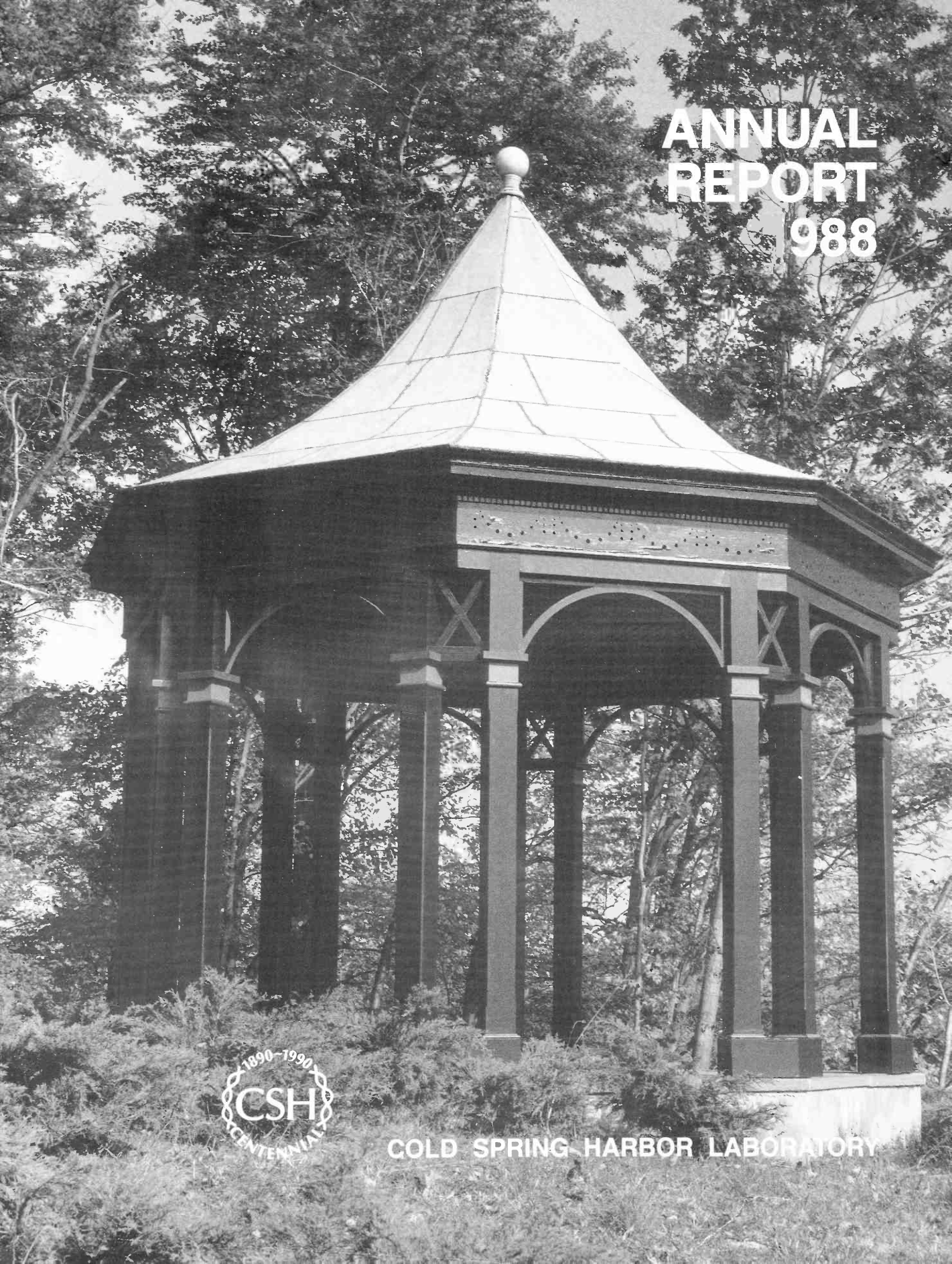
ANNUAL REPORT 1988

COLD SPRING HARBOR LABORATORY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ANNUAL REPORT 1988

Cold Spring Harbor Laboratory
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Front cover: Davenport House/formerly the Carnegie Dormitory/originally Mather House, 1884. Restored, 1979–1980.
(Photo by Margot Bennett)
Back cover: Jones Laboratory, overlooking harbor. Erected in 1893 for marine biological work by the Laboratory's founder, John D. Jones. Renovated in 1972.
(Photo by Susan Zehl)

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(*Back row*) T. Maniatis, D. Botstein, S. Strickland, J.D. Watson, B.D. Clarkson, G. Cutting, J.E. Darnell, Jr., T. Whipple, J.R. Warner (*Middle row*) L.J. Landeau, R. Landau, T.J. Knight, Mrs. G.N. Lindsay, E.R. Kandel, D. Pall, T.J. Silhavy (*Front row*) W.H. Page (Honorary), J. Klingenstein, O.R. Grace, Mrs. S. Hatch, D.L. Luke III Not shown: G.M. Browne, W.M. Cowan, Mrs. C.E. Dolan, W. Everdell, Mrs. H.U. Harris, Jr., Mrs. G. Montgomery, F.M. Richards, W.S. Robertson, D.D. Sabatini, H. Sampson.

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DIRECTOR'S REPORT

We have now, as a great nation, set as a national objective the working out of the human DNA sequence. Similar to the 1961 decision made by President Kennedy to send a man to the moon, the United States has committed itself to a highly visible and important goal. Although the final monies required to complete the human DNA sequence of some 3 billion base pairs will be an order of magnitude smaller than that needed to let Americans explore the moon, the implications of the Human Genome Project for human life are likely to be far greater. A more important set of instruction books will never be found by human beings. When finally interpreted, the genetic messages encoded within our DNA molecules will provide the ultimate answers to the chemical underpinnings of human existence. They will not only explain how we function as healthy human beings, but also let us understand at the chemical level the multitude of genetic diseases, such as cancer, Alzheimer's, and schizophrenia, that demean the individual lives of so many millions of our citizens.

The possibility of knowing our complete set of genetic instructions seemed an undreamable scientific objective in 1953 when Francis Crick and I found the double helical structure of DNA. Then, there existed no way to sequence even very short DNA molecules, nor did there exist any possibility of obtaining the totality of human DNA as a collection of discrete pieces for eventual chemical analysis. Only years later, with the 1973 birth of the recombinant DNA revolution, was it possible to think of isolating individual genes. This breakthrough provided the incentive for Walter Gilbert and Fred Sanger to develop their powerful sequencing techniques that now make it almost routine to establish in a single experiment the sequences of some 300–500 base pairs (bp) within a given DNA molecule.

The first complete DNA sequences to be established by these procedures were those of the smaller DNA viruses—the monkey virus SV40 and the phage ϕX174, each of which contains some 5000 bp. These sequences became known by 1977, and within the next five years the tenfold larger DNAs of the bacteriophages T7 and λ were worked out. Today, the more than 100,000-bp DNAs of several plant chloroplasts and of the herpesvirus EBV have been established. The largest DNA now sequenced is that of cytomegalovirus (a herpesvirus), which contains almost a quarter of a million base pairs and has just been completed by Bart Barrel's group at the Laboratory of Molecular Biology in Cambridge.

Simultaneously, the sequences of a large number of individual genes have been worked out, with the total number of base pairs approaching 25 million. The most completely known organism to date is the intensively studied bacterium

Escherichia coli, with over 800,000 bp of its 4.8×10^6-bp genome already established. There now exist in the United States and Japan a number of labs geared up to complete the *E. coli* sequence in university-like environments, and there are good reasons for believing that success will come within the next decade. Today, DNA sequencing usually costs between three and five dollars per base pair, and so at most, 25 million dollars would be required; a large, but not unthinkable sum when spent over an extended interval. Most likely, as sequencing efficiencies improve, the *E. coli* sequence will finally cost a sum less than half this amount. Conceivably, we could know the complete sequences of one to several bacteria without the creation of a major research program specially aimed at working out complete genomes.

A completely different picture holds for the human genome, which is almost 1000 times larger and is distributed over 24 different chromosomes. Here, the cottage industry approach involving small groups of individuals, each working at a large number of different sites, seems very unlikely to succeed. The time involved would more than exceed the lifetimes of those who would like to work this way. To be truly exciting for both our best scientific minds and the average citizen, we must aim to complete the job over a 15-year time interval. Those who start the project should plan for themselves, not their scientific descendants, to complete it. From the beginning, we have to design game plans where economies of scale are sought and found. We shall, moreover, have to bring into existence sequencing facilities far larger than any that exist today and that of necessity more resemble industrial production lines than conventional university research laboratories.

The first serious proposal to start sequencing the human genome occurred at a meeting held in early 1985. Robert Sinsheimer, then Chancellor of the University of California at Santa Cruz, brought together a small group of scientists with the hope that the project might be centered in the Santa Cruz environment. Renato Dulbecco came away excited from that gathering, and when he flew to Cold Spring Harbor later that year to speak at the dedication of our Joseph Sambrook Laboratory, he spoke glowingly of the prospects for cancer research if we knew the sequence of our own DNA. By this time, the Department of Energy was seriously thinking about taking on the challenge. Toward this end, Charles DeLisi, then head of the DOE's Division of Health and Environmental Research, brought together in Santa Fe in February 1986 some 80 scientists with expertise in DNA research and human genetics. Sensing the need for large dedicated labs, he and his colleagues at the DOE proposed that their National Laboratories, particularly those at Livermore and Los Alamos, should be the center of the American, if not the worldwide, human genome effort.

Over the next several months, rumors that the DOE would soon commence a large-scale human genome program began to spread through the general biological community. The pros and cons for such a DOE-led project were first discussed before a more general audience here at Cold Spring Harbor in 1986. During the course of that June's Symposium on the "Molecular Biology of *Homo sapiens*," a special afternoon session took up the question. Although several of the more-senior scientists, like myself, Walter Gilbert, and Paul Berg, voiced the opinion that now was the time to start the project, much less enthusiasm, if not downright hostility, was voiced by many younger scientists. They feared that a megabillion dollar project would of necessity divert money away from single-investigator-initiated research grants and slow down the pace at which our country does high-quality biological and medical research. Also troubling to many was the thought that the DOE had never been a major supporter of recombinant-DNA-based research and possessed no strong set of administrators familiar with the world of

genetics. The intellectual competence for managing the Human Genome Project might never exist within a DOE whose leaders were invariably physical scientists and where biology of necessity always occupied a low position on its totem pole of priorities. Conceivably, strong managers could be brought in from the outside, but lacking such assurances, the safe course then seemed to me for NIH itself to take on the Human Genome Project, provided that new monies would be appropriated by Congress to fund it.

Soon the controversy reached the attention of the Board of Basic Biology and the Commission on Life Sciences of the National Academy of Sciences. After a joint meeting in Woods Hole in August 1986, a decision was made to appoint a special (National Research Council) committee to prepare a report as to what our nation should do next. Chaired by Bruce Alberts, known for his distrust of big labs for biology, the 15-member committee represented a diverse collection of viewpoints, including that of past strong vocal opposition to the project. Its six-month-long deliberations led to a unanimous report, "Mapping and Sequencing the Human Genome," which urged that the United States should commence the Human Genome Project, working cooperatively with those other nations who wished jointly to pursue the common goal.

Soon after the NRC Committee began its deliberation, it became apparent that within the meeting room the project itself was not really controversial—who could be against obtaining the much higher molecular genetic and physical maps of human DNA that would need to be on hand before the sequencing itself could begin. Such maps would be invaluable allies in the finding of key human disease genes. These mapping efforts would dominate the first five years of the project; only then would the production-line sequencing efforts commence. What had generated much of the initial opposition was fear that the project would be divorced from the main currents of biological research, focusing exclusive concern on human DNA sequences, most of which might prove uninterpretable unless equal attention was paid to the genomes of much simpler model organisms such as *E. coli*, the yeasts, the roundworm *Caenorhabditis elegans*, and the fruit fly *Drosophila*, as well as to the much more closely related mouse. There was also strong reservation about any project where the ultimate control of resources lay in the hands of administrators, like those who rule the DOE, as opposed to control by the scientific community itself. DOE's known propensity for overruling peer review panels had created much unease at the thought that they might direct the project.

In urging that the Human Genome Program start, the committee emphasized the need for technological improvements that lead to five- to tenfold improvements in the efficiency of current gene mapping, sequencing, and data analysis capabilities. Only when the true cost of sequencing falls to no more than 50 cents a base pair should extensive sequencing begin. With this proviso, the total costs of the project should not exceed 3 billion inflation-adjusted dollars. Federal funding was urged to rise quickly to 200 million dollars a year, with the project planned to be completed in approximately 15 years. The sequencing of the model genomes was urged to go hand in hand with, if not slightly ahead of, that of the human genome. Knowledge of the simpler structures of the genes of bacteria and budding yeasts should facilitate the task of distinguishing the amino acid coding regions (exons) of the human genome from the much more prevalent noncoding (intron) components.

In addressing the question of whose genome would be sequenced, the committee pointed out that no two individuals (except for identical twins) contain exactly the same DNA sequences because of mutations, particularly in noncoding

regions, that have occurred during the course of evolutionary time. The Human Genome Program would be producing a prototype blueprint laying out the basic organization and sequence of the genes of our 24 chromosomes. Most likely, it will be a composite of regional sequences from the chromosomes of many different individuals already being mapped in major laboratories. Later, the nature and extent of the variation from one person to another can be ascertained when large numbers of sequences from specific regions are compared.

To ensure that the project be scientist-directed, the formation of a strong human genome advisory committee, chaired by a leading scientist, was deemed indispensable. It should both participate in the planning process and provide oversight on ongoing programs. A majority of the committee, furthermore, felt that the Human Genome Program should be assigned to a single federal agency, but it did not rule out dual management by both the DOE and NIH as long as there existed a unified scientific advisory committee.

Parallel with the deliberations of the NRC panel, the Office of Technology Assessment (OTA) of the U.S. Congress was commissioned by the House Committee on Energy and Commerce to prepare a report that later was to be entitled "Mapping Our Genes—The Genome Project: How Big, How Fast?" The Congressional interests on which they focused centered on (1) assessing the scientific and medical reasons for genome projects, (2) the potential funding—at what level and through what mechanisms, (3) how to coordinate activities if several federal agencies are involved, and (4) how to strike a proper balance between the virtues of international scientific collaboration and the need to promote the United States competitive position in biotechnology. Unlike the NRC report, the OTA document did not offer specific recommendations, viewing as its purpose the informing of Congress on the options for future action. Despite this aim for neutrality, reading of the OTA report left the unmistakable message that some form of human genome program was bound to proceed and that Congress had a real role in seeing that it started off in the right direction.

In fact, in their 1988 Federal budget request the DOE had asked for 15 million dollars, and later received 10 million dollars, to start their human genome effort. There was no formal request from NIH for genome studies, but during the spring 1987 Congressional appropriation hearings, its director Jim Wyngaarden was asked how much they would need to have a meaningful program. His reply was 50 million dollars. Later, in mid-May, David Baltimore and I visited key mentors of the House and Senate Appropriation Committees on behalf of the Delegation for Basic Medical Research, of which I was the official spokesman. We emphasized the need for a multihundred-million-dollar increase in AIDS research monies as well as indicated that a 30-million-dollar appropriation would let NIH start a serious human genome effort. In the summer, when the respective appropriation subcommittees marked up and then reconciled their NIH budgets, 18 million dollars for genome studies was added to the NIGMS (National Institutes for General Medical Studies) budget. By then, Ruth Kirschstein, the Director of NIGMS, had sent out RFAs (requests for grant applications) on genome studies, with the funds for the program hopefully to come from new monies added to the NIGMS budget.

The HHS (Health and Human Services) appropriation, with its NIH component, only became signed into law by President Reagan in early December, two months along into the 1988 fiscal year. The 17.4 million dollars finally allocated for genome studies allowed Jim Wyngaarden to convene quickly an 18-member Ad Hoc Advisory Committee on Complex Genomes to propose priorities for the NIH Genome Program. Meeting in Reston, Virginia, in late February, it was ably

chaired by David Baltimore and broadly backed the main features of the prior NRC report. In its final recommendations it strongly supported Jim Wyngaarden's proposal to establish an Office of Human Genome Research to be headed by a new Associate Director of NIH. A key feature of the Office was to be a permanent expert Advisory Committee to work with the head of the genome effort to keep the various genome efforts on target. Emphasis was also given to the need during the early phases of the program for major training efforts that would prepare prospective scientists with the new technologies needed to generate and then assemble the massive amounts of new information that would flow out of the genome programs.

At the Reston meeting, I strongly urged that the Associate Director position be filled by an active scientist, as opposed to an administrator, arguing that one person had to be visibly seen in charge and that only a prominent scientist was likely to reassure simultaneously Congress, the general public, and the scientific community that scientific reasoning, not the pork barrel, would be the dominant theme in allocating the soon to be large genome monies. Then I did not realize that I could be perceived as arguing for my own subsequent appointment. For many years, my most visible role had been that of an administrator dominated by the fund-raising activities needed to keep Cold Spring Harbor Laboratory at the forefront of DNA-based science. Whether I was still a real scientist was not at all clear.

So, I felt uneasy when I heard rumors that I was to be offered the position of Associate Director of NIH. My job here as the Director was already more than full-time, and if I ran the genome effort, I would hold simultaneously two demanding positions. Yet, if I turned down the job, it was not clear that any prominent scientist still active in the lab would take on the task. Lacking a real boss, the NIH program might have the same severe drawbacks that had worried us about how the DOE would run their genome studies. So, when in early May Jim Wyngaarden asked me to come down to Building 1 to talk about working for NIH, I knew I would accept. By then I had also realized that I would only once have the opportunity to let my scientific life encompass a path from double helix to the three billion steps of the human genome.

I soon also had the reassurance of very capable assistance in the running of the Bethesda office. Because I would not be a full-time employee, the head of the Office of the Human Genome was to be Elke Jordan, then on Ruth Kirschstein's staff at NIGMS, in charge of its Genetics Program, as Deputy Director and later Associate Director for Program Activities. I had first met Elke in the mid-1960's when she worked on phage λ in Matt Meselson's lab at Harvard. Assisting her was to be Mark Guyer, also an experienced NIGMS administrator and a product of Norman Davidson's lab at Caltech before spending several years in the biotechnology industry. So, I would have no reason to feel apprehensive that I would have no real role in the day-to-day operations of the Human Genome Program. My major task clearly was to help formulate a workable strategy for establishing the human genome sequence.

Officially, I started working for our government in early October, having secured permission from my Trustees here at Cold Spring Harbor to begin a commuting life where I would try to spend the beginning of each week at NIH. Already by then, Jim Wyngaarden had sent suggestions to the HHS Secretary as to possible members of the Program Advisory Committee on the Human Genome. The 12 members finally chosen were much to our liking and had Norton Zinder as their Chairman. Its composition reflected a broad range of expertise, with strong representation from the world of pure science that had initially reacted so

negatively when the Human Genome Program was first discussed. Their presence on the Advisory Board was a strong message to the world of biology that NIH would not bring forth a narrowly construed effort catering exclusively to the immediate needs of the human genetics community. And by having three members from industry, we sought to reassure Congress that our nation's competitive position in biotechnology would not be neglected.

Beginning with my opening press conference at NIH, and later through other meetings with the press, I made clear my concern with the ethical implications of an ever-increasing knowledge of human genes and of the respective genetic diseases that result from imperfections in our genetic messages. This knowledge undoubtably will lead to much deeper understanding of many of the worst diseases that plague human existence. Thus, there are strong ethical reasons to pursue this genetic knowledge as fast as possible and with all our might. On the other hand, the knowledge that some of us as individuals have inherited disease-causing genes is certain to bring unwanted grief unless appropriate therapies have been developed. So, it is imperative that we begin to educate our nation's people on the genetic options that they as individuals may have to choose between.

I believe we should put real money behind these convictions and suggested that at the start at least 3% of our genome-targeted funds should go to grants in the ethics area. In doing so, we must be aware of the bad misuses of the then very incomplete knowledge of human genetics that went under the name of eugenics during the first part of this century. There exists real fear among many individuals that genetic reasons will again be used to make the lives of our underprivileged even more disadvantaged. So, we must work to ensure that the right laws come into existence at both the federal and state levels to prevent spying into the privacy of an individual's DNA by either prospective employers or insurers, or government agencies. If we fail now to act, we are bound to witness unwanted and unnecessary abuses that eventually will create a strong popular backlash against the human genetics community. We only have to look at how the Nazis used the German professors of human genetics to justify their genocide programs, first against the mentally ill and then against the Jews and the Gypsies. We need no more vivid reminders that science in the wrong hands can do incalculable harm.

At its inception, the Office of the Human Genome had essentially only an advisory function with the legal authority for the initial distribution of NIH genome funds belonging to NIGMS. All the 1988 monies in fact were distributed by peer-reviewed NIGMS grants before I went to Bethesda, and the 1989 appropriation of 27.6 million dollars will likewise be distributed by NIGMS. For both the 1988 and 1989 monies, special ad hoc genome study sections were convened, with several specifically responding to requests for grants aimed at technology innovation. Although the rumor is going around the biological world that our monies allow all approved applications to be funded, this is not true. Only approximately 30% of the grants so far approved will in fact be funded, a figure not that different from that which used to hold for more regular study sections. Our funding level is, however, above the disastrous 15–18% level now predicted for much of NIGMS. So, we are bound to feel more than the occasional ill will from those less fortunate. We must strive to see that the quality of funded genome research remains high and that the larger sum of money to be available in fiscal 1990 is spent wisely.

It was clear from the first study section that I sat in as an observer that totally distributing our funds through conventional research grants would work only during our first several years of operation. Now we need to encourage many

different approaches to genome mapping, sequencing, and information storage and retrieval. Over the next few years, as we begin to make real choices as to how to proceed, we will need the legal authority to distribute our monies in much more targeted ways, creating the peer review mechanisms for funding large groups to completely map and then sequence chromosome-sized sections of DNA. Jim Wyngaarden had told me when I first came to work with him that he wanted to change our Office into a "Center," which would have the authority to make grants, as soon as we had sufficient funds to start a targeted program. The inclusion in President Reagan's 1990 budget of 100 million dollars for genome studies gave him the go-ahead signal to request the HHS Secretary to authorize our upgrading into a "Center." This request has just been approved by Secretary Louis Sullivan, and in October we will become a "Center." Now we are busily recruiting the new individuals that will let us have by early next year a staff of some 20 persons.

There still is uncertainty over how the plans we develop through our Program Advisory Committee will be coordinated with the DOE program. Last summer, a congressional bill was proposed authorizing a multi-agency coordinating committee that would add to the number of meetings I would need to attend, but which did not address the question whether NIH or DOE would play the role of leader. To circumvent what we feared would be an unneeded further level of bureaucracy, NIH and DOE signed in the fall of 1988 a memorandum of understanding which created a unified NIH-DOE Genome Committee, with its members drawn from a subset of our respective advisory bodies. I see as the most important immediate task of the joint committee the drawing up of a joint National Genome Plan to be submitted to Congress at the time it considers our fiscal 1991 appropriation. We have already scheduled our first planning meeting to be held here in Cold Spring Harbor the last days in August, with a smaller, follow-up meeting planned for early October in San Diego. I most look forward to these meetings where for the first time, the question before us is no longer whether to start a targeted genome program, but how to best mount one.

By now I am convinced that labs must be created over the next five years large enough to oversee over the subsequent decade the detailed physical mapping and sequencing of individual chromosomes. Groups of about ten individuals are probably the appropriate size for a cost-effective analysis of the typical bacterial and yeast chromosomes, which tend to range in size from one to several megabases (10^6). For the human chromosomes, which average in size over 100 megabase pairs, it may be necessary to put together groups of some 50 trained personnel. Their collective output of finished sequences would need to be approximately a phage λ equivalent of DNA (40 kilobases) per working day. The informational aspects of the genome program will, with time, become more important as an ever-increasing set of new DNA sequences begin to be compared with the sequences previously obtained. So, although the key talents needed to start a successful genome program are likely to be those of the recombinant DNA chemist, our effectiveness at the end of the program may depend more on the computer skills that are applied.

The idea that the various human chromosomes will become divided up between different labs is far from today's conventional wisdom. Seeming to argue against this approach is the fact that most chromosomes are already being both genetically and physically mapped in a large number of high-quality labs. Deciding which one of these groups should be given the funds to complete the maps of their respective chromosomes at first sight appears an impossible political task. But closer inspection reveals that these gene mappers are primarily

interested in locating their own specific disease genes. Once their gene is mapped, they will want to go on to its subsequent cloning and are very likely to discontinue mapping per se. To my knowledge, the number of individuals wanting to make complete high-resolution physical maps of their respective chromosome is less than the fingers on two hands. Badly duplicative efforts only exist for 21, the smallest human chromosome, which has the added incentive of containing a gene that leads to increased susceptibility to Alzheimer's disease. Here, we may witness a truly competitive race to clone the overlapping sets of DNA fragments that are needed to commence sequencing. Up till now, the only chromosomes where serious efforts have been started to make complete sets of overlapping DNA fragments are 21, 22, 19, 16, 11, and X. Our real problem will not be in deciding between alternative proposals for total mapping and sequencing, but in actually persuading intelligently led groups to focus on those chromosomes that still have no champions.

The question must thus be asked whether we may all too painfully find that we will have the money but not the talented brains to bring the Human Genome Project home to completion within an acceptable time period. I think not, but in so arguing I want to focus on the chief motivation that attracts talented scientists to their goals. Contrary to popular misconception, it is seldom fame or financial benefit. To be sure, when one makes a great discovery, it is frequently rewarded by a major scientific prize and the prospect of a much better academic or industrial position. But the more important reward is the satisfying of one's curiosity about how nature operates, and for biologists this means deeper and deeper understanding of the nature of living organisms. The working out of a bacterial genome will let us know for the first time the total set of proteins needed for a simple cell to grow and multiply. As soon as we have the *E. coli* genome, we shall have in our possession the amino acid sequences of all those proteins that, for example, control its gene expression or function as channels through which ion and key molecules move. Then the *E. coli* cell will begin to be treated as an object that we realistically can hope someday to comprehend in its entirety, as we can today understand complex machines like the 747. A total understanding of *E. coli* of course will not fall out immediately from the possession of its instruction book, and hundreds of years are likely to pass before *E. coli* poses no further scientific challenges. But the mere statement that *how E. coli* functions will one day be completely known is an extraordinary scientific assertion.

Possession of the genomes of multicellular organisms like *C. elegans* (~100 megabases) and *Drosophila* (~150 megabases) will be equally important scientific landmarks. Their much more complex genomes provide the instructions for the extraordinarily complex set of events that allow fertilized eggs to develop into functional adults. Until a decade ago, how multicellular organisms develop was virtually a black box at the molecular level. Then a number of molecular embryologists began to clone the regulatory genes that control the passage from one developmental stage to another. And now a number of the key development steps in genetically well-characterized organisms are understood at the molecular level. But if we are to understand all the events that lead, say, to the differentiation of a nervous system, we will need to work from the whole set of genetic instructions. So, both the *C. elegans* and *Drosophila* worlds are starting to make plans for working out their respective DNA messages. The group farthest ahead is that of *C. elegans,* where virtually all its genome is available as cloned sets of overlapping DNA fragments.

The main mappers of *C. elegans*, John Sulston and Alan Coulson from Cambridge and Bob Waterston from Washington University, now are about to start

pilot sequencing efforts that they hope will bring the cost quickly down to less than a dollar a base pair. Optimally, production-line-type sequencing efforts will begin both in the UK and in our country within three to four years, raising the possibility of establishing the total *C. elegans* genome by the year 2000. This project will probably motivate the *Drosophila* world to see whether they can work to a similar timetable. Here again, I would hope that the final sequencing effort be shared between Europe and the United States, with the total final costs for these programs to be no more than 75 million dollars each. Inherent scientific interest in the smaller model organisms, which may well be extended to the small-genome plant *Arabidopsis*, could prove to be the key ingredient in attracting the appropriate high-level scientific talents to develop the production-line sequencing capabilities that we will need to tackle the human chromosomes.

