriculum Study teachers gain an insider's view of current biological research and of the future of modern biology teaching. As the Curriculum Study continues to grow, we strive to provide a support system for pioneer teachers on Long Island, who are retooling biology education for the next century.

Through our collaboration with the Josiah Macy, Jr., Foundation, we have extended our teacher-training and student programs to Macy-sponsored schools in inner-city New York and New Haven, Connecticut, as well as in rural Alabama and Arizona. In summer 1989, minority/rural students and teacher chaperones representing each of the Macy-sponsored programs convened for a 2-week workshop at the DNA Learning Center. The first week of the workshop provided a microteaching experience, where students and their instructors learned DNA manipulation techniques in preparation for implementing specialized laboratory

courses at their home schools. During the second week, the students sampled some New York culture, including the Metropolitan Museum, Bronx Zoo, Broadway, the New York Mets, and a whale watch off Montauk Point.



Students from New York, Connecticut, Alabama, and Arizona participated in a two-week workshop sponsored by the Joshia Macy, Jr. Foundation.

A related collaboration is with the Macy BioPrep program at the University of Alabama, at Tuscaloosa, where a *DNA Science Workshop* has been held each year since 1987. With our assistance, the BioPrep staff has outfitted their own Vector van, which carries DNA restriction and bacterial transformation experiments to schools in rural Alabama. Since spring of 1988, the mobile laboratory has visited 62 schools where BioPrep teachers have instructed 2000 students.

The 1986 workshop held at the University of California at Davis prompted the creation of a state-supported instructional program. A mentor/teacher program was established at San Francisco State University to give high school teachers training in recombinant DNA techniques and access to working researchers who serve as their mentors. Our workshop is taught at three locations in California, and they have also "cloned" our Vector van approach to teaching. A minivan supplied by Genentech, Inc.—named Helix I—carries equipment to participating schools, where teachers and 600 students have performed DNA experiments.

A 1987 workshop, conducted in cooperation with the North Carolina Biotechnology Center, provided the initial impetus for what has become the nation's most extensive state-supported program in molecular biology education. Lead teachers, selected from throughout North Carolina, were trained at the 1987 workshop and then returned to their regions to assist local scientists in conducting eight local workshops in summer 1988 that reached an additional 172 teachers. By the end of 1989, the number of teachers trained increased to 325.

The program also makes available, on a rotating basis, eight equipment sets to help teachers begin to implement DNA laboratories. By the end of the 1989–1990 school year, 100 schools, representing two thirds of the school districts in the state, will have used an equipment set. One small measure of the program's success is the case of Celeste Posey, a senior at the North Carolina School of Science and Mathematics, who, working under the mentorship of a teacher trained at the 1987 workshop, took fifth place in the 1989 Westinghouse Talent Search.

Another ongoing collaboration is with the Institute for Genetics Education at the University of Wisconsin-Madison, where the *DNA Science Workshop* is one of several modules devoted to the study of genetics and its ethical implications. Reception of the workshop in 1988 was so enthusiastic that it has been incorporated as a standard part of the Institute's summer program.

As the year ended, we began a collaboration with the Howard Hughes Medical Institute. Respected as the largest nongovernment contributor to medical research, Hughes is now aiding the effort to revitalize science education. So, we were pleased when the Vice President of Hughes, Joseph Perpich, asked us to help establish a DNA science program in their home area of Montgomery County, Maryland. During the next year, we will be working with Hughes to train Montgomery County educators in laboratory-based DNA science teaching.

The Evaluation Program

As of fall 1989, we have personally instructed nearly 1100 high school and college instructors in DNA science workshops. The majority have completed both a pre-survey at the beginning of the workshop and a post-survey at the end of their week-long training. In fall 1988, we began a mail survey to follow up on 252 teachers who had completed the workshop prior to 1988. The response of our "alumni" was overwhelming; 90% returned completed surveys.

We have just completed a preliminary analysis of the survey data. The findings document a number of behavioral changes that resulted from workshop participation and suggest ways to promote innovation in the science classroom.

Seven in ten teachers had presented new topics on molecular biology and attempted at least one new laboratory during the school year following the workshop. Three in ten had implemented laboratories on DNA transformation (36%) and DNA restriction analysis (28%).

Many participants carried on networking activities to educate other teaching professionals about molecular biology, including presentations (36%), lab demonstrations (24%), presentations at professional meetings (11%), and training workshops (11%).

A majority of workshop participants (54%) attempted to secure funds or equipment with which to implement new laboratories. Seven in ten of those who tried were successful, reporting a total of $189,000 in monetary support and $88,000 in equipment/reagent donations.

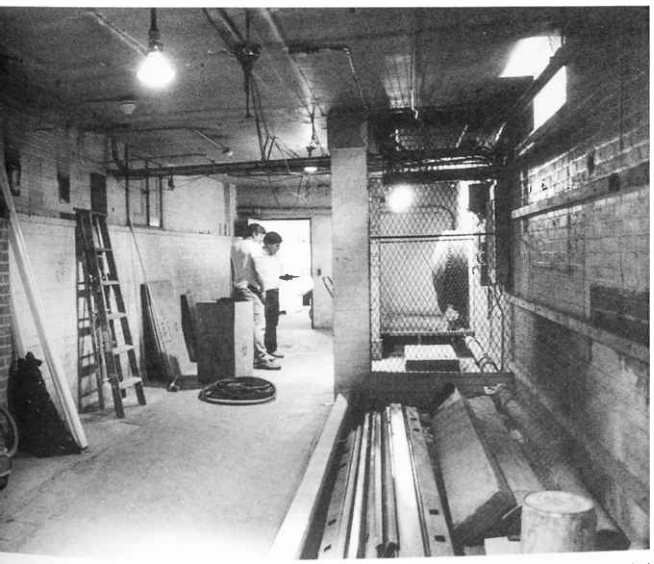
The highly innovative science teacher feels more respected in the community and has more positive attitudes about his/her students' parents and school administration. This finding emphasizes that the more innovative teacher is not a "lone wolf," but feels connected within a social system.

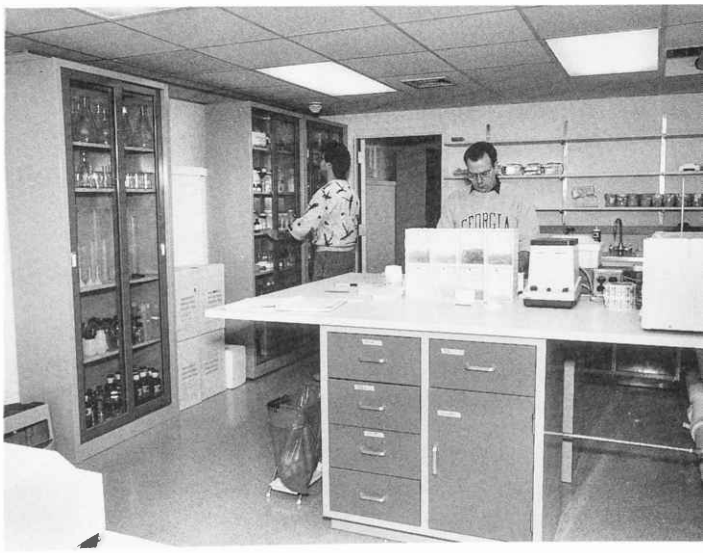
This silent background of positive attitudes about students, school system, and community allows the innovator to overcome infrastructure constraints to laboratory teaching—insufficient time, space, and equipment. The innovative teacher makes greater use of all information sources, except television, and incorporates into his/her teaching more information from primary sources—education and scientific journals.

We will have a complete set of data on 1988 participants later this fall. These new data will give us an opportunity to test a predictive model of innovative behavior. We will see if a discriminant function developed from the 1987 data set can identify innovators among the 1988 cohort.

Materials Development

In September, we completed a $24,500 renovation of our downstairs space to create a small prep and research laboratory. Supported by a grant from the Banbury Fund, the renovation gives us much needed private space to adapt research techniques to the needs of educators. The prep lab is fitted with two small autoclaves that free us from endless trips to the Laboratory to sterilize reagents and agar plates.



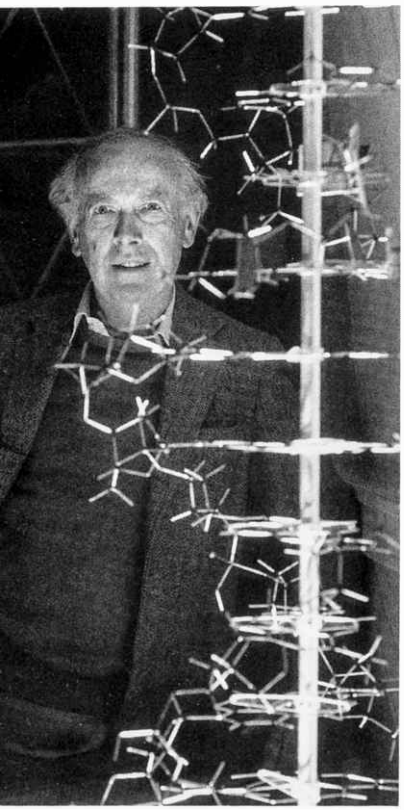


Basement area before and after renovation into a research lab.

We are developing a second set of laboratory exercises that articulate with and build upon those introduced during the *DNA Science Workshop*. These experiments will be published in a second edition of our *DNA Science* lab/text planned for publication in 1991. Envisioned as the basis for a second-level course, the new protocols will introduce three powerful techniques of molecular biology—Southern hybridization, DNA sequencing, and polymerase chain reaction. Our approach is to collaborate with a corporate partner that has specific expertise in the technology and work with them to optimize research-grade kits.

In spring 1989, we began a collaboration with United States Biochemical Corporation (USB) and Perkin-Elmer Cetus to develop polymerase chain reaction (PCR) for educational purposes. Of great interest is a kit that allows students to amplify a segment of their own DNA. We regard this as an ideal "entry-level" experience in DNA manipulation, combining the involvement of an individually performed experiment with the economy of an instructor demonstration. Although students prepare their own sample of DNA, student samples are run together in separate lanes of an agarose gel. Thus, one or at most two gels would be necessary for an entire class.

United States Biochemical, with expertise in PCR, DNA sequencing, and nonisotopic detection systems, is an ideal corporate partner to assist in the development of these second-level experiments. Furthermore, USB has expressed interest in helping us to develop instructional materials that make use of video and interactive computer software. This multimedia approach will complement laboratory-based instruction exceeding the capabilities of either used alone.



James D. Watson

Exhibit Development

A world-class museum program was established with the installation of *The Search for Life: Genetic Technology in the 20th Century*, on loan from the National Museum of American History of the Smithsonian Institution. The exhibit chronicles the study of heredity from Darwin to DNA and confronts the visitor with the promise and concern of genetic technology. Since its opening in September 1988, over 15,000 visitors have toured *The Search For Life.*

We now face the challenge of designing and executing new exhibits, revolving around the Human Genome Project, that must be readied to replace the Smithsonian exhibit. The establishment of *Exploring the Human Genome* exhibit at the DNA Learning Center will mark one of the first major efforts to spark public imagination about this important endeavor. Cold Spring Harbor Laboratory is an especially fitting host for such an exhibit. The Laboratory's director, James Watson, was the codiscoverer of the structure of DNA and is associate director of the National Institutes of Health in charge of the Human Genome Project.

As a small institution, we can never hope to attract audiences the size of those that visit the Smithsonian or the Museum of Natural History. Therefore, we intend to extend our educational reach by "franchising" or leasing exhibits to other science museums. Several factors should encourage franchising:

Cold Spring Harbor Laboratory has an established reputation in molecular genetics, and the DNA Learning Center is the world's only museum dedicated to biotechnology education. Few museums have a staff of molecular biology interpreters or such access to expert assistance.

The cost of lease or franchise will be far less than it would be to develop an exhibit from scratch.

Modular design will enable exhibit components to be configured for almost any display area.

Exhibit elements will incorporate the latest audiovisual and computer technology. Interactive features will encourage use of a number of senses to stimulate interest and understanding.

***DNA Detective*: Variability in Human Molecular Genetics**

The DNA Detective/DNA Diagnosis exhibit and the DNA manipulation laboratories are the first elements of a coordinated interpretive program on the Human Genome Project that captures the importance and excitement of human molecular genetics. Each case module consists of three backlit visual displays that highlight an actual case study involving DNA fingerprinting. Using a montage of photographs and newspaper reports, the first display presents the facts of the case and sets the stage for the DNA fingerprint data. The second display is composed of tempered glass panels with the stylized DNA fingerprints of individu-

als involved in the case. The observer slides the panels to juxtapose fingerprints—a match results in an obvious color and pattern change of the overlapping "bar codes." The third display describes the resolution of the case.

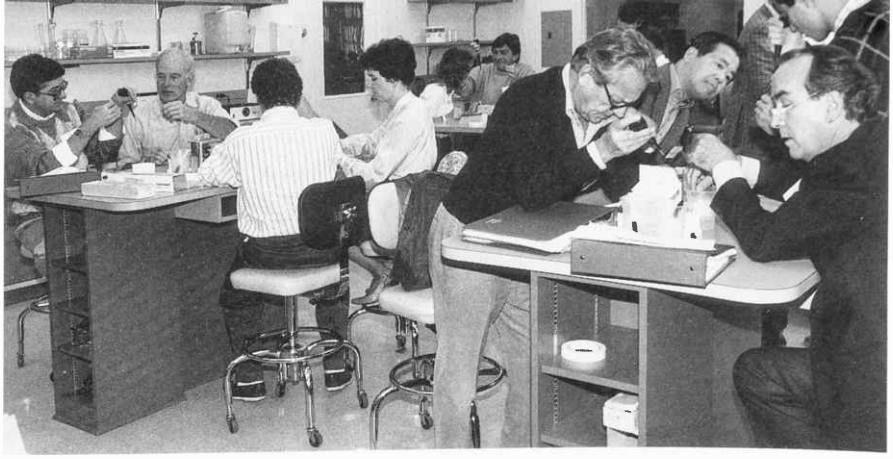
Dr. and Mrs. Richard J. Roberts at opening of the DNA Detective exhibit.

The serialization of cases and the ease of exchanging materials between one or more modules makes it cost-effective to create a rotating "gallery" of DNA fingerprint cases. The Technology Center of Silicon Valley in San Jose, California plans to install a unit for its opening in 1990, and several other museums have expressed interest. The initial cases illustrate various applications of DNA fingerprinting and, whenever possible, historical precedents in law, medicine, and society:

Ghana Immigration (1985). This case from Britain was the first to introduce DNA fingerprinting in a court of law. The data were used to prove the maternity of an English woman and her child, who wished to emigrate from Ghana. Original case materials were provided by Alec Jeffreys, University of Leicester.

Murder at Rodman Dam (1988). DNA fingerprints were used to help convict the suspect in a double murder/rape case. This was the first case involving DNA fingerprint evidence in which the death penalty was handed down. Original case materials were provided by Cellmark Diagnostics and the Florida State Attorney's Office.

Thoroughly Bred? (1989). In this case, the paternity of a thoroughbred race horse was in doubt. DNA fingerprints showed that the foal was not sired by a famous race horse, but by his less illustrious son. Original case materials were provided by Lifecodes Corporation.



Corporate executives attending the Baring Brothers conference used a sophisticated new technique called polymerase chain reaction to perform a DNA diagnosis for sickle cell anemia.

Staff

The expanding programs of the DNA Learning Center (DNALC) are a testament to the dedication and hard work of the staff. Education manager, John LeGuyader, continues to shoulder most of the daily teaching responsibilities in the *Bio2000* Laboratory. John's research and teaching experience enabled him to quickly assume the role of senior instructor for the *DNA Science Workshop program*. His organizational skills are now being put to effective use managing our Curriculum study and Macy-school collaborations.

In January 1989, Susan Lauter (formerly Susan Zehl) left the Laboratory's Public Affairs Department to join our permanent staff as designer. While a member of the Public Affairs Department, Sue played an important part in the development of the DNA Literacy Program. She has already launched us into the age of computer-aided design, using our Sun computer and plotter to generate exhibit concepts and artwork for our textbook, *DNA Science: A First Course in Recombinant DNA Technology*. Sue's first exhibit design, *DNA Detective*, is currently on display at the DNALC.

Anne Zollo has the unenviable task of overseeing the smooth running of the DNALC. Her many responsibilities include scheduling laboratory and exhibit visits, juggling travel schedules, and managing the bookstore. Perhaps most important, Anne has adapted well to the occasionally frenetic pace of activity—without losing her composure.

The teaching load was lightened by the arrival of part-time volunteer Kelly Flynn. She is a perfect addition to our teaching staff—with a degree in biology from Cornell University and experience in the laboratory of Amar Klar, a former CSHL staff scientist. Kelly has also made an important contribution to the summer workshop program, so we are very pleased that she will be helping us again this summer.

Carrie Abel began part-time work at the DNALC in June 1989, assisting Sue with artwork for the textbook. Carrie has an art degree from Syracuse University and currently teaches at C.W. Post. Her artistic and organizational talents have proven essential to the timely completion of the project.

Interns, ranging in age from high school sophomores to graduate students, provide critical assistance to our teaching staff. Deserving special mention is John

Kruper, who completed his doctorate in science education at the University of Illinois at Chicago, in October 1989. Currently a postdoctoral fellow at the University of Chicago, John has had primary responsibility for our evaluation program, which tracks the many hundreds of teachers who have participated in *DNA Science Workshops* over the years.

Chai Chen (aka "Sol,"), presently a senior at Rensselaer Polytechnic Institute, joined us for the summer 1989 *Vector* tour. Sol quickly adapted to life on the road as a DNA gypsy and kept the workshops running smoothly. Lab aide Steve Malloy, a senior at Cold Spring Harbor High School, has been joined by Steven Friedenberg and Jeff Hwang, both sophomores at Half Hollow Hills East High School. Their efforts are essential to the smooth functioning of the *Bio2000* Laboratory.

Sandy Ordway, who together with Anne Meier helped coordinate our volunteer program, is now a part-time DNALC staff member. Sandy continues to help with museum visitation while providing much needed assistance with our evaluation program. Working together, the staff are helping the DNALC continue to grow, striving to reach its potential as an "exploratorium" of DNA.

PUBLICATIONS

Micklos, D. and J. Kruper. 1989. Retooling biology education for the gene age: Preliminary results of a nationwide study of innovative teaching behavior among high school science teachers. Cold Spring Harbor Laboratory, New York.

Micklos, D. and M. Bloom. 1989. DNA restriction analysis. *Carol. Tips* **53**.

Micklos, D. and G. Freyer, eds. 1990. *DNA Science: A First Course in Recombinant DNA Technology*. Cold Spring Harbor Laboratory Press, New York. (In press.)

Micklos, D. and G. Freyer. 1989. A laboratory introduction to DNA restriction analysis, *Biotechnology Education* **1:** 16.

Vector Workshop Sites 1985–89

ALABAMA	University of Alabama, Tuscaloosa	1987, 1988, 1989
ARIZONA	Tuba City High School	1988
CALIFORNIA	University of California, Davis	1986
CONNECTICUT	Choate Rosemary Hall, Wallingford	1987
FLORIDA	University of Florida, Gainesville	1989
GEORGIA	Fernbank, Inc., Atlanta	1989
ILLINOIS	Argonne National Laboratory, Chicago	1986, 1987
	Wheaton College*	1988
INDIANA	Butler University, Indianapolis	1987
IOWA	Drake University, Des Moines	1987
	Murray State University	1988
MANITOBA	Red River Community College, Winnipeg	1989
MARYLAND	Annapolis Senior High School	1989
	McDonogh School, Baltimore	1988
MASSACHUSETTS	Beverly High School	1986
	Dover-Sherborn High School	1989
	Randolph High School, Boston	1988
	Winsor School, Boston	1987

MICHIGAN	Michigan State University, East Lansing*	1989
	Troy High School	1989
MISSOURI	Washington University, St. Louis	1989
NEW HAMPSHIRE	St. Paul's School, Concord	1986, 1987
NEW YORK	Albany High School	1987
	Bronx High School of Science	1987
	Cold Spring Harbor High School	1985, 1987
	DNA Learning Center	1988, (3), 1989
	Huntington High School	1986
	Irvington High School	1986
	State University, Purchase	1989
	State University, Stony Brook	1987, 1988, 1989
	Wheatley School, Old Westbury	1985
NORTH CAROLINA	North Carolina School of Science, Durham	1987
OHIO	Cleveland Clinic	1987
	Ohio State University, Wooster*	1989
PENNSYLVANIA	Duquesne University, Pittsburgh	1988
	Germantown Academy, Philadelphia	1988
	Gwenyde Mercy College, King of Prussia*	1988
SOUTH CAROLINA	Medical University of South Carolina, Charleston	1988
	University of South Carolina, Columbia	1988
TEXAS	University of Houston*	1989
VERMONT	Champlain Valley Union High School	1989
VIRGINIA	Jefferson School of Science, Alexandria	1987
WASHINGTON	Department of Public Health, Seattle*	1988
WEST VIRGINIA	Bethany College	1989
WISCONSIN	Marquette University, Milwaukee	1986, 1987
	University of Wisconsin, Madison	1988, 1989

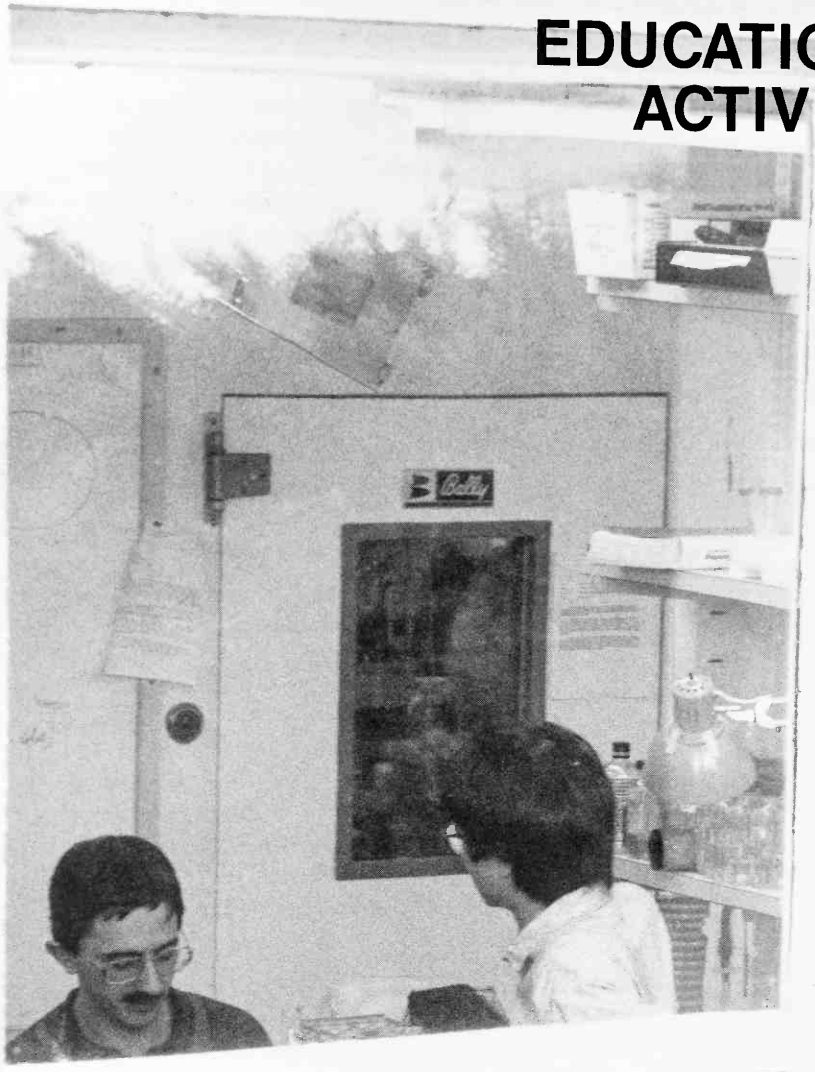
* Two-day workshop, all others five days.

Curriculum Study Membership 1989-90

Cold Spring Harbor Central School District*
Commack Union Free School District
East Williston Union Free School District*
Great Neck Public Schools*
Oyster Bay-East Norwich Central School District*
Half Hollow Hills Central School District
Harborfields Central School District
Herricks Union Free School District*
Huntington Union Free School District
Island Trees Union Free School District
Jericho Union Free School District*
Lawrence Public Schools
Lindenhurst Public Schools
Locust Valley Central School District
Manhasset Public Schools
Northport-East Northport Union Free School District*
North Shore Central School District
Plainedge Public Schools
Plainview-Old Bethpage Central School District
Port Washington Union Free School District
Portledge School
Sachem Central School District at Holbrook
South Huntington Union Free School District
Syosset Central School District*

* Founding members

EDUCATIONAL ACTIVITIES



Postgraduate Courses

The summer program of Postgraduate Courses at Cold Spring Harbor Laboratory is aimed at meeting the special need for training in interdisciplinary subjects that are either so new or so specialized that they are not adequately treated by universities. Our aim is to provide intensive study in the most recent developments and techniques in these subjects and to prepare students to enter directly into research in a particular area. To ensure up-to-date coverage of current research work, we bring together a workshop staff from many laboratories around the world and supplement this staff with a series of seminar speakers.

Cloning and Analysis of Large DNA Molecules

April 9–April 22

INSTRUCTORS

Burke, David, Ph.D., Princeton University, New Jersey
Gemmill, Robert, Ph.D., Southwest Biomedical Center, Scottsdale, Arizona
Sternberg, Nat, Ph.D., E.I. du Pont de Nemours & Company, Wilmington, Delaware

ASSISTANTS

Paulien, Sylvie, Ph.D., Southwest Biomedical Center, Scottsdale, Arizona
Ruether, James, M.S., E.I. du Pont de Nemours & Company, Wilmington, Delaware

This course covered the theory and practice of manipulating and cloning high molecular weight DNA. Lectures and laboratory work dealt with the use of bacteriophage P1 and yeast artificial chromosome (YAC) cloning systems, the isolation and manipulation of high molecular weight DNA from mammalian cells

for cloning (including the size-selection of 200-300 kb DNA fragments), and the analysis of high molecular weight DNA by pulse field gel (PFG) separation techniques. P1 and YAC recombinant DNA molecules were produced, introduced into cells (*E. coli* and yeast, respectively), and reisolated after appropriate clone selection and colony screening procedures. Comparison of DNA separation capabilities was made between the five major PFG techniques commonly in use. All aspects of Southern blot analysis of DNA were covered including sample digestion with restriction enzymes, separation on pulse field gels, Southern transfer, hybridization, and interpretation of results. Students gained hands-on experience through participation in ongoing efforts to prepare physical maps of portions of human chromosomes 3 and 12. Lectures by outside speakers on topics of current interest supplemented the laboratory work.

PARTICIPANTS

Ando, Asako, Ph.D., Tokai University, Japan
Bruns, Gail, Ph.D., Children's Hospital, Boston, Massachusetts
Drabkin, Harry, M.D., University of Colorado, Boulder
Ganal, Martin, Ph.D., Cornell University, Ithaca, New York
Goedert, Michel, Ph.D., MRC Laboratory, Cambridge, England
Henson, Victoria, Ph.D., Jackson Laboratory, Woods Hole, Massachusetts
Kagan, Jacob, Ph.D., Temple University, Philadelphia, Pennsylvania
Lafuse, Willliam, Ph.D., Ohio State University, Columbus
Petit, Christine, Ph.D., Institut Pasteur, Paris, France
Todd, Sean, B.S., University of Texas, Austin
Francis, David, B.A., University of California, Davis
Lamb, Allen, Ph.D., University of North Carolina, Chapel Hill
Marks, M. David, Ph.D., University of Nebraska, Lincoln
Scherer, Stephen, B.S., Hospital for Sick Children, Toronto, Canada
Stern, Marc-Henri, M.S., NCI-Naval Medical Oncology, Bethesda, Maryland
Wicking, Carol, M.S., St. Mary's Hospital, London, England

SEMINARS

Hieter, P., Johns Hopkins Medical School. Yeast methods for cloning and manipulating large DNA.
McClelland, M., University of Chicago. Cutting chromosomes.
Livak, K., E.I. du Pont Experimental Station. Novel DNA mapping technologies.
Garza, D., Washington University School of Medicine. Mapping the *Drosophila* genome with yeast artificial chromosomes.
Allshire, R., Cold Spring Harbor Laboratory. Introduction of *S. pombe* chromosomes into mouse cells and analysis of human telomeres.
Murray, A., University of California. Chromosome behavior in *Saccharomyces cerevisiae*.
Carle, G., INSERM, Universite de Nice. Field inversion gel electrophoresis.

Protein Purification and Characterization

April 9–April 22

INSTRUCTORS

Marshak, Dan, Ph.D., Cold Spring Harbor Laboratory, New York
Selk, Linda, Ph.D., California Institute of Technology, Pasadena
Smith, John, Ph.D., Massachusetts General Hospital, Boston

ASSISTANTS

Clawson, Leigh, Ph.D., California Institute of Technology, Pasadena
Chang, Yie-Hwa, Ph.D., Massachusetts General Hospital, Boston

This course was intended for scientists who are not familiar with techniques of protein isolation and characterization. Students learned the major techniques in protein purification by actually performing four separate isolations including: (i) a

regulatory protein from muscle tissue; (ii) a fusion protein from *E. coli*; (iii) a DNA-binding protein from nuclei of tissue culture cells; and (iv) a chemically synthesized peptide. A variety of chromatographic, electrophoretic, and bulk fractionation techniques were employed including: ion exchange, gel filtration, hydrophobic interaction, affinity-based adsorption, and immunoaffinity chromatography; polyacrylamide gel and two-dimensional gel electrophoresis and electroblotting; precipitation by salt and pH; and HPLC analysis. Methods of protein characterization were discussed including amino acid analysis, protein sequencing, and mass spectrometry. Emphasis was placed on methods of protein purification rather than automated instrumental analysis. Guest lecturers discussed protein structure, modifications of proteins, and methodologies for protein purification. Applications of protein biochemistry to various areas of research in molecular biology were discussed.

PARTICIPANTS

Baumann, Gotz, Ph.D., Sandoz, Ltd, Basel, Switzerland
D'Urso, Gennaro, B.A., Fred Hutchinson Cancer Center, Seattle, Washington
Das, Gokul, Ph.D., Cold Spring Harbor Laboratory, New York
Holz, George, Ph.D., Tufts University, Medford, Massachusetts
Jarrell, Kevin, Ph.D., Harvard University, Cambridge, Massachusetts
Kleinberger, Tamar, Ph.D., Tel Aviv University, Israel
Leibowitz, Jacob, Ph.D., University of Alabama, Tuscaloosa
Loh, Johnson, B.S., Yale University, New Haven, Connecticut
Lossky, Marie, Ph.D., Brandeis University, Waltham, Massachusetts
Ludwig, Steven, Ph.D., University of Georgia, Athens
Myers, Jeff, B.A., University of Pennsylvania, Philadelphia
Ogunjobi, O., Ph.D., University of Edinburgh, Scotland
Shepherd, Gordon, B.A., Massachusetts General Hospital, Boston
Slepecky, Norma, Ph.D., Syracuse University, New York
Sowden, Mark, B.S., University of Oxford, England
Swenson, Katherine, Ph.D., Harvard Medical School, Boston, Massachusetts
Weinrich, Scott, Ph.D., University of California, San Francisco

SEMINARS

Aebersold, R., University of British Columbia. Electro-blotting of proteins.
Hunkapillar, M., Applied Biosystems, Inc. Protein sequencing.
Kadonaga, J., University of California, San Diego. DNA binding proteins.
Kent, S., California Institute of Technology. Peptide synthesis.
Pace, N., Texas A&M University. Physical measurements of proteins.
Paterson, Y., University of Pennsylvania. Antigenic sites on proteins.
Robbins, P., Massachusetts Institute of Technology. Glycoproteins.
Rose, G., University of Pennsylvania, Hershey Medical Center. Protein folding.
Wilson, K., Applied Biosystems, Inc. Chromatography of proteins–HPLC and capillary electrophoresis.
Wold, F., University of Texas, Houston. Posttranslational modifications.

Molecular Embryology of the Mouse

June 9–June 29

INSTRUCTORS

Lovel-Badge, Robin, Ph.D., NIMR, London, England
McMahon, Andy, Ph.D., Roche Institute of Molecular Biology, Nutley, New Jersey

CO-INSTRUCTORS

Cooke, Lesley, M.Sc., ICRF, Oxford, England
Mann, Jeff, Ph.D., Roche Institute of Molecular Biology, Nutley, New Jersey

This course is designed for molecular biologists, biochemists, and cell biologists interested in applying their expertise to the study of mouse embryonic development. Lecture and laboratory components of the course emphasized both the practical aspects of working with mouse embryos and the conceptual basis of current embryonic research. The following procedures and their potential applications were described: isolation and culture of germ cells and pre-implantation and post-implantation embryos, embryo transfer, establishment and genetic manipulation of embryo-derived stem cell lines, germ layer separation, chimera formation, nuclear transplantation, microinjection of DNA into eggs, retroviral infection of embryos, microinjection of cell lineage tracers, *in situ* hybridization and immunohistochemistry. Guest lecturers discussed current research in the field.

PARTICIPANTS

Bodine, David M., Ph.D., National Institutes of Health, Bethesda, Maryland
Capon, Daniel, Ph.D., Genentech, Inc., South San Francisco, California
Christofori, Gerhard, Ph.D., University of Basel, Switzerland
Cossu, Giulio, M.D., University of Rome, Italy
Dandolo, Luisa, Ph.D., Institut Pasteur, Paris, France
Doglio, Luisa, Ph.D., University of Chicago, Illinois
Donoviel, Dorit, B.A., University of Washington, Seattle
ffrench-Constant, Charles, Ph.D., Cambridge University, England
Gardiner, Edith, Ph.D., Yale University, New Haven, Connecticut
Lawler, Jack, Ph.D., Brigham and Women's Hospital, Boston, Massachusetts
Marth, Jamey, Ph.D., University of British Columbia, Vancouver
Nose, Toshiaki, Ph.D., Mitsubishi-Kasei Institute, Tokyo, Japan
Quaranta, Vita, M.D., Research Institute of Scripps Clinic, La Jolla, California
Vassar, Robert, B.A., University of Chicago, Illinois

SEMINARS

McMahon, A., Roche Institute of Molecular Biology. Preimplantation development.
Waelsch, S., Albert Einstein College of Medicine. Mouse genetics–an historical perspective.
Beddington, R., ICRF Developmental Biology. Postimplantation development and cell lineages.
McLaren, A., MRC Mammalian Development Unit. Germ cells.
Hogan, B., Vanderbilt University Medical Center. Extraembryonic membranes and molecules.
Wassarman, P., Roche Institute of Molecular Biology. Fertilization.
Mann, J., Roche Institute of Molecular Biology. Parthenogenesis.
McLaren, A., MRC Mammalian Development Unit. Sex determination I.
Lovell-Badge, R., National Institutes for Medical Research. Sex determination II.
Robertson, L., Columbia University. Manipulation of es cells.
Lovell-Badge, R., National Instututes for Medical Research. Teratocarcinomas.
Solter, D., Wistar Institute. Imprinting.
Rossant, J., Mount Sinai Hospital, Toronto. Trophoblast in development.
———. Gene targeting.
Jaenisch, R., Whitehead Institute. Mouse models of human disease.
Hastie, N., MRC, Western General Hospital. Approaches to reverse genetics.
Krumlauf, R., NIMR, London. Homeobox genes and mouse development.
Rinchik, G., Mammalian Genetics and Development Section. Genetic resources.
Papaioannou, G., Tufts University. Chimaeras in development.
Cebra-Thomas, J., Princeton University. T-complex.
McMahon, A., Roche Institute of Molecular Biology. Gene expression in development.
Costantini, F., Columbia University. Gene expression in transgenic mice.
Strickland, S., State University of New York. Maternal RNA and the mouse oocyte.
Stewart, C., Roche Institute of Molecular Biology. Growth factors in development.
Cepko, C., Harvard Medical School. Cell lineage in the nervous system.
Noden, D., Cornell University. Neural crest.
McKay, R., Massachusetts Institute of Technology. Early neural tube development.
Williams, D., Children's Hospital, Boston. Hematopoietic stem cells.
Curran, T., Roche Institute of Molecular Biology. Transcription factors.

Advanced Bacterial Genetics

June 9–June 29

INSTRUCTORS

Berget, Peter, Ph.D., Carnegie Mellon University, Pittsburgh, Pennsylvania
Maurer, Russell, Ph.D., Case Western Reserve University, Cleveland, Ohio
Weinstock, George, Ph.D., University of Texas, Houston

ASSISTANTS

Jin, Xiaomei, B.S., University of Texas, Houston
Kean, Leslie, Ph.D., Case Western Reserve University, Cleveland, Ohio
Regan, Mary, B.S., Carnegie Mellon University, Pittsburgh, Pennsylvania

This laboratory course demonstrated genetic approaches that can be used to analyze biological processes and their regulation, as well as detailed structure/function relationships of genes. Techniques that were be covered included: isolation, complementation, and mapping of mutations; use of transposable genetic elements; construction of gene fusions; cloning of DNA; restriction enzyme mapping; Southern blotting; macrorestriction mapping of genomes by pulse-field electrophoresis; and DNA sequencing. The course consisted of a set of experiments incorporating most of these techniques, supplemented with lectures and discussions. The aim was to develop in students the ability to design a successful genetic approach to any biological problem.

PARTICIPANTS

Bramanti, Thomas, D.D.S., University of Texas, San Antonio
Farinha, Mark, B.S., Queen's University, Kingston, Ontario, Canada
Fernandes, Prabhavathi, Ph.D., The Squibb Institute, Princeton, New Jersey
Hannaert, Veronique, M.S., Institute of Cellular and Molecular Pathology, Brussels, Belgium
Hughes, Carrie, Ph.D., University of Minnesota, Minneapolis
Ingham, Colin J., Ph.D., University of Wisconsin, Madison
Kaiser-Rogers, Kathleen, B.S., University of North Carolina, Raleigh
Lamb, Andrew J., B.S., University of Aberdeen, Scotland
Maloney, Peter, Ph.D., Johns Hopkins University, Baltimore, Maryland
Masure, H. Robert, Ph.D., University of Washington, Seattle
Rappa, Philip, B.S., Hunter College, New York, New York
Rouviere, Pierre, Ph.D., University of Utah, Salt Lake City
Sherman, Michael, Ph.D., Harvard Medical School, Boston, Massachusetts
Songer, J. Glenn, Ph.D., University of Arizona, Tucson
Williams, Huw, Ph.D., University of Wales College of Cardiff
Ziegler, H. Kirk, Ph.D., Emory University, Atlanta, Georgia

SEMINARS

Kaplan, S., University of Texas Medical School. Photosynthetic membrane biogenesis in *Rhodobacter spaeroides*.
Bassford, P., University of North Carolina. Genetic approaches to studying protein export in *E. coli*.
Radman, M., Institut Jacques Monod. Mismatch repair systems and the maintenance of genetic information.
Youngman, P., University of Pennsylvania. Use of transposons as tools for genetic manipulation in sporulating gram positive bacteria.
Walker, G., Massachusetts Institute of Technology. Genetic analysis of nodulation by *Rhizobium*.

Molecular Approaches to Ion Channel Function & Expression

June 9–June 29

INSTRUCTORS

White, Michael, Ph.D., University of Pennsylvania, Philadelphia
Snutch, Terry, Ph.D., University of British Columbia, Vancouver, Canada
Leonard, John, Ph.D., University of Illinois, Chicago
Goldin, Al, M.D., Ph.D., University of California, Irvine
Margiotta, Joseph, Ph.D., Mt. Sinai Medical Center, New York, New York
Dionne, Vince, Ph.D., University of California, San Diego

ASSISTANT

Gilbert, Mary, M.S., University of British Columbia, Vancouver, Canada

Application of the techniques of molecular biology to neurobiology has provided novel approaches and a new level of sophistication in the examination of many neurobiological problems. This intensive laboratory/lecture course is designed to introduce students to the application of these techniques to the study of ion

channels. Students concentrated initially on basic aspects of this approach, and then proceeded to more integrated studies. The course dealt with the following topics: MRNA isolation and handling; preparation of *in vitro* transcripts for expression and use as gybridization probes; *Xenopus* oocytes as an expression system; characterization of newly expressed channels in oocytes using immunoprecipitation, voltage- and patch-clamping; design and implementation of recording equipment; monitoring levels of and changes in channel expression using Northern blot analysis. There were opportunities during the final week of the course for students to undertake special projects of their own design using the methods taught in the course.

PARTICIPANTS

Barr, Eliav, M.D., Johns Hopkins Hospital, Baltimore, Maryland
Bennett, Eric S., B.S., University of Rochester, New York, New York
Davies, Noel W., Ph.D., University of Leicester, England
Fiszman, Monica, Ph.D., National Institutes of Health, Bethesda, Maryland
Garcia-Martinez, Esperanza, M.S., Universidad de Colima, Mexico
Haber, Róberta J., Ph.D., NCI, National Institutes of Health, Bethesda, Maryland
Hsiung, Hansen, Ph.D., Lilly Corporate Center, Indianapolis, Indiana
Izumo, Seigo, M.D., Children's Hospital, Boston, Massachusetts
Jensen, Abigail, B.A., University of Wisconsin, Madison
MacDonald, John, Ph.D., University of Toronto, Canada

SEMINARS

White, M., University of Pennsylvania. Biosynthesis in assembly of the ACh receptor
Snutch, T., University of British Columbia. Cloning around with channels.
Leonard, J., University of Illinois. Expression of NMDA receptors in *Xenopus* oocytes.
Goldin, A., University of California. Structure-function studies of the NA^{+} channel.
Beam, Kurt, Colorado State University. Developmental and genetic regulation of Ca^{++} channels in muscle.
Enyeart, J., Ohio State University. Hormonal regulation of Ca^{++} channels.
Dionne, V., University of California. Molecular studies of GABA receptors.
Kinnamon, S., Colorado State University. How do we taste?