The first serious efforts to clone overlapping fragments of human chromosomes have just begun, with most of the American effort so far centered in our National Laboratories. Both at Livermore and at Los Alamos, cell-sorting instruments have been adapted to chromosome separation, with the resulting semipurified chromosomes used to prepare "chromosome-specific libraries" of cloned DNA fragments inserted into cosmid vectors. Ordering of these cloned human DNA fragments (~50 kilobase pairs in length) into the overlapping sets (contids) presents a greater technical problem than present when working with either *C. elegans* or *Drosophila* libraries. Over half of human DNA comprises highly repetitive sequences that greatly hinder the detection of true overlaps between DNA fragments. Recently, the assembly problem has begun to seem less daunting due to the ability to clone much larger DNA fragments (> several hundred kilobases) in yeast artificial chromosomes (YACs) using techniques developed at Washington University by Maynard Olson. Also encouraging is the ability to pinpoint at the megabase level of resolution the chromosomal location of any DNA fragment through in situ hybridization to stretched out metaphase chromosomes.

Assembly of almost complete sets of overlapping DNA fragments for the first several chromosomes may well have to occur over the next two to three years if we are to complete the human genome sequence within the next 15 years. The option of letting the job spread out over, say, 10 years does not exist if we are to keep costs under control. So, hopefully, the three main DOE groups at Berkeley, Los Alamos, and Livermore will show they are up to the goals they have staked out for themselves through assembling soon the overlapping DNAs of chromosomes 21, 16, and 19 in forms ready for sequencing. There is no assurance, however, that it will be the DOE labs that will put together the first useful overlapping maps. Not only is chromosome 21 up for grabs, but the almost equally small size of chromosome 22 has already made it a tempting target for scientists at Caltech and Massachusetts General Hospital. Competition, at least initially, will also exist between the several groups in Italy, London, and Houston that have all aimed to assemble the X chromosome map. In contrast, there is likely to be cooperation from the start between the labs at the Salk Institute and in London that aim to make the overlapping map of chromosome 11. How fast other groups will come into existence for the overlap mapping of the remaining human chromosomes is not obvious. We do know, however, that groups of two to four scientists are not big enough to conquer the average-size chromosome within a reasonable time. Instead, funds for groups of around ten may have to be committed for a realistic chance of success. Even when we really know how to succeed, sums of 2–5 million dollars are likely to be consumed for every chromosome successfully mapped.

These sums will thus be a modest entry fee for those nations who wish to be real participants in the Human Genome Program. So far, only the United States, the United Kingdom, and Italy have announced definite programs, but there are good reasons for believing that France, the USSR, Japan, and possibly Canada will join. Whether Germany will mount an effort is problematic because of the negative connotations that human genetics research still brings to the German psyche. How to ensure that we as nations work together instead of indulging in costly competitive races for the same chromosomal objectives is not yet settled. Although a number of prominent molecular biologists and human geneticists have banded together to form "The Human Genome Organization" (HUGO), it is not yet a free-standing organization capable of taking the steps that will make it a real, as opposed to paper, organization. Although the decision was made to have three regional offices, in the United States, Europe, and Japan, the funds have yet to be found to attract a Secretary General who would be responsible for coordinating the efforts of the regional offices. Now, I suspect, a truly effective Secretariat will cost initially about 1.5 million dollars per year, with the sum rising to possibly 3 million dollars when the Human Genome Program is in full operation, say five years from now. Initially, the HUGO founders hope to find private or foundation donations for much of this sum, arguing that most governments will join in only when a seasoned administrator is already in place.

HUGO's coming into existence will greatly facilitate the free and open exchanges of data that we would all like to be features of the Human Genome Program. Knowing the sequences of, say, half the human chromosomes without having access to the other half would be unbearably frustrating. Optimally, soon after new sequences are established, they will be added to a worldwide accessible database. Complicating this matter will be the fact that labs that are generating the sequences, before passing them on to others, will naturally want to work out the genes located within them and find clues for where they function or how they are expressed. In putting real meaning into their DNA sequences, it is highly likely that they will obtain the first indication of many, until then undiscovered, important human proteins that will provide clues to the functioning of the human body. Moreover, there are bound to be commercial implications from the first learning of many of these new human proteins.

Thus, it would be naive to expect that any extensive human sequence data will be released by a sequencing group until it had a reasonable time to explore its implications. It would not be unreasonable for the scientists concerned to ask, say, for a year delay before passing their results on to an openly accessible database. This is already the custom followed in the several labs that already have sequenced big DNA pieces (e.g., a viral genome). The 4×10^7-bp sequence of bacteriophage T7 DNA worked out at Brookhaven National Laboratory by Bill Studier and John Dunn only became available after the many months needed to first interpret it and prepare it for publication in an established journal. With the human genome, we could easily find that we are generating sequences faster than they can be scientifically interpreted by their finders, leading to apparently unacceptable delays in general release. So we as a nation may have difficulty in setting the limits of time that DNA sequences can remain the private property of those who find them. In trying to be fair to all sides, we must remember that the chief reason many labs will take on production-level sequencing is not high technology development but the desire to be the first to see the genetic messages for a given chromosome and to have the opportunity to exploit the countless surprises these sequences will present.

Clearly, it will be easier psychologically for a lab to release their own sequences

if they can be exchanged for others of equal dimension. Sharing of the human DNA database is much more likely to occur if large-scale mapping and sequencing efforts are undertaken by all those major industrial nations that will want to use these data. Now it is too early to ask what we should do if we identify one or more countries with the economic clout to join in but that apparently intend to free-load on the traditions of scientific openness of other nations. I do not like even contemplating such a possibility, since our Congress is bound to act outraged by such one-sided behavior and want to move us toward a more nationalistic approach to science. I would hate this alternative, since it is so counter to the traditions that have allowed me to admire and enjoy the scientific life. But, if the major nations in the world fail to see that the human genome belongs to the world's people, as opposed to its nations, then we may be in for a fight that no one of sense can want.

HIGHLIGHTS OF THE YEAR

DNA Learning Center Dedicated

The fantastic pace of discovery in the various fields of DNA science requires extraordinary measures to keep the general public informed. The first large-scale effort in the world was the dedication and formal opening of our DNA Learning Center in the village of Cold Spring Harbor on September 18th. Dr. Robert E. Pollack, former Cold Spring Harbor scientist and Dean of Columbia College at Columbia University, delivered the keynote address, entitled "Reading DNA," to a standing-room-only crowd in Grace Auditorium. He stressed the importance of public education in DNA, because of the vast potential impact DNA technology holds for the future.

Over 750 invited guests arrived that day to preview the Smithsonian Institution's exhibit *The Search for Life: Genetic Technology in the Twentieth Century*. David Micklos, Director of the Learning Center, and Jack Richards, Director of Building and Grounds, along with their staffs, performed miracles to get the Learning Center renovated and the exhibit installed in time for the opening. Since that date, there has been steady progress and improvement with the installation of an office suite in the basement and a one-of-a-kind bookstore. The bookstore and exhibit have been ably run by a team of local volunteers, directed by Mrs. Anne Meier and Mrs. Sandy Ordway. A new exhibit produced at the Learning Center, entitled *DNA Detective*, had its debut in spring 1989. This exhibit highlights and demonstrates the use of recent DNA technology for identification in various legal suits and for diagnosis of genetic disease. Several museums have already expressed serious interest in having their own *DNA Detective* exhibits.

Judging from the huge number of Learning Center visitors, from senior citizens to elementary school students, who have performed experiments in the *Bio2000* teaching laboratory and viewed the exhibit, I am convinced that we have set an impressive example for other institutions to follow.

Dr. Robert E. Pollack

Guest cabins under construction

Upper Campus Development Moves Ahead

The Upper Campus is starting to show hints of what it will look like upon completion. The first group of six guest cabins was finished in April of this year, just in time for the first spring meeting. Each of the Adirondack-style winterized cabins accommodates eight visiting scientists in four bedrooms and has a common meeting room and two bathrooms. The six cabins are interconnected by raised walkways that give them a small community feel. So far, scientists who have stayed in the cabins have reacted positively to this new on-grounds housing—not surprisingly, when compared to the alternative many-mile van trek to local motels. Tentatively, we plan to start the construction at a nearby site of a new group of six cabins in mid-1990.

Neuroscience Center Takes Shape

To make way for the 20 million dollar Neuroscience Center, we said goodbye to the L-shaped Page Motel. Named for Arthur W. Page, its two unheated wings had served us well for 32 and 35 summers, respectively, but in a mere 30 minutes, it was all reduced to a pile of rubble by an earth mover. Thankfully, the Page name lives on at the Laboratory in the Arthur W. and Walter H. Page Laboratory dedicated last year and now a home for the plant genetics program.

In place of the Motel, there now stands the first giant leap on our way to opening the Neuroscience Center. The 150-car two-level garage was completed this spring and will now act as a staging area for construction of the Beckman Neuroscience Laboratory and accompanying Dolan Hall. Presently an imposing structure, the garage will be much less conspicuous when completed; from most vantage points, the lower level will seem underground.

Major Gifts for the Neuroscience Center

Looking to complete the funding for the Neuroscience Center, we are elated to acknowledge votes of confidence from both local and national sources. The Dolan Family Foundation of Oyster Bay donated 2 million dollars for the Visitor Lodge,

Neuroscience Center under Construction

which will henceforth be known as Dolan Hall. This 60-room facility is anxiously awaited, as the soaring meetings attendance is sending greater numbers to area motels. The Dolan family is no stranger to the Laboratory. Mrs. Helen Dolan, a member of the Board of Trustees, and Charles F. Dolan, Chairman and CEO of Cablevision Systems, have both been dedicated friends of the Laboratory.

On the national scene, we were able to attract the attention of the highly respected W.M. Keck Foundation of Los Angeles. Their two million dollar grant will fund construction of the W.M. Keck Foundation Structural Biology Laboratory. This facility, located on the lower level of the Beckman Neuroscience Laboratory, will house the molecular structure analysis facilities. Using X-ray crystallography and other methods, researchers here will study in detail the three-dimensional structures of key protein and nucleic acid molecules.

I was particularly pleased to acknowledge the recent major gift from Mrs. Lita Annenberg Hazen, our newest trustee. To honor her support, the tower at the focal point of the Center's courtyard will be called Hazen Tower. No ordinary structure, Hazen Tower will fit in well with the character of Cold Spring Harbor Laboratory, with its open walls revealing a double-helical staircase at its core.

Cornerstones Dedicated

We heralded in the construction of the Beckman Neuroscience Laboratory and Dolan Hall with the dedication of two cornerstones on May 12, 1989. With the help of quickly installed ramps and walkways, we were able to hold the ceremony on the just-completed parking garage. Representatives from most major contributors were present, with the notable exception of Arnold and Mabel Beckman, who due to Mrs. Beckman's illness were unable to attend. Arnold Beckman had the foresight to realize the importance of the Center and made his critical contribution early in the development stage. I was saddened to hear a few weeks later that Mabel had passed away. My sincerest sympathies to Arnold.

Joseph Perpich of the Howard Hughes Medical Institute, Charles Dolan, and A. Terry Anderson were among the speakers. Ms. Anderson represented the Nassau County Executive's Office and the Nassau Industrial Development Agency, which helped the Laboratory acquire 20 million dollars in industrial development bonds

J. D. Watson (*r.*) at cornerstone laying

from Morgan Guaranty Trust Co. of New York to cover interim construction costs. As a keynote speaker, Dr. Robert Horvitz of the Massachusetts Institute of Technology, the only person ever to take three neurobiology courses at the Laboratory in one summer, remarked, "I believe we are laying the cornerstone today not only for a new building, but also for a new era in neuroscience and medicine."

Second Century Campaign Goes Public

Amidst the furious pace of construction, we also managed to embark on the first large-scale public appeal for support in the Laboratory's history. As of April 1989, we announced that 28 million of the 44 million dollar Second Century Campaign goal had been raised from our trustees, private foundations, and a few corporations. To raise the remaining 16 million dollars, we knew it would be necessary to expand our base of support.

On April 22, 1989, we kicked off this public phase of the campaign in grand fashion. Over 100 campaign volunteers and guests—some long-time benefactors, others new—gathered in Grace Auditorium to receive their task from David L. Luke III, whom we are grateful to have as chairman of the campaign. I doubt the Laboratory's fund-raising message has ever been put so simply and elegantly as when David said, "I think we can recognize that if we want to find answers for tomorrow for problems that cannot be solved today, there has to be commitment to organizations who are dedicated to pushing back the frontiers of knowledge. We are such an institution."

To aid the campaign volunteers, the two newest campaign tools, both highlighting the Laboratory's cancer research, were presented and well-received: "A Vision of Hope," a 12-minute video produced by Zebra Productions, and "The Good Fight," a full-color brochure written and designed by Daniel Schechter and Margot Bennett of our Public Affairs Office. Together, these will serve as an easy introduction for those unfamiliar with our work. Following the presentation, volunteers dined with some of the scientists in Bush Lecture Hall, which, thanks to clever decorating, became an elegant dining hall.

G. Morgan Browne and David L. Luke III

We Begin to Teach Courses in the Fall and Spring

Expanding our role as the world's largest center for postgraduate biology education, we have added four courses—two in spring, two in fall—to our already packed course and meetings schedule. Making possible these new offerings were the funds given to us two years ago specifically designated for teaching by the Howard Hughes Medical Institute. Our now seven-month respite from visitors will dwindle to only 5 months as we develop a more year-round program. The first of these new courses, "Macromolecular Crystallography," was offered last October and co-organized by Laboratory scientist Jim Pflugrath. Another organizer was Johann Diesenhofer, who just after the course ended learned he had been awarded the 1988 Nobel Prize for chemistry in recognition of his role in the elucidation of the three-dimensional structure of the photoreaction center responsible for photosynthesis. Taught largely in Bush, which proved a perfect spot for the needed large number of graphic terminals, this course will again be offered this year.

Two more courses were initiated early this spring, both of which also covered the newest experimental techniques. They were "Cloning and Analysis of Large DNA Molecules" and "Protein Purification and Characterization," the latter co-organized by Dan Marshak. This coming fall, the fourth new course will focus on *Schizosaccharomyces pombe*, an organism that has quickly jumped to the forefront of research on the cell life cycle and its control. Entitled the "Molecular Genetics of Fission Yeast," this course will be co-organized by David Beach.

Fifty-third Symposium Focuses on Cellular Signaling

The record-breaking attendance at the 1988 Symposium on the *Molecular Biology of Signal Transduction* verified the words of summarizer H.R. Bourne, who said, "The 53rd Cold Spring Harbor Symposium came at a perfect time." The more than 440 scientists who packed Grace Auditorium and spilled over into the closed-circuit viewing room in Bush saw 119 speakers lecture on areas from learning and memory, to signaling pathways, to cellular membranes, to gene expression and cell proliferation. Having been in attendance at the 26th Symposium on

Mechanisms of Cellular Regulation in 1961, it gave me great pleasure to see how far the world has come and also what lies just ahead. The fierce pace of lectures was briefly interrupted by a wonderful Dorcas Cummings Lecture on learning and memory by Eric Kandel, a Laboratory trustee and pioneer in the biology of learning. Using his favorite lab subject, the large sea slug, *Aplysia*, as an example, he gave the LIBA members and guests an overview of the chemical changes that occur during learning. This was followed by 21 of the traditional dinner parties for visiting scientists and friends at our neighbors' homes.

Timely and Exciting Banbury Conferences

In his first full year as Director of the Banbury Conference Center, Jan Witkowski has firmly established that Banbury meetings will continue to be up-to-the-minute, exciting, and, on occasion, controversial. As a result of a December 1988 meeting on the *Polymerase Chain Reaction*, a technology in its relative infancy, a Current Communications volume on PCR will be the first thorough treatment of this extraordinary procedure. The November meeting on *DNA Technology and Forensic Science* was attended by experts from all sides of the issues, including Alex Jeffreys, inventor of "DNA fingerprinting." The resulting Banbury Report is sure to be a seminal publication in the field. Early this year, our Congressional Aides' meeting in January on *The Ethos of Scientific Research* offered a rare chance for scientists and legislators to get together and speak their minds openly on fraud in science. The sessions ranged from philosophical debate on the nature of scientific research to the practicalities of dealing with misconduct. The discussions were vigorous, but by the end of the meeting each side got to see the other's position, if not sometimes agree with it.

Baring Brothers Co. conference at Banbury Center

Baring Brothers & Co. Hosts Business Leaders at Banbury

We were very fortunate to have Baring Brothers & Co., Inc., co-sponsor a meeting at our Banbury Center last October for senior executives. Douglas E. Rogers, a director of Baring Brothers, and I organized the meeting, which proceeded to be an overwhelming success for both the scientists and the corporate visitors. The executives from companies in the pharmaceutical, biotechnology, and related fields came not only to stay informed, but also to look for possible research collaborations and new venture ideas. Seemingly a miniature rehearsal for the upcoming 54th Symposium on immunology, nine of the world's leading immunologists gathered at Banbury to address some 30 company leaders. From allergies to autoimmunity, the experts brought the guests up to date on all the latest research findings. In addition, the executives "got their feet wet" in bench molecular biology, performing a DNA restriction analysis experiment at the DNA Learning Center. A similar meeting is already scheduled for this coming October.

Uplands Farm Purchased from Nature Conservancy

After several years of leasing the 12-acre Uplands Farm lot from the Long Island Chapter of the Nature Conservancy, we closed on a contract in May to purchase the land. Our Uplands Farm experimental station consists of a cytogenetics lab, planting fields, a research greenhouse tall enough for a winter corn crop, two residences, and fertile fields for growing maize. This, along with the dedication in 1987 of the Page laboratory, completes our several-year efforts to expand the plant research program, which focuses on the genetic manipulation of corn.

Strengthening of Our Administrative Capabilities

The number of scientists here at Cold Spring Harbor Laboratory has been steadily increasing as the new facilities provided by the Sambrook and Page Laboratories become fully utilized. At this time, there are 130 persons doing full-time science, a number that increased by a further 25 during this visitor-filled summer. The Lab's administrative center, the George Lane Nichols Building, is correspondingly a much busier site. Our Administrative Director Morgan Browne has recently strengthened our ability to handle this onrush of new business by appointing two senior administrators: Chuck Haibel, as Director of Purchasing, and Tony Napoli, as Director of Personnel. Chuck has spent his entire career in the purchasing field, most recently as the Purchasing and Facilities Manager for Praxis Biologics, Inc., a start-up biotechnology firm in Rochester, New York. Tony came from Gulf and Western Industries, where he was Vice President of Human Resources for a consumer products division.

By now there is no way we could have handled in Nichols the increasing tasks of our Development Office. Thus, last fall, we renovated the until now largely vacant basement floor of Wawepex for Konrad Matthaei and his staff of the Development Office. Graced by a new porch that faces the harbor, the suite of offices was finished just in time to make the final preparations to begin the Second Century Campaign.

Chuck Haibel, Konrad Matthaei, and Tony Napoli

Undergraduate Research Program

Despite the lack of National Science Foundation funding this past year, the Undergraduate Research Program (URP) continued to hold its own. We received 130 applications for a final 19 spots (the same as last year), two of which were filled by students from Switzerland and England. Without NSF funding, Winship

Herr had a harder time keeping the program, begun in 1959, running smoothly. But with help from the Alfred P. Sloan Foundation, the Burroughs Wellcome Fund, and the Robert Olney Fund, he was more than able to put together another excellent group of URPs.

LIBA Garden Party a Throwback to Bygone Era

Thanks to the unending efforts of George W. Cutting, Jr., who can seemingly always be seen giving a Laboratory tour to potential donors, membership in LIBA has soared now to 725 members. Many of the new members come as a result of a benefit garden party, held June 10th of this year on the Airslie lawn in the tradition of Marshall Field's benefit gala back in 1932. A warm summer evening greeted the 350 new friends of the Laboratory, all of whom received LIBA membership as part of their benefit donation. The party, organized by Mrs. Jane Greenberg, was followed by 26 dinner parties at local residences for all the guests.

At the LIBA annual meeting in January, Edward Pulling was elected as Honorary Director, after serving 20 years as a LIBA director and 17 years as chairman. His diligence and concern as chairman of our building committee can be seen in the new buildings and the face lifts of old ones that have come into existence over the past 20 years. I'm sure his work at Cold Spring Harbor is far from finished.

Last year's more than 300,000 dollars in contributions to LIBA made possible LIBA Fellowships for Joseph Colasanti, Bernard Ducommun, Ann Sutton, and Peter Yaciuk. In addition, the new Investigator Start-Up Fund awarded grants to Dave Frendewey and Tom Peterson. Remaining contributions went toward the Unrestricted Fund, giving the Laboratory most-needed additional resources to adapt to scientific progress.

Robertson Research Fund: A Key Endowment

It is exceedingly difficult for a young scientist to get government or other funding when he or she has no prior results to put in a grant application. For this reason, we created the CSHL Outstanding Junior Fellow Award with support from the Robertson Research fund, our biggest endowment. The award provides salary support for the Fellow, as well as a research technician. This past year, the awards sponsored Adrian Krainer and Carol Greider, who study RNA splicing and the enzyme telomerase, respectively.

Other major commitments of the fund were the full support of our Postdoctoral Fellows, one Visiting Scientist, and two graduate students. The plant research program and X-ray crystallography group also received funds while they were in the process of securing independent research support. And Robert Franza received invaluable assistance from the Fund to bridge the gap between his two NIH awards. Robertson monies also helped keep the formal seminar program running by providing backup honoraria and travel support for otherwise unfunded speakers.

Michael Wigler Elected to the National Academy of Sciences

Citing his "distinguished and continuing achievements in original research," Michael H. Wigler was elected this year into the prestigious National Academy of Sciences. Although his scientific accomplishments are many, he is probably best known for his co-isolation of the first human oncogene. In 1981, building on work he started

at Columbia University, Mike and his associates at the Laboratory searched the DNA of human cancer cells and found the *RAS* oncogene that had helped give rise to a prostate cancer cell. Later they showed that this oncogene arose through a single nucleotide change in the sequence of a normal *RAS* gene that codes for a GTP-bound membrane protein involved in signal transduction. Since 1981, Mike's research team in Demerec Lab has discovered several additional important oncogenes and key clues to how these genes function. Today, they also intensively study the functioning of the RNA gene equivalents in simple yeast cells.

Dr. Michael H. Wigler

1988 Changes in Our Scientific Staff

We keenly felt the loss of two Senior Staff Scientists, James Feramisco and Amar Klar, both of whom served the Laboratory with distinction for ten years. In 1978, Jim, who was working on his Ph.D. at the University of California, Davis, attended a seminar given by me there on changes in cell architecture that occur with malignancy. Soon after, he wrote me for a postdoctoral position in the cell biology group and came the next year to McClintock Lab. Within three years of his coming, he became head of the cell biology research group and, in 1984, a senior scientist. Jim now is at the Cancer Center of the University of California Medical School, San Diego, where he continues to do research on the biochemistry of oncogenic proteins.

Amar Klar also departed this year. Fresh from a postdoctoral period at Berkeley, Amar joined Jim Hicks and Jeff Strathern in 1978 to form the yeast genetics group, in the then Davenport Lab, that so revolutionized our ideas on how yeast cells switch their sex. Their cassette model is increasingly realized as one of the great achievements of 20th century genetics. Amar now continues his high-powered genetics approach at the Frederick Cancer Research Facility in Maryland.

Senior Staff Investigators William Welch and Mark Zoller both accepted new positions in San Francisco, California. Bill, whose research at Cold Spring Harbor focused on the response of mammalian cells to physiological stress, now is Associate Professor of Medicine at the University of California, San Francisco. At the Department of Cardiovascular Research, Genentech, Inc., Mark now works on the development of second-generation tissue plasminogen activator (TPA) for dissolving blood clots.

Leaving their positions as Staff Investigators were Ashok Bhagwat, from Rich Robert's lab, who is now Assistant Professor of Chemistry at Wayne State University in Detroit, Michigan, and Daniel Broek, from Mike Wigler's lab, who is Assistant Professor at the Comprehensive Cancer Center, University of Southern California, Los Angeles. Also leaving Mike's lab was Scott Powers, who is presently Assistant Professor at the Robert Wood Johnson Medical School, Department of Biochemistry, Piscataway, N.J. Margaret Sharma, who worked with Terri Grodzicker, accepted an appointment as Assistant Professor at the University of Tennessee Health Science Center in Memphis.

New Staff Members

Newly appointed to positions as Senior Staff Investigators are Arne Stenlund and Kim Arndt. Arne, whose Ph.D. was awarded from Uppsala University in Sweden, completed his postdoctoral work with Mike Botchan in the Department of Molecular Biology at the University of California, Berkeley. He works in James Lab and is particularly interested in the replication and transcription of bovine papillomaviruses. In 1981, Kim received his Ph.D. in biochemistry from the

University of Pennsylvania and then worked with Gerry Fink at the Whitehead Institute at the Massachusetts Institute of Technology before joining us in the early part of this year. He is studying the interactions of proteins with DNA at the molecular level, combining his expertise as a biochemist with the techniques of today's yeast genetics.

New Visiting Scientists

With the arrival of Nicholas Dyson, the joint program between Ed Harlow's laboratory and Amersham International to produce monoclonal antibodies for research purposes was ensured a smooth transition.

It seems most appropriate that in this year of "glasnost," we have four scientists from that USSR visiting us. Konstantin Galactionov, a native of Leningrad, is on leave from that city's Academy of Science, the Institute of Cytology. He is working with David Helfman in McClintock Laboratory on cell biology and molecular cytology. Valentin Shick and Grigory Yenikolopov come to us from the Institute of Molecular Biology at Moscow's Academy of Science, and Mart Ustav comes from the Molecular Biology Institute of Estonia.

Departing Visiting Scientists

After completing their studies at the Lab, several visiting scientists departed for new institutions or returned to former ones. Andre-Patrick Arrigo accepted a position as Professor at the Institut National de la Sante et de la Recherche Medicale (INSERM) at Montpelier, France. Barbara Knowles and Davor Solter, both Professors at Wistar in Philadelphia, returned to that institute after spending their sabbatical leaves here. A native of Lithuania, Donaldas-Jonas Citavicius returned home after working in Michael Wigler's laboratory for six months. Margaret Raybuck, who worked with Ed Harlow in the monoclonal antibody facility, went back to Amersham International in England. After spending their sabbatical leaves with us, Steve Munroe and Paul Young, both Associate Professors in biology, moved on: Steve to Marquette University in Milwaukee and Paul to Queen's University in Kingston, Ontario, Canada.

Staff Promotions

At the end of the year, the Laboratory's Trustees approved the promotions of David Beach and Ed Harlow from Senior Staff Investigators to Senior Scientists, or "Rolling-5" rank. A native of England and a graduate of Cambridge University, David received his Ph.D. in 1977 from the University of Miami in Florida studying developmental neurobiology. During the last five years at the Laboratory, he has focused his studies on eukaryotic cell-cycle control in fission yeast. Recently, he has expanded the scope of that work considerably to include research on the molecular basis of cell-cycle control in mammalian cells. In 1982, Ed, coming from the University of Oklahoma, completed his doctorate in biochemistry at the Imperial Cancer Research Fund, London. Instrumental in setting up the new monoclonal antibody facility in Sambrook Lab, he has developed a host of antibodies to study adenovirus E1A proteins and the human p53 tumor antigen. This year, Ed and his research 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.

Guilio Draetta and Elizabeth (Betty) Moran, formerly Staff Investigators, have accepted positions as Senior Staff Investigators. A native of Italy, Guilio completed

his M.D. in 1981 at the University of Naples, and then stayed on to pursue postdoctoral studies. In 1983, he went to the National Institutes of Health in Bethesda, Maryland, where he worked at the National Cancer Institute prior to accepting an appointment as Robertson Fellow at Cold Spring Harbor in 1986. Since then, he has worked with David Beach in Demerec Lab on cell-cycle control in yeast and vertebrates. Betty came to us in 1983 as a postdoctoral fellow in Mike Mathews' lab, after completing her doctorate in microbiology at the New York Medical College. She studies the role of adenovirus E1A protein in regulating transformation and viral transcription.