Molecular Neurobiology of Human Disease

June 9–June 19

INSTRUCTORS

Black, Ira, Ph.D., Cornell University Medical Center, New York
Breakefield, Xandra, Ph.D., E.K. Shriver Center, Waltham and Harvard Medical School, Boston, Massachusetts
Gusella, James, Ph.D., Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts

This intensive seminar course explored the molecular and cellular basis of abnormal neural function. It focused on basic scientific studies that have provided insight into the etiology and pathogenesis of neurologic and psychiatric diseases. Emphasis was also placed on new techniques in neuroscience and molecular genetics. Topics included: linkage analysis using DNA polymorphisms; strategies for identifying and cloning defective neural genes; onc genes in neural tumors; gene therapy using viral vectors; creating animal models using embryonic stem cells and transgenic animals; genetic defects in demyelination and lipidoses; viral neurotropism; molecular events in development and degeneration; cellular events in neural regeneration and brain grafting; neuroimmunology, ion channels in epilepsy; and brain imaging.

PARTICIPANTS

Anderson, Mary Dilys, M.S., Yale University, New Haven, Connecticut
Baez, Mel, Ph.D., Lilly Research Laboratories, Indianapolis, Indiana
Barbany, Gisela, M.D., Karolinska Institute, Sweden
Bayney, Richard, Ph.D., Molecular Therapeutics, Inc., West Haven, Conecticut
Bejjani, Bassem, M.D., E.K. Shriver Center, Waltham, Massachusetts
Cook, David, B.S., Yale University, New Haven, Connecticut
Davar, Gudarz, M.D., Massachusetts General Hospital, Boston
Feldblum, Sophie, Ph.D., University of California, Los Angeles
Gary, Todd, B.A., Vanderbilt University, Nashville, Tennessee
Janson, Lars, B.S., University of Uppsala, Sweden
Lee, Jung Kyo, Ph.D., Massachusetts General Hospital, Boston
Perry, Sharon E., Ph.D., Vanderbilt University, Nashville, Tennessee
Phelps, Creighton, Ph.D., National Institute on Aging, National Institutes of Health, Bethesda, Maryland
Reiner, Orly, M.S., Weizmann Institute, Israel
Robertson, David, M.D., Yale Medical School, New Haven, Connecticut
Sahin, Mustafa, B.S., Yale Medical School, New Haven, Connecticut
Skinner, Karen, Ph.D., National Institutes of Health, Bethesda, Maryland
Straub, Richard, B.A., Cornell University, New York
Tobia, Annette, Ph.D., Squibb Corporation, Princeton, New Jersey
Tombaugh, Geoffrey, B.A., Stanford University, California
Veruki, Margaret, B.S., University of Rochester, New York
Watanabe, Takashi, M.D., University College of London, England
Weiss, Joachim, M.D., Washington University, St. Louis, Missouri
Zhou, Feng, Ph.D., Indiana University, Indianapolis

SEMINARS

Gusella, J., Massachusetts General Hospital and Harvard Medical School. Search for HD gene.
Hoffman, E., Boston Children's Hospital. Duchenne muscular dystrophy gene and protein.
Racaniello, V., Columbia University College of Physicians & Surgeons. Poliovirus.
Stevens, J., University of California School of Medicine. Herpes latency in the nervous system.
Johnston, B., Columbia University College of Physicians & Surgeons, New York. Lysosomal diseases.
Gravel, R., Toronto Hospital for Sick Children. Genetic defects in lysosomal enzymes.
Selkoe, D., Brigham and Women's Hospital. Molecular etiology of Alzheimer's disease.
Gusella, J. Massachusetts General Hospital and Harvard Medical School. Familial Alzheimer's disease.
Wallace, D., Emory University. Mitochondrial inheritance.
Rappaport, J. National Institutes of Health. Structure and function HIV I *tat* gene.
Ledbetter, D., Baylor College of Medicine. Chromosome deletion causing lissencephaly in humans.
Herrup, K., E.K. Shriver Center. Neuron movement and

numbers in murine development.
Bernards, R., Massachusetts General Hospital. Molecular biology of tumor progression and neuroblastoma.
Harlow, E., Cold Spring Harbor Laboratory. Oncoproteins.
Mallet, J., Centre National de la Recherche Scientifique. Genes implicated in psychiatric illness.
Breakefield, X., E.K. Shriver Center. MAO deficiency state in man.
Colman, D., Columbia University College of Physicians & Surgeons. Biogenesis of myelin membrane.
Milner, R., Salk Institute. Genetic defects in myelin.
Young, A., University of Michigan. Neuropathogenesis of HD.
Wexler, N., Columbia University. Molecular and human aspects of HD.
Rossant, J., Mt. Sinai Hospital Research Institute. Creating animal models using embryonic cells.
Sutcliffe, G., Research Institute, Scripps Clinic. New genetic strategies for finding disease genes.
Gage, F., University of California School of Medicine. Brain grafting.
Black, I., Cornell University Medical Center. Developmental neuroplasticity.
Raichle, M., Washington University School of Medicine. Brain imaging.
Aguayo, A., Montreal General Hospital. Regeneration in the adult mammalian CNS.
Sanes, J., Washington University Medical Center. Cell lineage in the CNS.
Rakic, P., Yale University School of Medicine. Altered neural cell migration following environmental or genetic insults.
McNamara, J., Duke University Medical Center. Epilepsy.
Dingledine, R., University of North Carolina School of Medicine. NMDA receptors.
Fields, H., University of California Medical School. Pain.

Genetic Approaches to Human Disease Using DNA Markers

June 21–June 25

INSTRUCTORS

Lander, Eric, Ph.D., Whitehead Institute and Harvard University, Cambridge, Massachusetts
Page, David, Ph.D., Whitehead Institute and Massachusetts Institute of Technology, Cambridge

It has recently become feasible to map genes underlying some human diseases and traits—even when the molecular basis is unknown—by studying individuals from natural populations and using DNA markers (such as RFLPs). This intensive lecture course explored the possibilities and difficulties of applying these methods to the study of various human diseases and traits, including simply-inherited disorders (such as cystic fibrosis), complex disorders (such as heart disease or

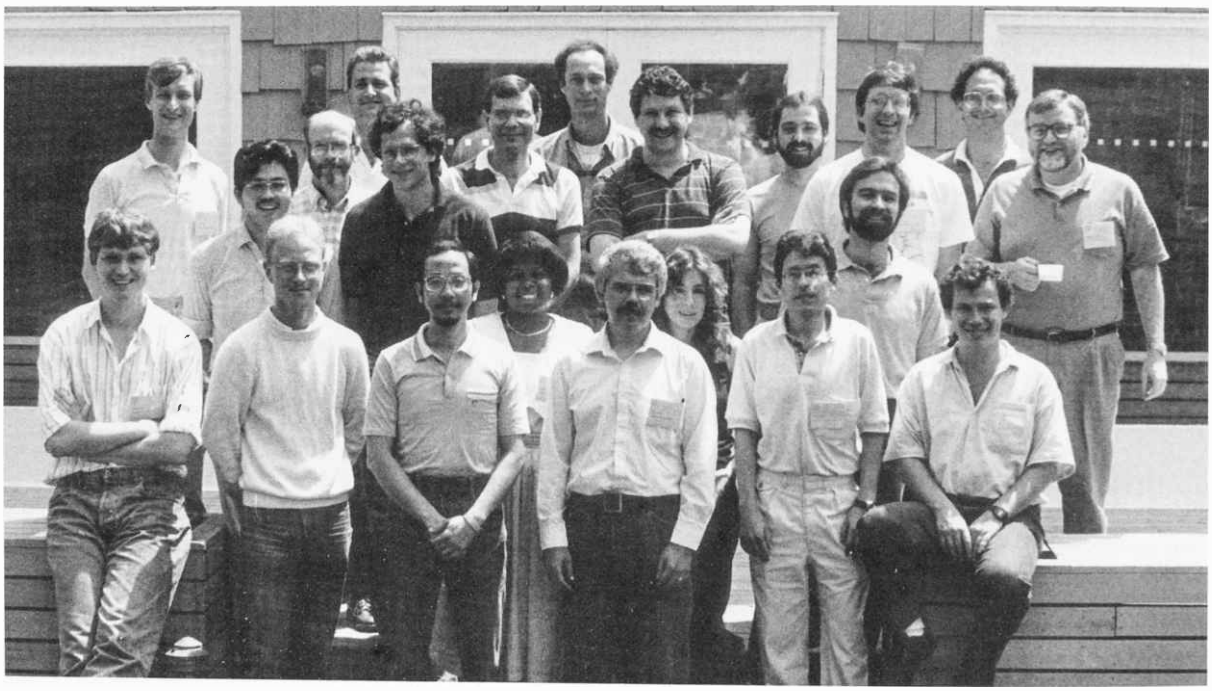
psychiatric conditions), inherited and spontaneous cancers, and developmental abnormalities (such as sex determination). Understanding such biological systems requires an interplay among transmission genetics and cytogenetics in mammals, human population genetics, and molecular biological techniques. Accordingly, the course concentrated on ways to employ genetics to dissect complex biological problems in natural populations.

PARTICIPANTS

Baron, Miron, M.D., New York State Psychiatric Institute, New York
Bear, John, Ph.D., Memorial University of Newfoundland, Canada
Blake, David, M.D., Columbia University, New York, New York
Chen, Wei-Jane, M.D., Harvard School of Public Health, Boston, Massachusetts
Chiang, Huey-Jenn, Ph.D., California Institute of Technology, Pasadena
Crowe, Raymond, M.D., University of Iowa, Iowa City
DeVries, Niek, M.D., Academic Hospital, Holland
Fyer, Abby, M.D., Columbia University, New York, New York
Giuffra, Luis, M.D., Yale University, New Haven, Connecticut
Gumerlock, Paul, Ph.D., University of California, Davis
Hogan, Kirk, M.D., University of Wisconsin, Madison
Janson, Lars, B.S., University of Uppsala, Sweden
Julian, Bruce, M.D., University of Alabama, Birmingham
Levinson, Douglas, M.D., Medical College of Pennsylvania, Philadelphia
McAlmon, Karen, M.D., University of California, San Diego
Perry, Sharon, Ph.D., Vanderbilt University, Nashville, Tennessee
Raymond, Vincent, Ph.D., Centre de Recherche Laval Robert-Giffard, Quebec, Canada
Straub, Richard, B.A., Cornell University, New York

SEMINARS

King, M.C., University of California. Genetic epidemiology and breast cancer.
Burke, D., Princeton University. Analyzing and cloning large DNA molecules.
Gilliam, C., Columbia University of Physicians & Surgeons. Linkage analysis of neurological disorders.
Glaser, T., Massachusetts Institute of Technology. Wilm's tumor and the genetics of recessive oncogenes.
Tanzi, R., Harvard University. The genetics of Alzheimer's disease.
Haines, J., Harvard University. Linkage analysis: Computer methods.
Nussbaum, R., Pennsylvania State University. The curious genetics of the Fragile X Syndrome.
Puck, J., Pennsylvania State University. X-linked immunodeficiencies and the uses of somatic cell genetics.
Lincoln, S., Whitehead Institute. Constructing genetic linkage maps.



Computational Neuroscience: Vision

July 2–July 15

INSTRUCTORS

Hildreth, Ellen, Ph.D., Massachusetts Institute of Technology, Cambridge
Movshon, J. Anthony, Ph.D., New York University, New York

ASSISTANTS

Geggenfunter, Karl, B.A., New York University, New York
Horswell, Ian, B.A., Massachusetts Institute of Technology, Cambridge
Poirson, Allen, B.A., Stanford University, California

Computational approaches to neuroscience have produced important advances in our understanding of neural processing. Prominent successes have come in areas where strong inputs from neurobiological, behavioral, and computational approaches can interact. Through a combination of lectures and hands-on experience with a computer laboratory, this course examined several areas, including feature extraction, motion analysis, binocular stereopsis, color vision, higher-level visual processing, visual neural networks, and oculomotor function. The theme was that an understanding of the computational problems, the constraints on solutions to these problems, and the range of possible solutions can help guide research in neuroscience.

PARTICIPANTS

Basri, Ronen, B.S., Weizmann Institute, Israel
Beusmans, Jack M., M.S., University of California, Irvine
Chichilnisky, Eduardo, B.A., Stanford University, California
DePriest, Derryl, B.A., University of Rochester, New York
Elliott, David, B.S., Massachusetts Institute of Technology, Cambridge
Freeman, William, M.S., Massachusetts Institute of Technology, Cambridge
Galun, Merav, M.S., Hebrew University, Israel
Harris, Julie, B.S., University Laboratory of Physiology, Oxford, England
He, Jijiang, M.S., University of Alabama, Birmingham

Kiper, Daniel, M.A., New York University, New York
Knill, David, B.S., Brown University, Providence, Rhode Island
Levitt, Jonathan, M.A., New York University, New York
Mansfield, John, B.S., University Laboratory of Physiology, Oxford, England
O'Keefe, Lawrence, M.S., Florida State University, Tallahassee
Pollick, Frank, M.S., University of California, Irvine
Rothwell, Bart, M.A., University of California, Los Angeles
Sha'ashua, Amnon, M.S., Weizmann Institute, Israel
Stoner, Gene, B.A., Salk Institute, San Diego, California
Stuck, Elizabeth, M.S., University of Minnesota, Minneapolis
Treue, Stefan, B.S., Massachusetts Institute of Technology, Cambridge
Turk, Matthew, M.S., Massachusetts Institute of Technology, Cambridge
Wuerger, Sofia, M.S., New York University, New York
Zabrodsky, Haggith, M.S., Hebrew University, Israel
Zhang, Jun, B.S., University of California, Berkeley

SEMINARS

Frisby, J., University of Sheffield. Computational and empirical studies of human stereo vision.
Sejnowski, T., Salk Institute. Neural network models of stereo vision.
Adelson, E., Massachusetts Institute of Technology. Early vision and the measurement of motion.
Newsome, W., Stanford University School of Medicine. Single neurons and the perception of motion.
Wandell, B., Stanford University. Computational and empirical studies of color vision.
Lee, H., Eastman Kodak Company. Lightness scales and chromaticity convergence.
Bulthoff, H., Brown University. The psychophysics of cue integration.
Hildreth, E., Massachusetts Institute of Technology. The computational study of vision.
———. C and HIPS.
———. LISP and OBVIUS.
———. Computational models of edge detection.
———. Computational models of the recovery of 3-D structure and motion.
Desimone, R., National Institute of Mental Health. ATTENTION! Neural mechanisms in extrastriate visual cortex.
Lisberger, S., University of California School of Medicine. Visual tracking.
Grimson, E., Massachusetts Institute of Technology. Computational models of stereo vision.
———. Models of object recognition.
Grzywacz, N., Massachusetts Institute of Technology. Single neurons as complex processors: The example of visual motion computations.
———. Theories for how neurons interact to estimate the visual velocity field.
Landy, M., New York University. Psychophysical observations of early visual processing.
———. Psychophysical observations of structure-from-motion.
Morgan, M., University College London. The psychophysical study of visual processing.
———. Mechanisms of feature analysis in human vision.
Movshon, J., New York University. Empirical studies of visual processing.
———. C and HIPS.
———. The physiology of early vision.
———. Physiological studies of motion analysis.
———. Physiological studies of binocular stereopsis.

Molecular Cloning of Eukaryotic Genes

July 3–July 23

INSTRUCTORS

Alt, Fred, Ph.D., Columbia University, New York, New York
Roberts, Tom, Ph.D., Dana Farber Cancer Institute, Boston, Massachusetts
Yancopolous, George, M.D., Ph.D., Regeneron Pharmaceuticals, Tarrytown, New York

ASSISTANTS

Morrow, Maureen, M.A., Columbia University, New York, New York
Wood, Ken, M.A., Dana Farber Cancer Institute, Boston, Massachusetts
Young, Fay, M.D., Columbia University, New York, New York

This laboratory and lecture course covered the principles of recombinant-DNA technology and the application of these procedures to the study of eukaryotic genes. The isolation, characterization, and expression of specific eukaryotic genes, including strategies for isolating genes that encode rare mRNA sequences such as subtractive hybridization, will be emphasized. Among the topics covered were:

construction of cDNA libraries, construction of bacteriophage λ genomic DNA libraries, rapid screening of DNA libraries with gene-specific hybridization probes, purification and characterization of recombinant clones using restriction endonuclease and blot hybridization analyses, PCR technology, and reintroduction and expression of cloned genes in heterologous systems. Expression of cDNA clones in bacteria and insect virus systems were taught. Guest lecturers discussed the application of molecular cloning procedures to the study of specific eukaryotic gene systems.

PARTICIPANTS

Abraham, Robert, Ph.D., Mayo Clinic, Rochester, Minnesota
Aggarwal, Aneel, Ph.D., Harvard University, Cambridge, Massachusetts
Barrandon, Yann, M.D., Harvard Medical School, Boston, Massachusetts
Cohen, Carl, Ph.D., St. Elizabeth's Hospital, Boston, Massachusetts
Gifford, David, Ph.D., Massachusetts Institute of Technology, Cambridge
Hall, Michael, Ph.D., Jules Stein Eye Institute, Los Angeles, California
Jamnadass, Ramni, M.S., International Laboratory for Research on Animal Diseases, Kenya, Africa
Rauch, Joyce, Ph.D., Montreal General Hospital, Canada
Reich, Eva-Pia, Ph.D., Yale University, New Haven, Connecticut
Sarkar, Rita, Ph.D., Madras University, India
Schultz, Maggie, Ph.D., Hahnemann University, Philadelphia, Pennsylvania
Slezynger, Thelma, Ph.D., Universidad Simon Bolivar, Venezuela
Soyfer, Valery, Ph.D., Ohio State University, Columbus
Thomas, Patricia, Ph.D., Oklahoma Medical Research Foundation, Oklahoma City
Thompson, Nancy, Ph.D., Rhode Island Hospital, Providence
Winchester, Robert, M.D., New York University School of Medicine

SEMINARS

Alt, F., Columbia University. Myc family genes/B cell differentiation.
Roberts, T., Dana Farber Cancer Institute. Signal transduction.
McKnight, S., Carnegie Institute. DNA binding specificity and genetic function of C/EBP.
Oste, C., Perkin-Elmer Cetus. Polymerase chain reaction technology.
Hood, L., California Institute of Technology. Ig gene superfamily/New advances in biotechnololgy.
Yancopoulos, G., Regeneron Pharmaceuticals. Molecular genetics of pre B-cell differentiation.
Schimke, R., Stanford University School of Medicine. Gene amplification and related topics.
Rothstein, R., Columbia University. Gene cloning in yeast systems.
Tucker, P., University of Texas Southwestern. Regulated expression of Ig genes.
Kiang, S.C., Mt. Sinai Medical School. IL-6 regulation of Ig gene expression.
Cantor, C., Columbia University. Mapping the human genome.
Smith, C., Columbia University. Functional elements of chromosome structure.
Goff, S., Columbia University. Mutagenesis of retrovirus genomes.
Harlow, E., Cold Spring Harbor Laboratory. EIA complexes with the retinoblastoma protein.
Aruffo, A., Massachusetts General Hospital. Cloning cDNAs encoding cell surface proteins by transient expression in cos cells.
Kaufman, R., Genetics Institute. Cleavage of the P220 subunit of the cap-binding protein complex inhibits mRNA accumulation, and not translation initiation in intact cells.
Calame, K., Columbia University. Regulation of Ig gene expression.
Kucherlapati, R., Albert Einstein College of Medicine. Gene replacement in mammalian cells.
Mulligan, R., Whitehead Institute for Biomedical Research. Retroviral vectors/Gene transfer into stem cells.
Jarvis, D., Texas A & M University. Baculovirus vectors.
Mandel, G., State University of New York, Stony Brook. Electrophysiology to clone genes.
Herr, W., Cold Spring Harbor Laboratory. Structure and function of transcription factors.
DePinho, R., Albert Einstein College of Medicine. Transgenic models for development.

Molecular and Developmental Biology of Plants

July 3–July 23

INSTRUCTORS

Maliga, Pal, Ph.D., Waksman Institute, Rutgers University, Piscataway, New Jersey
Gruissem, Wilhelm, Ph.D., University of California, Berkeley
Varner, Joseph, Ph.D., Washington University, St. Louis, Missouri

ASSISTANTS

Cosowsky, Laurey, M.S., Waksman Institute, Rutgers University, Piscataway, New Jersey
Hajdukiewitz, Peter, M.S., Waksman Institute, Rutgers University, Piscataway, New Jersey
Staub, Jeffrey, M.S., Waksman Institute, Rutgers University, Piscataway, New Jersey

This course provided an intensive overview of current topics and techniques in plant biology, with emphasis on molecular and developmental biology and genetics. It was designed for scientists with working knowledge of molecular techniques who are either working with plant systems or wish to. The course consisted of a rigorous lecture series, a hands-on laboratory, and informal discussions. Different guest speakers provided both an in-depth discussion of their work and an overview of their specialty. The laboratory covered established and novel techniques in plant biology, including cell and tissue culture techniques, gene transfer techniques, assays for transient gene expression, nucleic acid manipulations, *in vitro* chloroplast transcription, footprint analysis, *in situ* detection RNA and protein, genetics of *Arabidopsis*, genetics and cytogenetics of maize.

PARTICIPANTS

Avni, Adi, M.S., Weizmann Institute, Israel
Haider, Syed, M.S., University of Washington, Seattle
Hershberger, Jane, Ph.D., Stanford University, California
Huttner, Eric, Ph.D., INRA, Versailles, France

Lai, Shoupeng, M.S., University of Maryland
Leyser, Ottoline, B.A., University of Cambridge, England
Marrs, Kathleen, B.A., University of Illinois
Paje-Manalo, Leila, Ph.D., University of New Hampshire
Pereto, Juli, Ph.D., University of Pennsylvania, Philadelphia
Schena, Mark, Ph.D., University of California, San Francisco
Serrano, Elba, Ph.D., University of California Los Angeles Medical School
Thomas, George, Ph.D., National Institutes of Health, Bethesda, Maryland
Valles, Maria-Pilar, B.A., Centro de Investigacion, Barcelona, Spain.
Van der Mark, Frits, Ph.D., Centre for Cytotechnology, Netherlands
Vance, Nan, Ph.D., United States Dept. of Agriculture, Corvallis, Oregon
Wang, Yi-Chang, Ph.D., Biotechnica International, Cambridge, Massachusetts

SEMINARS

Sussex, I., Yale University. Plant development.
Varner, J., Washington University. Cell wall chemistry and architecture.
Lamb, C., Salk Institute. Plant response to pathogens.
Fromm, M., Plant Gene Expression Center. Assays for transient gene expression.
Gruissem, W., University of California. Plastid differentiation during plant development.
Boynton, J., Duke University. *Chlamydomonas* organelle genetics.
Miki, B., Plant Research Center. Microinjection.
Ahlquist, P., University of Wisconsin. Plant viruses.
Mottinger, J., University of Rhode Island. Maize cytogenetics.
Dooner, H., Advanced Genetic Sciences. Transposable elements.
Zambryski, P., University of California. Agrobacterium biology and applications.
Staskawicz, B., University of California. Plant host-pathogen interaction.
Theologis, A., Plant Gene Expression Center. Hormonal control of gene expression.
Quail, P., Plant Gene Expression Center. Phytochrome.
Maliga, P., Waksman Institute. Organelle genetics.
Messing, J., Waksman Institute. Regulation of gene expression.
Chua, N.-H., Rockefeller University. Regulation of nuclear gene expression.
Chory, J., Salk Institute. Transduction pathways.
Keegstra, K., University of Wisconsin. Protein targeting.
Freeling, M., University of California. Mutants to study plant development.
Levings, C., North Carolina State University. Mitochondrial genes, CMS.
Nasrallah, J., Cornell University. Self-incompatibility.
Meyerowitz, E., California Institute of Technology. Arabidopsis I.
Hoisington, D., University of Missouri. RFLP mapping.
Somerville, C., Michigan State. Arabidopsis II.

Neurobiology of *Drosophila*

July 3–July 23

INSTRUCTORS

Bate, Michael, Ph.D., University of Cambridge, England
Campos-Ortega, Jose, Ph.D., University of Cologne, Federal Republic of Germany
Palka, John, Ph.D., University of Washington, Seattle

ASSISTANTS

Currie, Douglas, Ph.C., University of Cambridge, England
Dickinson, Michael, Ph.C., University of Washington, Seattle

This laboratory/lecture course provided an introduction to current research in neuronal function and development in *Drosophila*. It was intended for researchers at all levels who may want to use *Drosophila* as an experimental system for studying neurobiology.

The course began with a crash course on *Drosophila* genetics and other techniques which make *Drosophila* research distinctive, such as cytogenetics and DNA transformation. The main emphasis, however, was on studies of the nervous system.

The course covered basic electrophysiological techniques, as applied to mutants with altered ion channels or sensory physiology. In the developmental section, processes of neurogenesis including determination and pathway formation were examined. The course familiarized students with various preparations, including the embryonic nervous system, imaginal discs, and adult nervous system. It also reviewed the different approaches being used in attempts to unravel the molecular basis of neural development.

PARTICIPANTS

Auld, Vanessa, B.S., University of Toronto, Canada
Campbell, Shelagh, M.S., University of Connecticut, Storrs
Chia, William, Ph.D., University of Bristol, England
Hovemann, Bernd, Ph.D., University of Heidelberg, Federal Republic of Germany
Hummon, Margaret, Ph.D., Ohio University, Athens
Kimura, Hideo, Ph.D., The Salk Institute, San Diego, California
Kramer, Helmut, Ph.D., University of California School of Medicine, Los Angeles
Selleck, Scott, Ph.D., Washington University, St. Louis, Missouri
Smith, Hazel, Ph.D., University of Warwick, England
Wolff, Tanya, B.S., Purdue University, West Lafayette, Indiana

SEMINARS

Aldrich, R., T. Schwarz, Stanford University, and J. Palka, University of Washington. Physiology.
Ganetzky, B., University of Wisconsin. Genetics, emphasis on "channel mutants."
Orr-Weaver, T., Whitehead Institute. P-element transformation.
Hartenstein, V., University of California. Embryogenesis.
Campos-Ortega, J., University of Cologne. Embryonic neurogenesis.
Truman, J., University of Washington. Postembryonic neurogenesis.
Hartenstein, V., University of California, J. Palka, University of Washington, and M. Bate, University of Cambridge. Peripheral nervous system and muscle.
Goodman, C., University of California. Molecular aspects of neural differentiation.
Ready, D., Purdue University, G. Rubin, University of California, and I.A. Meinertzhagen, Dalhousie University. Visual system.
Heisenberg, M., California Institute of Technology, and C.P. Kyriacou, University of Leicester. Brains and behavior.

Cellular and Molecular Biology of Learning and Memory

July 17–July 30

INSTRUCTORS

Byrne, John, Ph.D., University of Texas Medical School, Houston
Kandel, Eric, Ph.D., Columbia University College of Physicians & Surgeons, New York, New York
Pearson, Keir, Ph.D., University of Alberta, Canada
Squire, Larry, Ph.D., University of California School of Medicine, San Diego

This lecture course provided an introduction to cell and molecular biological approaches to learning and memory. Suited for graduate students in molecular biology, neurobiology and psychology as well as research workers who are interested in an introduction to this new 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 five selected areas: 1) an introduction to modern behavioral studies of learning and memory; 2) the cell biology of neuronal plasticity and the regulation of gene expression by experience; 3) cellular and molecular mechanisms of simple forms of learning and memory in invertebrates and vertebrates; 4) cellular and molecular mechanisms of long-term potentiation; and 5) neural approaches to human learning and its abnormalities.

PARTICIPANTS

Adam, Geza, Ph.D., Hungarian Academy of Sciences, Szeged, Hungary
Barbour, Boris, B.S., University College of London, England
Barzilai, Ari, Ph.D., Columbia University, New York, New York
Blau, Susana, Ph.D., Nathan Kline Institute, Orangeburg, New York
Haas, Kurt, B.S., Albert Einstein College, Bronx, New York
Gonczy, Pierre, B.S., Rockefeller University, New York, New York
Hewes, Randall, B.A., University of Washington, Seattle
Hoch, Werner, B.S., University of Heidelberg, Federal Republic of Germany
Knowlton, Barbara, B.A., University of Southern California, Los Angeles

Kolb, Stephen, Ph.D., University of Texas, Austin
Kubo, Tai, Ph.D., Columbia University, New York, New York
Kuhl, Dietmar, Ph.D., Columbia University, New York, New York
Martin, Donna, B.S., University of Michigan, Ann Arbor
Mestel, Rosemary, Ph.D., University of California Los Angeles Medical Center
Nazif, Fidelma, B.S., University of Texas, Houston
Pacheco, Mauro, Ph.D., University of Colima, Mexico
Raymond, Jennifer, B.A., University of Texas, Houston
Ruff, Naomi, B.S., NCI, National Institutes of Health, Bethesda, Maryland
Shors, Tracey, Ph.D., University of Southern California, Los Angeles
Suzuki, Wendy, B.A., The Salk Institute, San Diego, California
Takita, Masatoshi, M.S., Tokyo Institute of Technology, Japan

SEMINARS

Kandel, E., Columbia University. Introduction to the cellular study of learning.
Holland, P., Duke University. Introduction to learning theory.
Gould, J., Princeton University. Ethological approaches to learning.
Byrne, J., University of Texas Medical School. Overview of membranes and synaptic transmission.
Schulman, H., Stanford University. Overview of second messenger systems and their role in learning.
Ganetzky, B., University of Wisconsin. Introduction to the study of genes and behavior.
Brunet, J.-F., and P. Pfaffinger, Columbia University. Cloning of genes important to learning.
Curran, T., Roche Institute. Modulation of gene expression in the vertebrate nervous system.
Adler, J., University of Wisconsin. Genes and the behavior of bacteria.
Kenyon, C., University of California. Genes and the behavior of *C. elegans*.
Kandel, E., Columbia University. Learning in *Aplysia* I. Habituation.
———. Learning in *Aplysia* II. Sensitization.
Byrne, J., University of Texas Medical School. Learning in *Aplysia* III. Classical conditioning.
Quinn, C., Massachusetts Institute of Technology. Genetic approaches to study associative learning in *Drosophila*.
Nicoll, R., University of California. Long-term potentiation I.
———. Long-term potentiation II.
Morris, R.G., University of Edinburgh. Role of the Hippocampus and Hippocampal long-term potentiation in learning.
Crow, T., University of Texas Medical School. Cellular correlates of associative learning in Hermissenda.
Pearson, K., University of Alberta. Neuronal correlates of motor learning.
Lisberger, S., University of California. Plasticity in the vestibulo-occular reflex.
Thompson, R., University of Southern California. Cellular locus and correlates of the classical conditioning of the nictitating membrane response.
Greenough, W., University of Illinois. Morphological correlates of learning and experience.
Squire, L., University of California. Memory in humans and non-human primates.
Posner, M., Washington University. Neuropsychology of cognition.
Price, D., Johns Hopkins University. Alzheimer's diseases.

Advanced Molecular Cloning and Expression of Eukaryotic Genes

July 25–August 14

INSTRUCTORS

Botchan, Michael, Ph.D., University of California, Berkeley
Kingston, Robert, Ph.D., Harvard Medical School, Boston, Massachusetts
Myers, Richard, Ph.D., University of California, San Francisco
Rio, Donald, Ph.D., Whitehead Institute, Cambridge, Massachusetts

ASSISTANTS

Misra, Sima, Ph.D., Whitehead Institute, Cambridge, Massachusetts
Robbins, Alan, Ph.D., E.I. du Pont de Nemours, Wilmington, Delaware
Taylor, Ian, Ph.D., Harvard Medical School, Boston, Massachusetts

This course focused on how to manipulate cloned eukaryotic genes to probe questions on their structure, expression and function. As a model system, we examined *cis*- and *trans*-acting components involved in the regulation of eukaryotic gene expression. Students learned the theoretical and practical aspects of constructing genomic and cDNA libraries. Expression libraries from various organisms were screened with recognition site probes for specific DNA-binding proteins. A variety of transfection techniques were used to introduce cloned DNA molecules that have been manipulated *in vitro* into *Drosophila* and vertebrate cells in culture. Mutants were generated by oligo-directed and random mutagenesis procedures and characterized by DNA sequencing. The expression pattern of wildtype and mutant DNAs were analyzed by S1 nuclease, RNase protection and enzymatic assays. Techniques and theory for detecting and characterizing the interaction between regulatory DNA sequences and *trans*-acting protein factors were presented. Guest lecturers discussed present problems in eukaryotic molecular biology as well as technical approaches to their solutions. Experience with basic recombinant-DNA techniques was a prerequisite for admission to this course.

PARTICIPANTS

Attie, Alan, Ph.D., University of Wisconsin, Madison
Behrens, Margarita, M.S., Instituto de Investigaciones Biomedicas, Madrid, Spain
Brisebois, Josee, M.S., McGill University, Montreal, Canada
Buchman, Timothy, Ph.D., Johns Hopkins Hospital, Baltimore, Maryland
Chilton, Beverly, Ph.D., Texas Tech University, Lubbock
Cross, James, D.V.M., University of Missouri, Columbia
Korsgren, Catherine, B.A., St. Elizabeth's Hospital, Boston, Massachusetts
Kronidou, Nafsika, B.A., Dartmouth College, Hanover, New Hampshire
Louvi, Angeliki, B.S., University of Athens, Greece
Lu, Bing, M.S., Albert Einstein College, Bronx, New York
Macdonald, Mary, B.S., University of British Columbia, Vancouver, Canada
Marshallsay, Christopher, B.S., Friedrich Miescher Institut, Basel, Switzerland
Petch, Leslie, B.S., University of North Carolina, Chapel Hill
Sandrin, Mauro, Ph.D., University of Melbourne, Australia
Xu, Lin, M.S., Albert Einstein College, Bronx, New York
Yakobson, Bracha, M.S., Weizmann Institute, Israel

SEMINARS

Rio, D., Whitehead Institute. Regulation of *Drosophila P* element transposition.
Myers, R., University of California. Molecular genetics of Huntington disease.
Ptashne, M., Harvard University. Regulation of transcription—A general view.
McKnight, S., Carnegie Institution. Clues from the structures of transcription factors.
Guarente, L., Massachusetts Institute of Technology. Eukaryotic transcription: A conserved process from yeast to man.
Sharp, P., Massachusetts Institute of Technology. Multi-factor

transcription complexes.

Maniatis, T., Harvard University. Mechanisms of inducible and tissue-specific gene expression.

Treisman, R., I.C.R.F., London. Induction of gene expression following growth factor stimulation.

Courey, A., University of California. Transcriptional activation by the glutamine-rich domains of human SP1.

Herr, W., Cold Spring Harbor Laboratory. Structure and function of eukaryotic transcription factors.

Kingston, R., Harvard Medical School. Transcriptional control by heat in human cells.

Evans, R., Salk Institute. Molecular genetics of steroid and thyroid hormone receptors.

Yeast Genetics

July 25–August 14

INSTRUCTORS

Hieter, Phil, Ph.D., Johns Hopkins University, Baltimore, Maryland
Rose, Mark, Ph.D., Princeton University, New Jersey
Winston, Fred, Ph.D., Harvard Medical School, Boston, Massachusetts

ASSISTANTS

Chapman, Karen, B.A., Johns Hopkins University, Baltimore, Maryland
Hirschhorn, Joel, B.A., Harvard Medical School, Boston, Massachusetts
Meluh, Pamela, B.A., Princeton University, New Jersey

The major laboratory techniques used in the genetic analysis of yeast were studied, including the isolation and characterization of mutants, tetrad analysis, complementation, and mitotic recombination. Micromanipulation used in tetrad analysis was carried out by all students. Molecular genetic techniques, including yeast transformation, gene replacement, analysis of gene fusions, and electrophoretic separation of chromosomes were applied to the analysis of yeast DNA. Indirect immunofluorescence experiments were done to identify the nucleus,

microtubules, and other cellular components. Lectures on fundamental aspects of yeast genetics were presented along with seminars given by outside speakers on topics of current interest.

PARTICIPANTS

Amberg, David, B.A., Dartmouth Medical School, Hanover, New Hampshire
Arwood, Laura, Ph.D., North Carolina State University, Raleigh
Austin, Judith, M.S., University of Wisconsin, Madison
Chakravarti, Debabrata, M.S., Albert Einstein College, Bronx, New York
Chirico, William, Ph.D., Rockefeller University, New York, New York
Hershey, John, Ph.D., University of California, Davis
Hilgen, Sharon, B.A., University of North Carolina, Chapel Hill
Leberer, Ekkehard, Ph.D., University of Toronto, Canada
Moore, Claire, Ph.D., Tufts University, Medford, Massachusetts
Nakamura, Yoshikazu, Ph.D., University of Tokyo, Japan
Peltz, Stuart, Ph.D., University of Wisconsin, Madison
Prentice, Holly, M.S., Massachusetts General Hospital, Boston
Rienhoff, Hugh, M.D., Fred Hutchinson Cancer Center, Seattle
Rocco, James, B.S., Mt. Sinai School of Medicine, New York, New York
Sinensky, Michael, Ph.D., Eleanor Roosevelt Institute, Denver, Colorado
Schiavi, Susan, Ph.D., Harvard Medical School, Boston, Massachusetts

SEMINARS

Mitchell, A., Columbia University. Control of meiosis in yeast.
Carlson, M., Columbia University. Glucose repression in yeast.
Wigler, M., Cold Spring Harbor Laboratory. RAS control pathways in yeast.
Guthrie, C., University of California School of Medicine. snRNPs and splicing.
Broach, J., Princeton University. Plasmid persistance.
Herskowitz, I., University of California. A genetic hierarchy responsible for yeast cell type.
Petes, T., University of Chicago. Meiotic recombination in yeast.
Hinnebusch, A., National Institutes of Health. Translational control of transcriptional regulator.
Guarente, L., Massachusetts Institute of Technology. Eukaryotic transcription: A conserved process from yeast to man.
Sherman, F., University of Rochester. Post-translational modification of cytochrome c.
Pringle, J., University of Michigan. Cellular morphogenesis during the yeast cell cycle.
Fox, T., Cornell University. Translational control of yeast mitochondrial genes and escape to the nucleus.
Novick, P., Yale University. Protein export in yeast.
Kurjan, J., Columbia. Cell-cell interaction involved in yeast mating.
Hieter, P., Johns Hopkins Medical School. Chromosome transmission fidelity in yeast.
Fink, G., Whitehead Institute. Molecular biology of cell fusion in yeast.
Sternglanz, R., State University of New York. DNA topoisomerases.
Rose, M., Princeton University. Genes affecting nuclear fusion in yeast.
Winston, F., Harvard Medical School. Analysis of transcription mutants in yeast.

Molecular Probes of the Nervous System

July 25–August 14

INSTRUCTORS

Carlson, Steve, Ph.D., University of Washington, Seattle
Evans, Christopher, Ph.D., Stanford University, California
Levitt, Pat, Ph.D., Medical College of Pennsylvania, Philadelphia

ASSISTANT

Hallden, Gunnel, Ph.D., University of California, Berkeley

This course was designed for scientists from various disciplines who are interested in understanding the power and pitfalls of antibodies and nucleotide probes as biochemical and anatomical reagents. A series of evening lectures addressed basic and advanced immunology concepts and the use of molecular probes to investigate current issues in neurobiology. The primary emphasis of the course was to acquire practical laboratory experience through daily exercises using an extensive number of techniques, including generation and characterization of monoclonal antibodies to synthetic peptides and complex neural antigens, immunocytochemistry, *in situ* hybridizaton, immunoassays (ELISA & RIA), affinity chromatography and Western blotting. Approaches that combine the use of molecular probes were highlighted by performing expression library screening and exercises employing double labeling strategies. We also designed biological assays in tissue culture to demonstrate functional relevance of specific molecules.

PARTICIPANTS

Antonelli, Marta, Ph.D., Universidad de Buenos Aires, Argentina
Belting, Heinz-Georg, B.A., Yale University, New Haven, Connecticut
Brown, Abraham, Ph.D., Columbia University, New York, New York
Buchner, Klaus, Ph.D., Freie Universitat, Berlin, Federal Republic of Germany

Helms, Jill, D.D.S., University of Connecticut, Storrs
Krulewski, Thomas, M.S., Mt. Sinai School of Medicine, New York, New York
McKeon, Tina, Ph.D., University of Vermont, Burlington
Rinaman, Linda, Ph.D., University of Pennsylvania, Philadelphia
Savio, Tiziana, Ph.D., Universitat Zurich, Switzerland
Xiang, Chang, Ph.D., Lehigh University, Bethlehem, Pennsylvania
Sretavan, David, Ph.D., Rockefeller University, New York, New York
Wayne, Denise, Ph.D., Washington University Medical School, St. Louis, Missouri

SEMINARS

Fleischman, J., Washington University School of Medicine. Igs and super family.
Kurt-Jones, Evelyn, Brigham Women's Hospital. T-cells.
———. Interleukins.
Burrows, P., University of Alabama School of Medicine. B cell differentiation.
Lagenaur, C., University of Pittsburgh School of Medicine. Cell surface interactions in neural development.
Lee, V., Hospital of the University of Pennsylvania. Neurofilament structure and regulation.
Chesselet, M.-F., Medical College of Pennsylvania. In-situ hybridization histochemistry as a tool to study neurotransmitter interactions in the basal ganglia.
Sharff, M., Albert Einstein College of Medicine. Somatic mutations.
Buckley, K., Harvard Medical School. Synaptic vesicle proteins.
Hockfield, S., Yale University School of Medicine. The effect of activity on the molecular anatomy of the developing brain.
Alt, F., Columbia University College of Physicians & Surgeons. Ig gene rearrangements.
Pintar, J., Columbia University College of Physicians & Surgeons. Gene expression in development.
Dodd, J., Columbia University College of Physicians & Surgeons. Axon pathfinding.
Evans, C., Stanford Medical School. Peptide processing and expression.
Levitt, P., Medical College of Pennsylvania. Circuit formation in CNS development.
Carlson, S., University of Washington. Proteoglycans as synaptic components.