Accepting a new position as Staff Investigator is Jeff Kuret, formerly a postdoctoral fellow working under Mark Zoller. In 1984, Jeff completed his Ph.D. in pharmacology at Stanford University in California. After two and a half years of postdoctoral studies with Phil Cohen at the University of Dundee in Scotland, Jeff came to Cold Spring Harbor to continue work on protein kinases.

Newly designated Staff Associate Maureen McLeod joins only two other such appointees at the Laboratory. Created last year, the position provides a transitional stage between the postdoctoral period and an independent staff position. The postdoctoral fellow's potential for technical proficiency, scientific creativity, and independent thought is emphasized. Maureen, who received her Ph.D. in 1984 from SUNY, Stony Brook, works with David Beach on cell-cycle regulation.

Postdoctoral Fellows

Leaving the Laboratory after completion of their postdoctoral terms were: Susan Alpert to the University of California Medical School, San Francisco; Thomas Baumruker to Sandor Research Institute, Austria; Vicki Bautch to the University of North Carolina, Chapel Hill, North Carolina; Robert Cafferkey to the Scripps Clinic, La Jolla, California; Steven Cheley to the Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts; Roger Cone to Tufts University School of Medicine, Division of Molecular Medicine, Boston, Massachusetts.; Shimon Efrat to the Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, New York; Michaela Fairman to the Cancer Research Campaign (CRC), Molecular Embryology Group, Department of Zoology, Cambridge, England; Joan Harper to the Biological Sciences Department at Columbia University, New York; Christine Jolicoeur to the University of California, San Francisco; Christa Lechelt to the Max Planck Institute in Germany; Brenda Lowe to the U.S.D.A. Agricultural Research Service, Albany, California; Lee Mizzen to the Lung Biological Center at the University of California, San Francisco; Avudaiappan Maran to the Long Island Jewish Medical Center, New Hyde Park, New York; Peter Whyte to the Fred Hutchinson Cancer Research Center, Seattle, Washington; Gregory Prelich to the Department of Genetics, Harvard Medical School, Cambridge, Massachusetts; Shreenath Sharma to the University of Tennessee Health Science Center, Memphis; Vijay Sharma to the Department of Civil Engineering, University of Manitoba, Winnipeg, Canada; and Paul Walton to the University of California, San Diego.

Graduate students Paul Chomet and Carmela Stephens completed their doctoral dissertations; Paul went to the Department of Genetics at the University of Berkeley in San Francisco and Carmela to the Children's Medical Hospital in Boston. After receiving his doctoral dissertation with Mike Wigler, Scott Cameron entered the Massachusetts Institute of Technology–Harvard Medical School Program in Cambridge.

Employees Recognized for 15 or More Years of Service

In 1988, we were pleased to acknowledge the contribution of valued employees who have worked at the Laboratory with such great dedication and enthusiasm. In recognition of 15 or more years of service, momentos were presented to the following: Carol Caldarelli, housekeeper; Susan Cooper, Director of Libraries and Public Affairs; Barbara Cuff, buildings and grounds secretary (now retired); Nancy Ford, Director of Publications; Terri Grodzicker, Assistant Director for Academic Affairs; Doug Haskett, plumber; Laura Hyman, Assistant to the Director of Libraries; Jack Richards, Director of Buildings and Grounds; William Keen, Comptroller; John Maroney, Assistant Administrative Director; Phyllis Myers, senior laboratory technician; Richard Roberts, Assistant Director for Research; Madeline Szadkowski, McClintock Laboratory secretary; Hans (Buck) Trede, grounds foreman; and Barbara Ward, meetings and courses Registrar. Awards will be given each future year to staff members reaching 15 years of service.

Our Very Effective Board of Trustees

As our board members work with me in preparing for our 1990 Centennial Year, I realize how fortunate I and the Laboratory are in having the backing of such distinguished men and women. They never cease to amaze me with their intelligent, loving enthusiasm for our many diverse activities. Sadly, the conclusion of two consecutive three-year terms as trustee made it necessary for Ralph Landau to leave our board. Few individuals equal Ralph in his achievements as a creative engineer, industrialist, and academic economist, and we are indeed fortunate that we have had his penetrating mind and warm heart on our side. Happily, Ralph's position on our board will be taken over by his talented daughter, Laurie, also a resident of nearby Asharoken, who, after acquiring both veterinary and business degrees at the University of Pennsylvania, has become an expert in aquatic veterinary medicine and helps run a summer program in this area at the Marine Biological Laboratory in Woods Hole. Leaving also at the conclusion of his most recent two consecutive terms was Townsend Knight, who carries on the distinguished legal career of his Jones' family forebears and whose seasoned advice we increasingly count on in this age of litigation. And for the same statutory reasons, we lost the invaluable services of Mary Jean Harris, who played an important role in the founding of the DNA Learning Center. We have also lost the dedicated help of Harvey Sampson, a trustee since 1984, who, because of the change in his main residence from Cold Spring Harbor to St. Thomas in the Virgin Islands, thought it appropriate to resign this past winter. We thank him for his many contributions of time and support and hope he will somehow continue to find other ways to improve our existence.

Joining the board last year was W. Maxwell Cowan, the Vice President for Research at Howard Hughes Medical Institute. Max, a distinguished anatomist and neurobiologist, has been involved with our neurobiology program since its inception in the early 1970s and made an unforgettable presentation at our 1987 Shearson Conference on "The Brain" where he produced an intact human brain for examination. Our newest trustee, elected this May, Lita Annenberg Hazen, has also long been involved with neurobiology. She founded the Neurosciences Institute at the Rockefeller University, as well as being the sponsor of a distinguished series of yearly meetings on neurobiology that attract neuroscientists of the highest distinction.

Our Scientific and Administrative Staff Lets Me Shuttle Back and Forth between My Two Jobs

I would have never dared accept the genome job at NIH was it not for the fact that our Lab is essentially in very good scientific, physical, and financial health. So, I go back and forth to NIH not fearing that my absence is seriously harming our efforts. But others must work harder to compensate for my inability to be at my desk. Rich Roberts has done a first-class job as our Assistant Director for Science, keeping our scientific budget balanced and seeing that we continue to recruit the new scientists that do not let us go stale. And Terri Grodzicker, our Assistant Director for Academic Affairs, oversees most competently our ever-changing series of meetings and courses, simultaneously acting as the main editor of our fast-growing *Genes and Development* journal. Equally important, I continue to know that Jack Richards, as Director of Buildings and Grounds, performs far beyond the call of ordinary responsibilities, seeing that our buildings and grounds are attractive and functional despite the great burden he bears in keeping our 20 million dollar neurobiology construction project almost invisible to all.

Equally important has been my reliance on Susan Cooper, who does an extraordinarily good job in running our Public Affairs Office. Her vast experience and competence let her present our activities to the outside world in ways that please instead of embarrass. She has also put together the very competent staff that makes her offices in Grace so reassuring to be in.

Likewise in tip-top shape are our administrative offices headed by Morgan Browne, who over the past four years has most competently presided over our transition from an institution that was beginning to aspire to be first-class into one that now knows how to act like one. At this moment, Morgan, working with our most effective comptroller, Bill Keen, handles more than 70 million dollars in our various accounts—40 million dollars of which are endowment funds, an unimaginable sum to all when I arrived as the Director 21 years ago. Then we had an endowment of only 20,000 dollars, about all of which legally belonged to LIBA.

Enjoying the Beauty of Bungtown Road

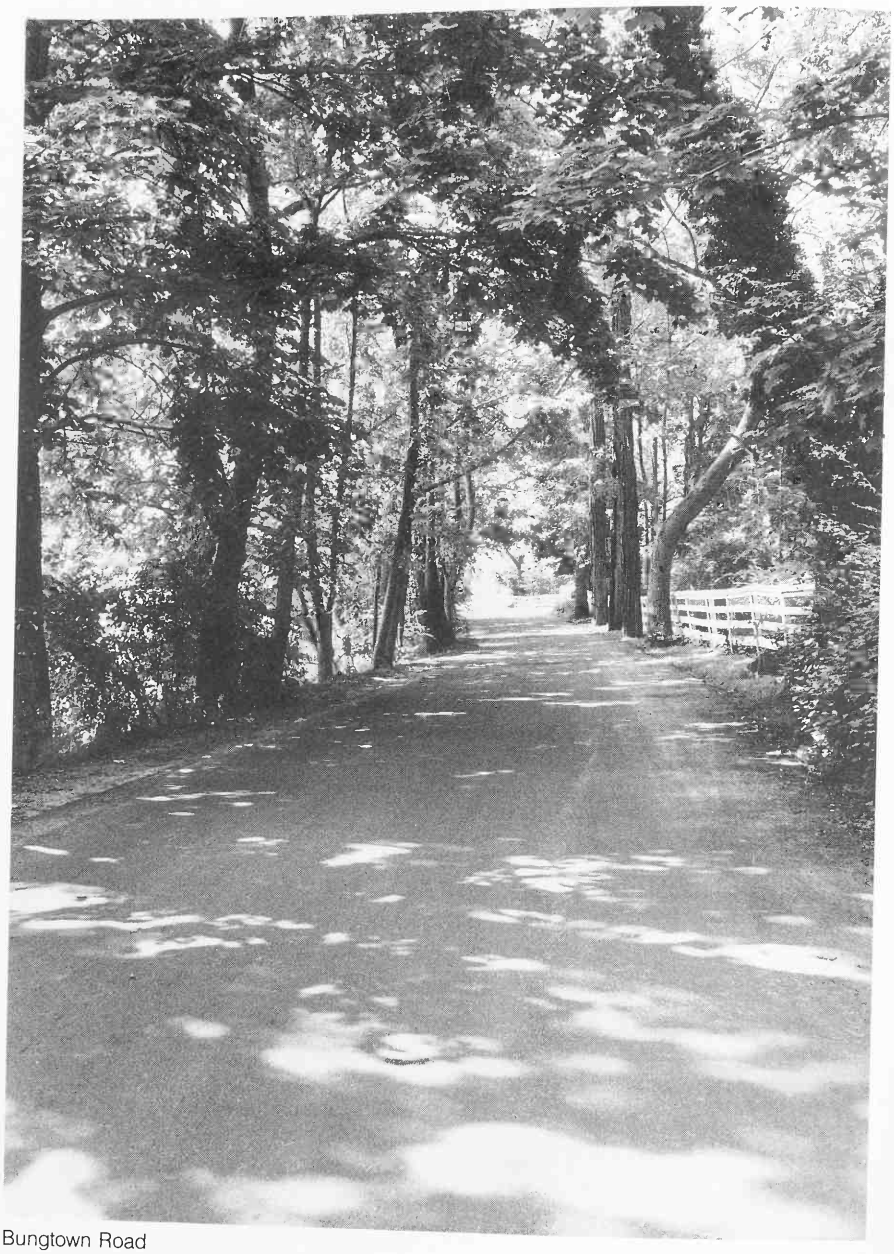
A prime goal of mine for many years has been to restore the inherent beauty of Bungtown Road, along which much of our Laboratory stretches. When I first came here in the summer of 1948, this then sylvan path had a rural quality that reflected what Long Island must have been like in the 19th century. Since then, our immediate part of Long Island has lost its potato fields and all too many of its parkland-like great estates. Our immediate environment along the inner harbor fortunately remains unspoiled, and we have the potential for looking almost as perfectly tranquil as we were during the whaling ship days. But increasingly defacing our appearance has been the ever-increasing number of telephone poles, which grew larger in size and number as we increased the pace of our science and teaching. So, I have long dreamed of putting all of our communications and electrical wiring underground and then to repave Bungtown Road, most of which had become a giant pothole. Happily, these jobs at last are done, and I now can walk to and from my house thrilled by beauty similar to that which must have existed when there were sheep on our fields to produce the fleece for the woolen mill that once existed at the intersection of Bungtown Road and 25A.

I know there is talk that as I once commuted between Harvard and Long Island,

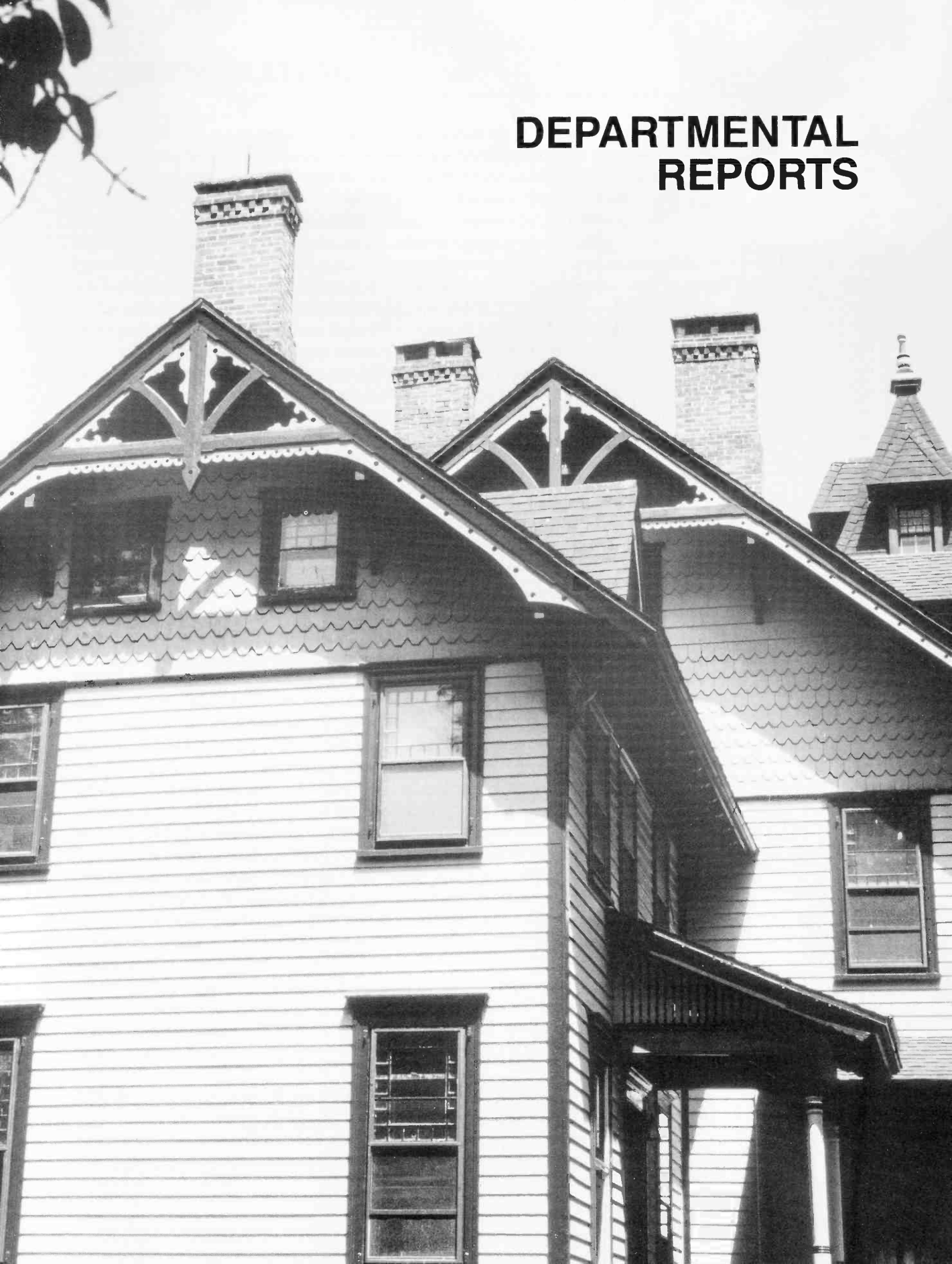
only to eventually move full-time here, my days on the PanAm Shuttle portend my going on full-time to Washington. But a Bungtown Road restored and the new pond beside it make my every return here a delight. No one need further imagine that the corridors of real power will tempt me.

August 2, 1989

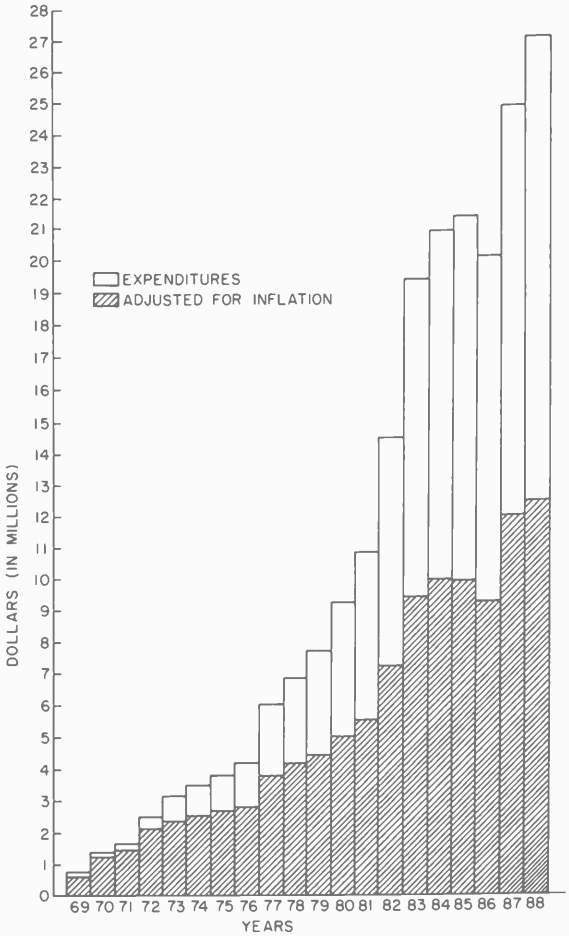
James D. Watson

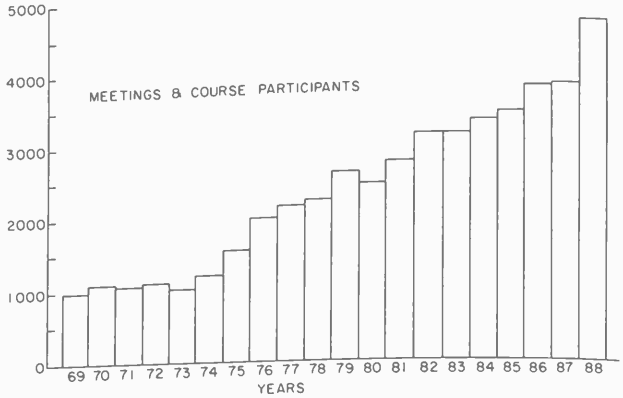


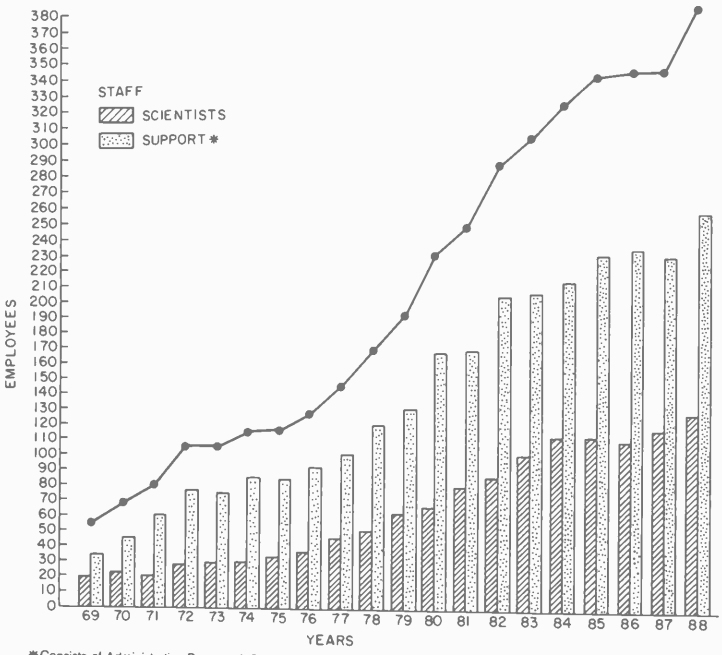
Bungtown Road



DEPARTMENTAL REPORTS







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

ADMINISTRATION

The past year was a good one financially for the Laboratory as revenues once again comfortably exceeded expenses before depreciation. For the first time, four NIH-supported Program Project Grants—for research on cancer, oncogenes, and HIV and for core support—were in place for most of the year. The goal of fully funding depreciation, however, remained elusive. This future cost of replacing facilities and equipment will increase as the present capital programs are completed and technological obsolescence of equipment continues to accelerate. It is extremely important that we provide adequately for these requirements.

The overall financial position of the Laboratory is strong. The value of endowment and similar funds rose from $31.3 million at year-end 1987, to about $36 million at the end of 1988, and to nearly $40 million by mid-1989. The appreciation has been the result of substantial reinvested income, good market performance, and additional contributions. These funds reduce the Laboratory's dependence on the "soft money" of short-term government grants. They contribute greatly to our independence and ability to recruit and maintain a world-class scientific staff.

In many respects, 1988 was an extraordinary year at the Laboratory. The research was unusually productive even by past standards, and the education programs, already unique in the world, continued their rapid growth. Construction of the Neuroscience Center began, the DNA Learning Center opened, our first ever capital campaign was launched, and Dr. Watson assumed leadership of the national effort to sequence the human genome. The administrative departments supported all this and should feel great pride and satisfaction in their accomplishments.

Meetings and Food Service deserve special recognition for their handling of the large increase of visitors attending the many functions here. This past spring, attendance at meetings had to be limited because of capacity restrictions. This is a period of transition when many of our infrastructure facilities, such as overnight housing and kitchen and dining, have become seriously overtaxed. New or refurbished facilities are just now becoming available, as is the case with our six new guest houses providing overnight accommodations for 48 scientists. Other facilities, such as Dolan Hall, are in the construction phase. Some are still being planned, as is the expansion and refurbishment of Blackford Hall. Older facilities such as the Page Motel have been demolished to make room for new construction. Barbara Ward, Maureen Berejka, Jim Hope, and their staffs now organize, house, and feed more than 5000 visitors annually with good grace and a minimum of inconvenience. But they look forward to the day when all of our infrastructure facilities will be on a par with the comfort and convenience of Grace Auditorium.

One cannot speak of Cold Spring Harbor or Banbury Center meetings without noting the superlative job Herb Parsons does with audiovisuals. There is no service at the Laboratory that is performed better or more reliably or that is so widely praised by the visiting scientists who depend on Herb for their presentations.

Susan Cooper assumed the leadership of Public Affairs this past year, in addition to being Director of the Library. The visibility and reputation of the Laboratory were further enhanced in local and national print and television, always with taste and sensitivity. Supporting the "Second Century" capital drive, Susan's

staff produced the excellent *Good Fight* brochure, which describes cancer research at the Laboratory. Collaborating with Zebra Productions, they fashioned a wonderful new video that highlights the Lab's scientists describing their own feelings about doing science and living at Cold Spring Harbor. The *Harbor Transcript* newsletter was given a new look, greater frequency, and a larger circulation. The DNA Learning Center opening and the Neuroscience Center cornerstone ceremony were great successes, and planning moved ahead for the many forthcoming centennial events.

1988 was Tony Napoli's first year heading the Personnel department. Under his direction, the personnel staff computerized and updated the personnel records and brought order to salary administration. Management of on- and off-grounds housing was also improved. Perhaps most importantly, they have enhanced the sense of community and caring at the Laboratory. Chuck Haibel, also in his first year at the Laboratory, brought new spirit and professionalism to the Purchasing department and modernized and improved shipping and receiving. The new Development department, under Konrad Matthaei, gave new impetus to both annual and capital giving, as described elsewhere in this Report.

Bill Keen deftly managed the transition to a much faster MAI Basic Four business computer with higher memory capacity. Already, management of the operating budget has improved. The arrival of Barbara Wang permitted Bill to spend more time managing cash and preparing the long-term forecasts and planning documents needed for the Lab's expansion programs. This past spring, a $20 million issue of tax-exempt Civic Facility Revenue Bonds was completed through the Nassau County Industrial Development Agency. Secured by a letter of credit from Morgan Guaranty Bank, the Bonds are rated AAA by Standard and Poors and assure the Laboratory the lowest possible interest rate on borrowed funds. They provide great flexibility in managing the cash flow and timing of our large capital construction program.

In recent years, commercial relations have become increasingly important at the Laboratory, and John Maroney has been most effective in supervising and managing these activities. He has been particularly helpful in acting as liaison between the scientific staff and our patent attorneys and in processing inquiries from commercial interests regarding licensing and research support arrangements. Recently, he shaped and drafted the Laboratory's new Professional Misconduct Policy, now required by Federal Granting Agencies. Besides helping enormously as my assistant, John has also pursued a law degree at Touro College, and we congratulate him on his graduation in June.

Jack Richards, Art Brings, and Susan Schultz are others we could not do without. Nor could the Board of Trustees and its 14 committees function without the very careful and personal attention of Roberta Salant.

The expansion of the Laboratory is an exciting but considerable challenge. Additional staff will soon be required for whom we have no physical space. Additional funds will be needed, the sources of which are not yet identified. There are programs and facilities to be designed which are now barely in the conceptual stage. Nevertheless, I am sure that the administrative departments will get the job done, and we should view the future with anticipation and not alarm.

G. Morgan Browne

BUILDINGS AND GROUNDS

Maintaining the physical plant is becoming more challenging as the Laboratory grows in both size and complexity. The Buildings and Grounds Department has perservered to create a pastoral environment for research and to have a facility that functions effectively on a daily basis. Each day, all of our trades respond to many problems, including leaks, lack of heat or air conditioning, and telephone repairs. Our workers strive to repair all of our systems so that the important work of the Laboratory is not disrupted. The Department has also updated mechanical systems in an effort to improve reliability.

Neuroscience Center

One of the several milestones achieved during 1988 was, most notably, the groundbreaking for the Neuroscience Center. The project is divided into three phases of construction: Phase 1, Excavation and Demolition; Phase 2, Parking Garage and Site Work; and Phase 3, Laboratory and Lodge. Designs for the complex were finalized and put into a construction documentation format so that bids could be obtained. The contractor selected for Phases 1 and 2 is A.D. Herman Construction Company, who was also contracted for the construction of Grace Auditorium. Nasco Associates, Inc., an outside cost-consulting firm, has been employed to keep track of all the expenses.

Another milestone was the demolition of the 33-year-old Page Motel, located on the site of the Neuroscience Center. In preparation for the bulldozer, the primary electrical loop from the Buildings and Grounds office to the Treatment Plant was completed so that the loop that ran underneath the Page Motel could be disconnected. In October, a 30-ton bulldozer was brought onsite and within 45 minutes, the motel met its demise. In the weeks to follow, the landscape was changed into a barren crevasse, and by year's end, the back west retaining wall for the parking garage and most of the columns were in place.

Log Cabins

On the hill overlooking the site of the future Neuroscience Center is the new complex of six heated log cabins. Construction of the cabins was begun in March. Each cabin will contain four double bedrooms, two baths, and a common day room. With the help of an outside contractor, the exterior of the cabins, including the roof, was completed. Our carpenters then began work on the interiors, including the insulation, ceilings, trim, and cabinets. Coordinating with the carpenters, our plumbers and electricians began to install piping and wiring, and our painters began the staining and painting. While construction was going on, the Grounds crew worked on the landscaping, and Wendy Hatch and the Building Commission designed the interior blinds, curtains, bedspreads, and paint colors. All six cabins will be completed for the 1989 Spring meetings.

Other Alterations and Renovations

Demerec: Because of badly deteriorating copper pipes, the Demerec well water loop that heats and cools the building was completely repiped by our plumbers. This massive project was completed in 10 weeks and has resulted in much less time spent for repairs.

Wawepex: The lower level of the Wawepex building was completely reworked to accommodate the new Development Office. The design included a new porch on the east side and a new stairway to the Blackford lawn and the Buildings and Grounds Office.

Grace Auditorium: Plans for the lower-level bookstore and office conversions were designed and executed. Some of the boiler room equipment was relocated to make room for the bookstore, and construction was completed on schedule.

Delbrück: The Delbrück Teaching Laboratory was completely renovated. The furnishings were removed and replaced with new benches, bookshelves, and equipment. The walls were painted, and air-conditioning and heating systems were installed.

Uplands Farm: The heating controls in the Uplands greenhouse have been improved to provide for more accurate temperature control. In addition, a new tractor, pickup truck, and farm implements were purchased for the Farm Research Group.

DNA Learning Center: The auditorium area at the Center was redesigned and renovated to accommodate the "Search for Life" exhibit from the Smithsonian Institute. The project included the creation of a bookstore, a holding room, basement offices, and laboratory and storage areas. In addition, a new heating and cooling system was installed, the electrical system was rewired, and two new control panels and a transformer were installed. The project was completed just in time for the opening of the exhibit in September.

A Word of Thanks

Buck Trede deserves much praise for a job well done in keeping the grounds looking attractive even though we keep digging holes and trenches in every corner of the Lab grounds. The custodial department is performing better than ever thanks to Danny Jusino, who took over as our Custodial Foreman.

We wish to extend our heartfelt thanks for the fine service of two of our employees who left during the year: Barbara Cuff and Willie Gardner. Barbara, who was our office secretary for more than 18 years, was replaced by Dottie Smith, a most vivacious and well-organized person. What a lucky break for us! Willie, who was our Custodial Foreman, departed from our doors after having been at the Lab for over 6 years. We miss them, and we wish them much happiness.

Jack Richards

DEVELOPMENT

To address the growing financial requirements of the Laboratory, the development function, formerly operating as a part of the Public Affairs Department, was given its own identity. Charged with increasing our support from the private sector,

both by expanding *annual* contributions and by directing and coordinating the Laboratory's first *capital* drive, the Development Department was established as a separate entity early in 1988. By June, we had set up shop on the ground floor of Wawepex, the building Mr. Jones built in 1850 to serve as a granary for his whaling fleet.

Our *Annual Giving Program* is sponsored by the Long Island Biological Association under the very able direction of Chairman George W. Cutting, Jr., and its 28 directors. This organization of "Friends of the Laboratory" is responsible, through its Associate Program and its membership, for the largest source of unrestricted annual giving. (A complete report of their activities may be found in *Financial Support of the Laboratory*, later in this Annual Report.) The Corporate Sponsor Program is an important source of restricted funding for the Laboratory, as we share the latest biotechnical information with some 30 leading corporations. (A report of this Program also appears later in this Annual Report.) As our facilities and staff expand, in order to maintain our position among the preeminent basic research institutions in the world, it is imperative that we expand our base of annual support by upgrading our own gifts and introducing many more friends to this remarkable "village of science."

In 1985, Dr. Watson and the trustees gave considerable thought as to what would be needed to keep Cold Spring Harbor Laboratory in the forefront of basic research and education in the field of molecular biology for the *second century* of our existence. Because capital campaigns are pivotal points in the life of an institution, indicating the direction of future growth and vitality, the trustees decided to embark on a capital campaign coinciding with the Laboratory's centennial. *Capital campaigns* are quite distinct and apart from *annual giving programs*, in that they are mounted to meet specific new needs of an institution that cannot be met through its annual operating budget.

Trustee Treasurer, and Chairman of Westvaco, David L. Luke III was selected to chair the first public capital campaign ever undertaken in the Laboratory's 100-year life, the Second Century Campaign. Under his thoughtful and careful guidance, planning continued throughout 1985, and in January of 1986, the private solicitation of leadership gifts from trustees and foundations and from corporations closest to us was begun. The Campaign goal is to raise $44,000,000 in gifts and pledges by December 31, 1991: $31,500,000 for facility construction and renovation, $10,500,000 for staff and student endowments, and $2,000,000 for program and unrestricted endowment.

At year's end, through the prodigious efforts of Mr. Luke, Dr. Watson, Morgan Browne, and the trustees, over $28,000,000 had been given or pledged to the Second Century Campaign and plans were well along to bring the Campaign to the public. (Campaign contributions are listed under Financial Support of the Laboratory.)

We thank again and again all those who have given so generously to the Campaign, and we urge those who have not yet made a commitment to consider as handsome a gift as possible. (Methods of giving either outright or in trust or by will are outlined under Financial Support of the Laboratory.) Your generous support honors our distinguished reputation and ensures our vigorous future.

Konrad Matthaei

LIBRARY SERVICES

Staff Changes

In February 1988, I was given responsibility for the Office of Public Affairs, and I continue as Library Director, doing budgeting, personnel, and original cataloging. Fortunately, I am blessed with a staff that has been willing to tighten the reins. Genemary Falvey, head of library services, has taken on additional day-to-day responsibilities. She is handling the frontline information needs in an exemplary fashion. As Genemary absorbs more of the work, so must her highly motivated staff. Wanda Stolen, a Laboratory veteran of 5 years, does all online interlibrary loans and bindery preparation like a professional, while handling other public and technical services. Jeannette Romano, who has been with us since August, is responsible for acquisitions, claiming materials not received, and informational references.

Celebrating more than 15 years of service, Laura Hyman continues to demonstrate her ability to adapt to change. Working for two incredibly challenged departments, Laura handles the finances for both departments, shares events planning for Public Affairs, and coordinates archives and records management for the library. She continues in the library, training, structuring special projects, and helping as needed. Supporting Laura's expanding role, Lynn Kasso ably handles all library correspondence. She is the key operator for the labwide mailing list (CLAM) and organizes archives, fulfilling all requests for historical materials.

Archives Renovation

The library attic was completely renovated to house the Laboratory Archives, which includes the Carnegie Collection of historic books and journals. In addition, two major gifts to the Laboratory, the Harris and Fricke collections, have been cataloged for the Archives. The Harris collection was recently augmented by additional gifts from Mrs. Noelle Glenn, who purchased the Harris/De Tomasi house. The gifts include sculpture, paintings, and prints by Jane Davenport Harris De Tomasi. Jane Davenport's work will be exhibited during the Cold Spring Harbor Laboratory Centennial.

Rearrangement of Library Collection and Offices

In September, our current book and reference collections were moved to the lower level of the library, providing needed quiet study space on this level. A VCR and monitor were installed in Reference Room I, thanks in part to a generous gift from Mr. Henry Platt. A collection of videotapes, including some of the staff seminars, has been assembled. To provide additional study space on the main level, the offices of the Library Director were moved to the second floor. Genemary Falvey and the library assistants remain on the first floor. The second floor now also houses the Marketing and Journal Departments, the office of the Director of Publications, and the library administrative offices. These new arrangements have made operations more efficient for all of the departments involved.

Reference Services Thriving

Automated interlibrary loan service continues to provide rapid retrieval of requested books and periodicals. The use of our computerized search service

increased by 81% in 1988. The annual report collection was expanded this year to include many more businesses and institutions to aid the Development Office in their fund-raising effort.

Storage Facility

Our storage facility continues to function very well, with overnight retrieval of requested volumes. In emergencies, same-day retrieval is possible. Although the number of volumes requested increased by 148%, some titles have not been accessed since we opened the storage facility in 1985. Accordingly, we are withdrawing these titles from our collection to make room for other inactive titles and to give us much needed shelf space in the main library. Space continues to be the major drawback in library service. The future must bring a serious plan for expansion.

Permanent Collection Remains Stable

In 1988, our monograph and journal collections increased by only 1%. This was achieved by judicious trimming and updating of the current book collection. Careful examination of the journal renewals and cancellation of some titles allow the purchase of others to keep pace with the Laboratory's changing directions.

Susan Cooper

PUBLIC AFFAIRS

Public Affairs at Cold Spring Harbor Laboratory had its official inception in 1983 under the able direction of David Micklos. Initially the stepchild of the Library and Publications departments and labeled "Information Services," it evolved over a period of five years to encompass fund raising, media relations, audiovisual/ photographic services, and public relations materials. A new tone was set: A forum for communication with the Laboratory's constituencies was established, necessitating a wide range of expanded services.

Department Structure Changes

Structural and staff changes were numerous in 1988. In February, David assumed full-time responsibility for the DNA Learning Center, while Susan Cooper coupled her library and events-planning assignments with media relations and public/community affairs. The Corporate Sponsor program was passed to Jan Witkowski at the Banbury Center, and fund-raising activities were coordinated under Konrad Matthaei, Director of Development.

A New Staff for Public Affairs

Since the written word is the focal point of public relations activities, we were pleased to hire a talented science writer, Daniel Schechter, a graduate of MIT's writing program. In 1988, he was responsible for most of the text of three *Harbor*

Transcripts, Visitor's Guide to the Search for Life, The Good Fight, various brochures for public affairs and development, as well as a dozen or more press releases. In addition to preparing copy, Dan has handled the editorial and design functions necessary to present these materials. In June, Emily Eryou left the Library to join us as public affairs assistant, replacing Ellen Skaggs. Emily is an extremely capable editor, as well as a writer in her own right. Along with managing the day-to-day activities of the department, she created the Lab-wide calendar and wrote the 1988 Undergraduate Research Program report and articles for the *Harbor Transcript*.

Sue Zehl, who joined the staff in 1985 and established a design concept to take the Laboratory into its second century, resigned her position to pursue exhibit design at the DNA Learning Center. However, during the year, several new pieces were created: the first four-color poster for the 1989 Symposium on *Immunological Recognition*; the first combined meetings and courses calendar poster; *Walking Tour: The History, Science, and Architecture of CSHL*, written by Elizabeth Watson; and the very handsome *The First Hundred Years,* a small history of the Laboratory written by David Micklos. To replace Sue's substantial skills, which include photography, design, and layout, we were fortunate to hire Margot Bennett, a graduate of Cooper Union and a former employee of *Newsday*. Margot's design skills were immediately applied to planning the layout of *The Good Fight: Cancer Research at Cold Spring Harbor Laboratory*, our newest public relations tool. Rounding out the Public Affairs staff, Herb Parsons ably directs the audiovisual services for both the Laboratory and Banbury Center.

Press Coverage

Press activity at the Laboratory has never been greater. The appointment of James D. Watson to the NIH Office of Human Genome Research, the Laboratory's continuing scientific discoveries, plans to establish a Neuroscience Center at the beginning of our second century, and the opening of the DNA Learning Center have resulted in a genuine desire to heighten public awareness. These events have individually and collectively attracted coverage by major national newspapers, several national news magazines, and the television networks. There has also been a discernible increase in local coverage both in the press and on television.

Special Events

In 1988, Public Affairs had responsibility for various events, both large and small. In April, students and friends honored Jim Watson on the occasion of his 60th birthday with a daylong meeting of reminiscences, a festschrift, a scrapbook, and a banquet. The planning and coordination of the DNA Learning Center dedication in September was a major media affair. We also hosted five Banbury seminars, a program in conjunction with Lloyd Harbor and its five residing nonprofit organizations. Finally, the Public Affairs department established bimonthly guided walking tours for the public, conducted numerous special tours, and gave talks to local groups.

Along with these activities, planning got under way for the centennial celebration in 1990, including design and acquisition of appropriate commemorative items, identification of special events to be held, and the commissioning of two books to mark our century of achievement. The plan for the central event includes

the re-creation of the first biology class, fireworks, tall ships, and an array of whaling-era activities.

We look forward to the challenge presented by the plans for the centennial celebration. The implementation of this multifaceted, two-year-long event will require the entire staff to marshall its various skills to ensure that this milestone is observed in grand fashion.

Susan Cooper

PUBLICATIONS

After the upheavals of 1987, 1988 was a year of consolidation for the Publications staff. Eighteen new books were published, the largest annual total ever. Of these, 16 books arose from meetings held at the Banbury Center and in Grace Auditorium, on subjects as diverse as cancer, aging, vaccines, parasites, and plant genetics. Over 600 leading scientists contributed the papers included in these volumes. Acquiring articles from these busy people for assembly into books that are timely and attractive is a huge task demanding persistence and diplomacy from the editorial and production staff. Their reward is the special value placed on the proceedings of Cold Spring Harbor Laboratory meetings in the literature of science. Book production was made faster and cheaper this year by the development in-house of microcomputer-based typesetting systems, and as a result, the purchase price of smaller books was reduced by as much as 20%.

A laboratory manual, *Antibodies*, by Ed Harlow and David Lane, and a monograph on the nematode, *Caenorhabditis elegans*, edited by William Wood, were also published. The first, a unique guide to immunological techniques, became a best-seller even before publication. The second is a widely praised, scholarly work of lasting value to the small but growing community of scientists using the worm to study genetics, neuroscience, and cell biology. These are two very different volumes, but each, like the conference proceedings, is an example of the kind of book the Laboratory has a reputation for doing well.

That reputation was enhanced by the rise in stature of the journal, *Genes & Development*. In its second year, it became known as a place for good, often outstanding papers—remarkably rapid acceptance in a scientific community generally skeptical about new journals. This success can be credited to Mike Mathews' insistence, as Editor, on the highest standards for published papers and the staff's commitment to rapid refereeing and publication. The journal's circulation increased steadily throughout the year. In December, after almost 2 years as Editor, Mike happily resumed full-time laboratory work and we welcomed Terri Grodzicker as his successor.

A videotape, *Transgenic Techniques in Mice*, giving instruction on the genetic manipulation of embryos, was released in December as the first in a planned series of visual guides to complex laboratory methods. Created by Roger Pedersen and Janet Rossant, the tape subsequently won a national educational award.

The 1987 Annual Report and 13 books of meetings abstracts were also published on the Laboratory's behalf during the year. As our publications increased and diversified and journal subscriptions grew, the Customer Service

and Marketing Departments coped well with increasing work. Following Susan Cooper's departure for Public Affairs, Charlaine Apsel took charge of both departments. The marketing effort was augmented by the opening of a bookstore in Grace Auditorium, for the supply of Laboratory publications and sundries to employees and visitors.

In this year of consolidation, there was also building for the future. At the year's end, several innovative books were in press and over 20 monographs and laboratory manuals had entered the planning stage. Joe Sambrook, Ed Fritsch, and Tom Maniatis continued intensive work, coordinated by Nancy Ford, on the second edition of their 1982 classic manual *Molecular Cloning*. Despite the many competing books now available, orders for the manual flooded in from June to December, in advance of publication in 1989. Plans were made for the launch in 1989 of a second journal, *Cancer Cells: A Monthly Review*, and an experienced science editor, Paula Kiberstis, joined us to take charge of it. The journal will review and comment on new findings in cancer biology and their significance for the diagnosis and treatment of disease. To publish *Genes & Development* and *Cancer Cells*, a Journal Department was founded, with Judy Cuddihy as Managing Editor.

Publishing at the Laboratory began in 1933, when the proceedings of the Annual Symposium first appeared. With his personal interest in books, Dr. Watson established a first-class publishing program when he became Laboratory Director. Many books originated in the meetings and courses held here and set a standard for excellence in content and production. Our aim now is to build further and diversify the program, increasing its revenues for the Laboratory, while retaining its reputation for quality. In 1988, much investment was made in that future, and to signal our intention, the decision was taken as the year ended to enter 1989 under the new banner of "Cold Spring Harbor Laboratory Press."

John R. Inglis

RESEARCH

TUMOR VIRUSES

The DNA tumor viruses, adenovirus and simian virus 40 (SV40), have now been studied at Cold Spring Harbor Laboratory for 20 years. These viruses interact with cells in two ways. They replicate in the cells and tissues of their natural hosts, releasing numerous progeny that can perpetuate the infectious cycle. Alternatively, they may form a close liaison with the chromosomes of other cells, resulting in subtle perturbations of cellular gene function that can lead to transformation and tumorigenesis. Both the overt and covert activities of the viruses rest upon interactions of viral products—DNA, RNA, and proteins—with the cellular machinery responsible for synthesizing macromolecules and for regulating cell growth and behavior. The libretto for such a large repertoire of activities is compressed into the relatively small genomes of the two viruses, which are fully sequenced and can readily be manipulated by recombinant DNA techniques. Consequently, investigations of the viruses' activities continue to afford new insights, and not a few surprises, into the operation of normal cell processes such as DNA replication, transcription, and protein synthesis, as well as the mode of action of oncogenes and the sequelae of transformation. The progress reports that follow summarize recent developments in these areas.

With the arrival from Berkeley of Arne Stenlund, the Tumor Virus Group sustains its links with two alumni—Ulf Pettersson and Mike Botchan, in whose labs Arne was a graduate student and postdoctoral fellow, respectively—and also returns to a project that lapsed 5 years ago with the departure of Louise Chow and Tom Broker. Arne joined the Laboratory late in the year and will be continuing his studies of bovine papillomavirus. This is another DNA tumor virus, distantly related to SV40 but with some features that are all its own, and it promises a new dimension to the investigations in future years.

DNA SYNTHESIS

B. Stillman E. White, S. Brill, J.F.X. Diffley, S. Din, M. Fairman, T. Melendy, T. Tsurimoto, F. Bunz, K. Fien, Y. Marahrens, G. Prelich, S. Smith, R. Cipriani, S. Longinotti, S. Penzi

During the past year, we have continued to seek an understanding of the mechanism and regulation of DNA replication in eukaryotic cells. The DNA tumor virus, SV40, continues to be our main focus, but these studies increasingly lead toward an appreciation of the replication of cellular chromosomes. As a consequence, our studies on the replication of DNA in the yeast *Saccharomyces cerevisiae* are expanding, since this biological system combines biochemistry with the power of genetics.

SV40 DNA Replication

F. Bunz, K. Fien, S. Din, M. Fairman, T. Melendy, G. Prelich, T. Tsurimoto, S. Longinotti, B. Stillman

A cell-free system from human 293 cells capable of replicating plasmid DNAs containing the SV40 origin of DNA replication has been used as an experimental system to identify cellular proteins involved in DNA replication and to determine the

mechanism of replication. This cell-free system requires the SV40-encoded large tumor antigen (T antigen), but for the most part, DNA replication relies upon cellular proteins. To identify these cellular proteins and understand the mechanism of DNA replication and how it may be regulated throughout the cell cycle, we have biochemically fractionated the cellular extract into a number of essential components and have purified several replication proteins. The functions of these cellular proteins in DNA replication have been determined, and studies have begun to determine if they are growth-regulated or if they play a role in the switch from the G_1 phase to the S phase of the cell cycle.

INITIATION OF REPLICATION

SV40 T antigen binds to specific DNA sequences within the origin of DNA replication, and correct binding is essential for DNA replication. In addition to this essential function, T antigen also has ATPase and DNA helicase activities, both of which are required for DNA replication in vitro (see collaborative work with Y. Gluzman's laboratory, Molecular Biology of SV40). It has also been shown by J. Hurwitz's laboratory (Memorial Sloan-Kettering Cancer Center) that T antigen, in the presence of ATP, induces local unwinding of the SV40 origin sequence upon binding to the core origin sequences.

A cellular protein called replication factor A (RF-A) was purified and is essential for SV40 DNA replication in vitro. This protein contains multiple subunits of 70,000, 34,000 and 11,000 daltons and binds preferentially to single-stranded DNA. Recent work has demonstrated that RF-A functions in both initiation and elongation of DNA replication. Kinetic analyses of SV40 DNA replication and origin unwinding in the absence of DNA replication have lead to the identification of at least two distinct steps during initiation: The first step is the formation of a DNA-protein complex containing SV40 T antigen, which, for reasons of analogy to the well-characterized *Escherichia coli* chromosome initiation reactions, we have called the open complex. The second stage of initiation at the SV40 origin is the formation of an unwound complex that requires RF-A. This intermediate probably represents the DNA-protein complex that is recognized by the DNA polymerase α/DNA primase holoenzyme for starting DNA synthesis at the origin (see Fig. 1).

A series of monoclonal antibodies have been produced that recognize the 70,000- and 34,000-dalton subunits of RF-A. These antibodies have been useful in determining the function of RF-A in replication but have also proved to be invaluable reagents for studying the cell biology of the protein. RF-A is a nuclear protein, and its levels in the nucleus do not change throughout the cell cycle in any significant way. Recently, we have demonstrated that the 34,000-dalton subunit is phosphorylated at multiple sites, and we are investigating the role that protein phosphorylation may have in the function of RF-A.

ELONGATION OF SV40 DNA REPLICATION

We have previously described the purification of a replication factor required for the complete replication of SV40 DNA in vitro and the identification of this 36,000-dalton protein as the proliferating cell nuclear antigen (PCNA). PCNA was shown to be a cell-cycle-regulated protein (originally called cyclin) and also to be equivalent to the polymerase δ auxiliary protein. Polymerase δ has recently been recognized as a second replicative DNA polymerase present in eukaryotic cells, the other being the well-characterized DNA polymerase α. The role of PCNA in DNA replication is discussed below.

Another replication factor, RF-C, has been purified as an essential replication protein and is required for the elongation of DNA replication from the SV40 origin. RF-C is a multisubunit protein and appears to contain a number of protein subunits. The purified factor contains protein subunits of 37,000 and 41,000 daltons, each of which migrates as a doublet in acrylamide gels, but also associated with the activity is a series of higher-molecular-mass proteins in the range of 100,000–140,000 daltons. RF-C binds to both single- and double-stranded DNAs, and studies on its function during replication have revealed a role with PCNA in chain elongation.

The roles of both PCNA and RF-C in DNA replication were investigated by analyzing the products of DNA replication in reconstituted reactions either in the presence or in the absence of each factor. Surprisingly, omission of each factor alone yielded the same biochemical phenotype, namely, an interesting defect in elongation of replication. In the absence of either factor alone, initiation at the origin proceeded normally, but subsequent chain elongation was abnormal. The leading-strand products (those synthesized continuously in the 5′ to 3′ direction) were absent, whereas the lagging-strand products (those synthesized discontinuously as Okazaki fragments) were shorter than normal and were displaced from

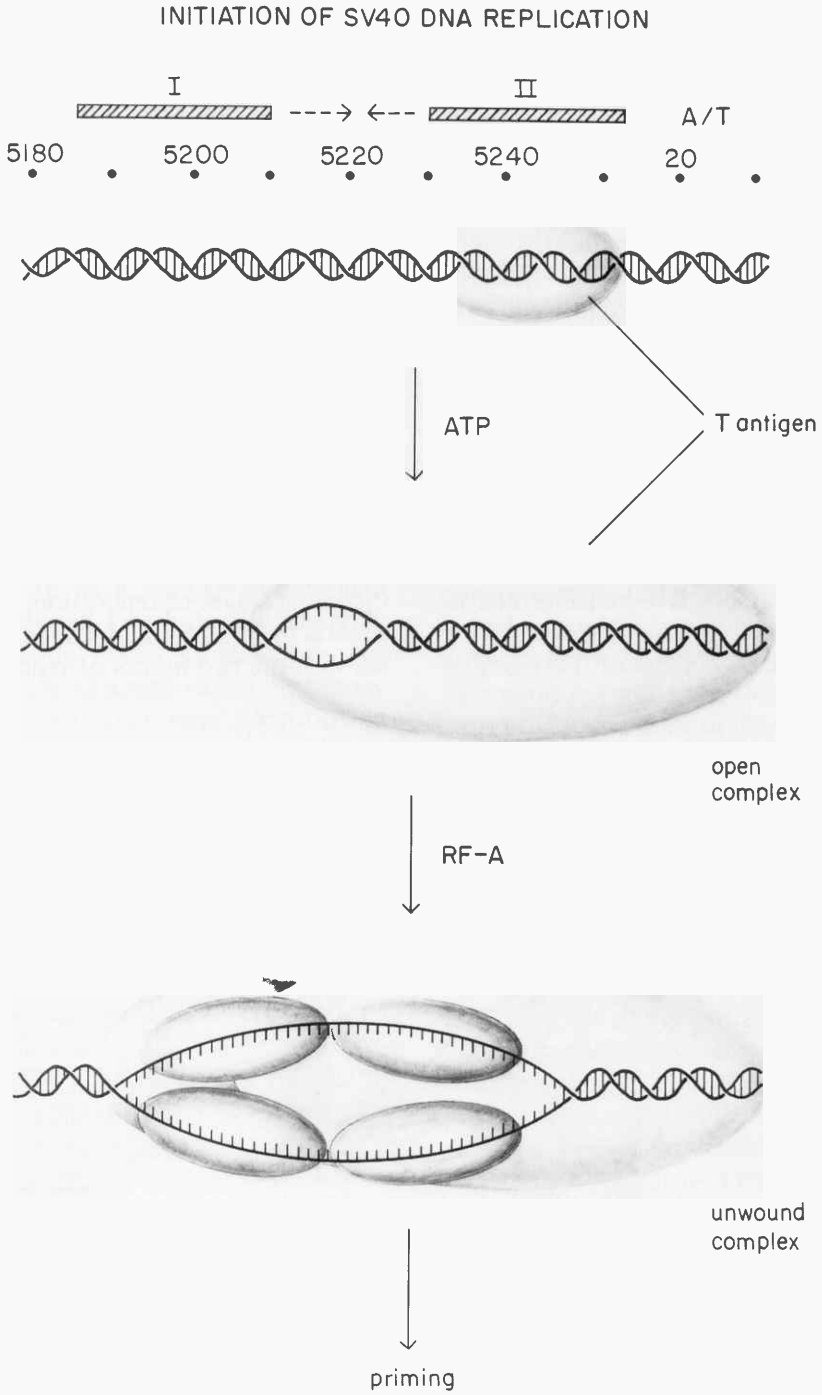


FIGURE 1 Proposed scheme for initiation of DNA replication at the SV40 origin. Two steps prior to priming of DNA synthesis at the origin are shown, based on kinetic analysis of initiation. The unwound DNA in the open complex has been reported by Boroweic and Hurwitz (*EMBO J. 7:* 3149 [1988]).

the template DNA. Furthermore, the replication products that remained bound to the template DNA were predominantly located at or near the replication origin. These results suggested that in the absence of PCNA, RF-C, or both, replication at the origin occurred normally, but subsequent elongation

was defective. Therefore, it was concluded that under normal circumstances, PCNA and RF-C are required for a post-initiation step in replication, presumably as part of a large complex of proteins that form the replication machinery at a fork.

The discovery that PCNA is a processivity factor for DNA polymerase δ raised the possibility that this DNA polymerase was involved in replicative DNA synthesis. Since, in the absence of PCNA, DNA synthesis only occurred on the lagging-strand template and not on the leading-strand template, we proposed that DNA polymerase δ was the polymerase required for synthesis of leading strands at a replication fork and that PCNA is required for the switch from DNA synthesis at the replication origin to DNA-polymerase-δ-dependent synthesis during elongation. To extend this model further, we have suggested that DNA polymerase α, with its associated DNA primase activity, is responsible for synthesizing the RNA-primed Okazaki fragments on the lagging-strand template. A model of this mode of synthesis, involving an asymmetric distribution of the replicative DNA polymerases α and δ, is shown in Figure 2. The replication protein, RF-C, is also required for the switch from the initiation mode to the elongation mode of DNA replication, and we have suggested that it also functions at a replication fork.

We have begun to test the dual polymerase model for synthesis of leading and lagging strands at a eukaryotic replication fork by testing replication by purified polymerases on artificial replication templates. Initial results demonstrate that polymerase α activity is stimulated by both RF-A and RF-C, whereas polymerase δ activity is stimulated by RF-A and RF-C, but only in the presence of PCNA. These results are consistent with the dual polymerase model shown in Figure 2. We also note that RF-A, which is required for origin unwinding by T antigen during the initial stages of replication, also seems to play a role at the replication fork and thus may be a bridge between the two modes of replication.