Molecular Neurobiology: Brain Development and Function

August 1–August 14

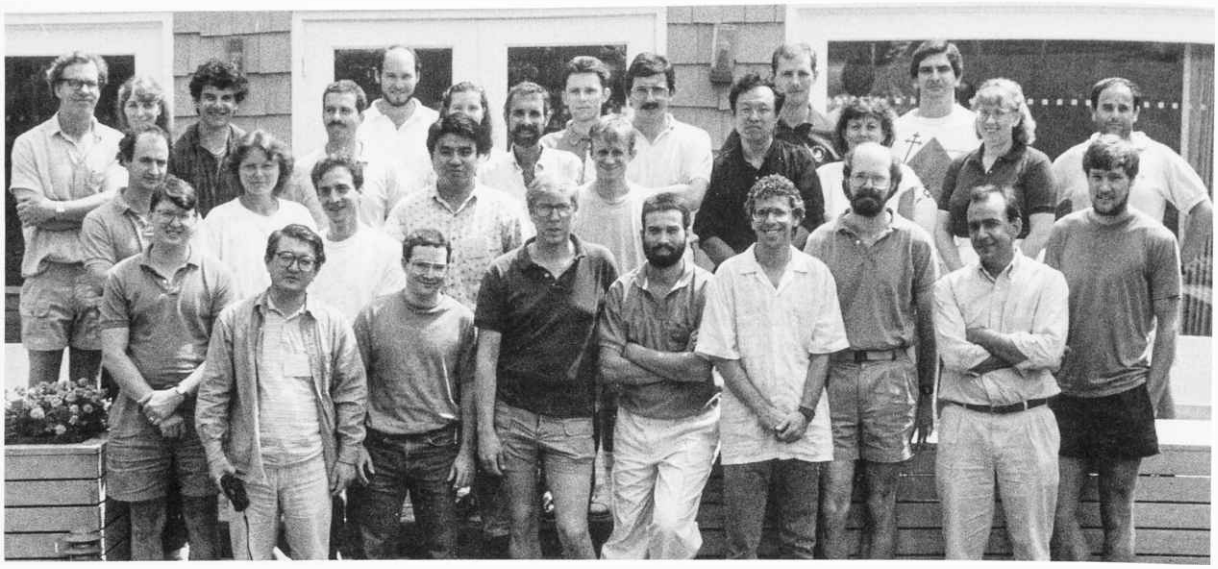
INSTRUCTORS

McKay, Ronald, Ph.D., Massachusetts Institute of Technology, Cambridge
Patrick, James, Ph.D., Baylor College of Medicine, Houston, Texas
Reichardt, Louis, Ph.D., University of California, San Francisco
Schwarz, Thomas, Ph.D., Stanford University, California

This lecture course presented both basic concepts and currently exciting research problems in molecular neurobiology. It focused on approaches and methods now used to study the development and function of the nervous system. Topics covered included gene expression, receptor structure and function, ion channel cloning, second messenger systems, learning, sensory transduction, behavioral genetics, neural induction, cell lineage, immortal cell lines, cell adhesion, onco-genes, neurite outgrowth. The course provided the opportunity to discuss this rapidly expanding research area with invited lecturers. Students from a wide variety of backgrounds (graduate students to faculty) were encouraged to apply.

PARTICIPANTS

Aldrich, Eric, B.A., George Washington University, Washington, D.C.
Allen, Kathryn, B.S., Institute of Neurology, London, England
Allsopp, Timothy, Ph.D., Max Planck Institut, Tubingen, Federal Republic of Germany
Berman, Joshua, B.A., Mt. Sinai School of Medicine, New York, New York
Delay, Rona, B.S., Colorado State University, Fort Collins
Eide, Anne-Lill, M.D., University of Oslo, Norway
Gregor, Paul, M.S., Weizmann Institute, Israel
Hart, Ian, B.S., University College, London, England
Hayashi, Motoharu, M.S., Kyoto University, Japan
Higashinakagawa, Toru, Ph.D., Mitsubishi Kasei Institute, Tokyo, Japan



Karlstrom, Rolf, B.S., University of Utah, Salt Lake City
Kessler, Daniel, B.S., Rockefeller University, New York, New York
Leith, Erich, Ph.D., Worcester Foundation, Shrewsbury, Massachusetts
Noda, Tetsuo, Ph.D., Whitehead Institute, Cambridge, Massachusetts
Paganetti, Paolo, M.S., Brain Research Institute, Zurich, Switzerland
Prendergast, George, Ph.D., New York University, New York
Rubin, Michael, Ph.D., Rockefeller University, New York, New York
Singer, Matthew, B.A., University of California, Berkeley
Skinner, Karen, Ph.D., National Institutes of Health, Bethesda, Maryland
Smith, Paul, Ph.D., Institute of Neurology, London, England
Stoeckli, Kurt, M.S., Max Planck Institute, Martinsried, Federal Republic of Germany
Weinstein, David, M.S., Columbia University College of Physicians & Surgeons, New York, New York

SEMINARS

Hayes, T., Massachusetts Institute of Technology. The regulation of cell growth.
Myers, R., University of California. The molecular genetics of Huntington's disease.
Stillman, B., Cold Spring Harbor Laboratory. DNA replication in mammalian cells.
Bargmann, C., Massachusetts Institute of Technology. Chemosensory neurons and chemotaxis in *C. elegans*.
Zipursky, L., University of California School of Medicine. Genetic analysis of visual system development in *Drosophila*.
McKay, R., Massachusetts Institute of Technology. Differentiation pathways of mammalian CNS stem cells.
Bridgemen, P., Washington University School of Medicine. Dynamics and structure of the nerve growth cone cytoskeleton.
Scheller, R., Stanford University. The cellular and molecular biology of neuropeptides.
Beach, D., Cold Spring Harbor Laboratory. Control of the cell division cycle.
Bently, D., University of California. Axon outgrowth in insects.
Reichardt, L., University of California School of Medicine. Factors regulating neurite outgrowth in mammals.
Pfeffer, S., Stanford University. Protein sorting and membrane traffic in eukaryotic cells.
Ihle, J., St. Jude's Children's Research Hospital. The role of hematopoetic growth factors in growth and differentiation.
Harlow, E., Cold Spring Harbor Laboratory. Interaction of oncogenes with cellular control pathways.
Patrick, J., Baylor College of Medicine. Ligand-gated ion channels.
Schwarz, T., Stanford University School of Medicine. A family of K^+ channels found by genetics.
Reed, R., Johns Hopkins School of Medicine. Sensory signal transduction and models for neurogenesis.
Siegelbaum, S., Columbia University College of Physicians & Surgeons. Molecular mechanisms regulating the electrophysiological response of *Aplysia*.
Curran, T., Roche Institute. Transcription factors on the brain: *Fos, Jun* and the AP-1 binding site.
Kennedy, Mary, California Institute of Technology. The enzymology of Ca^{++} regulated phosphorylation in the brain.
Beer, E., University of California. Molecular genetics of neural development in *Drosophila*.
Madison, D., Stanford University. Second messengers and LTP in hippocampus.
Konishi, M., California Institute of Technology. Birdsong: From behavior to molecules.

Molecular Cloning of Neural Genes

August 16-September 4

INSTRUCTORS

Eberwine, Jim, Ph.D., Stanford University, California
Evinger, Marian, Ph.D., Cornell University Medical Center, New York
Schachter, Beth, Ph.D., Mt. Sinai School of Medicine, New York, New York

ASSISTANTS

Inman, Irene, M.S., Stanford University, California
Sanchez, Mercedes, Ph.D., Mt. Sinai School of Medicine, New York, New York

This intensive laboratory/lecture course provided scientists with a basic knowledge of the use of the techniques of molecular biology in examining questions of neurobiological significance. Particular emphasis was placed on using and discussing methods for circumventing the special problems posed in the study of the nervous system; for example, examination of low abundance mRNAs in extremely heterogeneous cell populations.

The laboratory work included mRNA quantitation methods (nuclease protection, etc.), preparation of hybridization probes, library construction (lambda ZAP and IST procedure), plaque screening techniques (probe hybridization, antibody interaction), DNA sequencing, PCR amplification, RNA amplification, and DNA mediated gene transfer. A major portion of the course was devoted to *in situ* hybridization and *in situ* transcription technologies. The lecture series, presented by invited speakers, focused on emerging techniques and how they may be applied to the study of the nervous system.

PARTICIPANTS

Crews, Lynda, B.S., Emory University, Atlanta, Georgia
Finnell, Richard, Ph.D., Washington State University, Pullman
Fisher, Marilyn, Ph.D., University of Virginia, Charlottesville
Harsh, Griffith, M.D., University of California, San Francisco
Houenou, Lucien, Ph.D., Bowman Gray School of Medicine, Winston-Salem, North Carolina
Howard, Marthe, Ph.D., Columbia University, New York, New York
Leifer, Dana, M.D., Children's Hospital, Boston, Massachusetts
MacKenzie, Robert, Ph.D., Abbott Laboratories, Abbott Park, Illinois
Martuza, Robert, M.D., Massachusetts General Hospital, Boston
Moran, Ofira, M.S., Sackler School of Medicine, Israel
Sanders-Bush, Elaine, Ph.D., Vanderbilt University, Nashville, Tennessee
Schlosshauer, Burkhard, Ph.D., Max Planck Institut, Tubingen, Federal Republic of Germany
Trapido-Rosenthal, Henry, Ph.D., University of Florida, Gainesville
Unsicker, Klaus, M.D., University of Marburg, Federal Republic of Germany
Villarroel, Alvaro, M.S., State University of New York, Stony Brook
Zaidi, Nikhat, Ph.D., University of Pittsburgh, Pennsylvania

SEMINARS

Goodman, R., Tufts University. Regulated expression of neuropeptide genes.
Hahn, B., University of Colorado. Molecular cloning and the definition of a gene product with brain-specific function.
Stevens, C., Yale University. Site-directed mutagenesis as a tool for understanding channel gating.
Abood, M., Stanford University. Molecular analyses of opioid systems.
Cepko, C., Harvard Medical School. Lineage analysis using retrovirus vectors.
Hockfield, S., Yale University. The effects of activity on the molecular anatomy of the developing brain.
Ciaranello, R., Stanford University. Molecular cloning of neurotransmitter receptors.
Singer, R., University of Massachusetts Medical School. Subcellular organization of nucleic acid sequences revealed by high resolution in situ hybridization.
Jonassen, J., University of Massachusetts Medical School. Endocrine regulation of pituitary hormone gene expression.
Claudio, T., Yale University. Biogenesis and cell surface distribution of acetylcholine receptor stably expressed in cell lines.
Evans, C., Stanford University. Use of antibodies in molecular cloning procedures.
Helfman, D., Cold Spring Harbor Laboratories. Regulation of alternative splicing in the central nervous system.
Bailey, M., Milligen/Biosearch. Automated DNA synthesis.

Macromolecular Crystallography

October 11–October 24

INSTRUCTORS

Pflugrath, James, Ph.D., Cold Spring Harbor Laboratory, New York
McPherson, Alex, Ph.D., University of California, Riverside
Remington, S.J., Ph.D., University of Oregon, Eugene

Crystallography and X-ray diffraction yield a wealth of structural information unobtainable through other methods. This intensive laboratory/computational course focused on the major techniques used to determine the three-dimensional structures of macromolecules. It was designed for scientists with a working knowledge of protein structure and function, but who were new to macromolecular crystallography. Topics that were covered included protein purification, crystallization, crystal characterization, data collection (film and area detector methods), data reduction, anomalous dispersion, phase determination, molecular replacement and averaging, electron density interpretation, structure refinement, molecular graphics, and molecular dynamics. Participants learned through extensive hands-on experiments, informal discussions, and lectures on current applications of these and related procedures given by outside speakers.

PARTICIPANTS

Anderson, Dwight, Ph.D., University of Minnesota, Minneapolis
Cerritelli, Susana, Ph.D., Brookhaven National Laboratory, Upton, New York
Chang, Yie-Hwa, Ph.D., Massachusetts General Hospital, Boston
Chaudhuri, Gautam, Ph.D., Chicago Medical School, Illinois
Cockle, Stephen A., Ph.D., Connaught Laboratories, Canada
Dewan, John, Ph.D., New York University, New York
Ganguli, Subrata, M.S., University of Illinois, Chicago
Gittis, Apostolos, Ph.D., Johns Hopkins University, Baltimore, Maryland
Honegger, Annemarie, Ph.D., Rorer Biotechnology, King of Prussia, Pennsylvania
Ilag, Leodevico, B.S., University of Tennessee, Memphis
Matthews, John, Ph.D., Naval Research Laboratory, Washington, D.C.
Milligan, Daniel, B.A., University of California, Berkeley
Nanni, Raymond, Ph.D., Rutgers University, New Brunswick, New Jersey
Sun, Wei-Hsiang, Ph.D., Massachusetts Institute of Technology, Cambridge
Szabo, Linda, B.S., Eastman Kodak, Rochester, New York
Williams, Neal, B.S., University of Sydney, Australia

SEMINARS

McPherson, A., University of California. Protein purification.
———. Crystallization of macromolecules.
Carter, C., University of North Carolina. Experimental design for efficient screening of crystal growth conditions.
Sweet, R., Brookhaven National Laboratory. Introduction to crystallography.
———. X-ray sources and optics.
McPherson, A., University of California. Preliminary crystal characterization.
Remington, S.J., University of Oregon. Data collection I. Oscillation photography.
Pflugrath, J., Cold Spring Harbor Laboratory. Data collection II. Area detector madness.
Remington, S.J., University of Oregon. MIR phase refinement.
Jones, T.A., University of Uppsala. Electron density interpretation.
Remington, S.J., University of Oregon. Improving phases: Solvent flatening, phase combination.
Saper, M.A., Harvard University. Molecular replacement.
———. Molecular averaging.
———. Human histocompatibility antigen structure: A close look of the peptide binding site.
Hendrickson, W.A., Columbia University. Application of multiwavelength anomalous diffraction to direct macromolecular structure determination.
Gronenborn, A., National Institutes of Health. Determination of protein structure by 2D and 3D NMR.
Brunger, A., Yale University. Refinement and molecular replacement with X-PLOR.
Tronrud, D., University of Oregon. Refinement with TNT.
Arnold, E., Rutgers University. Solution and analysis of the human rhinovirus 14 structure: New insights into viral structure-function relationships.

Molecular Genetics of Fission Yeast

October 24–November 6

INSTRUCTORS

David Beach, Ph.D., Cold Spring Harbor Laboratory, New York
Peter Fantes, Ph.D., University of Edinburgh, Scotland
Jerry Hyams, Ph.D., University of College, London, England
Maureen McLeod, Ph.D., State University of New York, Brooklyn

ASSISTANTS

Carolina Alpha, Ph.D., University College London, England
Emma Warbrick, M.A., University of Edinburgh, Scotland

The fission yeast (*Schizosaccharomyces pombe*) is increasingly being used as a model organism for the study of basic aspects of cell biology. This course introduced the student to all aspects of fission yeast biology, but with particular emphasis on genetic manipulation (both classical and with recombinant DNA) and the use of the organism for the study of cell biology. Topics covered included chemical mutagenesis and mutant analysis, transformation and gene replacement techniques, isolation of nuclei, preparation of nuclear DNA, plasmid recovery from yeast into bacteria, cell cycle methods, cytology and immunocytochemical techniques. In addition to hands-on experience, participants had the opportunity to learn through informal group discussions and a lecture series designed to complement the experimental section.

PARTICIPANTS

Allen, Jerry, B.S., University of Texas, Dallas
Cande, W. Zacheus, Ph.D., University of California, Berkeley
Damagnez, Veronique, B.A., C.N.R.S., Villejuif, France
Davey, John, Ph.D., University of Birmingham, England
DeCaprio, James, Ph.D., Dana Farber Cancer Institute, Boston, Massachusetts
Dunphy, William, Ph.D., University of California, San Diego
Elble, Randolph, Ph.D., Cornell University, Ithaca, New York
Fikes, John, Ph.D., Massachusetts Institute of Technology, Cambridge
Gonda, David, Ph.D., Massachusetts Institute of Technology, Cambridge
Hengst, Ludger, B.S., Max-Planck-Institut, Gottingen
Pichova, Alena, Ph.D., Czechoslovak Academy of Sciences
Pidoux, Alison, B.A., Imperial Cancer Research Fund, London
Selinger, David, B.S., University of Illinois, Urbana
Subramani, Suresh, Ph.D., University of California, San Diego
Thornton, Roy J., Ph.D., Massey University, New Zealand
Wang, Teresa, Ph.D., Stanford University, California

SEMINARS

Fantes, P., University of Edinburgh. Introduction to fission yeast.
Wise, J., University of Illinois. Roles of small ribonucleoprotein particles in RNA splicing and protein secretion.
Hyams, J., University College London. Fission yeast cytology.
Gasser, S., ISREC, Lausanne. Genome organization and nuclear structure in fission yeast.
McLeod, M., Downstate Medical School, Brooklyn. Regulation of sexual differentiation in fission yeast.
Wigler, M., Cold Spring Harbor Laboratory. *S. pombe* as a surrogate surrogate.
Young, P., Queens University, Ontario. Sodium transport in fission yeast.
Beach, D., Cold Spring Harbor Laboratory. Cell cycle control.
Clark, L., University of California, Santa Barbara. Centromeres in fission yeast.
Klar, A., National Cancer Institute, Frederick. Regulation of fission yeast mating-type interconversion.
Yanagida, M., Kyoto University. The nucleus and its division.

Seminars

Cold Spring Harbor Seminars were initiated to provide a semiformal avenue for communication between the various research groups at the Laboratory. They are particularly useful for research personnel who have joined the Laboratory during the summer. The seminars also afford a necessary opportunity for the graduate students and postgraduate staff to develop their skills in defending, organizing, and presenting their research. In addition to those listed below, seminars were given by many others involved in research at this laboratory.

1989

January

Larry Feig, Tufts University, Medford, Massachusetts: Inhibition of the cellular *ras* pathway by a dominant inhibitory *ras* mutant.

Peter Cornelius, East Carolina University, Greenville, North Carolina: Regulation of lipoprotein lipase and glucose transport in 3T3-L1 cells by tumor necrosis factor.

Michael Harrington, California Institute of Technology, Pasadena, California: Characterizing disease-associated proteins using two-dimensional gel electrophoresis and amino acid sequence analysis—What are the current challenges?

Jinez Morata, Universidad Autonima de Madrid, Spain: Further explorations of the Bithorax complex of *Drosophila*.

Mike Mulligan, Stanford University, California: Transcription, initiation, and RNA processing in maize mitochondria.

Nina Federoff, Carnegie Institution of Washington, Baltimore, Maryland: Developmental regulation of the maize controlling element *Suppressor-Mutator*.

Doug Engel, Northwestern University, Evanston, Illinois: Erythroid cell gene regulation.

February

Drew Schwartz, Indiana University, Bloomington: Regulation of *Ac* function—Gene-controlled demethylation of the promoter region.

B.R. Brinkley, University of Alabama, Tuscaloosa: Centromere structure and evolution—Repetitive microtubule binding segments in the kinetochore of mammalian chromosomes.

Sidney Strickland, State University of New York, Stony Brook: Use of antisense and chimeric RNAs to study mRNA recruitment in mouse oocytes.

Jeff Schell, Max-Planck Institut, Cologne, Federal Republic of Germany: Genes involved in the control of plant development.

Andreas Zimmer, University of Gottingen, Federal Republic of Germany: New strategies in developmental biology—In vivo mutagenesis as a tool to dissect mouse development.

Diane M. Robins, Columbia University, New York, New York: Regulation and evolution of an androgen-dependent mouse gene.

Tom Cline, Princeton University, New Jersey: Molecular genetics of the primary events in *Drosophila* sex determination.

Masafumi Tanaka, Cold Spring Harbor Laboratory, New York: Differential activation of transcription by ubiquitous and cell-specific transcription factors Oct-1 and Oct-2.

March

Bill Clouston, Howard Florey Institute of Experimental Physiology and Medicine, Melbourne, Australia: Tissue-specific expression of an angiotensinogen minigene in transgenic mice.

Vadim Nikiforov, Institute of Molecular Genetics, Moscow, Union of Soviet Socialist Republics: Genetics of bacterial RNA polymerase.

Hong Ma, California Institute of Technology, Pasadena, California: The genes controlling flower development in *Arabidopsis*.