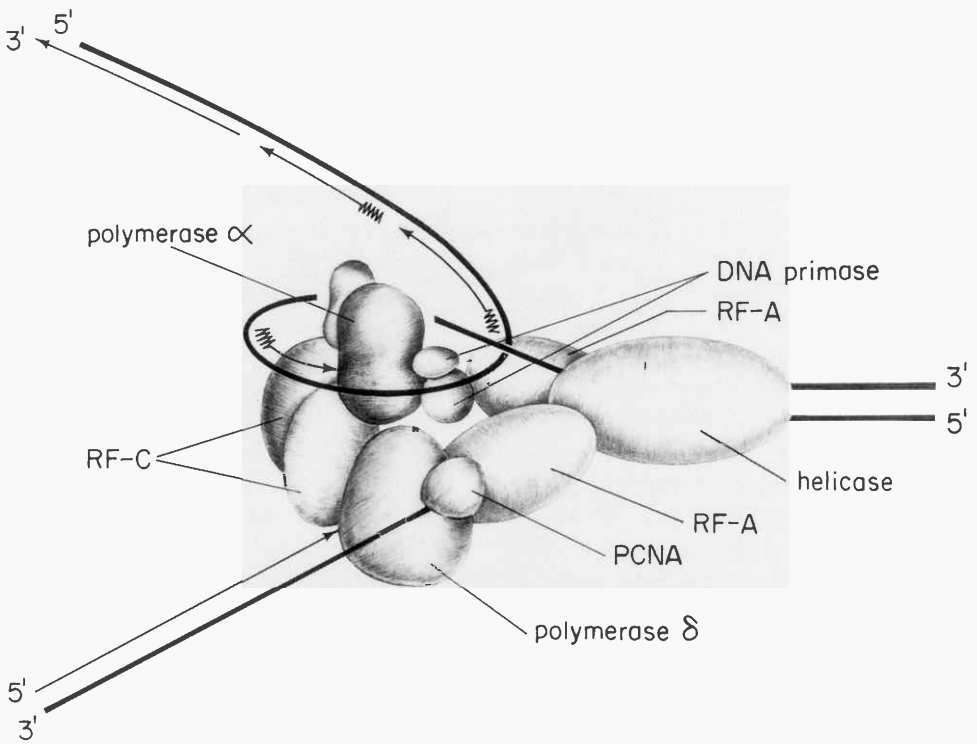


FIGURE 2 Proposed model for the asymmetric distribution of polymerases α and δ at a eukaryotic replication fork. The factors PCNA, RF-A, and RF-C were identified as essential components of the replication apparatus based on studies with SV40 as a model system.

Replication-dependent Chromatin Assembly

S. Smith, B. Stillman

In addition to our studies on SV40 DNA replication, we have previously described a cell-free system that supports assembly of the replicating plasmid DNA into a chromatin structure that resembles the structure of mammalian cell chromosomes. The chromatin assembly occurs concomitantly with DNA replication and requires the addition of a nuclear extract obtained from human cells to the DNA replication reactions. Biochemical fractionation of the nuclear extract has resulted in the identification of a single component required for replication-dependent chromatin assembly in vitro. We have purified this component, chromatin assembly factor I (CAF-I), as a multisubunit protein complex that is required for chromatin assembly during DNA replication. CAF-I is distinct from the previously characterized chromatin assembly factors, nucleoplasmin and N1, that were identified in *Xenopus* egg extracts. These factors act as histone storage and carrier proteins, but CAF-I appears to perform some other function in the assembly of correctly spaced chromatin because the histones are supplied in a soluble form. The minichromosomes assembled de novo by CAF-I consist of correctly spaced nucleosomes containing the four core histones H2A, H2B, H3, and H4, and thus resemble chromatin found in vivo. The focus of current efforts is to understand the role of the CAF-I multisubunit protein in the replication-coupled chromatin assembly process. In addition, we will use this in vitro system to investigate the influence of site-specific DNA-binding transcription factors on chromatin assembly, replication, and structure.

Yeast DNA Replication

J.F.X. Diffley, S. Brill, Y. Marahrens, B. Stillman

As an approach to understanding chromosomal DNA replication and its control, we have been studying the yeast *S. cerevisiae*. Yeast has several advantages for studying DNA replication, including well-characterized genetics, the availability of a large number of cell-cycle-arrested mutants, and the availability of well-characterized origins of DNA replication (ARSs). Our approach to understanding the mechanism and control of yeast chromosomal DNA replication has been threefold. First, we have been characterizing proteins that interact specifically with one ARS, ARS1. Second, we have been analyzing the sequences required for ARS1 function both on plasmids and in their normal chromosomal location. And finally, we have been using our knowledge of the enzymology of SV40 replication in vitro to identify analogous yeast factors that will ultimately allow genetic analysis of the roles of these proteins in cellular DNA replication.

Proteins that interact specifically with yeast ARSs are likely to have important roles in controlling DNA replication. Two proteins that interact specifically with ARS1 have been identified, characterized, and purified. These proteins are designated *A*RS *b*inding *f*actors 1 and 2 (ABF1 and ABF2). ABF1 is a sequence-specific DNA-binding protein that binds to a single site within a region of ARS1 important but nonessential for ARS function. ABF1 binds at or near many but not all ARSs, including ARS2, the 2 μM origin, and three of the four ARSs associated with transcriptional silencing of mating-type information at the *HML* and *HMR* loci. In addition to its potential role in initiation of DNA replication, a role for ABF1 in transcriptional silencing has clearly been indicated by deletion analysis of one of these silencers (*HMR E*)

We have purified the protein to homogeneity, raised polyclonal antiserum to the purified protein, and used this antiserum to screen an expression library. A single clone was obtained that contained a full-length copy of the ABF1 gene. The sequence of the insert contained within this recombinant phage revealed several interesting features of the encoded ABF1 protein. First, ABF1 appears to be similar to a growing number of transcription factors with extremely high asparagine content, including the *Drosophila* deformed gene product, the yeast heat-shock transcription factor (HSTF), and RAP1, another yeast protein involved in transcriptional silencing. In fact, the similarity to RAP1 extends far beyond amino acid composition. These two proteins are 30% identical over 60% of the protein. The possible significance of this with respect to transcriptional silencing is discussed below. Finally, ABF1 appears to contain an interesting variation on the zinc finger DNA-binding motif typified by that found in the transcription factor TFIIIA. In the TFIIIA-like zinc finger, a single molecule of zinc is

coordinated by two cysteine and two histidine residues each in short conserved structures that are separated by a short linker. In ABF1, the cysteine and histidine regions are conserved, although the length of the linker between them is tenfold longer than in TFIIIA. We have used chemical modification to demonstrate that sequence-specific DNA binding by ABF1 requires both unmodified cysteine residues and the presence of zinc, arguing that this putative zinc finger is an important functional domain of ABF1. With the ABF1 gene in hand, the role of ABF1 in initiation of DNA replication and general cellular function can now be investigated.

ABF2 has a number of biochemical properties which suggest that it may have an important role in the initiation of DNA replication. The binding of this protein to ARS DNA, in fact, is reminiscent of the binding of several prokaryotic initiator proteins. ABF2 binds to several (at least five) discrete sites within ARS1 separated by A+T-rich sequences that are essential for complete ARS function, similar to the binding of the *E. coli* dnaA protein binding adjacent to the A+T-rich 13-mer sequences. Our current efforts are aimed at isolating the ABF2 gene and further characterizing the interactions between ABF2 and ARS DNA.

To better understand initiation of yeast replication, we have undertaken a systematic mutagenesis of ARS1. Mutant ARSs are being tested both indirectly by their ability to support the stable propagation of episomes and directly using the recently developed two-dimensional gel techniques to quantify replication frequencies of mutant ARSs when placed back at their normal chromosomal locus. Initial deletion analysis of sequences flanking the essential 11/11 ARS consensus sequence has defined a critical sequence that contains a 9/11 match to the ARS consensus sequence, consistent with the view that ARSs are composed of multiple, properly spaced and oriented ARS consensus sequences. Interestingly, these correspond to the sequences specifically located between ABF2-binding sites at several ARSs tested. Further analysis of ARS1 coupled with more extensive ABF2 footprint analysis should lead to insights into initiation of DNA replication.

Finally, we have recently begun searching for yeast proteins that can complement human 293 cell fractions in various aspects of SV40 DNA replication in vitro. Initially, we have concentrated on searching for the yeast homolog of the single-stranded DNA-binding protein, RF-A, since we can follow its activity with the relatively simple origin unwinding assay. Following a purification protocol similar to that used for the purification of human RF-A, a structurally and functionally similar protein has been purified to apparent homogeneity. The yeast RF-A (yRF-A), like its human counterpart, is composed of three subunits (70, 36, and 11 kD) and also like human RF-A, single-stranded DNA-binding activity resides in the largest subunit. Furthermore, yRF-A substitutes completely for the human protein in the unwinding assay. As with the ARS-binding factors, the availability of purified yRF-A will allow the isolation of the genes encoding these proteins. Subsequent genetic analysis will determine the role of yRF-A in yeast replication and, by inference, in human replication.

The approaches outlined above should ultimately lead to a more complete understanding of how chromosomal DNA replication is accomplished. Understanding how these proteins are regulated throughout the cell cycle will lead to a deeper understanding of how cell proliferation is controlled.

ABF1, Nuclear Lamins, and Transcriptional Silencing

J.F.X. Diffley, B. Stillman

Wild-type yeast contain three cassettes of mating-type information on chromosome III. Mating type is determined by expression of the cassette at the *MAT* locus, whereas the cassettes at *HML* and *HMR* are transcriptionally inert. *HML* and *HMR* contain the promoter elements required for active expression, but they are maintained in an inactive state by the concerted action of *cis*- and *trans*-acting elements. Sequences known as *E* and *I* lying on either side of each of the silent cassettes are required to maintain the silent state. One of these, *HMR E*, has been shown to function in a distance- and orientation-independent manner and, by analogy to transcriptional enhancers, they have been termed silencers. ABF1 (see above) binds to three of the four silencers (*HML I*, *HMR E*, and *HMR I*), and another DNA-binding protein, RAP1, binds to two of the four silencers (*HML E* and *HMR E*). In addition to being transcriptional silencers, these sequences function on plasmids as ARSs, and at least one, *HMR E*, exhibits a mitotic partitioning function in addition

to its ARS activity. Transcriptional silencing also requires the action of the four *SIR* genes. None of the *SIR* genes are essential for viability nor do they encode either ABF1 or RAP1, although evidence points to action of the *SIR* genes through ABF1 and RAP1.

The molecular mechanism of silencing is, at present, obscure, although two observations that we have made may help to elucidate this pathway. First, as mentioned above, ABF1 and RAP1 exhibit extensive homology with each other. Interestingly, this homology does not include the putative zinc finger of ABF1, suggesting that regions outside the DNA-binding domains of these proteins may be conserved. Since the ABF1 and RAP1 sites at *HMR E* are functionally redundant, and since these proteins act in silencing through the *SIR* genes, we suggest that the conservation between ABF1 and RAP1 includes sequences involved in interaction with one or more of the *SIR* gene products. The second observation we have made involves the *SIR4* gene. *SIR4* is a large protein with several genetically defined subdomains. During the course of comparing the predicted ABF1 protein sequence with other proteins, we noticed that within a long region of predicted α-helix in the carboxy-terminal subdomain of *SIR4* were 12 in-phase heptad repeats that typify the central rod of the intermediate filament (IF) proteins. If the amino acids of the heptad repeat are designated $(a\text{-}b\text{-}c\text{-}d\text{-}e\text{-}f\text{-}g)_n$, residues a and d are generally hydrophobic, aliphatic amino acids and form the basis for coiled-coil interactions between IF protein monomers. Homology searches with this sequence revealed that the strongest similarity was with the central rod of the human nuclear lamins A and C, which form a filamentous structure known as the nuclear lamina subjacent to the nuclear envelope. Although lamins A and C are highly homologous to other IF proteins, the 95-amino-acid region of homology between *SIR4* and the lamins contains the 43-amino-acid region found only in lamins A and C and not in other members of the IF family.

These observations suggest a mechanism for transcriptional silencing. Since *SIR4* homology with the lamins is within the heptad repeats that are clearly implicated in IF dimerization and higher-order multimerization, we propose that the carboxyl terminus of *SIR4* is inserted into the nuclear lamina by direct interaction with as yet unidentified yeast homologs of the human nuclear lamins. A complex including *SIR4* and some or all of the other *SIR* gene products would then form and interact directly with ABF1 and RAP1 bound at the *E* and *I* regions through conserved amino acid sequences in these two sequence-specific DNA-binding proteins. The proposed interaction between ABF1/RAP1 and the lamina-bound *SIR* complex would account for the partitioning phenomena seen with *HMR E*-containing plasmids, since in yeast, unlike higher eukaryotes, the integrity of the nuclear envelope is maintained during a closed mitosis. How association with the lamina would affect transcriptional silencing is suggested by the results of Sedat and coworkers, who showed that specific and almost universally heterochromatic regions of *Drosophila* salivary gland polytene chromosomes are associated with the nuclear envelope. Thus, the lamina may represent a transcriptionally inert region of the nucleus. We are currently investigating the validity of this model by looking at the subnuclear localization of the *SIR* gene products and the silencer sequences.

Biological Function of the 19-kD Product of the Adenovirus E1B Oncogene: Disruption of IFs and the Nuclear Lamina

E. White, R. Cipriani

The adenovirus E1A and E1B genes cooperate to transform primary rodent cells and are responsible for regulating adenovirus gene expression during productive infection. E1B encodes two major gene products, the 19,000-dalton (19K) and 55,000-dalton (55K) tumor antigens. We have been primarily interested in determining the function of the 19K protein in transformation and productive infection.

Initially, we approached the function of the 19K protein by examining the effect of mutations in the E1B 19K coding region on the virus life cycle. It was found that 19K viral mutants were not defective for virus replication but were defective for transformation, and possessed a multitude of phenotypes. These phenotypes included the degradation of host-cell and viral DNA (*deg* phenotype), enhanced and unusual cytopathic effect (*cyt* phenotype), the formation of large plaques (*lp* phenotype), and a host-range (*hr*) phenotype whereupon the mutant viruses replicate more efficiently than the wild-type virus. Manifesta-

tion of the 19K mutant phenotypes was E1A-dependent, genetically defining an interaction between the E1A proteins and the E1B 19K protein. The main conclusions from this genetic analysis were, first, that the 19K protein functioned to protect DNA from degradation during infection and, second, that it acted as a negative regulator of E1A-dependent viral early gene transcription. As the 19K protein was found in the cytoplasm and the nuclear envelope/lamina, the effect of the 19K protein on gene expression and DNA stability was likely to be indirect, possibly via an effect on mRNA metabolism, chromatin and nuclear structure, or on the E1A proteins.

Unfortunately, the pleiotropic nature of the mutant phenotypes made it difficult to determine the primary function of the 19K protein in infected cells. Therefore, we decided to look for a biological function of the 19K protein by using plasmid expression vectors to produce the 19K protein in cells, outside the realm of a productive infection.

CONSTRUCTION OF E1B PLASMID EXPRESSION VECTORS

The E1B 19K protein is a fairly abundant protein in adenovirus-infected and -transformed cells. Hence, we chose to express the 19K protein under the control of strong, heterologous promoters, as opposed to the normal E1B promoter. The mouse metallothionein and the cytomegalovirus promoter-enhancer were used to drive expression from the 19K coding region, flanked by SV40 small t intron and polyadenylation sequences. The pMT19K and pCMV19K plasmids contained an additional point mutation that introduces a stop codon at the second position of the overlapping E1B 55K reading frame, which did not affect the amino acid sequence of the 19K protein. The E1B 19K protein should therefore be the only E1B protein expressed from this plasmid, and it is the only E1B protein product immunologically detectable. These plasmids, when introduced into HeLa and COS cells by calcium phosphate precipitation, transiently express the E1B 19K protein to levels comparable to those found in infected and transformed cells. The 19K protein localized to the cytoplasm and nuclear envelope of transfected cells, closely resembling its localization in transformed cells and in infected cells at early times postinfection.

Two other E1B plasmid expression vectors were constructed that encode either all of E1B (pCMVE1B) or just E1B 55K sequences (pCMV55K). pCMVE1B expresses both the 19K protein and 55K and related proteins after transient transfection, whereas pCMV-55K expresses only the 55K and related proteins. The E1B 55K protein was detected by indirect immunofluorescence with a 55K-specific monoclonal antibody and was found to colocalize with p53 in a perinuclear spot in the cytoplasm, resembling the localization of E1B 55K and p53 proteins in transformed cells.

We have used these expression vectors for probing the function of the individual E1B proteins in (1) the cooperation with E1A in the transformation of primary cells and (2) as a means for examining the effect of E1B proteins on regulation of gene expression, cell growth, and architecture.

TRANSFORMATION OF PRIMARY RODENT CELLS WITH E1A AND E1B

The ability of the E1B 19K protein to cooperate with E1A to transform primary baby rat kidney (BRK) cells was determined by DNA-mediated gene transfer. BRK cells were transfected with plasmids encoding E1A alone (pE1A), E1A plus E1B (either pE1 or pE1A plus pE1B), and E1A (pE1A) plus the E1B 19K protein (pCMV19K). Transfection of BRK cells with E1A alone resulted in the appearance of a few small, not very dense, and often abortive foci that were difficult to establish in long-term culture. These cells grew slowly to low saturation density, and their morphology was very flat and often resembled the morphology of the primary BRK cells. Transfection of BRK cells with pCMV19K alone did not result in foci formation, nor did transfection of cells with the T24 Ha-*ras* plasmid. Transfection of plasmids encoding E1A plus E1B resulted in the appearance of large numbers of dense foci with very transformed morphologies that were easily established in culture. Cotransfection of pE1A plus pCMV19K plasmids greatly increased the frequency of focus formation over transfection of pE1A alone, resulting in about half the number obtained with E1A plus an intact E1B gene. The morphology of the E1A-E1B 19K transformants was different from that of cell lines expressing only E1A, and they were readily established into cell lines that grew to high density. Therefore, the E1B 19K protein was responsible for promoting focus formation, producing morphological changes associated with the transformed phenotype, and enabling cells to grow rapidly and to high density.

E1B 55K protein contributes to the transformation process as well by promoting the frequency of transformation and further altering cell morphol-

ogy. Cotransfection of pE1A plus pCMV55K plasmids also resulted in the appearance of foci that can be established in long-term culture. Therefore, both E1B proteins contributed to the transformation process, but expression of either one along with E1A enabled the formation of foci and long-term growth in culture.

TRANSIENT EXPRESSION OF THE E1B 19K PROTEIN DISRUPTS IFs AND THE NUCLEAR LAMINA

Transfection of either HeLa or COS cells with the pCMVE1B expression vector resulted in a subtle alteration in cell morphology. We therefore tested the possibility that expression of an E1B gene product could affect cell architecture. HeLa cells were transfected with pCMV19K or pCMVE1B, and at 48 hours posttransfection, the cells were fixed and stained for double-label indirect immunofluorescence with a polyclonal antibody directed against the E1B 19K protein, in conjunction with monoclonal antibodies specific for the IF-related protein vimentin or the nuclear lamins.

In normal HeLa cells or HeLa cells transfected with carrier DNA, the IFs exist as long filaments that extend from the vicinity of the nuclear envelope to the cell periphery (Fig. 3). Drastic alterations in the distribution of vimentin containing IFs were observed in many of the cells that expressed the 19K protein. Routinely, 10–50% of cells transfected with the pCMV19K plasmid expressed the 19K protein and 12–50% of those had severe perturbations in the IF system. The IFs became detached from the nuclear envelope and the plasma membrane, formed large clusters in the cytoplasm, and often appeared fragmented (Fig. 3). The 19K protein was often observed to colocalize with the disrupted IFs in the cytoplasm (Fig. 3).

Vimentin shares extensive amino acid and secondary structure homologies with the nuclear lamin proteins. Furthermore, the 19K protein is known to associate with the lamina physically. We therefore investigated the possibility that the 19K protein might also disturb nuclear lamina structure as well as cytoplasmic IFs. pCMV19K-transfected HeLa cells were stained by double-label indirect immunofluorescence with antibodies directed against the E1B 19K protein and lamins A and C. Lamin antibodies stain the nuclear envelope-lamina in a uniform fashion, but in 7% of the cells that expressed the 19K protein, small areas of the lamina were devoid of lamin staining (Fig. 3). As in the case of vimentin staining, the 19K staining of the lamina was coincident with that of the lamins (Fig. 3).

The effect of the 19K protein is specific for IFs and the lamina, since 19K expression did not disturb the organization of microtubules or the actin cytoskeleton. Furthermore, disruption of IFs and the lamina also occurred in cells infected with wild-type adenovirus but not in cells infected with an E1B 19K gene deletion mutant. Therefore, 19K-dependent IF and lamina disruption is specific for those cytoskeletal elements, it is a normal occurrence in productively infected cells, it is the result of expression of the 19K protein alone, and it is not a transfection artifact.

DISRUPTION OF IFs IN TRANSFORMED BRK CELL LINES

The arrangement of IFs and the nuclear lamina was investigated in BRK cell lines that were immortalized by E1A alone or transformed by E1A plus E1B, E1A plus pCMV19K, E1A plus pCMV55K, or E1A plus *ras*. As expected, the cell lines that expressed the E1B 19K protein displayed perturbations in the arrangement of IFs, whereas in those lines that did not express the 19K protein, the arrangement of IFs appeared normal. What was surprising, however, was the degree of IF disruption. In primary BRK cells, BRKs immortalized by E1A, and BRKs transformed by E1A plus pCMV55K or E1A plus *ras*, the IFs appeared as continuous filaments radiating out from the nuclear envelope to the cell perimeter. IF disruption was observed in BRK cells transformed by pE1A plus pCMV19K or pE1A plus pE1B plasmids. In two of the pE1A-pE1B cell lines, all of the cells showed gross abnormalities in the arrangements of IFs. The types of IF distributions in these cell lines fell into three categories: cells in which the IFs appeared short and very disorganized, with 19K staining evident in areas of the most pronounced disorganization; cells where vimentin appeared in large perinuclear aggregates coincident with the localization of the 19K protein; and cells that appeared predominantly devoid of vimentin staining. Whether this absence of vimentin staining represents the total lack of vimentin IFs or is a consequence of epitope masking is not yet known.

Finally, despite the disruption of the vimentin network in E1A- and E1B-transformed BRK cell lines, tubulin and actin distributions appeared normal. Therefore, BRK cells transformed by adenovirus DNA sequences displayed gross perturbations in the vimentin IF network, and this was di-

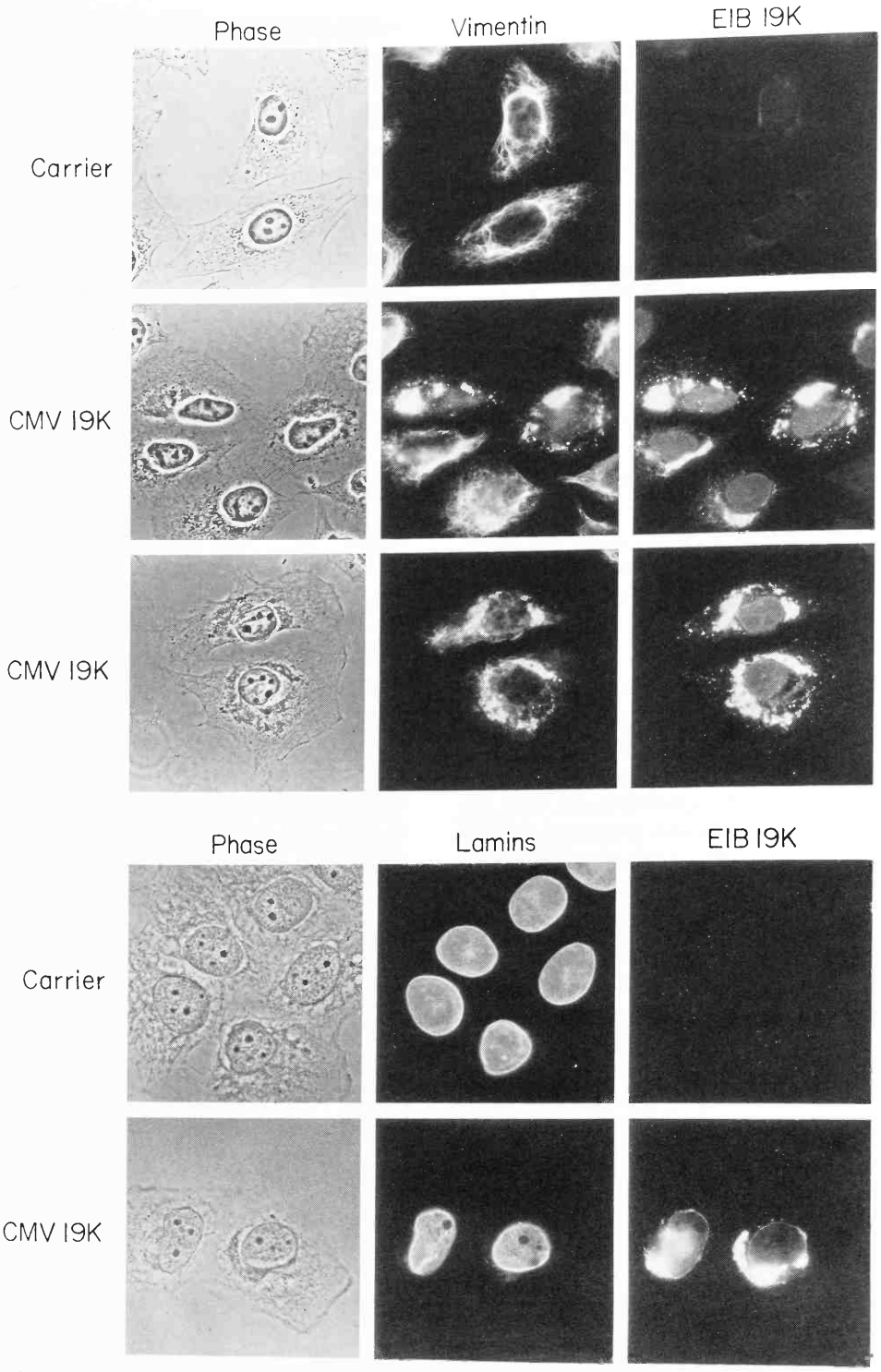


FIGURE 3 Disruption of IFs and the lamina by 19K expression in transient assays. HeLa cells were transfected either with carrier DNA or with pCMV19K plasmid DNA, and at 48 hr posttransfection, cells were fixed in either paraformaldehyde (upper panels) or methanol (lower panels). Double-label indirect immunofluorescence was performed with either anti-vimentin and anti-E1B 19K antibodies (upper panels) or anti-lamins and anti-E1B 19K antibodies (lower panels). The phase-contrast and immunofluorescent staining for vimentin or lamins and the E1B 19K protein is shown for the same set of cells in each horizontal row.

rectly correlated with expression of the E1B 19K protein.

In conclusion, the 19K E1B-transforming protein of adenovirus functions to specifically disrupt IFs and the nuclear lamina of transfected, infected, and transformed cells. This is likely to be the main, if not the only, function of the 19K protein. Therefore, the role of the 19K protein in the prevention of abnormal cytopathic effect, DNA degradation, and increased early gene transcription in productively infected cells, and in transformation are likely consequences of IF and lamina disorganization. Once having determined a function of the E1B 19K protein, we can now begin to address (1) how the 19K protein disrupts IFs and the lamina and (2) why disruption of these structures prevents the occurrence of the pleiotropic phenotypes in E1B mutant infected cells and promotes oncogenic transformation.

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MOLECULAR BIOLOGY OF SV40

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I. Mohr

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

volved in the specific recognition of the SV40 origin of replication.

Our previous studies on the origin-specific DNA-binding domain of large T antigen utilized a series of deletion mutants coding for amino-terminal fragments of different lengths and allowed us to map the carboxy-terminal boundary of this domain at amino acid 246. To delineate this boundary, DNA-binding activity was measured by immunoprecipitating protein-DNA complexes and examining the labeled DNA fragments coprecipitated from a mixture of specific and nonspecific DNA. Truncated T-antigen proteins containing the amino-terminal 272 or 266 amino acids bound origin-containing DNA with a low efficiency compared to shorter proteins. These proteins, beginning with an amino-terminal fragment 259 amino acids in length and culminating with a protein of 246 amino acids, displayed a resurgence in specific DNA-binding activity. We have since confirmed and further quantitated these findings by means of a nitrocellulose-filter-binding assay using individual gel-purified fragments containing the wild-type SV40 origin of replication, site I or site II, and equimolar mixtures of them. The 246-amino-acid protein bound to all fragments tested with an approximately two-fold greater efficiency than the 259-amino-acid protein, and they both displayed efficient site I binding at concentrations of protein lower than that required to achieve efficient site II binding. This filter-binding assay will be employed to determine precisely the dissociation constants for T-antigen binding to the various origin fragments under a variety of conditions. Initial results indicate that the dissociation constant is surprisingly high. Limited proteolysis of T antigen in other laboratories has generated a fragment that spans residues 131–371 which specifically recognizes the SV40 origin of replication (Simmons, *J. Virol 57:* 776 [1986]). We have utilized this amino-terminal boundary and produced a protein containing amino acids 132–246 that contains all of the information necessary to recognize the SV40 origin of replication.