Joe Gall, Carnegie Institution of Washington, Baltimore, Maryland: Proteins associated with transcription units of lampbrush chromosomes.

Phil C. Andrews, Purdue University, West Lafayette, Indiana: Pancreatic prohormone processing.

Jim Rothman, Princeton University, New Jersey: Mechanism of intracellular protein transport.

Jim Peacock, CSIRO-Div. of Plant Industry, Canberra, Australia: Enhancer and promoter interactions in a plant gene expression system.

Ron Morris, Rutgers University, New Jersey: Three genes involved in mitosis in *Aspergillus*.

Jonathan Jones, John Innes Institute, Norwich, England: Genetic properties of *Ac* in tobacco.

April

Barry Stripp, East Carolina University, Greenville, North Carolina: Cloning, sequencing, and expression of New Castle disease virus genes.

Terry Platt, University of Rochester, New York: mRNA 3′-end formation in *E. coli* and yeast.

Marc Van Montagu, Rijks Universiteit, Gent, Belgium: Progress in plant genetic engineering.

Sandra Rempel, University of Calgary, Canada: Estrogen regulation of c-*myc* proto-oncogene expression in proliferating chick oviduct.

Walter Schaffner, University of Zurich, Switzerland: Oct factors and lymphocyte-specific transcription.

Ruth Ann Nichols, Purdue University, West Lafayette, Indiana: Characterization of a *Drosophila* homolog of the vertebrate neuropeptide cholecystokinin.

Tom Peterson, Cold Spring Harbor Laboratory, New York: Molecular analysis of the maize *P* locus and *Ac* transposition.

Gunter Blobel, Rockefeller University, New York, New York: Structure and function of the nuclear lamina and pore complexes.

May

Rick Morimoto, Northwestern University, Evanston, Illinois: Transcription and function of the human hsp70.

Judith Scheppler, Emory University, Atlanta, Georgia: Down modulation of MHC class I antigens after HIV-1 infection.

Helen Blau, Stanford University, California: Plasticity of differentiated cells.

Daniela Rhodes, Medical Research Council, Cambridge, England: TFIIIA—Zinc fingers and DNA recognition.

Karl Riabowol, Cold Spring Harbor Laboratory, New York: Mechanisms and specificity of FOS complex activation.

Erich Grotewold, Cold Spring Harbor Laboratory, New York: Genes induced by protein synthesis inhibition in *Neurospora crassa*.

Greg Dressler, Max-Planck Institute, Gottingen, Federal Republic of Germany: Developmental expression of murine *Hox* and *Pax* genes.

June

Tom Marr, Los Alamos National Laboratory, New Mexico: Computational tools for physically mapping large segments of DNA.

Huseyin Mehmet, ICRF, London, England: Expression of c-*myc*.

Yung Yu, Medical Research Council, Cambridge, England: Molecular genetics of the human serum protein, complement component Ca.

Eric Richards, Cold Spring Harbor Laboratory, New York: Characterization of telomeric and centromeric DNA sequences from *Arabidopsis*.

Nahum Sonenberg, McGill University, Montreal, Canada: 5′-noncoding regions of eukaryotic mRNAs as effectors of translation—Poliovirus and HIV-1.

Chin-Hwa Hu, Oregon State University, Corvallis: Strand displacement and the molecular gymnastics in adenovirus replication.

Jacek Skowronski, Cold Spring Harbor Laboratory, New York: Latency and activation of the HIV-1 LTR in transgenic mice.

July

Emma Lees, Imperial Cancer Research Fund, London, England: Physical and functional properties of a transcriptional regulator—The E2 protein from human papillomavirus type 16.

Peter A. Peterson, Iowa State University, Ames: Characterization of maize transposable elements.

Nick Tonks, University of Washington, Seattle: Signal transduction and protein tyrosine dephosphorylation.

Anthony Craig, Uppsala University, Sweden: Comparison of mass spectrometric sequence information from peptides using different ionization methods.

Michael Hubbard, University of Dundee, Scotland: Phosphoprotein phosphatases and cellular regulation—Reversible targeting of protein phosphatase 1 to glycogen.

Peter Baciu, West Virginia University, Morgantown: Changes in nuclear protein phosphorylation during granulocytic differentiation of HL-60 cells.

Valery N. Soyfer, Ohio State University, Columbus: Lysenko—The crash of Soviet genetics in the age of Stalin and Khruschev.

August

Catherine Thompson, The Salk Institute, La Jolla, California: Structural and functional relationship of thyroid and steroid hormone receptors.

September

Jackie Lees, ICRF, London, England: Functional analysis of the mouse estrogen receptor.

Peter Starlinger, University of Cologne, Federal Republic of Germany: Experiments on the transposable element *Ac* of *Zea mays*.

Christiane Zock, Institute of Genetics, University of Cologne, Federal Republic of Germany: Mitigator sequence in the major late promoter of Ad12.

Ulrich Müller, Princeton University, New Jersey: Activation of AP1 transcription factor by E1A and cAMP.

Angus Wilson, Kings College, University of London, England: Enhancement, replication, and silencing—Three ways to control β-globin gene activation.

October

Gil Morris, Cold Spring Harbor Laboratory, New York: Regulation of PCNA expression.

Doug Melton, Harvard University, Boston, Massachusetts: The role of peptide growth factors in cell determination and patterning.

November

Lawrence A. Chasin, Columbia University, New York, New York: Mutants at the dihydrofolate reductase locus affecting DHFR RNA processing.

Scott C. Henderson, University of Western Ontario, Canada: The architecture of arborescent nuclei—The silk gland nuclear matrix.

Steve Bell, University of California, Berkeley: Species-specific promoter recognition properties of the RNA polymerase I transcription machinery.

Tim Tully, Brandeis University, Waltham, Massachusetts: Memory formation in normal and mutant *Drosophila*.

December

Richard McIntosh, University of Colorado, Boulder: Microtubule dynamics and chromosome movement.

Andrei Mirzabekov, Institute of Molecular Biology, Academy of Sciences of USSR, Moscow: A new oligonucleotide hybridization approach to sequencing DNA and sequencing the arrangement of proteins along DNA.

Gerald Fink, Whitehead Institute, Cambridge, Massachusetts: Genetic analysis of cell fusion in yeast—Protein kinases strike again.

Jonas Salk, Salk Institute, San Diego, California: Strategies for the control of HIV infection and/or disease using an envelope depleted in activated HIV immunogen.

Ben Bowen, Pioneer Hi-Bred International, Johnston, Iowa: The *R* gene as a novel visible marker for microprojectile transformation of corn.

David Price, University of Iowa, Ames: Factors affecting elongation by RNA polymerase II.

Mike Ruppert, Johns Hopkins Oncology Center, Baltimore, Maryland: The Gli family of human zinc finger proteins.

Undergraduate Research

An important aspect of the summer program at the Laboratory is the participation of college undergraduate students in active research projects under the supervision of full-time laboratory staff members. The program was initiated in 1959. Since that year, 317 students have completed the course, and many have gone on to creative careers in biological science.

The objectives of the program are to provide (1) a greater understanding of the fundamental principles of biology, (2) an increased awareness of major problem areas under investigation, (3) a better understanding of the physical and intellectual tools for modern research and the pertinence of this information to future training, and (4) a personal acquaintance with research, research workers, and centers for study.

The following students, selected from over 100 applicants, took part in the program, which was supported by the National Science Foundation, Burroughs Wellcome Fund, Baring Bros., Miles Inc./Bayer AG, and Cold Spring Harbor Laboratory.

Doug Adler, State University of New York, Binghamton
Research Advisor: **Betty Moran**
Cloning the retinoblastoma gene in baby rat kidney cells.

Lisa Bellavance, Drake University
Research Advisor: **Loren Field**
Organ dysgenesis in IV/-mice.

Ron Bose, University of Rhode Island
Research Advisor: **Kim Arndt**
Analysis of suppressors of *sit4*.

Ross Breckenridge, University of Cambridge
Research Advisor: **Dan Marshak**
Purification of HeLa p34 *cdc2* using a novel assay.

Ivan Brockman, Cornell University
Research Advisor: **Bruce Futcher**
whi4 is a mutant of *S. cerevisiae* that may actually be Whi1-1.

Franco Carlotti, University of Cambridge
Research Advisor: **Andy Rice**
Properties of mutants within the cysteine-rich region of the HIV *tat* protein.

Nancy Fan, Harvard University
Research Advisor: **Arne Stenlund**
Synchronization of bovine papillomavirus transformed mouse cells using centrifugal elutriation.

Ellen Gadbols, College of St. Catherine
Research Advisor: **Bruce Stillman**
Purification of a yeast protein equivalent to human RF-C.

Amy Kistler, University of Pennsylvania
Research Advisor: **David Helfman**
Generation and purification of beta skeletal muscle tropomyosin and two novel carboxy-terminal β tropomyosin chimeras.

Karen Kopecek, Franklin and Marshall College
Research Advisor: **Jeff Kuret**
The feasibility of using direct expression cloning to determine downstream components of signal transduction pathways.

James Lister, Pomona College
Research Advisor: **Nouria Hernandez**
Analysis of the A/T-rich region of the U6 snRNA promoter by site-directed mutagenesis.

Steven Palmer, Wabash College
Research Advisor: **Dafna Bar-Sagi**
Isolation of the membrane phospholipase A2 gene from rat brain cDNA in λgt11.

Nives Pecina, University of Zagreb
Research Advisors: **A. Krainer/G. Conway**
Reconstitution of snRNPs.

Mika Slovak, Reed College
Research Advisor: **David L. Spector**
Effects of transcriptional inhibition upon the snRNP network.

Martin Stoddart, University of Cambridge
Research Advisor: **Mike Mathews**
Secondary structure determination of virus-associated RNAs (VA RNAs).

Karen Zito, Indiana University
Research Advisor: **Winship Herr**
Expression pattern of Oct-1 and Oct-2 in mice.

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, students can engage in such introductory programs as Nature Detectives, Seashore Life, and Pebble Pups, as well as more advanced programs such as Marine Biology and Nature Photography.

During the summer of 1989, a total of 480 students participated in the Nature Study Program. Most classes were held outdoors, when weather permitted, or at the Uplands Farm Nature Preserve, the headquarters of the Long Island Chapter of the Nature Conservancy. The Laboratory has equipped and maintains classrooms/laboratories as well as a darkroom at Uplands Farm. This facility is used as a base for the students' exploration of the local environment. Field classes are held on Laboratory grounds, St. John's Preserve, Fire Island National Seashore, Caumsett State Park, the Cold Spring Harbor Fish Hatchery, and the Muttontown Preserve, as well as other local preserves and sanctuaries. Students in Marine Biology participated in a whale watch aboard the Finback II, operated by the Okeanos Ocean Research Foundation Inc., Hampton Bays, New York.

The two-day Adventure Education class took students on a 22-mile bike hike to Caumsett State Park and a 12-mile canoe trip on the Nissequoque River.

PROGRAM DIRECTOR

William M. Payoski, M.A., Adjunct Professor,
Nassau Community College

INSTRUCTORS

Maria Anfora, M.A., science teacher, West Babylon School District
Ruth Burgess, B.A., Cold Spring Harbor Nursery School
James Dunleavy, B.A., science teacher, St. Anthony's High School
Cheryl Littman, B.A., science teacher, Northport School District
Linda Payoski, B.A., science teacher, Uniondale High School
Marjorie Pizza, B.A., science teacher, Glen Cove School District

COURSES

Nature Bugs
Nature Detectives
Advanced Nature Study
Introduction to Ecology
Frogs, Flippers, and Fins
Pebble Pups
Bird Study
Fresh Water Life
Seashore Life
Marine Biology
Nature Photography
Adventure Education
Astronomy

FINANCIAL STATEMENT

FINANCIAL STATEMENT

BALANCE SHEET
year ended December 31, 1989
with comparative figures for year ended December 31, 1988

ASSETS

	Operating Funds						
	Unrestricted			Endowment & Similar Funds	Land, Building & Equipment Funds	Total All Funds	
	Undesignated	Designated	Restricted			1989	1988
Cash and cash equivalents	$1,011,950	400,000	575,418	8,927,394	19,803,202	30,717,964	14,660,307
Marketable securities	91,252	—	—	5,849,228	105,196	6,045,676	2,920,614
Accounts receivable:							
Publications (less allowance for doubtful accounts of $95,000 in 1989 and $24,000 in 1988)	1,884,531	—	—	—	—	1,884,531	230,056
Other	195,572	—	—	333,333	—	528,905	158,657
Grants receivable	—	—	2,024,479	—	—	2,024,479	853,813
Accrued interest receivable	—	—	—	153,978	—	153,978	232,526
Publications inventory	617,542	—	—	—	—	617,542	602,807
Other assets, principally prepaid expenses	361,082	—	—	—	576,333	937,415	512,401
Contract deposit	—	—	—	—	—	—	161,143
Investment in employee residences	—	—	—	—	853,524	853,524	747,156
Land, buildings and equipment:							
Land and improvements	—	—	—	—	3,248,451	3,248,451	2,579,440
Buildings	—	—	—	—	23,045,909	23,045,909	21,614,551
Furniture, fixtures and equipment	—	—	—	—	2,013,732	2,013,732	1,736,200
Laboratory equipment	—	—	—	—	4,752,761	4,752,761	4,550,493
Library books and periodicals	—	—	—	—	365,630	365,630	365,630
	—	—	—	—	33,426,483	33,426,483	30,846,314
Less accumulated depreciation and amortization	—	—	—	—	11,172,671	11,172,671	9,774,795
Land, buildings and equipment, net	—	—	—	—	22,253,812	22,253,812	21,071,519
Construction in progress	—	—	—	—	7,222,851	7,222,851	2,774,090
Total assets	$4,161,929	400,000	2,599,897	15,263,933	50,814,918	73,240,677	44,925,089

LIABILITIES AND FUND BALANCES

	Operating Funds			Endowment & Similar Funds	Land, Building & Equipment Funds	Total All Funds	
	Unrestricted						
	Undesignated	Designated	Restricted			1989	1988
Liabilities:							
Accounts payable and accrued expenses	$2,370,284	—	—	—	632,696	3,002,980	1,842,148
Notes payable	—	—	—	—	1,063,857	1,063,857	670,000
Loan payable	—	—	—	—	20,000,000	20,000,000	4,000,000
Deferred revenue	553,240	—	2,599,897	—	—	3,153,137	1,757,549
Total liabilities	2,923,524	—	2,599,897	—	21,696,553	27,219,974	8,269,697
Fund balances:							
Unrestricted—undesignated	1,238,405	—	—	—	—	1,238,405	4,355,654
Unrestricted—designated	—	400,000	—	—	—	400,000	—
Endowment and similar funds	—	—	—	15,263,933	—	15,263,933	3,058,581
Land, buildings and equipment:							
Expended	—	—	—	—	17,424,570	17,424,570	15,734,248
Unexpended—Donor restricted	—	—	—	—	10,843,739	10,843,739	7,958,695
Unexpended—Board authorized	—	—	—	—	850,056	850,056	5,548,214
Total fund balances	1,238,405	400,000	—	15,263,933	29,118,365	46,020,703	36,655,392
Total liabilities and fund balances	$4,161,929	400,000	2,599,897	15,263,933	50,814,918	73,240,677	44,925,089

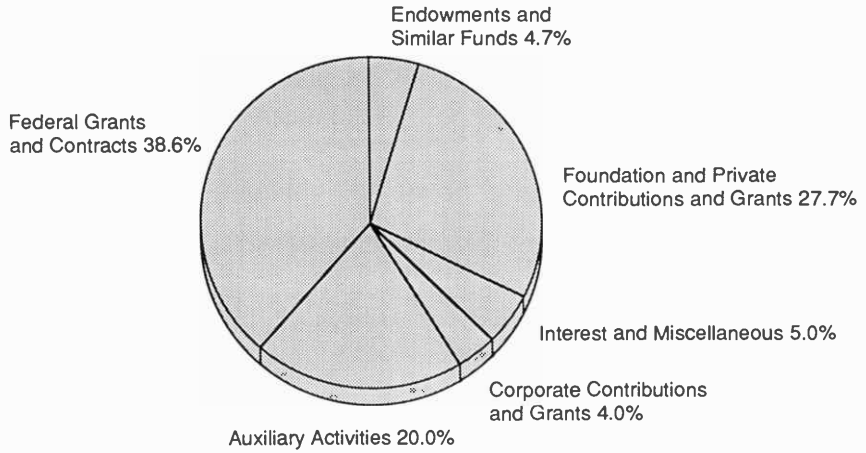
STATEMENT OF SUPPORT, REVENUE, AND EXPENSES AND CHANGES IN FUND BALANCES
year ended December 31, 1989
with comparative figures for year ended December 31, 1988

	Operating Funds					Total All Funds	
	Unrestricted			Endowment & Similar Funds	Land, Building & Equipment Funds		
	Undesignated	Designated	Restricted			1989	1988
Support and revenue:							
Public support	$876,134	—	3,618,335	5,549,775	1,914,925	11,959,169	10,921,519
Government grant awards	—	—	9,412,256	—	—	9,412,256	8,102,876
Indirect cost allowances	6,456,143	—	—	—	—	6,456,143	5,643,433
	7,332,277	—	13,030,591	5,549,775	1,914,925	27,827,568	24,667,828
Other revenue:							
Program fees	1,298,031	—	—	—	—	1,298,031	956,795
Rental income	93,036	—	—	—	—	93,036	89,658
Publications	4,449,690	—	—	—	—	4,449,690	1,640,491
Dining services	1,336,698	—	—	—	—	1,336,698	1,118,037
Rooms and apartments	774,769	—	—	—	—	774,769	544,753
Distribution from Robertson Funds	136,800	—	840,000	—	—	976,800	930,000
Investment income	243,355	—	—	873,428	1,456,130	2,572,913	1,121,483
Miscellaneous	262,109	—	—	—	—	262,109	396,453
Total other revenue	8,594,488	—	840,000	873,428	1,456,130	11,764,046	6,797,670
Total support and revenue	15,926,765	—	13,870,591	6,423,203	3,371,055	39,591,614	31,465,498
Expenses							
Program services:							
Research	—	—	11,049,896	—	—	11,049,896	9,138,957
Summer programs	846,398	—	2,011,855	—	—	2,858,253	2,421,858
Publications	3,934,216	—	—	—	—	3,934,216	1,719,187
Banbury Center conferences	131,100	—	285,581	—	—	416,681	521,103
DNA Education Center programs	31,195	—	318,391	—	—	349,586	363,339
Total program services	4,942,909	—	13,665,723	—	—	18,608,632	14,164,444
Supporting services:							
Direct research support	495,853	—	—	—	—	495,853	485,915
Library	367,448	—	—	—	—	367,448	339,915
Operation and maintenance of plant	3,706,217	—	—	—	—	3,706,217	3,259,354
General and administrative	3,067,652	—	—	54,439	—	3,122,091	2,890,857
Dining services	1,377,351	—	—	—	—	1,377,351	1,167,405
Interest	—	—	—	—	1,149,302	1,149,302	339,055
Total supporting services	9,014,521	—	—	54,439	1,149,302	10,218,262	8,482,501

Depreciation	—	—	—	—	1,399,409	1,399,409	1,286,159
Total expenses	13,957,430	—	13,665,723	54,439	2,548,711	30,226,303	23,933,104
Excess of support and revenue over expenses before designation	$1,969,335	—	204,868	6,368,764	822,344	9,365,311	7,532,394
Designation:							
Funds designated for neuroscience program	(400,000)	400,000	—	—	—	—	—
Excess of support and revenue over expenses and designation	1,569,335	400,000	204,868	6,368,764	822,344	9,365,311	7,532,394
Other changes in fund balances:							
Transfer to unexpended plant funds	(4,353,951)	—	(70,000)	—	4,423,951	—	—
Capital expenditures	—	—	(202,268)	—	202,268	—	—
Transfer to restricted funds	—	—	67,400	(67,400)	—	—	—
Transfer to endowment funds	(332,633)	—	—	5,903,988	(5,571,355)	—	—
Net increase (decrease) in fund balance	(3,117,249)	400,000	—	12,205,352	(122,792)	9,365,311	7,532,394
Fund balance at beginning of year	4,355,654	—	—	3,058,581	29,241,157	36,655,392	29,122,998
Fund balance at end of year	$1,238,405	400,000	—	15,263,933	29,118,365	46,020,703	36,655,392

Copies of our complete, audited financial statements, certified by the independent auditing firm of KPMG, Peat, Marwick & Co., are available upon request from the Comptroller, Cold Spring Harbor Laboratory.