Addition of the monoclonal antibody employed in the immunoprecipitation assays (Pab419) to DNA-binding reactions containing full-length T antigen from HeLa cells resulted in an increased retention of the site II fragment on nitrocellulose filters. Preliminary results indicate that the DNA-binding activity of the truncated proteins is unaffected by the presence of antibody. Furthermore, Pab419 had no effect on the ability of the protein to bind to single-stranded DNA. This raises the interesting possibility that certain monoclonal antibodies may stabilize particular conformations of T antigen and thus influence the protein's activity in vitro. These studies will be pursued by making use of the battery of monoclonal antibodies we have at our disposal.

The 246- and 259-amino-acid proteins have been purified from bacteria, HeLa cells have been infected with an adenovirus vector, and insect cells have been infected with a baculovirus vector. The truncated proteins produced in insect cells and bacteria behave similarly in a DNA-binding assay. Both proteins bind first to the wild-type origin fragment, then to site I, followed by the site II fragment. The mammalian proteins, however, bind first to the wild-type fragment, followed by the site II fragment. Binding to the site I fragment is seen only at high protein concentrations. DNA-binding studies comparing the full-length proteins are currently in progress.

The helicase activity of large T antigen requires the protein to interact with single-stranded DNA. The 246-amino-acid truncated derivative that bound efficiently to origin sequences was also able to interact with a partial duplex helicase substrate. This binding was competed by single-stranded DNA in a 1:1 ratio. SV40 origin sequences contained within a pBR322-based plasmid effectively competed for binding, whereas pBR322 failed to compete at a mass ratio of 100:1. Various RNAs (α globin, poly[A], poly[U], poly[C]) only competed at mass ratios that exceeded 100:1. Poly(G), however, competed more efficiently than single-stranded DNA. This may be due to the large amount of secondary structure present in this polyribonucleotide. Full-length T antigen from HeLa cells behaved similarly in these competition experiments, with the exception that pBR322 lacking origin sequences was an effective competitor.

All of these truncated proteins lack the putative metal-binding motif found between residues 302 and 320. It is therefore unlikely that this structure plays a pivotal role in the recognition of specific or nonspecific DNA sequences. We have observed that micromolar concentrations of zinc efficiently promote the oligomerization of T-antigen monomers. This oligomerization can be reversed with either EDTA or DTT, both of which are capable of chelating metal ions. Furthermore, a truncated protein that contains the amino-terminal 246 amino acids and lacks the putative metal-binding motif behaves predominately as a monomer. A protein containing the amino-terminal 360 amino acids, however, also

behaves predominately as a monomer. Thus, the mere presence of the putative metal-binding motif is not sufficient to promote oligomerization. Atomic absorption spectroscopy performed in collaboration with D. Geidroc and J. Coleman (Dept. of Molecular Biophysics and Biochemistry, Yale University) has revealed 1 g atom of zinc that is resistant to removal by dialysis associated with pure preparations of T antigen. Further experiments are in progress to probe the nature of this association and to address whether or not T antigen is a zinc metalloprotein. It is worth noting that the region of T antigen involved in specific DNA binding contains numerous cysteine and histidine residues that may also be involved in coordinating a metal ion. The 246-amino-acid protein that lacks the metal-binding motif is currently being analyzed for its zinc content and should further address this question. Proteolysis studies will also be performed on the full-length protein to determine if a stable folded domain containing a coordinated metal ion can be isolated. Finally, we will size-fractionate T antigen in the presence of various concentrations of metal ions and assay individual fractions for origin-specific DNA binding, single-stranded DNA binding, helicase activity, and in vitro DNA replication activity.

Last year, we described the use of a bacterial expression system to produce T antigen that lacks mammalian posttranslational modifications. Full-length T antigen purified from *E. coli* directs levels of in vitro DNA synthesis 10–15% of those directed by T antigen purified from a mammalian source. The purified protein binds to fragments containing the wild-type SV40 origin of replication or site I with the same efficiency as mammalian T antigen. However, T antigen from bacteria fails to bind to fragments containing only site II at protein concentrations where its mammalian counterpart binds efficiently.

Further biochemical characterization of T antigen from *E. coli* has revealed that its ability to function in an origin-specific unwinding assay parallels its apparent inability to bind specifically to site II. The nature of these defects may thus account for the low replication levels observed. The *E. coli*-produced protein possesses levels of helicase activity comparable to that of mammalian T antigen, providing evidence that both efficient site II binding and helicase activity are required to achieve origin-specific unwinding. The aforementioned conditions, however, are not sufficient for unwinding to occur. This is supported by the existence of a mutant T antigen containing a single lesion at amino acid 224. This mutant retains wild-type levels of helicase activity and binds to site II, yet it is still defective in its ability to catalyze the unwinding of origin-containing plasmids.

Our previous studies on phosphorylation of T antigen have clearly implicated this posttranslational modification with a role in regulating SV40 DNA replication. Briefly, we demonstrated that T antigen treated with calf intestinal alkaline phosphatase (CIAP) displayed an increase in its ability to direct SV40 origin-specific DNA synthesis in vitro. This was accompanied by at least a fourfold increase in binding to site II, an essential element within the SV40 origin of replication, and a twofold increase in binding to site I. The protein's intrinsic ATPase activity remained unaltered (Mohr et al., *EMBO J. 6:* 153 [1987]). Interestingly, CIAP treatment of T antigen successfully removed 80% of the ^{32}P label from the polypeptide. The remaining 20% remained covalently attached to the protein following subsequent treatment with bacterial alkaline phosphatase in 0.1% SDS (unpublished observations). Work in other laboratories has established that whereas CIAP removes phosphate from serine residues on T antigen, the threonine residues resist the action of this enzyme (Shaw and Tegtmeyer, *Virology 115*: 88 [1981]; Grasser et al., *J. Virol. 61:* 3373 [1987]; Klausing et al. *J. Virol. 62:* 1258 [1988]). There are two phosphothreonine residues present on T antigen, Thr-124 and Thr-701. Proteins containing the conservative substitution of alanine for threonine at residue 701 are indistinguishable from wild-type in plaque assays, in vivo replication assays, and DNA-binding assays performed in crude extracts. Mutants at Thr-124, however, fail to form plaques, do not replicate SV40 DNA in vivo, and fail to bind to site II (Schneider and Fanning, *J. Virol. 62:* 1598 [1988]). We have obtained the coding sequences for these mutant T antigens from Dr. Fanning and have overexpressed these proteins in adenovirus vectors. The immunoaffinity-purified mutant proteins will be tested in several quantitative in vitro assays (replication, DNA binding, helicase, unwinding). Pure protein will also allow us to address the effect mutating this critical threonine residue has on the interactions that occur between T-antigen molecules in solution and those that occur when the protein is bound to DNA. Finally, it will also facilitate comparisons between full-length T antigen produced in *E. coli* and the mammalian Thr-124 mutant. The similarity between these two proteins is striking, as they both replicate

DNA poorly and bind to DNA fragments containing the wild-type origin or site I, yet fail to bind to site II.

Recently, it has been demonstrated that ATP can alter the DNA-binding pattern of T antigen. It has been reported that efficient binding to site II at 37°C is ATP-dependent, and the limits of protection as assayed by DNase I footprinting are extended over the AT-rich region of the origin (Deb and Tegtmeyer, *J. Virol. 61:* 3649 [1987]; Boroweic and Hurwitz, *Proc. Natl. Acad. Sci. 85:* 64 [1988]) in the presence of this nucleotide. This phenomenon has also been observed in electron micrographs of T antigen–DNA nucleoprotein complexes (Dean et al., *Proc. Natl. Acad. Sci. 84:* 8981 [1987]). We will initially examine two replication-defective T antigens for their ability to respond to ATP in this footprinting assay. One mutation has wild-type levels of helicase activity and binds to both origin sites, yet fails to unwind origin-containing plasmids. The second mutant is defective in ATP hydrolysis. Full-length T antigen from *E. coli* will also be tested in this assay. It will be extremely important to assess the effect of ATP on the ability of this protein to bind to site II. The continued analysis of these mutant T antigens will allow us to pinpoint the defect in the initiation of replication and will lend support to the biological significance of these partial biochemical reactions. Furthermore, the distribution of their phenotypes reinforces the complex nature of the initiation reaction and the multiplicity of functions provided by T antigens.

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ADENOVIRUS GENETICS

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The adenovirus oncogene E1A (early region 1A) is a multifunctional protein that affects many aspects of cellular function, such as transcription and induction of cellular DNA synthesis and proliferation. Expression of E1A can lead to immortalization or transformation of different cell types. Several E1A proteins are expressed as a result of alternative splicing of the primary transcripts, and different proteins may express some but not all of the E1A functions. We have been interested in several aspects of E1A function. First, we have been analyzing the growth factors induced in primary epithelial cells by E1A proteins. Second, we have been using E1A mutants to define the role of growth factors in E1A-induced cell proliferation. We have also continued work using viral vectors that express E1A and SV40 T antigen to immortalize a variety of primary cell types.

Role of the Adenovirus E1A 12S Gene Product in Stimulation of Epithelial Cell Proliferation, Immortalization, and Growth-factor Production

T. Grodzicker, M. Cleary, P. Hinton, M. Quinlan

In our pursuit of the identification of the 12S-induced growth factor, we have been testing the ability of known growth factors to stimulate quiescent primary baby rat kidney (BRK) cells to proliferate. These cells do not respond to TGFβ, PDGF, EGF, bradykinin, or bombesin. TGFβ also does not inhibit the proliferative response of the primary epithelial cells to the 12S-conditioned medium. However, the BRK cells do proliferate in the presence of bFGF or TGFα. Since we know that our growth factor(s) does not bind to the EGF

receptor, we have eliminated the possibility that TGFα is present in the conditioned medium. In addition, 12S-conditioned medium induces a morphological alteration of NIH-3T3 cells that resembles that which is produced by the conditioned medium from cells transformed by the Kaposi sarcoma oncogene, a member of the FGF family (provided by C. Basilico, NYU School of Medicine). We have been pursuing purification of the growth factor from conditioned medium of 12S virus-infected immortalized epithelial cells in collaboration with Dan Marshak (see Protein Chemistry, this section).

The 12S protein encoded by the adenovirus E1A region induces cellular DNA synthesis in and proliferation and immortalization of primary rat epithelial cells in the presence or absence of serum, as well as the production of a growth factor(s) that stimulates epithelial cell proliferation. We have undertaken a mutational analysis of the 12S gene to determine the sequences required for these functions. We found that a region near the carboxyl terminus of the 12S protein was required for growth factor induction. No activities have been defined previously for this region. Furthermore, we showed that growth factor production was necessary for epithelial cells to survive past their normal life span in culture and to become immortalized. The ability to induce growth factor production required prior expression of E1A activities encoded by the amino terminus of the 12S protein, including activation of quiescent cells into the cell cycle, and an unknown activity that required expression of the first 13 amino acids of the gene. In addition, examination of the subcellular localization of mutant 12S polypeptides suggested new regions that affect the nuclear localization of E1A proteins.

Use of Retroviruses That Carry Immortalizing or Transforming Genes

L. Arrigoni, R. Cone

An epithelial-cell-transforming virus could be of great use, both in the culture of epithelial cell lines and in the study of carcinogenesis. Since the adenovirus E1A gene has been shown to partially transform some epithelial cells from primary rat cell cultures, we constructed retrovirus vectors containing either the 12S or 13S E1A cDNA sequences to facilitate the transfer of these genes into a variety of primary cell types. The 12S E1A virus induced proliferation and immortalization of epithelial cells in rat kidney, liver, heart, pancreas, and thyroid primary cultures. In the two cases tested, heart and liver cultures, E1A-immortalized cells were nontumorigenic, but they could be completely transformed by subsequent introduction of the *ras* oncogene. To our surprise, the 13S virus had a greatly reduced immortalization potential. This may be related to the fact that the 12S E1A protein is required for the complete induction of the cellular DNA replication machinery in the quiescent human epithelial cells in which adenoviruses normally replicate.

We have utilized a retrovirus vector encoding the adenovirus E1A oncogene and the neomycin phosphotransferase gene to establish a differentiated human thyroid cell line capable of expressing HLA class II antigens in collaboration with T. Davies (Mt. Sinai Medical Center). Human fetal thyroid was collagenase-digested, cultured as a monolayer, and infected directly with 12S or 13S E1A-containing retrovirus constructs. Infected clones were selected in a hormone-supplemented medium containing bovine TSH (10 mU/ml), 10% fetal bovine serum, and 0.5 mg/ml G418 antibiotic. A rapidly growing clone (designated 12S) was chosen for detailed analysis over 11 months of continuous culture. The 12S clone was sensitive to >10 μU/ml bovine TSH when assessed by extracellular accumulation of cAMP, but bovine TSH had no influence on [^{3}H]thymidine incorporation over a 72-hour period. One 12S E1A line, in particular, has additionally retained the ability to inducibly express class II histocompatibility antigens, in response to α-interferon, a property that makes the line useful for the study of human thyroid autoimmune disease.

During this past year, in collaboration with D. Williams (Children's Hospital, Boston), we have continued to examine the use of oncogene-containing retrovirus vectors for the establishment of differentiated cell lines. T.M. Dexter showed some time ago that long-term bone marrow cultures provide an in vitro environment capable of supporting proliferation of hematopoietic stem cells, analogous to the role of bone marrow in vivo. These cultures are very complex, containing a variety of adherent stromal cells and nonadherent hematopoietic cells that are dependent on the stromal cells for continued growth in culture. To simplify the study of stromal cell–hematopoietic cell interactions, we have established a number of murine bone marrow stromal cell lines from long-term Dexter-type cultures by infection of the adherent layer with retroviruses encoding E1A

or SV40 T antigen. Three of five lines immortalized with SV40 T antigen were able to support proliferation of the most primitive murine hematopoietic cell known, the CFU-S cell (colony forming unit-spleen), whereas none of the E1A-immortalized lines were able to do so. Assays were performed by cocultivating fresh nonadherent bone marrow cells with feeder layers of immortalized stromal cell lines and quantitating the number of CFU-S cells per culture at 1-week intervals for up to 5 weeks. CFU-S cells are quantitated by virtue of their ability to home to the spleen and form macroscopic hematopoietic colonies there after intravenous injection into irradiated recipient mice.

One clone, U2, supported CFU-S proliferation at levels comparable to those of primary murine adherent cells (200 per flask for up to 5 weeks). Evidence from a number of laboratories suggests that proteins on the surface of stromal cells may be involved in the homing and proliferation of CFU-S cells. Future efforts will be directed toward identifying proteins on the surface of U2 cells that may be involved in these processes.

Structure-Function Relationships in the Adenovirus E1A Gene

E. Moran, P. Yaciuk, M. Corrigan

Our work continues to focus on how the structural features of the E1A gene products determine their biological activities, particularly their ability to induce quiescent cells to enter the cell cycle. In past years, we have shown that the E1A proteins contain three highly conserved amino acid regions that are important for biological activity. We have also found that the first two of these are involved in the cell-cycle regulating and transforming activities of the E1A products. By last year, we had mapped the boundaries and defined most of the essential amino acids of domain 2. Our delineation of the minimal essential sequence of domain 2 led us to the recognition that this domain represents a conserved amino acid motif common to the transforming proteins of several divergent classes of DNA tumor viruses. We further demonstrated that the homologous domain from the papovavirus SV40 T antigen can substitute functionally for adenovirus E1A domain 2 and, additionally, that either of these domains can associate specifically with a common cellular protein. These results suggested strongly that the transforming proteins of different classes of DNA tumor viruses act at least partly through a similar biochemical mechanism. The identification of the common cellular target as the product of the retinoblastoma tumor suppressor gene (see E. Harlow, Protein Immunochemistry, this section) verified the common identity of the cellular target, and suggested a model for the mechanism of DNA-tumor-virus-induced release from normal cellular growth controls.

Although domain 2 plays an essential role in E1A-transforming functions, we have found that it is not sufficient for these functions. A large deletion (PS*dl*) near the amino terminus, removing all of conserved domain 1 and many of the surrounding sequences, is also unable to participate in host-cell transformation. The defect in this mutant is not due to tertiary disruption of domain-2 structure, because this peptide is able to cooperate in *trans* with a domain-2 deletion peptide (CX*dl*). This *trans*-cooperating activity argues that each domain remains structurally intact in its individual peptide and demonstrates the extraordinary autonomy of each region.

Much of our work this year has focused on defining the essential sequences in E1A upstream of domain 2, understanding the relationship between this region and E1A domain 2, and probing the structural relationships between E1A and other DNA-tumor-virus-transforming proteins. Since at least two separable regions of E1A can cooperate to enact transforming functions, we considered whether each region has a separate function or whether they act in concert, perhaps in a single complex, to enact a single function.

As stated above, sequences in domain 2 are essential for detectable association with the retinoblastoma tumor suppressor gene product. Deletion of the intervening sequences between conserved domains 1 and 2 does not disrupt this association. Deletions extending further upstream, to within conserved domain 1, do, however, disrupt or destabilize this association, so that the association is not detectable by coimmunoprecipitation. This raises the possibility that the essential function contributed by the upstream region is participation in binding the retinoblastoma product. One could imagine that the *trans*-cooperation effect is the result of an association between the domain-1 peptide, the domain-2 peptide, and the retinoblastoma product.

Our attempts to demonstrate by coimmunoprecipitation the formation of a heterodimer or complex between the E1A peptides expressing separate regions have yielded only negative results, even under con-

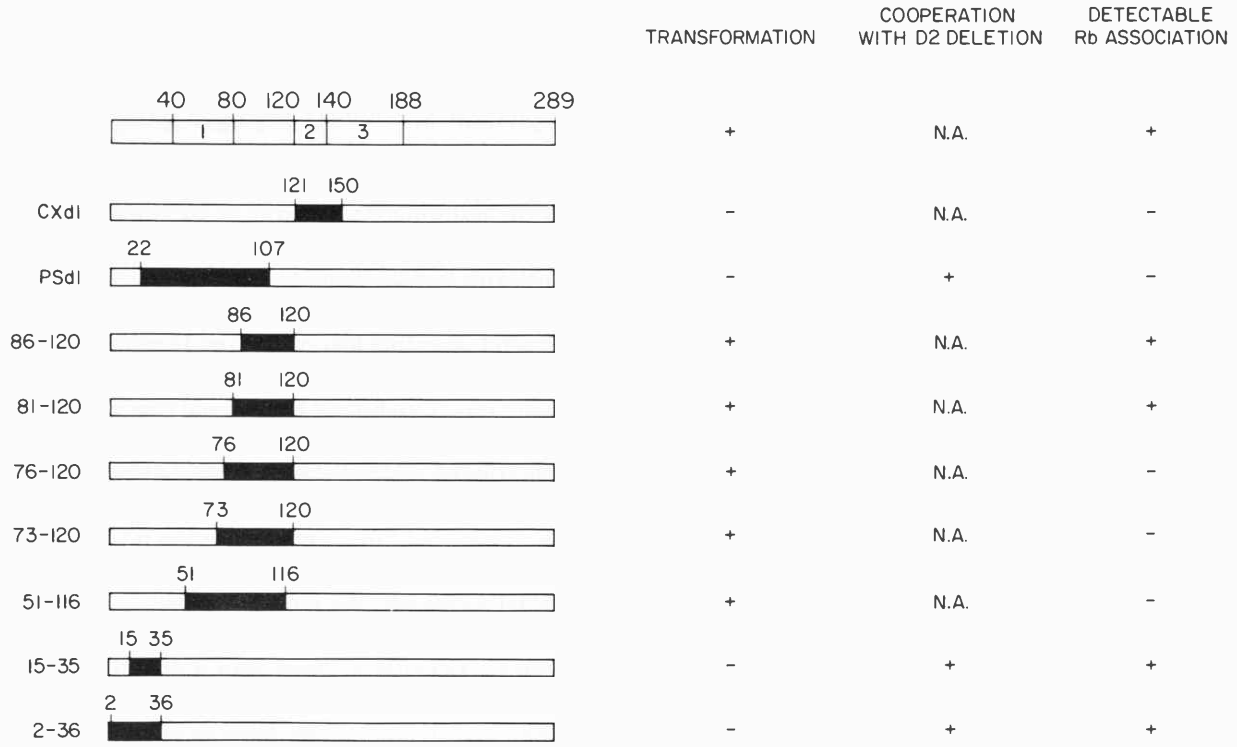

FIGURE 1 The first bar in the diagram above represents the 289R E1A protein product. The residues bordering the three highly conserved regions are noted above the bar. The black regions in the smaller bars below represent deleted sequences. The table to the right summarizes the phenotype of the deletion mutants. The first column indicates the ability of each mutant to cooperate with an activated *ras* oncogene to transform primary cells. The second column indicates the ability of each mutant to cooperate in this assay with an E1A domain-2 deletion mutant. Determination of domain-2-cooperating activity is not applicable (N.A.) in the case of the domain-2 deletion mutant itself (CX*dl*) or in cases where the mutant product has a positive function on its own. The third column indicates the ability of each mutant to bind the cellular retinoblastoma tumor suppressor gene product stably enough for detection in a coimmunoprecipitation assay from infected cells.

ditions that do not disrupt the association between the wild-type E1A products and host-cell proteins. Although these experiments do not rule out the possibility that there is a necessary association that is too transient or unstable to be detected in this assay, the available evidence does not support a model predicting formation of a single active complex. It therefore remains possible that each region contributes a relatively independent function.

To determine the actual active site in the upstream E1A region, we have made systematic deletions extending from the upstream boundary of domain 2 toward the amino terminus or from the extreme amino terminus toward domain 2. We used similar analyses in the past to show that the functional boundaries of domains 2 and 3 correspond very closely with the boundaries of the highly conserved sequences. Surprisingly, though, the results of the present analysis indicate that most of conserved domain 1 is actually dispensable for at least part of E1A-transforming function. The truly essential sequences in this region appear to lie near or in the relatively unconserved region preceding conserved domain 1.

The upstream sequences essential for transformation do not appear to coincide with the upstream sequences necessary for stable association with the retinoblastoma product. We have characterized mutants with deletions extending from the border of domain 2 well upstream into conserved domain 1 (in fact, removing 30 of the 40 amino acids recognized as comprising domain 1) that are not severely defective for E1A-transforming function. These mutants do not show detectable association with the retinoblastoma gene product. Conversely, deletions confined to the relatively unconserved region preceding domain 1 abolish E1A-transforming function but continue to bind the retinoblastoma

gene product indistinguishably from wild-type E1A. These mutants also *trans*-cooperate with domain-2 deletion mutants. These results suggest that the transforming function required from the E1A region upstream of domain 2 is separate from the function promoting association with the retinoblastoma product.

It appears that the sequences upstream from domain 2 constitute several different regions. The nonconserved region (residues 80–120) between conserved domains 1 and 2 is dispensable for retinoblastoma product binding and essentially all other known E1A activities. This region is highly variable both in sequence and in length in the E1A gene products of various adenovirus serotypes.

The highly conserved region that extends from approximately residue 40 to residue 80 may play a significant role in E1A transformation functions, but it is not absolutely essential. Residues from at least 50 onward in domain 1 are not required for transformation, although this region appears to be required for stable association with the retinoblastoma product. Nevertheless, considering the known properties of the retinoblastoma product, it is unlikely that the association between the E1A and retinoblastoma products is not required for E1A function. We suggest that whereas the second domain constitutes an essential active site for transformation and retinoblastoma product association (consistent with our demonstration that a single amino acid substitution in domain 2 abolishes transformation activity and retinoblastoma product binding), the first conserved region, at least from residues 50 to 80, functions more as a stabilizer of this association, or of E1A structure or function, than directly as an active site.

Failure to detect retinoblastoma association with domain-1 deletion mutants may not reflect lack of functional association in vivo, since these mutants retain appreciable transformation activity. This would be consistent with the properties of the E7 transforming gene of the human papillomaviruses. The E7 gene product has a transforming activity similar to that of the E1A products, and a region highly homologous to E1A domain 2. However, the E7 protein shows only limited homology with E1A conserved domain 1. Deletion of E1A residues 51–116 removes most of the sequences that are not homologous between E1A and E7, making the E1A deletion product look very similar to the wild-type E7 product. The transforming activity and lack of detectable retinoblastoma product association in the 51–116 deletion mutant mimic the properties of the E7 product in that retinoblastoma product association with the E7 product has not been detected by coimmunoprecipitation of products expressed intracellularly, but has been detected in vitro. We are currently assessing the ability of the 51–116 product to associate with the retinoblastoma product in vitro.

Although most of conserved domain 1 is not essential for transforming activity, there does appear to be an essential upstream region. It has been known for several years that loss of the first 14 residues from the E1A proteins does not destroy the E1A-transforming function, but we have found that a deletion from residues 15 to 35 abolishes this activity. The boundaries of this deletion lie entirely upstream of the recognized conserved boundaries of domain 1. Although loss of function in a deletion mutant does not mean necessarily that the deletion removes an essential active site, the mutant peptide in this case is as stable as the wild-type E1A proteins, and cooperates efficiently in *trans* with a domain-2 deletion product, so its defect is not due to disruption of domain-2 structure. The deletion product associates very stably with the retinoblastoma product, so this also is not the function lost by this deletion. This mutant demonstrates clearly that association with the retinoblastoma product is not sufficient for E1A-transforming activity.

Since the E1A products associate with several cellular products, it is possible that at least one other association is required for E1A-transforming activity, or there may be an alternative biochemical function required. We are continuing work to determine the biochemical nature of the activity required and to understand the structural and functional relationships between E1A active sites and those of the transforming proteins of other classes of DNA tumor viruses.

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PROTEIN IMMUNOCHEMISTRY

E. Harlow S. Allan, C. Bautista, K. Buchkovich, L. Duffy, N. Dyson, B. Faha, M. Falkowski, A. Giordano, Q. Hu, C. McCall, M. Raybuck, W. Reece, C. Stephens, P. Whyte, N. Williamson

The Protein Immunochemistry Laboratory is divided into two units, one doing basic research and the other serving as a central facility for the production of monoclonal antibodies. The monoclonal antibody facility is supervised by Carmelita Bautista and is ably staffed by Margaret Falkowski and Susan Allan. During the past year, this facility has produced over 700 hybridoma cell lines that secrete antibodies specific for 15 different antigens. This work has been done in close collaboration with many other groups at Cold Spring Harbor Laboratory, and the resultant antibodies are currently being used by many of the Cold Spring Harbor research groups.

The research in the Protein Immunochemistry Laboratory has focused on understanding the biochemical activities of the adenovirus E1A proteins. In particular, we have concentrated on understanding the ability of E1A to transform cells in culture. E1A has been studied intensely over the last several years, mostly concentrating on two of its functions: *trans*-activation and transformation. Studies from a number of laboratories have shown that these two activities map to different regions of the E1A proteins, supporting the hypothesis that these activities are distinct biochemical functions. Two regions of the E1A protein sequence are required for transformation, and these regions correspond to amino acid sequences that are highly conserved between E1A proteins from various serotypes of adenoviruses. These sequences are known as conserved regions 1 and 2, and studies from many laboratories have shown that these regions are important for E1A-mediated transformation (see, e.g., E. Moran in the Adenovirus Genetics section).

Our studies and those of other laboratories have shown that when the E1A proteins are introduced into mammalian cells, they form stable protein/protein complexes with cellular polypeptides. At present, there are ten cellular proteins that are known to bind either directly or indirectly to E1A. The proteins are known by their relative molecular weights of 300K, 130K, 107K, 105K, 90K, 80K, 60K, 50K, 40K, and 28K. Much of the work in the Protein Immunochemistry Laboratory centers on understanding the function of these various E1A/cellular protein complexes.