SOURCES OF REVENUE
YEAR ENDED DECEMBER 31, 1989



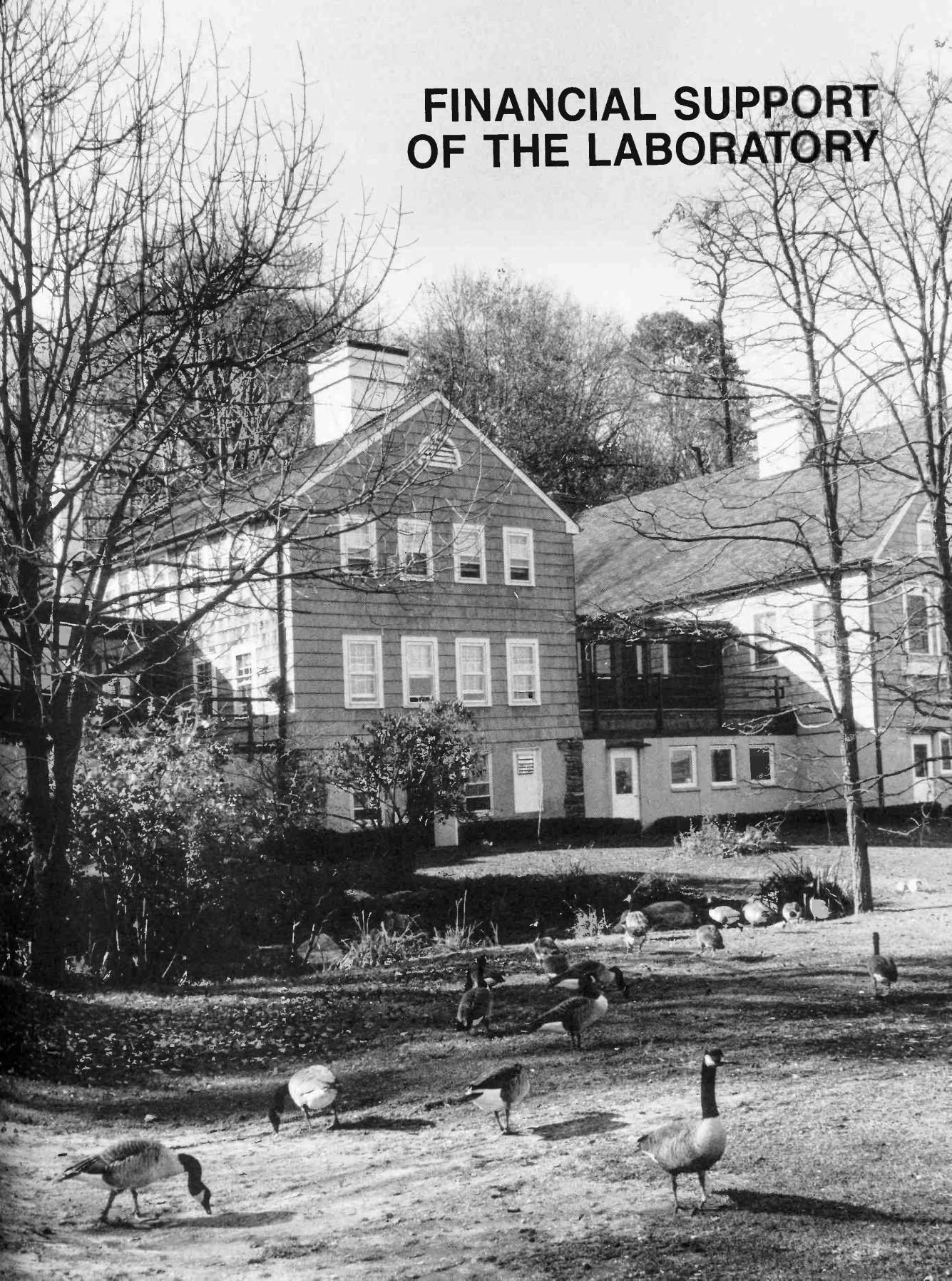
COMPARATIVE OPERATING HISTORY
1985-1989
(Dollars in Thousands)

	1985	*1986*	*1987*	*1988*	*1989*
Income:					
Main Lab:					
Grants and Contracts	$9,071	9,439	10,409	10,799	13,062
Indirect Cost Reimbursement	4,406	4,533	4,779	5,707	6,412
Other	2,235	2,572	2,727	3,205	4,034
CSH Press	1,433	1,357	1,556	1,641	4,450
Banbury Center	724	963	982	976	1,012
DNA Learning Center	—	—	349	660	622
Total income	17,869	18,864	20,802	22,988	29,592
Expenses:					
Main Lab:					
Grants & Contracts	9,071	9,439	10,409	10,799	13,062
Operation & maintenance of plant	2,284	2,442	2,791	3,010	3,412
General & Administrative	1,951	1,889	1,975	2,102	2,377
Other	2,076	2,124	2,633	3,049	3,165
CSH Press	1,030	1,060	1,311	1,719	3,934
Banbury Center	685	868	947	910	1,038
DNA Learning Center	—	—	260	590	635
Total expenses	17,097	17,822	20,326	22,179	27,623
Excess before depreciation and designation of funds	772	1,042	476	809	1,969
Depreciation	(1,077)	(1,093)	(1,127)	(1,286)	(1,399)
Designation of funds	—	—	—	—	(400) (2)
Net operating excess (deficit)	$(305)	(51)	(651)	(477)	170

(1) The above amounts are presented on a combined basis for all funds for which Cold Spring Harbor prepares operating budgets.

(2) Funds designated to underwrite future direct and indirect expenses of the neuroscience program.

FINANCIAL SUPPORT OF THE LABORATORY



SOURCES OF SUPPORT

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 its annual support is derived from Federal grants and contracts. Contributions from the private sector are tax exempt under the provisions of Section 501(c)(3) of the Internal Revenue Code. In addition, the Laboratory has been designated a "public charity" and, therefore, may receive funds resulting from the termination of "private" foundations.

Over the years, the Laboratory has earned a reputation for innovative research and high-level science education. By training young scientists in the latest experimental techniques, it has helped spur the development of many research fields, including tumor virology, cancer genes, gene regulation, movable genetic elements, yeast genetics, and molecular neurobiology. This continual development of new research programs and training courses requires substantial support from private sources.

Because its endowment is small, because government support is highly competitive and the uses of research grants are restricted, the Laboratory depends on **annual** contributions from the private sector; foundations, corporations, and individuals for its central institutional needs.

The Second Century Campaign seeks to raise $44M in **capital** funds by December 1991 for construction of new facilities, renovation of existing facilities, and for staff and student endowment. This is the Laboratory's first public capital campaign and it marks its Centennial.

METHODS OF CONTRIBUTING TO COLD SPRING HARBOR LABORATORY

Gifts of money can be made directly to Cold Spring Harbor Laboratory.

Securities You can generally deduct the full amount of the gift on your income tax return, and, of course, you need pay no capital gains tax on the stock's appreciation. We recommend any of the following methods:

(1) Have your broker sell the stock and remit the proceeds to Cold Spring Harbor Laboratory.

(2) Deliver the stock certificates to your broker with instructions to him to open an account for Cold Spring Harbor Laboratory and hold the securities in that account pending instructions from Cold Spring Harbor Laboratory.

(3) Send the *unendorsed* stock certificates directly to the Laboratory: Comptroller, Cold Spring Harbor Laboratory, Box 100, Cold Spring Harbor, New York 11724. In a separate envelope send an *executed* stock power.

Pooled Income Funds Combine gifts from a number of donors in a pool for attractive investment and tax purposes.

Appreciated real estate or personal property Sizable tax benefits can result from such donations; the Laboratory can use some in its program and can sell others.

Life insurance and charitable remainder trusts can be structured to suit the donor's specific desires as to extent, timing, and tax needs.

Bequests Most wills probably need to be updated. Designating Cold Spring Harbor Laboratory as beneficiary ensures that a bequest will be utilized as specified for continuing good.

Conversion of private foundation to "public" status on termination This may be done by creating a separate fund within Cold Spring Harbor Laboratory whereby the assets of the private foundation are accounted for as a separate fund and used for the purposes specified by the donor. Alternatively, the private foundation can be established as a "supporting organization of Cold Spring Harbor Laboratory."

For additional information, please contact the Director of Development, Cold Spring Harbor Laboratory, Box 100, Cold Spring Harbor, N.Y. 11724, or call 516-367-8840.

GRANTS

January 1, 1989–December 31, 1989

COLD SPRING HARBOR LABORATORY

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*Total Award*
FEDERAL GRANTS			
NATIONAL INSTITUTES OF HEALTH			
Program Projects	Cancer Research Center, Dr. Mathews	1/87–12/91	$16,787,416
	Cancer Center Support, Dr. Roberts	7/87–6/90	5,346,907
	PEBRA—HIV Grant, Dr. Herr	9/88–8/91	3,672,816
	Oncogene Program Project, Dr. Wigler	3/88–2/93	4,869,923
Research Support	Dr. Arndt	4/88–3/93	1,288,964
	Dr. Beach	12/84–1/93	1,481,482
	Dr. Beach	9/86–8/94	2,125,179
	Dr. Beach	7/88–6/93	1,488,740
	Dr. L. Field	4/87–3/92	568,521
	Dr. Franza	9/85–11/94	1,497,483
	Dr. Frendeway	4/87–3/92	775,814
	Dr. Futcher	4/88–3/93	1,115,434
	Dr. Garrels	1/85–12/89	2,028,833
	Dr. Gilman	9/87–8/92	608,291
	Dr. Greider	12/89–11/94	1,130,019*
	Dr. Helfman	9/85–3/94	2,109,264
	Dr. Hernandez	7/87–6/92	1,293,754
	Drs. Klar/Futcher	7/81–6/90	2,411,676
	Dr. Krainer	7/89–6/94	1,298,402*
	Dr. Kuret	7/89–6/94	1,312,808*
	Dr. Marr	9/89–9/90	126,428*
	Dr. Moran	3/88–2/93	547,214
	Dr. Peterson	4/88–3/93	704,475
	Dr. Pflugrath	4/88–3/91	573,503
	Dr. Rice	9/87–8/90	528,037
	Dr. Roberts	7/88–6/93	1,598,876
	Dr. Roberts	9/88–8/91	446,241
	Dr. Stillman	7/83–6/92	1,869,678
	Dr. Wigler	7/85–6/92	8,426,929
Equipment Support	B.R.S.G. Award	4/88–3/89	121,971
	Dr. Marshak	2/88–2/89	189,000
	Dr. Roberts	6/88–5/89	27,331
	Dr. Roberts	6/89–5/90	36,300*
Fellowships	Dr. Ballester	2/89–1/92	63,996*
	Dr. Brill	8/89–7/92	46,667*
	Dr. J. Field	10/86–9/89	63,996
	Dr. Kessler	8/88–7/91	63,996
	Dr. Meyertons	5/88–9/89	27,996
	Dr. Morris	11/86–10/89	82,008
	Dr. Steinhelper	3/89–2/92	77,254*
	Dr. Stern	11/88–11/91	63,996

* New Grants Awarded in 1989

Grantor	Program/Principal Investigator	Duration of Grant	Total Award
Training Support	Institutional, Dr. Grodzicker	7/78–4/94	2,364,146
Course Support	Advanced Bacterial Genetics, Dr. Grodzicker	5/80–4/93	559,540
	Cancer Research Center Workshops, Dr. Grodzicker	1/83–3/92	1,010,057
	Neurobiology Short Term Training, Dr. Hockfield	5/82–4/90	723,939
Meeting Support	Cancer Cell	1989	4,000*
	C. elegans	1987 & 1989	61,874
	Eukaryotic DNA	1989	1,000*
	Hepatitis B Virus	1989	3,000*
	Liver Gene	1989	16,250*
	RNA Processing	1988–1991	9,500
	Symposium	1989	24,000*
NATIONAL SCIENCE FOUNDATION			
Research Support	Dr. Herr	6/88–5/91	240,000
	Dr. Marshak	7/87–12/90	190,000
	Dr. Martienssen	7/89–6/92	300,000*
	Dr. Richards	7/89–6/92	285,000*
	Dr. Roberts	1/83–5/90	640,000
	Dr. Roberts	2/86–11/89	300,000
	Dr. Roberts	8/87–1/90	43,106
	Dr. Sundaresan	5/87–4/91	360,000
	Dr. Zoller	8/88–7/89	92,000
Equipment Support	Dr. Spector	3/87–2/89	95,000
Training Support	Undergraduate Research Program, Dr. Herr	6/89–5/90	42,000*
Course Support	Plant Molecular Biology	8/86–1/90	137,490
	Molecular Genetics Courses Scholarships Dr. Grodzicker	5/89–4/90	25,730*
Meeting Support	Cancer Cell	1989	10,000*
	C. elegans	1987–1989	22,819
	Eukaryotic DNA Replication	1989	6,000*
	Hepatitis B Virus	1989	5,000*
	Symposium	1989	2,000*
	RNA Processing Conference	4/88–3/91	30,000
DEPARTMENT OF ENERGY			
Meeting Support	Symposium	1989	10,000*

* New Grants Awarded in 1989

NONFEDERAL GRANTS

Grantor	Program/Principal Investigator	Duration of Grant	Total Award
Research Support			
Aaron Diamond Foundation	Dr. Anderson	12/88–11/91	200,000
American Cancer Society	Dr. Bar-Sagi	7/89–6/91	194,000*
	Dr. Hanahan	7/87–6/89	160,000
	Dr. Moran	1/88–12/90	90,500
	Dr. Spector	7/87–6/91	363,000
	Drs. Stillman, Gluzman, Welch, Gilman, Institutional Award	7/82–6/90	300,000
	Dr. Wigler	4/89–3/90	10,000*
	Dr. Wigler, Professorship	1986–2012	1,333,333
Amersham International plc	Dr. Harlow	11/86–10/91	799,635
Howard Hughes Medical Institute	Neurobiology Support	1987–1990	1,000,000
Japan Health Science Foundation	Dr. Roberts	12/89–2/90	20,000
J.N. Pew Jr. Charitable Trust	Plant Group Support	4/87–4/90	260,000
LIBA	Dr. Frendewey	4/88–3/89	25,000
	Dr. Peterson	4/88–3/89	25,000
Muscular Dystrophy Association	Dr. Helfman	7/86–6/89	94,500
	Dr. Mathews	1/87–6/90	100,718
Mellam Family Foundation	Dr. Franza	12/88–11/90	100,000
Monsanto Company	Cooperative Research	10/84–9/89	2,089,200
New England Biolabs	Dr. Roberts	1989	15,000*
Pfizer, Inc.	Dr. Wigler	1985–1990	500,000
Pioneer Hi-Bred International Inc.	Cooperative Research	8/85–4/91	2,500,000
Rita Allen Foundation	Dr. Herr	9/85–8/90	150,000
	Dr. Hernandez	10/89–9/90	30,000*
Samuel Freeman Charitable Trust	Freeman Laboratory of Cancer Cell Biology	7/89–6/94	1,000,000*
Fellowships			
American Cancer Society	Dr. Conway	10/88–9/91	63,000
	Dr. Ryan	7/88–6/90	90,000
	Dr. Chan	3/89–8/89	11,500*
	Dr. Pittenger	3/89–12/89	20,334*
	Dr. Laspia	7/89–6/92	112,200*
American Foundation for AIDS Research		6/86–5/91	500,000
Bristol-Myers Company	Fellowship Support	7/88–6/90	75,000
Bioseeds International	Plant Fellowship Support	9/86–8/89	79,500
Cancer Research Institute	Dr. Sturm	9/86–1/89	62,313
	Dr. Efrat	3/87–2/90	69,000
Damon Runyon-Walter Winchell Cancer Fund	Dr. Michaeli	9/88–8/89	25,642
	Dr. D. Roberts	1/89–12/91	69,000*
	Dr. Melendy	9/89–8/90	1,082*
Government of Canada	Dr. Tyers	7/87–6/90	100,000
Esther and Joseph A. Klingenstein Fund, Inc.	Dr. Marshak	7/87–6/89	51,000
Juvenile Diabetes Foundation International	Dr. Grant	7/89–6/92	76,140*
The Leukemia Society of America	Dr. Das	1987–1990	300,000
LIBA	Four Fellowships a Year	9/86–8/89	105,000
Life Science Research Foundation	Dr. Colicelli	9/89–8/92	105,000*
	Dr. D. Roberts	12/88–11/90	30,000
Merck Sharp & Dohme Research Laboratories	Graduate Student Support		

* New Grants Awarded in 1989

Grantor	Program/Principal Investigator	Duration of Grant	Total Award
Muscular Dystrophy Association	Dr. Lees-Miller	7/88–6/89	51,000
Rita Allen Foundation	Postdoctoral Support	1989	25,000*
Weizmann Institute of Science	Dr. Gerst	9/89–8/91	57,000
Training Support			
Baring Brothers & Co., Ltd.	Undergraduate Research Program	1989	23,325*
Burroughs Wellcome Foundation	Undergraduate Research Program	1987–1990	50,960
Grass Foundation	Neurobiology Scholarship Support	1980–1989	151,670
Lucille P. Markey Charitable Trust	Scholarship Support	1985–1989	220,000
Miles Research Center Molecular Therapeutics	Undergraduate Research Program	1989	4,500*
Robert H.P. Olney Memorial Fund	Undergraduate Research Program	1989	2,400*
Course Support			
Alfred P. Sloan Fund	Computational Neuroscience Course	1986–1989	140,000
Amersham International plc	Advanced Molecular Cloning and Expression of Eukaryotic Genes	1989	1,000*
Howard Hughes Medical Institute	Neurobiology Courses	1987–1990	1,000,000
Meeting Support			
Anheuser-Busch Companies, Inc.	Yeast Cell Biology Conference	1989	500*
Beckman Instruments, Inc.	Liver Gene Expression Conference	1989	500*
Bio-Rad Laboratories, Inc.	Liver Gene Expression Conference	1989	500*
Chisso Corporation	Liver Gene Expression Conference	1989	1,000*
Clontech Laboratories Inc.	Liver Gene Expression Conference	1989	500*
Codon	Liver Gene Expression Conference	1989	200*
ICN Biomedicals, Inc.	Translational Control Conference	1989	20,000*
Life Technologies, Inc.	Liver Gene Expression Conference	1989	300*
Lucille P. Markey Charitable Trust	54th Symposium: Immunological Recognition	1989	15,000*
March of Dimes Birth Defects Foundation	Liver Gene Expression Conference	1989	10,000*
Merck & Co., Inc.	*C. elegans* Conference	1989	1,000*
	Liver Gene Expression Conference	1989	500*
Nestle, S.A.	Liver Gene Expression Conference	1989	1,000*
New England Biolabs, Inc.	*C. elegans* Conference	1989	500*
Repligen	Liver Gene Expression Conference	1989	250*
Rockefeller Foundation	Vaccines Conference	1986–1990	45,000
Rhone-Poulenc, Inc.	*C. elegans* Conference	1989	250*
The Salk Institute Biotechnology/ Industrial Associates, Inc.	Liver Gene Expression Conference	1989	250*
Stratagene	Liver Gene Expression Conference	1989	500*
Syntex Research	Liver Gene Expression Conference	1989	750*
United States Biochemical Corporation	Liver Gene Expression Conference	1989	175*

* New Grants Awarded in 1989

DNA LEARNING CENTER

Grantor	Program/Principal Investigator	Duration of Grant	Total Award
FEDERAL GRANTS			
NATIONAL SCIENCE FOUNDATION	Teacher Enhancement Program	1987–90	415,928
NONFEDERAL GRANTS			
The Banbury Fund	Core Support	1989	30,000
Dentsu Inc.	Core Support	1989	10,010
Harweb Foundation	Core Support	1989	1,000
J.M. Foundation	Core Support	1989	25,000
The Ester A. and Joseph Klingenstein Fund, Inc.	Core Support	1989	25,000
Richard Lounsbery Foundation	Core Support	1989	50,000
Josiah Macy, Jr. Foundation	Core Support	1987–90	490,850
Teleflex Foundation	Core Support	1989	1,000
American Society for Microbiology, Michigan Branch	Vector Workshop	1989	1,539
Anne Arundel Public School, Maryland	Vector Workshop	1989	3,430
Bethany College, West Virginia	Vector Workshop	1989	10,110
Biology Teachers' Organization, Winnipeg, Canada	Vector Workshop	1989	10,870
Board of Cooperative Education Services, New York	Vector Workshop	1989	2,000
Center for Biotechnology, SUNY Stony Brook	Vector Workshop	1989	10,550
Champlain Valley High School, Vermont	Vector Workshop	1989	830
Cooperating School District of St. Louis Suburban Area, Inc.	Vector Workshop	1989	7,382
Dover-Sherborn High School, Maine	Vector Workshop	1989	373
Nassau Community College	Vector Workshop	1989	1,000
Project Share, Connecticut	Vector Workshop	1989	2,000
University of Wisconsin	Vector Workshop	1989	1,370
Commack School District	Curriculum Study	1989	500
East Williston School District	Curriculum Study	1989	500
Great Neck School District	Curriculum Study	1989	500
Half Hollow Hills School District	Curriculum Study	1989	1,500
Harborfields School District	Curriculum Study	1989	500
Herricks School District	Curriculum Study	1989	500
Huntington School District	Curriculum Study	1989	500
Island Trees School District	Curriculum Study	1989	2,000
Jericho School District	Curriculum Study	1989	500
Lawrence School District	Curriculum Study	1989	1,500
Locust Valley School District	Curriculum Study	1989	500
Manhasset School District	Curriculum Study	1989	500
Northport-East Northport School District	Curriculum Study	1989	500
North Shore School District	Curriculum Study	1989	500
Oyster Bay-East Norwich School District	Curriculum Study		