Cellular Proteins That Are Targets for Transformation by Adenovirus E1A

P. Whyte, N. Williamson, E. Harlow

At least ten cellular proteins are known to form complexes with the adenovirus E1A proteins. The three most abundant of these proteins have relative molecular weights of 300K, 107K, and 105K (p105-RB). To determine in which of the E1A activities

these proteins complexes might participate, the binding sites for these proteins on E1A were determined. The regions were mapped using a series of deletion mutants and were found to include two evolutionarily conserved regions encoded within the first exon of E1A. Amino acids 1–76 contained the binding site for the 300K protein and amino acids 121–127 contained the binding site for the 107K protein. The third cellular protein, p105-RB, appeared to interact with sequences from two noncontiguous regions of the E1A polypeptide chain. This interaction required the presence of amino acids 30 to 60 and 121 to 127. The location of the binding sites for these proteins coincided with the regions of E1A that are required for the transforming function of E1A. These results suggest that these interactions may be important elements of the transforming activity of E1A.

The 105K Protein That Binds to E1A Is the Product of the Retinoblastoma Anti-oncogene

P. Whyte, K. Buchkovich, M. Raybuck, E. Harlow [in collaboration with J. Horowitz, S. Friend, and R. Weinberg, Whitehead Institute]

The E1A proteins of adenovirus are required for viral transformation. These proteins form stable complexes with several cellular proteins, and mutational analysis has revealed that the binding sites for three of these cellular proteins coincide with the regions of E1A required for cellular transformation in collaboration with an activated *ras* gene. This correlation has led us to hypothesize that these cellular proteins are targets for E1A-mediated transformation.

We have demonstrated by peptide mapping and immunochemical analyses that one of the E1A-associated proteins, p105, is the product of the retinoblastoma gene (RB-1). The RB-1 gene was first identified as a locus responsible for predisposition to retinoblastoma. The disruption of both copies of this gene has been linked to the appearance of retinoblastomas and other related tumors, suggesting that the RB-1 gene product may be a component of a regulatory pathway responsible for inhibiting cellular proliferation. Since inactivation of genes such as RB leads to tumor growth, these proteins have been described as tumor suppressors or anti-oncogenes. The product of the RB gene was first identified by Lee et al. (*Nature 329*: 642 [1987]). These authors showed that the RB gene product was a nuclear phosphoprotein with a relative molecular weight of approximately 110K. The similar size and subcellular localization prompted us to compare the RB protein with the E1A-associated 107K and 105K polypeptides. These studies have shown that the RB polypeptide and the 105K protein comigrate on one-dimensional gels and yield identical patterns on Cleveland partial proteolysis experiments. In addition, antibodies specific for the 105K E1A-associated protein will bind directly to the RB protein made in vitro. Likewise, antibodies made to peptides whose sequence was deduced from the RB-1 cDNA confirm that the E1A proteins form a complex with the RB gene product, now known as p105-RB. The demonstration of the p105-RB/E1A complex is the first example of an association between an anti-oncogene and an oncogene.

The RB Protein Is a Common Target for Transformation by DNA Tumor Viruses

N. Dyson, L. Duffy, E. Harlow [in collaboration with K. Münger, B. Werness, and P. Howley, National Cancer Institute]

The RB gene is the best-characterized example of the tumor suppressor genes or anti-oncogenes. These genes are thought to function in the negative regulation of cellular proliferation. Recently, the RB protein has been shown to form stable protein/protein complexes with the transforming proteins of two DNA tumor viruses, the adenovirus E1A protein (see the previous report) and SV40 large T antigen (DeCaprio et al., *Cell 54*: 263 [1988]). To investigate the association between these and other proteins with the RB anti-oncoprotein, we have used assays that demonstrate complex formation in vitro. In these experiments, retinoblastoma proteins are synthesized in vitro in rabbit reticulocyte lysates. Radiolabeled RB proteins are then mixed with lysates containing a protein of interest. If complex formation occurs, the radiolabeled RB protein can be detected after immunoprecipitation using antibodies specific for the protein of interest.

Using this assay, we have shown that large T antigens from all of the commonly used polyomavirus-type viruses will bind to the human RB protein. These include the human BK and JC virus,

baboon SA12 virus, rhesus SV40, hamster lymphotropic virus, and the prototype mouse polyomavirus. Similar experiments using the mouse RB protein yielded analogous results. The observation that all of these viral large T antigens are capable of binding suggests that interactions with p105-RB are a common feature of these virus infections.

Neither the polyomaviruses nor the adenoviruses are thought to be associated with human cancer, but they can cause tumors in rodents. The assay described above has been used to demonstrate that the E7 oncoprotein of the human papillomavirus type 16 can form similar complexes with p105-RB. Human papillomavirus type 16 is found associated with approximately 50% of cervical carcinomas. These results suggest that these three DNA viruses may utilize similar mechanisms in transformation and implicate RB binding as a possible step in human papillomavirus-associated carcinogenesis.

Mapping the Regions of the RB Protein Required for the Interaction with Adenovirus E1A and SV40 Large T Antigen

Q. Hu, N. Dyson, E. Harlow

The protein product of the RB gene is thought to function in a pathway that restricts cell proliferation. Recently, transforming proteins from three different classes of DNA tumor viruses have been shown to form complexes with the RB protein (p105-RB). Genetic studies suggest that these interactions with p105-RB are important steps in transformation by these viruses. It is therefore possible that these viruses modulate p105-RB function through these associations.

To understand the function of the p105-RB/viral oncoprotein complexes better, we have mapped the regions of the RB protein that are necessary for association. Using polymerase chain reaction (PCR) technology, we have prepared and tested a series of mutants for their ability to form complexes with adenovirus E1A and SV40 large T antigen. Labeled RB proteins, synthesized using an in vitro translation system, were incubated with E1A-containing lysates or with purified T antigen, and complex formation was demonstrated by coprecipitation using anti-E1A or anti-T-antigen monoclonal antibodies. Two noncontiguous regions of RB were found to be sufficient for complex formation with the viral proteins. Similar portions of RB were required for complex formation with E1A or large T antigen, although we have observed slight differences in patterns of RB coprecipitation by E1A or T antigen with some of the mutants. At present, the two regions needed for binding include a fragment of 205 residues (amino acids 385–590) and one of 132 residues (amino acids 640–772). This second region contains the retinoblastoma deletion found in the J82 bladder carcinoma cell line, previously shown to be unable to bind to E1A.

Phosphorylation of the RB Protein during Specific Phases of the Cell Cycle

K. Buchkovich

The RB gene product (p105-RB) is a nuclear phosphoprotein found in a wide variety of tissues and cells. It is referred to as the product of an anti-oncogene or tumor-suppressor gene because of its hypothesized role as an inhibitor of cellular proliferation. Two lines of evidence support the hypothesis that p105-RB functions in the control of cell proliferation. First, extensive genetic studies of RB patients have demonstrated a correlation between the absence of the RB-1 gene and tumor formation (for review, see Benedict, *Adv. Viral Oncol. 7*: 19 [1987]). Second, the reintroduction of the RB-1 gene into cells lacking a functional p105-RB protein reduces the growth rate and tumorigenicity of these cells (Huang et al., *Science 242*: 1563 [1988]). The presence of p105-RB in various cell types suggests that its role is not tissue-specific but probably is performed throughout the body and throughout development. The presence of both the mRNA and protein in embryonic and rapidly proliferating cells raises the possibility of posttranslational control of the antiproliferative activity of p105-RB. Our results show that p105-RB exists in at least two forms generated by posttranslational modification. During the cell cycle, p105-RB is phosphorylated in a phase-specific manner. In the G_1 phase of the cell cycle, p105-RB exists in an unphosphorylated form. Beginning in the S phase, p105-RB is phosphorylated. We hypothesize that phosphorylation may be a reversible switch to ensure that p105-RB signals are given at the proper time during the cell cycle.

The 107K Cellular Protein That Binds to Adenovirus E1A also Binds to the Large T Antigens of SV40 and JC Virus

N. Dyson, K. Buchkovich, P. Whyte, E. Harlow

The association between the RB protein (p105-RB) and the large T antigen of SV40, the E1A proteins of adenovirus, or the E7 protein of human papillomavirus type 16 is thought to be an important step in transformation by these viral oncogenes. All three proteins share a small region of amino acid homology that is necessary for high-affinity binding with p105-RB. Mutations of this homology region have been shown to reduce drastically the frequency of transformation mediated by the E1A or large T oncogene. In addition to being required for high-affinity interactions with p105-RB, for E1A this small region is also sufficient for interaction with a second cellular protein of 107,000 daltons (107K).

Recently, we have shown that in SV40- or JCV-transformed human cells, immunoprecipitations of the large T antigens contain a polypeptide that comigrates with the cellular E1A-associated 107K protein. Partial proteolysis studies have confirmed that the 107K polypeptides coprecipitated with monoclonal antibodies specific for all three viral proteins are identical. Several experimental approaches have confirmed that the 107K proteins found in the large T antigen immunoprecipitations are detected as a result of complex formation. First, on sucrose gradients, the 107K protein copurifies with a portion of the large T antigen. Second, six monoclonal antibodies that recognize six different epitopes on T antigen all coprecipitate the 107K protein, indicating that the 107K polypeptide must be either closely related to T antigen or complexed with it. Third, complexes between T antigen and the 107K protein were formed following in vitro mixing. To distinguish the 107K protein from p105-RB, cells carrying homozygous deletions for the retinoblastoma locus were infected with adenovirus, and lysates were immunoprecipitated with anti-E1A antibodies. Full-length 107K protein was present in these cells, showing that the 107K and p105-RB proteins are encoded by separate genes. Although p105-RB and 107K are encoded by different genes, these polypeptides have several characteristics in common and may have similar or related structures at their binding sites for E1A. The demonstration of complexes between 107K and the large T antigens of SV40 and JCV suggests that these associations may represent another component of a common mechanism for transformation between adenoviruses and polyomaviruses.

Production of Monoclonal Antibodies Specific for the E1A-associated Cellular Proteins

L. Duffy, B. Faha, A. Giordano, P. Whyte, E. Harlow

Over the course of our studies with the E1A polypeptides, we have identified a number of cellular proteins that bind to E1A. Genetic and physical studies have shown that many of these interactions are likely to be important for E1A-mediated activities. One of the major methods used to study these interactions and the cellular proteins themselves has been to use immunochemical reagents. The production of antibodies that recognize these proteins directly has been an important part of our work. To prepare these reagents, E1A antibodies were purified and covalently attached to appropriate solid supports. Large-scale preparations were then used to immunoaffinity purify E1A and its associated proteins from transformed cells. The resulting proteins were injected into mice for the production of monoclonal antibodies. These studies have produced approximately 20 hybridomas, 2 of which have been used extensively. C36 is specific for the p105-RB retinoblastoma protein, and C160 recognizes the 60K cellular protein. These antibody production experiments are continuing as we attempt to prepare good reagents for all of the cellular proteins that bind to E1A. Only one of these cellular proteins has been studied in detail, the retinoblastoma p105-RB protein. The cDNA for this protein is available, and we have been attempting to express this protein in bacteria to facilitate antibody production.

Screening Human Tumor Cell Lines for Loss of Potential Anti-oncoproteins

W. Reece, P. Whyte, E. Harlow

One of the hallmarks of tumor suppressor genes is that both alleles of a candidate gene are mutated in certain tumors. This feature is best demonstrated in the case of the RB-1, where both copies of this gene

are mutated in all of the retinoblastoma tumors that have been characterized to date. The mutations of the RB-1 gene often lead to loss of the RB protein. If this characteristic of the RB-1 gene is common to other tumor suppressor genes, then candidate tumor suppressors may be able to be identified by the absence of a protein in tumor cell lines. All of the proteins that bind to the adenovirus E1A polypeptides are potential tumor suppressor genes; therefore, we have been screening human tumor lines for the absence of these proteins. Cell lines from tumors with known chromosomal changes were grown in tissue culture, infected with adenovirus, and immunoprecipitated with antibodies specific for E1A. One of the first cells that showed changes in the E1A-associated proteins was a bladder carcinoma cell line, known as J82. J82 cells produce a truncated retinoblastoma protein. After the initial identification of the mutated protein, the genetic lesion was characterized by J. Horowitz and colleagues at the Whitehead Institute. We have continued to screen additional lines, and approximately 100 cell lines have been analyzed using this technique. Several other potential mutations in the retinoblastoma gene have been identified, and we are currently studying several cell lines that show changes in the 107K E1A-associated protein.

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PROTEIN CHEMISTRY

D.R. Marshak G. Binns, D. Carroll, S. Coleman, N. Santoro, M. Vandenberg

The Protein Chemistry Laboratory is involved in research focusing on the structure and function of oncogene products of viral and cellular origin. The primary structure (amino acid sequence) of most oncogene products can be deduced from the sequence of cDNA molecules on the basis of the genetic code. However, the proteins that are made from the oncogenes are modified enzymatically

following translation. These modifications change the nature of the protein, both in structure and in function. Why does a cell use modifications to alter the function of proteins when the genes themselves can be turned on and off? Modification on proteins provides very fine regulation of function and is often reversible. Modifications are the fine tuning of the systems in cellular physiology and are critical to proper function. During the past year, our attention has focused on one particular type of modification, the phosphorylation of oncogene products by enzymes called protein kinases. In addition, we have obtained a new instrument, the plasma-desorption mass spectrometer, for the detailed analysis of proteins and their modifications. We continue to use state-of-the-art instruments to study the biochemistry of proteins.

Modification of Oncogene Products

D. Carroll, N. Santoro, M. Vandenberg, D. Marshak

The overall objective of our current research is to elaborate upon our earlier observations that have linked casein kinase II (CK-II) activity to the induction of cell growth and the phosphorylation of the viral oncoprotein SV40 large T antigen (LTag) and the adenovirus E1A-13S transcription product, as well as the nuclear oncoproteins c-Myc and c-Fos. To this end, we are taking advantage of several different biochemical strategies using purified E1A, LTag, and Myc protein and derivatization of phosphoserine to *S*-ethylcysteine to identify CK-II phosphorylation sites.

Purified, LTag and Myc proteins maximally incorporate 5 and 7 moles of phosphate per mole of protein, respectively, with K_m values of 300 (LTag) and 150 nM (Myc). The synthetic peptides E1A-001 and MYC-019, whose primary structure spans the predicted CK-II phosphorylation domains within the E1A and Myc proteins, respectively, can competitively inhibit CK-II-dependent phosphorylation of the full-length proteins with K_i values of around 500 nM.

In most of the nuclear oncoproteins we have predicted as being substrates for CK-II, the primary structure of the motif is characterized by clusters of serine residues. This is particularly true for LTag and Myc. Phosphorylation sites on LTag have been mapped last year in this lab and in other laboratories. It is clear that CK-II phosphorylates Ser-107, but there also is phosphorylation at Ser-111 and Ser-112 that have not been well defined. Recent developments in microchemical analysis have allowed for determination of specific phosphorylated serines. To sequence phosphorylation sites on the peptides, we have used β-elimination of phosphoserine with barium hydroxide, followed by reaction of the product, dehydroalanine, with ethanethiol. This results in the stoichiometric conversion of phosphoserine to *S*-ethylcysteine and the kinetics and extent of phosphorylation of serine can be quantitated accurately. This approach is unique in its ability to permit the identification of phosphorylated serines in regions characterized by the presence of multiple serines. Using synthetic peptides spanning the serine clusters found in Myc and LTag, we have derivatized the phosphorylated serines and determined the kinetics of CK-II-dependent phosphorylation. This approach has permitted the observation that the phosphorylation of adjacent serines is likely to be cooperative, with the negative charge contributed by the phosphate on one serine making the neighboring unphosphorylated serine a more likely substrate for CK-II. Such cooperativity could likely prove to be highly significant in identifying the mechanisms underlying phosphorylation-dependent regulation of these protein activities.

Further analysis of the kinetics of LTag phosphorylation demonstrated that the incorporation of phosphate into the intact protein was nonlinear. Increasing proportions of substrate (LTag) resulted in an increasing velocity of the enzyme. These data suggested that LTag was activating its own phosphorylation by CK-II. To test this hypothesis, we phosphorylated Myc protein at saturating levels with CK-II and added catalytic amounts of LTag. In the absence of LTag, the phosphorylation kinetics of Myc were linear, and in the presence of LTag, activation of CK-II phosphorylation of Myc was observed. Analysis of the sequence of LTag has allowed us to predict the domain of LTag that has the activating property. We are currently studying synthetic peptides from LTag as activators of the enzyme. Preliminary studies of COS I cells that overproduce LTag indicate that there is much greater CK-II activity in these cells than in the parent line, CV-1. Our observations may provide insight into the mechanism of LTag transformation of cells and the role of CK-II in viral oncogenesis.

Structure and Function of CK-II

D. Carroll, D. Marshak

We are continuing work in developing reagents and purifications to analyze CK-II structurally. Using purified CK-II from bovine liver, we have raised polyclonal antibodies in rabbits to the enzyme. These antibodies were quite difficult to elicit in rabbits, and efforts to immunize mice for monoclonal antibodies failed. The polyclonal antibodies react on dot-blot experiments on nitrocellulose, but attempts to do Western blots have been unsuccessful. This suggests that the antigenic site for these antisera may overlap two of the subunits of the protein, and gel electrophoresis that separates the subunits destroys the antigenicity. To develop more reagents for CK-II, we are trying to sequence the regulatory subunits to develop synthetic peptide antigens for immunization. In addition, we plan to use oligonucleotide probes to isolate cDNA clones of the regulatory and catalytic subunits of the enzyme. Brain tissue is a very rich source of CK-II, so we are purifying the enzyme from bovine brain. Initial purification steps indicate that the brain enzyme is similar but not identical to that of liver or lung.

Protein Kinases in Cell Growth

D. Carroll, D. Marshak, S. Coleman

Last year, we began to study the function of CK-II in cultured cells under various growth conditions. Experiments were conducted to study CK-II induction during the cell cycle. WI-38 cells, a human lung fibroblast cell line, were serum-starved to synchronize in G_0. After serum stimulation, CK-II levels and cAMP-dependent protein kinase activities were measured over a 48-hour time course. CK-II was induced sixfold by 15–30 minutes, with a corresponding eight- to tenfold decrease in cAMP-dependent protein kinase activity. These changes in kinase activities were independent of protein synthesis. At 16 hours, when thymidine incorporation into DNA began, cAMP-dependent protein kinase levels recovered to near basal levels, whereas CK-II showed a recovery followed by a second phase of induction. These results suggest that there is an early phase of CK-II induction by serum growth factors, coincident with the transcriptional induction of the early activatable genes, such as c-*myc* and c-*fos*. In addition, there appears to be a second phase of induction coincident with the S phase. We have followed up on these observations and have noted that there is an elevation in phosphate incorporated into the endogenous c-*myc* coincident with the 0.5- and 12-hour time points and that this increased phosphorylation can be blocked by the CK-II competitive substrate MYC-019. This observation underscores the likely role of CK-II in modifying growth-related proteins such as c-Myc. Our future experiments on CK-II in cell growth will focus on the cell cycle of mammalian cells.

Further experiments were conducted to link CK-II to other signal transduction pathways. Phorbol esters stimulate CK-II levels, presumably through a protein-kinase-C-mediated event. Preloading of cells with dibutyryl cAMP (which activates the cAMP-dependent protein kinase) blocks the phorbol ester induction of CK-II. Our working hypothesis in this area is that protein kinase C is a positive regulator of CK-II and that cAMP-dependent protein kinase is a negative regulator of CK-II. Protein tyrosine kinases have been shown by other laboratories to affect CK-II activities in growth-factor-stimulated cells. These effects are relatively modest and are indirect, probably involving other intermediate protein kinases. Experiments are now under way to evaluate the roles of *raf* kinase and *cdc2* kinase in the regulation of CK-II activity in signal transduction. During the coming year, we are hopeful that a new chapter in the ever-expanding story of signal transduction will be written linking CK-II to various cellular processes.

Mass Spectrometry of Proteins and Peptides

D. Marshak, G. Binns

During 1988, Cold Spring Harbor Laboratory became one of the first sites for a new type of instrument, a plasma desorption, time-of-flight mass spectrometer. This instrument is unique in its ability to measure the molecular weight (mass) of large macromolecules, including proteins, very accurately. Most laboratories would estimate the molecular weight of a protein by its relative mobility in an electric field, a process known as electrophoresis. Even under ideal circumstances, these size estimates

are only within about 1000 atomic mass units. With our new mass spectrometer, molecular weight can be measured to within 0.1 mass units for small proteins and 1–10 mass units for larger proteins. This capability becomes essential when searching for modifications of proteins. For example, much of our work involves phosphorylation of serine residues on proteins. This modification adds 80 mass units to a protein, a change that is easily demonstrated by the mass spectrometer. However, phosphorylated proteins often show anomalous mobility under electrophoresis. Thus, the use of mass spectrometry opens new doors to the analysis of modifications on proteins.

The mass spectrometer is especially helpful in the analysis of synthetic peptides. We synthesize peptides on a solid support, usually a modified polystyrene, using symmetric anhydride activation of *N*-α-t-Boc amino acids. This year, we have begun using a new solvent, *N*-methyl-pyrrolidone, with an addition of dimethylsulfoxide for the final coupling. This solvent system causes increased swelling of the polystyrene resin and more efficient coupling of the amino acid derivatives. The peptides are cleaved from the support using hydrogen fluoride (HF) in the presence of scavengers, such as anisole and thiocresol. Strong acids such as HF can lead to several unwanted side reactions including dehydration, oxidation, electro-

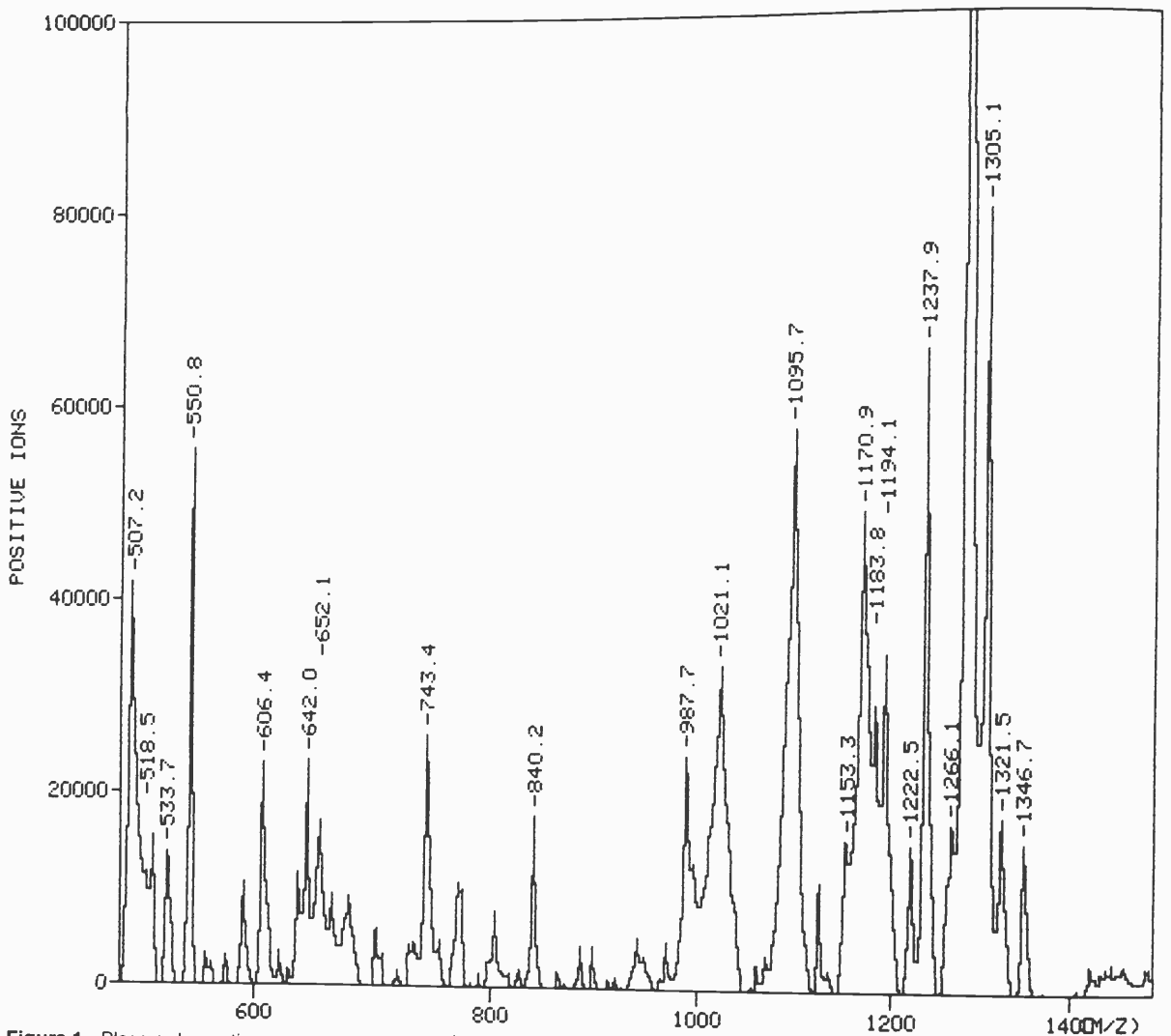

Figure 1 Plasma desorption mass spectrum of Val^5 angiotensin I. A partial mass spectrum was collected on a Bio-Ion spectrometer at 16 kV accelerating potential for 20 hr. The molecular ion $(M+H)^+$ peak at 1283 amu is off scale in order to show fragment ions from 500 to 1266 amu.

philic aromatic substitution, alkylation, condensation, and various miscellaneous free radical additions. The mass spectrometer allows us to screen the products of the synthesis and to eliminate the undesirable side products rapidly. The crude peptide product is subjected to preparative HPLC at high flow rates, 40–75 ml/min on columns that are 49–57 mm in diameter. Fractions that are collected from this chromatography are analyzed by mass spectrometry, and the desired fraction is identified in less than 1 hour. Thus, the purification of a synthetic peptide is reduced from 1–2 weeks to 1–2 days.

During the course of our analyses of synthetic peptides by mass spectrometry, we noticed that long runs on the instrument resulted in a discrete pattern of fragments from the peptide. Our lab and other investigators have used the plasma-desorption mass spectrometer to measure the mass of the whole molecule, but we now realize that the complete structure of a peptide can be deciphered from its fragmentation pattern. Thus, we can obtain both molecular weight and structural information from the mass spectrometer. Figure 1 shows the mass spectrum of an analog of angiotensin I, a peptide involved in the control of blood pressure. The peak corresponding to the intact molecule at a mass of 1283 is off scale in order to show the smaller peaks of fragment ions that describe the complete structure. We are very enthusiastic about the potential for structural analysis by mass spectrometry.

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PROTEIN SYNTHESIS

M.B. Mathews A.P. Rice, M. Kessler, M. Laspia, A. Maran, G.F. Morris, T. Pe'ery, C. Herrmann, K.H. Mellits, R. Galasso, H. Goodrich, L. Manche, R. Packer, M. Sullivan, P.A. Wendel

The Protein Synthesis group continues to investigate the control of gene expression at several levels. In addition to the adenovirus system, which for many years has been a mainstay of the group's work, studies of the AIDS human immunodeficiency virus type 1 (HIV-1) now form a substantial fraction of our research effort. There have also been some changes in personnel. Drs. M. Kessler and T. Pe'ery joined the lab as postdoctoral fellows in the latter part of the year, and Drs. A. Maran and E. Moran moved on. Betty Moran is continuing her studies of the transformation-related activities of the adenovirus E1A gene in James Lab, and her research report will be found in the Adenovirus Genetics section.