* New Grants Awarded in 1989

Grantor	Program/Principal Investigator	Duration of Grant	Total Award
Plainview-Old Bethpage School District	Curriculum Study	1989	500
Plainedge School District	Curriculum Study	1989	2,000
Portledge School District	Curriculum Study	1989	500
Port Washington School District	Curriculum Study	1989	500
Sachem School District	Curriculum Study	1989	500
Syosset School District	Curriculum Study	1989	500
Brunswick Appraisal Corp.	The Search for Life Exhibit	1989	100
Shogren Industries Inc.	The Search for Life Exhibit	1989	500

* New Grants Awarded in 1989

BANBURY CENTER

Grantor	Program/Principal Investigator	Duration of Grant	Total Award
FEDERAL GRANTS			
U.S. Department of Agriculture	RFLP's and the Molecular Biology of Plants Conference	9/88–8/89	3,000
U.S. Department of Justice	DNA Technology and Forensic Science Conference	10/88–9/89	5,000
Environmental Protection Agency	Mutation Induction and Heritability in Mammalian Germ Cells	1989	45,000*
OFFICE OF NAVAL RESEARCH	Computational Eye Movement Workshop	7/88–6/91	62,125
NONFEDERAL SUPPORT			
Alfred P. Sloan Foundation	Journalists and Congressional Workshops	1990–1992	150,000*
Alfred P. Sloan Foundation	Molecular Clocks of Evolution	1989	30,000*
FMC Corporation	Electrophoresis of Large DNA Molecules	1990	5,000*
ICI Agrochemicals	Recognition in Plant-Pathogen Interactions	1989	1,555*
ICPEMC	Mutation Induction and Heritability in Mammalian Germ Cells	1989	5,000*
Lifecodes Corporation	DNA Technology and Forensic Science Conference	1988	5,000*
Muscular Dystrophy Association	Dystrophin Meeting	1989	19,885*
Pioneer Hi-Bred International, Inc.	Recognition in Plant-Pathogen Interactions	1989	1,000*
Plant Cell Research Institute, Inc.	Recognition in Plant-Pathogen Interactions	1989	1,500*
Ross Laboratories	Programmed Cell Death—Concepts and Mechanisms	1989	1,000*
R. Brinkley Smithers	Molecular Genetics and Biology of Alcoholism	1989	50,000*
Smithkline Beecham Pharmaceuticals	Cloning the Antibody Repertoire	1990	35,000*
Anonymous	Molecular Genetics and Biology of Alcoholism	1989	27,000*

* New Grants Awarded in 1989

ANNUAL CONTRIBUTIONS

Unrestricted

Long Island Biological Association (LIBA)

The Long Island Biological Association is the oldest supporting organization for Cold Spring Harbor Laboratory. Over the years a most unique and productive partnership has developed wherein LIBA has become our "Friends of the Laboratory" and accounts for the largest amount of unrestricted annual giving for the Laboratory. (The detailed report of their activities appears later in this Annual Report.)

Memorial Gifts

Anonymous
Dr. and Mrs. Mortimer Arenstein
Ms. Regina Austin
Ms. Donna Bassin
Mr. and Mrs. Maurice Bauer
Mr. and Mrs. Harry Binder
Mrs. Dorothy E. Bird
Mr. and Mrs. Bruce R. Blovsky
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Mr. and Mrs. Alan Lowenstern
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Joseph W. Muldoon, Esq.
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Mr. and Mrs. Paul M. Peyser
The Irving Ratchick Family
Mr. and Mrs. Stephen Rogowsky
Mr. and Mrs. Burton L. Rozner
Mr. Sheldon Sacks
Mr. Gregory Scandrett
Dr. and Mrs. Charles F. Schiffer
Dr. and Mrs. Philip Schneider
Mr. and Mrs. Jacob Schreiber
Mr. and Mrs. Jack W. Schwartz
Ms. Linda Schwartz
Mr. and Mrs. Gerald L. Shak
The Eva Shapiro Memorial Foundation, Inc.
Mr. and Mrs. Bernard A. Sloane
Mr. Theodore Steichen
Ms. Laurie Stern
Mr. Ralph W. Stern
Mr. and Mrs. Leonard E. Stokes III
Mr. and Mrs. Paul Strauss
Dr. and Mrs. Allan H. Toffler
Mr. and Mrs. Daniel Tyson
Union Carbide Corporation
Mr. and Mrs. Steve Wormser
Mr. and Mrs. Terry Arthur Zima

In memory of . . .

Cathy Susan Bird
George A. Humphrey, Sr.
Helen Leckerling
Jeanne Leckerling
Gladys Montemarano
Joyce Montemarano
Irving Paeff
Robert L. Pieper
Beverly A. Raff
Mary Merritt Burke Ricker
Walter Sturm
Palmer B. Wald

Corporate Sponsor Program

Cold Spring Harbor Laboratory is renowned throughout the scientific world as a meeting place offering the most comprehensive series of conferences on molecular biology available anywhere. In 1989, over 5500 scientists attended meetings here, providing a unique opportunity for the exchange and discussion of data. Access to these meetings is an important resource for the biotechnology industry.

This meetings program would not be possible without the generosity of members of the Corporate Sponsor Program who each contributed $17,500 to help underwrite the costs of meetings in Grace Auditorium and to fund five meetings at the Banbury Center. The Corporate Sponsor meetings at Banbury Center cover topics in basic research as well as topics of special importance in biotechnology. In 1989, these five Banbury Center meetings included *Applications of Basic Research in Mammalian Development; Viral Proteinases as Targets for Chemotherapy; Recessive Oncogenes; Molecular Cytogenetics* and *The Molecular Genetics of Early* Drosophila *and Mouse Development*.

The benefits to the Sponsor companies were increased in 1989 to include the waiver of all fees for eight representatives at Cold Spring Harbor Laboratory meetings and the special Banbury Center conferences; gratis Cold Spring Harbor Laboratory and Banbury Center publications including *Genes & Development* and the new review journal, *Cancer Cells*; and acknowledgment in meeting abstracts and publications.

Each year Federal funding for meetings becomes progressively more difficult to find, and a greater burden falls on the corporate sector. The continuing success of the Corporate Sponsor program shows that companies are prepared to assume that burden to help promote the best in scientific research, and that the Program is fulfilling an important role. 31 companies participated in the Program in 1989, and the membership renewal rate continues to be 90%. Cold Spring Harbor Laboratory is very pleased to acknowledge once again those companies that make our meetings possible:

Alafi Capital Company
American Cyanamid Company
Amersham International plc
AMGen Inc.
Applied Biosystems, Inc.
Becton Dickinson and Company
Beecham Pharmaceuticals
Boehringer Mannheim Corporation
Ciba-Geigy Corporation/Ciba-Geigy Limited
Diagnostic Products Corporation
E.I. du Pont de Nemours & Company
Eastman Kodak Company
Genentech, Inc.
Genetics Institute
Glaxo Research Laboratories
Hoffmann-La Roche Inc.
Johnson & Johnson
Life Technologies, Inc.
Eli Lilly and Company
Millipore Corporation
Monsanto Company
Oncogene Science, Inc.
Pall Corporation
Perkin-Elmer Cetus Instruments
Pfizer Inc.
Pharmacia Inc.
Schering-Plough Corporation
Tambrands Inc.
The Upjohn Company
The Wellcome Research Laboratories, Burroughs Wellcome Co.
Wyeth-Ayerst Research

Summary of Annual Contributions

Unrestricted Annual Contributions

CSHL Associates (1/1/89–12/31/89)	$221,798	
LIBA Members (1/1/89–12/31/89)	52,194	
General	35,222	
Memorials	3,122	
		$312,336

Restricted Annual Contributions

DNA Learning Center	157,010	
Dorcas Cummings Fund	2,500	
		$159,510

Total Annual Contributions

$471,846

Peter Orth Concert Sponsors

Mary Lenore Blair, Chairman
Jim and Carol Swiggett, Co-Chairmen
Drs. Harold & Shirley Andersen
Mr. Bruce K. Bernann
Mr. and Mrs. G. Morgan Browne
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Mr. and Mrs. Bruce J. Rice
Mr. Paul Sheeline
Mr. Charles B. Updike
Mr. and Mrs. Robert P. Walton
Dr. and Mrs. James D. Watson
Middleton-White Imports, Ltd.
Nordberg Capital, Inc.
The Printery

SECOND CENTURY CAMPAIGN

January 1, 1986–December 31, 1989

Unrestricted Contributions

$6,977,367

Anonymous 1
Anonymous 2
Anonymous 3
Mr. S. Reed Anthony
Mrs. Donald Arthur, Jr.
Mr. Edmund Bartlett
Mr. and Mrs. Allen L. Boorstein
Mrs. Leonie T. Brigham
Bristol-Myers Squibb Foundation, Inc.
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Mr. and Mrs. Morris Karpen
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Mr. and Mrs. George N. Lindsay
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Endowment

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THE LONG ISLAND BIOLOGICAL ASSOCIATION (LIBA) and Its Relation to the Cold Spring Harbor Laboratory

Biological research at Cold Spring Harbor began in 1890 when the Brooklyn Institute of Arts and Sciences was looking for a place to set up a summer laboratory as the headquarters of its Department of Zoology. Representatives of the Institute were invited by Eugene S. Blackford, at that time the Fish Commissioner of the State of New York, to inspect a site on Bungtown Road across Northern Boulevard (25A) from the Fish Hatchery. The site was found to be ideal, and so the original Laboratory was organized with Mr. Blackford as president of the Board of Trustees. The land was leased from Mr. John D. Jones, whose family since 1819 had operated various industries including shipbuilding, coopering, and textile manufacture (later whaling, also) at the head of Cold Spring Harbor. Bungtown Road, which runs through the Lab property, got its name from the factory that specialized in making bungs—or stoppers—for barrels.

In 1892, the Laboratory's land was leased for a dollar a year from the Wawepex Society, which Mr. Jones had organized as a corporation for holding real estate and for investing funds for the propagation of fishes and for scientific research. In 1904 the Wawepex Society leased additional land to the Carnegie Institution of Washington, which wanted to locate a Department of Experimental Evolution in the Cold Spring Harbor area. Charles B. Davenport, who had been directing the Laboratory since 1896, assumed the additional duties of director of the Carnegie Institution's experimental station. Until 1934, Mr. Davenport lived in the large Victorian house that still stands at the corner of Bungtown Road and 25A. Built in 1882 by John D. Jones, the house was renovated and repainted in its original colors in 1979-80, when it was renamed Davenport House. Since 1934 it has served as a dormitory for Laboratory scientists.

The Long Island Biological Association was established in 1924 when the Brooklyn Institute decided to discontinue its research at Cold Spring Harbor and offered its laboratory to two universities. Fortunately, a local group of interested neighbors decided to assume responsibility for the Lab, and thus LIBA came into being. For 38 years LIBA actually operated the Laboratory in conjunction with the Carnegie Institution, but in 1962 it seemed advisable for the Laboratory to be reorganized as an independent unit. Therefore, the property on which it now stands was conveyed to it by LIBA, which, however, still retains reversionary rights. Today LIBA is one of twelve institutions participating in the support of the Laboratory, each institution being represented on the Laboratory's Board of Trustees.

LIBA has become an expanding group of "Friends of the Laboratory" who help support it through annual contributions.

A large part of the Laboratory's resources is obtained from governmental, corporate, and foundation sources.as a result of grant applications which are submitted by the individual scientists. Years ago, 85% of the funding came from governmental agencies, but presently less than 50% comes from these sources. Therefore the scientists must rely on an assortment of foundations, corporations,

Membership in LIBA requires a minimum annual contribution (tax deductible) of $25 to the Cold Spring Harbor Laboratory. Further information can be obtained from the Long Island Biological Association, Box 100, Cold Spring Harbor, N.Y. 11724, or by telephoning the Laboratory at (516) 367-8840.

and individuals for an increasing share of their support. The researchers compete for grants in their specific areas of study. If an award is made, a portion of the award is returned to the Laboratory in the form of indirect costs for overhead. It is important to remember that these grants are highly competitive, and even if a grant is given an outstanding score by scientific peers, the funding may not be available.

LIBA sponsors the Laboratory's Annual Giving Program, which is its largest source of unrestricted annual gifts. These gifts enable the Laboratory to respond quickly to urgent or unexpected needs. Also, primarily through LIBA Fellowships and funds to start up new laboratories, LIBA helps ensure that the Cold Spring Harbor Laboratory continues to attract the best and brightest young scientists.

The affairs of LIBA are handled by a board of 28 directors who are elected to office by the membership at an annual meeting. LIBA members are invited to bring their friends to lectures and open houses at the Laboratory.

CHAIRMAN'S REPORT

As the study of biology here at Cold Spring Harbor Laboratory approaches its centennial year, the Long Island Biological Association has completed its 65th and perhaps most significant year in the support of basic research. At the close of its fiscal year on September 30, 1989, membership reached 781, up 47% from 531 in 1988. Total contributions were $289,064 versus $261,525 in 1988. Included in our membership were 135 Cold Spring Harbor Laboratory Associates (contributors of $1,000 or more), up from 110 in 1988. This growth of support is of immense help to the Laboratory by giving it the resources to attract the finest young scientists. Additionally, this demonstration of broad-based advocacy is an important element in attracting foundation and corporate grants.

LIBA's increased membership was due in large part to the efforts of LIBA Director Mrs. Edward Greenberg. She conceived and planned a cocktail reception at Airslie, the home of Dr. and Mrs. James D. Watson, for members of the community who were not LIBA members. It was a joyous occasion attended by more than 400, and Jane and her committèe of . . .

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hosted 15 dinner parties for all attendees following the reception. Jim and Liz were wonderful hosts and Jim Hope and his staff provided an outstanding selection of hors d'oeuvres.

Dr. James D. Watson greets guests at the Airslie Garden Party for new LIBA members on June 10.

Special thanks also go to Mr. and Mrs. James D. Mooney, Mr. and Mrs. Donald L. Deming, and Mr. and Mrs. Joseph P. Downer, who also hosted receptions to introduce members of their communities to the exciting research at Cold Spring Harbor Laboratory.

In its continuing effort to increase the understanding of some aspects of molecular biology amongst its membership, the Annual Meeting on January 15 included a panel consisting of Drs. James D. Watson, Ed Harlow, Winship Herr, and Rich Roberts as well as Morgan Browne, the Lab's Administrative Director. The discussion was moderated by John Reese and the panel fielded questions from the membership about the Laboratory and its work.

Prior to this interesting discussion, Lawrence L. Davis, Chairman of the Nominating Committee, thanked retiring directors Mrs. Donald Arthur, Mrs. George N. Lindsay, Mr. William Payson, and Mr. Edward Pulling for their efforts on behalf of the Laboratory. Mr. Pulling, who retired after 20 years as a LIBA director, including 17 as its Chairman, was given special recognition by his election to Honorary Director. Five new directors were elected. They were: Mrs. Helen B. Chenery, Mr. John Cleary, Mrs. Donald Deming, Mrs. Edward Greenberg, and Mr. Edward McCann.

The Ambassador of the Year Award was presented to Mrs. Sinclair Hatch for her untiring efforts on behalf of the Laboratory. Wendy is a trustee of the Laboratory, a director of LIBA, and serves on the Executive, Development, and Finance & Investment Committees of the Lab, and also chairs the Major Gifts Committee of the Second Century Campaign. Her energies go far beyond these duties as she continually introduces new friends to the Lab and introduces staff and visiting scientists to the community.

In February, the Cold Spring Harbor Laboratory Associates were treated to one of the most interesting talks since the program's inception three years ago. John Hicks, who heads the FBI's DNA Fingerprinting Division, which was officially opened only a month before his talk, entertained us with stories of criminal investigations that were aided by DNA fingerprinting. A light supper was served in Blackford Hall following the program.

As in the past, the Associates were invited to hands-on laboratory workshops. These events are designed to give members a better understanding of molecular biology, DNA fingerprinting in particular, and the nature of the experimentation carried on daily at the Laboratory.

Dr. Jan Witkowski instructs participants at a DNA workshop.

Dr. Gustav Nossal addresses LIBA members at the Dorcas Cummings Memorial Lecture in June.

Again this year, the Dorcas Cummings Memorial Lecture was held during the Laboratory's symposium. LIBA was indeed fortunate to have Dr. Gustav Nossal, Director of the Eliza Hall Institute in Victoria, Australia, speak to the membership and the distinguished attendees from around the world. As the subject of the symposium was immunological recognition, his comments on some of the basic elements of immunology were most interesting, and were delivered with great humor.

Through the organizational efforts of Edward Pulling, more than 139 visiting speakers and laboratory scientists attended 19 symposium dinner parties following the lecture. This year's hosts were

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Mr. & Mrs. G. Morgan Browne
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These dinners are an annual highlight and are enjoyed by both the visiting scientists and the members of the community.

Under the leadership of Dr. James D. Watson, science at Cold Spring Harbor has flourished during the year. As one of its major educational functions is to host symposia, meetings, and high-level courses in molecular biology, 1989 was a spectacular success. At final count almost 5,600 visiting scientists participated.

Laboratory Highlights

By year's end, the number of staff scientists increased to 140. Some of the recent developments include

Mike Wigler was elected into the prestigious National Academy of Sciences. While his scientific accomplishments are many, Mike is probably best known for his co-isolation of the first human oncogene in 1981.

Ed Harlow and his team were the first to determine a physical link between a viral oncogene and an anti-oncogene (a cancer-suppressing gene), thereby providing a key piece of the cancer puzzle.

Through the generosity of LIBA members, LIBA Fellowships were awarded to Drs. Ashok Dubey, Anindya Dutta, Erich Grotewold, and Jeffrey Kazzaz. Drs. Carol Greider and Arne Stenlund were awarded New Investigator Start-up Fund grants.

Mrs. Walter C. Meier and Mrs. Craig B. Ordway gave generously of their time to the DNA Learning Center as they most capably organized, recruited, and directed a corps of volunteers to staff the Smithsonian's *Search for Life* exhibit this past year. Our sincere thanks go to all those LIBA members who staffed the exhibit.

The DNA Learning Center was dedicated in September 1988, the cornerstones for the Beckman Neuroscience Laboratory and Dolan Hall were laid in May 1989, and six new heated cabins were dedicated in August 1989, extending the course and meeting season to seven months of the year.

In addition to *Genes and Development*, the Lab now has a second journal. The inaugural issue of *Cancer Cells: A Monthly Review* was sent out in September.

The programs at the Banbury Center continued to draw together interested professionals from around the country to discussions on such topics as diseases and their possible problems of identification and treatment.

The most significant event of the year may have been the announcement in February of the Second Century Campaign by the Laboratory Board of Trustees. This capital campaign, the first in the Laboratory's history, is designed to meet the Lab's needs and enable it to continue its remarkable track record as it embarks on its second century.

The $44-million goal includes monies for the construction of the neuroscience laboratory and teaching facility, the residence hall and 10 cabins for visiting scientists, the refurbishing of McClintock Laboratory for cancer research, and the establishment of a number of endowment funds to enable the Laboratory to attract the very best young scientists. At the time of the announcement in April, more than $27 million had been committed; as we go to press in November, nearly $31 million has been pledged.

The Future

The Laboratory begins its 100th year in 1990. Numerous centennial special events are being planned. More and more, the Laboratory is receiving recognition as one of the leading institutions for scientific research. *Science Watch*, a newsletter that tracks trends and performance in basic research, proclaimed in its July 1989 headline "Cold Spring Harbor Packs Twice the Punch of Other Leading Independent Labs." The article goes on to say, "The observation that Cold Spring Harbor produces high-quality research will come as news to few. But what may surprise many is that, over the past decade and a half, papers from CSH have carried about twice the clout, measured by citations, as papers from several other premier independent labs." The reputation of this remarkable institution, already very high among scientists around the world, is growing among generous people who care about the effectiveness of their gifts.

As the Laboratory embarks on its second century, it will make the important transition from a summer to a year-round teaching institute specializing in DNA science. This will require new facilities. More importantly, we must continue to attract and retain exceptional scientists. Now is the proper time for us to reflect on past success and to insure another hundred years of excellence.

George W. Cutting, Jr., Chairman

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Karen Otto
Margaret Stellabotte
Diane Tighe

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Deon Baker
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Aldo Leo
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- Steven Guy
- Louis Jagoda
- Stanley Miecnikowski

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- Swinton Brown
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- Edward Haab
- Kevin Killeen
- John Marchetto
- Daniel Miller
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- Donald Rose
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- Catherine Burke
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- Dessie Carter
- Mary Genova
- Turid Mikalsen
- Barbara Robinson
- Cherie Thurn

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- Lane Smith

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- Paul Anzalone
- Frank Carberry

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Scientific Staff Departures During 1989

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