Adenovirus VA RNA and Translational Control

K.H. Mellits, L. Manche, P.A. Wendel, A. Maran, M.B. Mathews

Unlike most adenovirus genes, which encode proteins and are transcribed by RNA polymerase II, the adenovirus VA RNAs are noncoding and are transcribed by RNA polymerase III. Nevertheless, they play an equally important role in protein synthesis. These two RNAs, VA RNA_I and VA RNA_{II}, are both about 160 nucleotides long, and VA RNA_I in particular becomes very abundant at late times of infection. As described in previous years' reports, VA RNA_I is required for the efficient synthesis of proteins at late times of adenovirus infection, so that cells infected with a mutant adenovirus (*dl*331) that fails to make VA RNA_I synthesize proteins at less than one tenth of the normal rate. The mechanisms underlying this inhibitory process have been worked out in considerable detail. In the absence of VA RNA_I, translation is blocked by the action of a cellular protein kinase known as DAI, the double-stranded (ds) RNA-activated inhibitor of protein synthesis. In the presence of dsRNA, which is probably transcribed from the viral genome itself, this kinase phosphorylates the initiation factor eIF-2, thereby sequestering the recycling factor (GEF or eIF-2B) in a tight complex and blocking the initiation step of protein synthesis. We have now purified the DAI enzyme and are continuing our efforts to ascertain the mechanism by which VA RNA prevents the activation of DAI.

As a first step, we generated linker-scanning and deletion mutants within the 3′ half of the VA RNA_I molecule. The mutants were tested in a transient expression assay for their ability to correct the protein synthesis defect observed in *dl*331-infected cells. Based on the knowledge that VA RNA possesses short duplexed regions and that such short stretches of dsRNA are unable to activate DAI, we had speculated that the duplexed regions would play an important role in the function of VA RNA. Surprisingly, we found that activity was retained after deletion of a region near the center of the gene, nucleotides 72–85, which forms part of one of the longer duplexes. On the other hand, activity was drastically reduced by mutations located further to the 3′ side of this region. Analysis of the secondary structure of wild-type VA RNA_I using the nuclease sensitivity technique allowed us to derive a model for its secondary structure. Similar analysis of mutant RNAs suggested that a complex stem and loop structure located roughly in the center of the proposed secondary structure model is important for VA RNA's function. This central stem-loop structure is flanked by duplex regions that seem to be necessary for its stability. When the wild-type flanking sequences were replaced with foreign sequences, activity was retained provided that the foreign sequences restored base pairing. These findings emphasize that maintenance of duplex structure is more important than sequence conservation in some regions of the molecule, whereas in other regions, more intricate structural features are required.

We have begun to examine the interactions between DAI, VA RNA, and dsRNA at the biochemical level. Wild-type VA RNA binds to DAI and blocks the binding of dsRNA to DAI at concentrations similar to those that are effective in vivo. This result raises the possibility that VA RNA may function by competing, directly or indirectly, for the binding of dsRNA to the enyzme. To test this idea further, we are in the process of assaying the ability of mutant VA RNAs to bind to DAI and to compete with dsRNA for binding to DAI. Preliminary results suggest that there is no simple relationship between DAI binding and functional activity, but current data are limited by the difficulty of producing the mutant RNAs in adequate quantities. To alleviate this problem, we have cloned the VA RNA_I gene into a vector containing the T7 RNA polymerase promoter so that copious quantities of the RNA can be synthesized in vitro. Wild-type VA RNA made in this manner is fully functional, and we are now transferring the mutant VA RNA genes into the same vector so that suitably large quantities of these RNAs can be synthesized. In principle, the T7 system also offers the possibility of producing the wild-type transcript on a scale sufficient to attempt a determination of its tertiary structure using X-ray crystallographic methods, and we have initiated experiments toward this end.

Regulation of Gene Expression by E1B

C. Herrmann, M.B. Mathews

We have been studying the regulation of gene expression by a product of the adenovirus early region 1B. This region, together with E1A, is re-

sponsible for the ability of adenoviruses to transform cells. The E1B region encodes two major protein products of 55,000 and 19,000 daltons, which have both been implicated in the regulation of viral gene expression and in the complete transformation of primary rodent cells. Our attention has been focused on the smaller of these proteins, the 19K tumor antigen, and its role in increasing the expression of other genes. In the initial stages of this work, we employed infection and transient expression techniques to introduce the gene for the 19K protein into cells. We found that the 19K protein increased expression from several adenovirus promoters and from a cellular promoter in a sequence-independent manner. This increase occurred at or before the level of mRNA accumulation in the cytoplasm, consistent with elevated transcription from the various promoters. To facilitate further study, we have now constructed human cell lines that produce the 19K protein under the control of the inducible metallothionein promoter, allowing a high level of expression of the 19K protein. The 19K-expressing cell lines were characterized for 19K DNA, mRNA, and protein levels and were shown to complement 19K mutant viruses for their *cyt* (enhanced cytopathic effect) and *deg* (DNA degradation) phenotypes, indicating that the stably introduced gene is functional.

To examine the effect of the 19K protein on the expression of heterologous genes, a test promoter fused to the chloramphenicol acetyltransferase (CAT) gene was transfected into a 19K-expressing cell line or a control cell line, and expression from each promoter was assayed by measuring CAT activity. The promoters tested were the adenovirus early (E1A, E1B, E2e, E3, and E4) and late (MLP, IX, IVa2, and E2L) promoters, the SV40 early promoter linked to its enhancer, the human immunodeficiency virus type 1 (HIV-1) long terminal repeat (LTR), and the promoter for the cellular 70-kD heat-shock protein (hsp70). Without exception, these promoters showed greatly increased activity in the 19K-expressing cell line compared to the control line. Furthermore, we found that the 19K protein increased expression of the VA RNA_I gene which is transcribed by RNA polymerase III. These results indicate that the 19K protein acts in a nonspecific manner. The effect of the 19K protein appears to be limited to transfected genes, however, since we could not detect an increase in the synthesis of the endogenous hsp70 protein. We are currently examining the levels of other cellular proteins in the 19K-expressing and control cell lines by two-dimensional gel analysis.

We have been interested in determining the level of control at which the 19K protein acts to increase the level of expression of transfected genes. Our results show that one role of the 19K protein is to stabilize transfected plasmid DNA. In the absence of the 19K protein, the majority of the input DNA is degraded, especially at late times posttransfection. In 19K-expressing cells, however, a higher level of DNA is retained (approximately eightfold greater), suggesting that the increase in gene expression is due, at least partly, to an increase in plasmid DNA levels. The stabilization of plasmid DNA is not dependent on the transcriptional activity of the plasmid, since plasmids lacking promoters were stabilized to the same extent as plasmids containing actively transcribed promoters. Although we cannot rule out subsidiary effects at the transcriptional level, experiments using virus-infected cells indicate that the 19K protein does not have a direct effect on transcriptional activity as measured by a nuclear runoff assay; so it seems likely that the primary effect of the 19K protein in transfected cells is to increase the level of DNA, which in turn leads to a higher rate of transcription. Further experiments are under way to assay directly for a possible transcriptional action of the 19K protein in transfected cells.

Stabilization of plasmid DNA explains the nonspecific action of the 19K protein and is consistent with its role during viral infection, where it prevents the degradation of viral and cellular DNAs. We are in the process of testing other viral proteins that increase gene expression in a nonspecific fashion, to determine if stabilization of DNA is a general mechanism whereby gene expression can be regulated.

Regulation of PCNA

G.F. Morris, M.B. Mathews

The proliferating cell nuclear antigen (PCNA), also known as cyclin and DNA polymerase-δ auxiliary factor, is present in reduced amounts in nongrowing cells and is synthesized at a greater rate in the S phase of growing cells. (Its alias notwithstanding, PCNA is unrelated to the cyclins first discovered in sea urchins and clams and now detected in frogs and yeast; see Genetics Section.) This pattern of expression, coupled with the demonstration that PCNA

plays a role in DNA replication, suggested that the availability of PCNA may regulate DNA synthesis. To test this idea, we conducted an investigation of the synthesis, stability, and accumulation of PCNA throughout the cell cycle. HeLa cells were fractionated by centrifugal elutriation, a technique that separates cycling cells into nearly synchronous populations of cells at various positions in the cell cycle without the physiological stresses imposed by other methods of achieving cell synchrony. We found that there is an increase in the rate of PCNA synthesis, with a peak early in the DNA synthetic, or S, phase of the cell cycle, but the magnitude of the increase is only two- to threefold (Fig. 1, top). This change reflects similar changes in the amount of PCNA mRNA. The fluctuating synthesis of PCNA maintains this protein at a roughly constant proportion of the total cell protein and ensures that its cellular concentration doubles in the cell cycle as required for preservation of a steady state. Consistent with these findings, the stability of PCNA does not differ significantly from that of total cellular protein in the synchronized HeLa cells.

On the basis of these observations, we conclude that the synthesis of PCNA in cycling HeLa cells maintains PCNA in excess of the amount involved directly in DNA replication and that the amount of the protein neither fluctuates significantly with the cell cycle nor is limiting for DNA synthesis. It remains possible, however, that cells exhibiting more tightly controlled growth properties than HeLa cells regulate the synthesis of PCNA differently during the cell cycle. Nevertheless, consistent with its role in DNA replication, we found that a variable proportion of PCNA is tightly associated with the nucleus (Fig. 1, bottom). This fraction reaches a maximum value of about 35% of the total at the peak of S phase and presumably represents those molecules involved in replication complexes. We believe that the recruitment of PCNA into replication complexes can account for the large fluctuations in the immunofluorescent staining intensity previously reported for PCNA during the cell cycle.

To pursue our analysis of the expression of this protein at the molecular level, we have cloned the promoter for the PCNA gene. The cloned promoter directs the synthesis of RNA with the correct size for a properly initiated PCNA transcript in HeLa nuclear extracts, and it potentiates the synthesis of a reporter mRNA in transfected HeLa and 293 cells. We are presently working to define the *cis*-acting elements required for transcription of PCNA mRNA in both systems with a view to exploring the mechanisms whereby PCNA synthesis is increased by serum factors and by adenovirus infection.

Structure and Function of HIV-1 *tat*

A.P. Rice, H. Goodrich, M. Sullivan, R. Packer

Human immunodeficiency virus type 1 (HIV-1), the causative agent of acquired immune deficiency syndrome (AIDS), is a retrovirus with an unusually complex genetic structure. Besides encoding the usual structural proteins common to all nondefective retroviruses (*gag, pol, env*), HIV-1 contains at least six additional genes known as *vip, vpr, vpu, tat, rev,* and *nef.* The *tat, rev,* and *nef* genes specify regulatory proteins that work *in trans* to control HIV-1 gene expression. The *nef* protein acts to down-regulate HIV-1 replication. The *rev* protein controls the relative abundance of spliced and unspliced RNAs. The *tat* protein functions to increase the amount of HIV-1 gene expression. We are examining the mechanism of action of the *tat* protein in the regulation of HIV-1 gene expression.

The HIV-1 *tat* protein *trans*-activates gene expression by interacting with the so-called TAR element located within the viral long terminal repeat (LTR) sequence. The molecular mechanisms by which *tat* acts upon the TAR element are unclear, but as discussed in last years' report, they include increased transcription of mRNA from the LTR as a major (and possibly sole) component. This transcriptional regulation by *tat* has been proposed to involve antitermination. The *tat* protein from HIV-1 strain HXB2 consists of 86 amino acids encoded in two exons. The first exon encodes the first 72 amino acids and the second exon encodes the remaining 14. To gain further insight into structural features of the *tat* protein involved in *trans*-activation, we have carried out a mutational analysis of the HIV-1 *tat* gene. A *tat* cDNA was introduced into a SV40 late promoter-based expression vector, and site-directed mutagenesis was used to make a collection of 24 mutant proteins. The *trans*-activation activities of the mutant proteins were assayed in plasmid DNA cotransfection experiments.

We found that a deletion mutant containing only the first 57 amino acids retains approximately 30% activity, but further deletion to amino acid 48 abolishes all activity. We also have found that a mutation near the amino terminus of the protein, the deletion

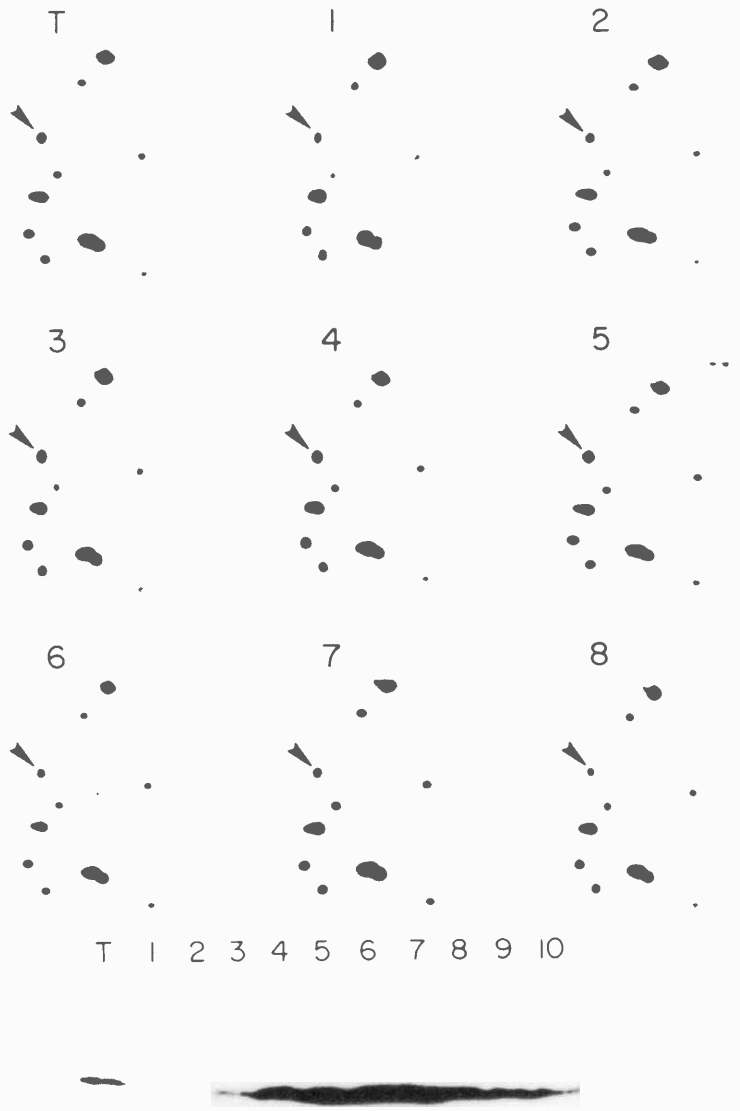

FIGURE 1 Regulation of PCNA. (*Top*) PCNA synthesis during the cell cycle determined by two-dimensional gel electrophoresis. Cells were fractionated by elutriation and pulse labeled with [^{35}S]methionine. Equal amounts of radioactive protein from each fraction were separated by two-dimensional gel electrophoresis in the Cold Spring Harbor Laboratory Quest facility. Separation in the first dimension was by isoelectric focusing from high pH to low pH (*right* to *left*). Separation in the second dimension was by SDS-polyacrylamide gel electrophoresis (*top* to *bottom*). Only the portion of interest of each autoradiogram is shown. The arrowhead denotes PCNA. Quantitation of PCNA synthesis by computer-aided densitometric scanning indicated that PCNA synthesis varied about twofold during the cell cycle, with a peak of synthesis at the beginning of S phase (Fractions 4 and 5). (*Bottom*) Chromatin-bound PCNA in cycling HeLa cells. Nuclei prepared from elutriated HeLa cell fractions were washed with saline solution to remove loosely bound PCNA. The tightly bound PCNA was released by incubation with DNase, resolved in an SDS-polyacrylamide gel, and electrophoretically transferred to a nitrocellulose membrane. PCNA was detected in each fraction by reaction with mouse monoclonal antibody to PCNA and ^{125}I-labeled rabbit anti-mouse antibody, followed by autoradiography. The chromatin-bound PCNA in unfractionated HeLa cells is shown in lane T. Lanes *1* through *10* show the bound PCNA observed as the cell progresses from G_1 (lane *1*) through S (peak in lane *6*) into the G_2 phase of the cell cycle (lane *10*).

of residues 3 through 6, greatly reduces activity. Our results with mutations at the amino and carboxyl termini agree with those of other workers in the field.

There are two regions located within the central region of the *tat* protein with notable sequence features; a cysteine-rich region and a region rich in basic residues. The cysteine-rich region contains seven cysteine residues between amino acids 22 and 37; the basic region contains eight lysine and arginine residues between amino acids 49 and 57. The basic region of the *tat* protein constitutes a nuclear localization signal, reminiscent of a similar sequence first identified in SV40 large T antigen. *tat* expressed and purified from *Escherichia coli* has been shown to bind two metal ions, either cadmium or zinc, per molecule and to form a homodimer, and it has been proposed that cysteine residues play an important role in these two processes. Several research groups, including our own, have shown that six of the seven cysteines are important for function, the exception being Cys-31. Mutation of any of the remaining six cysteine residues individually to either glycine or serine abolishes activity of the *tat* protein, consistent with the notion that these residues coordinate metal binding and dimerization and that this is a crucial property for function.

We have concentrated our mutagenesis on the noncysteine residues between amino acids 18 and 47, in most cases changing a single wild-type amino acid to alanine. Proteins with mutations at residues 23, 24, 46, and 47 were found to be as active as wild-type *tat* protein, but mutations between residues 26 and 41 greatly reduced the activity of the *tat* protein. The data suggest that the precise structure of this region of the *tat* protein, from residue 26 to 41, is crucial for function. We are currently conducting studies on wild-type and mutant *tat* proteins to correlate biochemical properties such as metal binding and dimer formation with functional properties.

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

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

Expression of the genes of the human immunodeficiency virus type 1 (HIV-1) is greatly stimulated by the *trans*-activator encoded by the viral *tat* gene. *tat* appears to interact with a sequence in the long terminal repeat (LTR) that lies downstream from the site of transcriptional initiation, to increase the levels of RNA. The *tat*-responsive element is called TAR, an acronym for the *trans*-activation response element. Although the detailed mechanism of *trans*-activation of HIV-1 gene expression by *tat* is unclear, and regulation has been suggested to occur at several levels, we and other investigators find the predominant mode of regulation by *tat* to be transcriptional. The 5′-untranslated region of HIV-1 mRNA, transcribed from the TAR region, is capable of forming a secondary structure, and mutations that alter this structure abolish *trans*-activation by *tat*. A model for the action of *tat* has been proposed which suggests that *tat* acts as an antiterminator, relieving a block to transcription elongation that occurs within the LTR.

We have made use of a recombinant adenovirus system, containing the HIV-1 LTR fused to the reporter gene chloramphenicol acetyltransferase (CAT) to study HIV-1 gene expression. As described last year, an advantage of this system is that infection with the recombinant adenovirus provides an efficient, controllable means to deliver an HIV-1 LTR/reporter gene fusion into human cells. Furthermore, recombinant adenoviruses can be used for large-scale infections, providing the opportunity to perform biochemical analysis. To determine the sequences required for *trans*-activation by *tat*, we introduced a number of deletions from the 5′ end of the LTR and several TAR region mutations into the HIV/adenovirus recombinant. HeLa cells expressing *tat* were infected with these viruses, and CAT expression and mRNA levels were measured. For comparative purposes, we also analyzed *trans*-activation of the HIV-1 LTR by the adenovirus E1A 13S gene product. This protein, which acts to increase transcriptional initiation, was introduced by coinfection with a wild-type adenovirus.

We found that *tat* increases the level of correctly initiated mRNA by more than 50-fold (Fig. 2). E1A also increased the level of HIV-1 RNA, presumably by increasing the rate of transcriptional initiation. A mutant containing a large deletion within the TAR region lacked the ability to respond to *tat* but was still *trans*-activated by E1A, indicating that the HIV-1 promoter does not absolutely require TAR to function. In addition to authentic full-length RNAs, a number of short transcripts were detected that initiated correctly at nucleotide +1 and appeared to terminate at +55 and +59. Physical characterization of the short transcripts indicated that they are

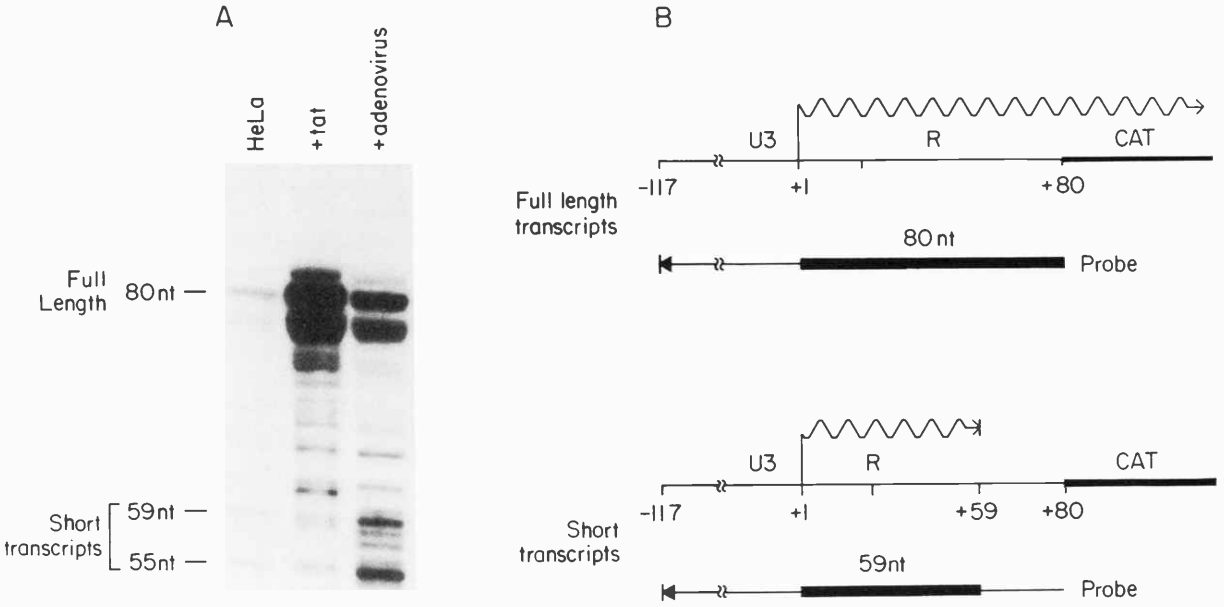

FIGURE 2 Analysis of RNA transcribed from the HIV-1 promoter in cells infected with the HIV-1 recombinant adenovirus. (*A*) A riboprobe-RNase protection assay of cytoplasmic RNA isolated from HIVCATad-infected HeLa cells (HeLa), HeLa cells expressing *tat* (+*tat*), and HeLa cells coinfected with HIVCATad and wild-type adenovirus (+adenovirus). (*B*) A schematic of the transcripts and probes. Transcripts are depicted as wavy lines, initiating from nucleotide +1 on the template DNA and transcribed in a rightward direction. U3 and R are regions of the HIV-1 LTR. The CAT gene begins at nucleotide +80. The probe was radiolabeled antisense RNA extending from nucleotide +80 to −117. Probe fragments protected by correctly initiated full-length mRNA and short transcripts are shown as thickened lines. An 80-nucleotide fragment is protected by full-length transcripts, whereas fragments of 55 and 59 nucleotides are protected by short transcripts.

not polyadenylated and are not produced by splicing, consistent with the idea that they result from premature termination. The level of full-length transcripts was increased in the presence of *tat* or of E1A. E1A also increased the level of short transcripts by severalfold, but *tat* did not. In neither case could the level of full-length transcripts be accounted for simply by extending the short RNAs observed in their absence. These results are consistent with a model that *tat* acts to increase the level of transcriptional initiation as well as the efficiency of reading through a proximal termination site. Interestingly, no short transcripts were detected with the TAR deletion mutant, nor was the basal level of gene expression increased. These observations provide no support for the idea that the TAR region acts as a terminator of transcription.

To determine the relative contributions of increased initiation and antitermination to the regulation of HIV-1 gene expression, we have used nuclear runon transcription assays to measure the distribution of RNA polymerase molecules engaged in transcription of the HIV-1 LTR. We find that *tat* causes a tenfold increase in the frequency of RNA polymerases engaged in transcription between nucleotides +1 and +80, suggesting that *tat* acts to increase the level of transcriptional initiation. We are currently examining the effect of *tat* on RNA polymerase distribution in promoter distal regions to assess the degree to which antitermination contributes to the regulation of HIV-1 gene expression.

We have also analyzed nested deletions of the LTR cloned into the recombinant adenovirus and found that nucleotides upstream of −104 are dispensable for *trans*-activation by both *tat* and E1A. Deletion of sequences upstream of nucleotide −48, removing both the core enhancer elements and the Sp1 sites, greatly reduced gene expression but still permitted some *trans*-activation by both *tat* and E1A. Thus, sequences lying downstream from −48 seem to be required for *trans*-activation by both *tat* and E1A. We will now use site-directed mutagenesis to identify

promoter elements that are required for *trans*-activation by both E1A and *tat*.

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NUCLEIC ACID CHEMISTRY

R.J. Roberts G.C. Conway, J. Harper, C. Marcincuk, S. Miceli, S. Munroe, M. Wallace

Isolation of the Spliceosome

G.C. Conway

The process of RNA splicing requires multiple factors that bind stably to pre-mRNA, forming a macromolecular complex called the spliceosome. My research goal is to isolate splicing factors and native spliceosomes. Density gradient and native gel analyses of in vitro splicing reactions can provide fractions enriched in splicing complexes. However, these fractions are still contaminated with endogenous particles that pre-exist in splicing extracts; in the case of native gel analysis, the splicing complexes have undergone some dissociation due to the electrophoretic conditions. My approach to the isolation of the spliceosome is to remove contaminating particles from nuclear extracts by density gradient fractionation prior to reconstituting an in vitro splicing reaction.

Nuclear splicing extracts have been layered onto sucrose gradients and material fractionated by sedimentation. There are three peaks of OD_{260} absorbing material: one sedimenting less than 30S, a 45S peak, and one sedimenting faster than 50S. The material that sediments faster than 30S contaminates spliceosome preparations isolated by density gradients and by native gels. It was hoped that fractions less than 30S would splice exogenously added RNA, thus providing a cleared splicing extract devoid of large particles. Unfortunately, neither of these fractions splices exogenously added pre-mRNA. However, fractions greater than 30S will complement fractions that are less than 30S in RNA splicing.

Interestingly, if nuclear extracts are incubated with ATP prior to gradient fractionation, fractions less than 30S will now splice RNA, and the 45S peak of material is reduced to one third of the original OD_{260} absorbance. This suggests that RNA and/or protein components are released or degraded from fast-sedimenting endogenous complexes. Likely splicing factors with a large OD_{260} value that might shift to upper gradient fractions are snRNPs (small nuclear ribonucleoprotein particles). The snRNPs are known to be involved in RNA splicing. Examination of the RNA species in gradient fractions indicates that the U1 and U2 snRNAs do in fact shift to upper gradient fractions after ATP incubation. The gradient distribution of two other splicing factors, SF2 and the heat-labile factor, has also been examined. These protein factors are present in fast-sedimenting fractions in unincubated extracts but shift to upper gradient fractions upon ATP incubation. Thus, it appears that splicing extracts contain fast-sedimenting complexes that possess splicing factors. Under splicing conditions, splicing factors are released from these complexes and remain at the top of density gradients. These top fractions constitute a cleared extract.

I have investigated splicing complex formation using cleared extracts and have found that spliceosomes formed in cleared extracts possess many properties different from those formed in standard nuclear extracts. These differences are apparently due to factors not necessary for splicing that bind to pre-mRNA in standard extracts but are missing from the splicing complexes formed in cleared extracts. For example, the spliceosome in cleared extracts sediments at 45S as opposed to the 50S to 60S complex formed in standard extracts. The kinetics of spliceosome formation in cleared extracts is also accelerated compared to those in standard extracts. These accelerated kinetics may be due to the prior release of splicing factors from endogenous complexes as well as reduced competition with endogenous heterogeneous nuclear RNA (hnRNA), which is missing in cleared extracts. Finally, the cleared splicing extract should be a powerful tool in the isolation of highly enriched spliceosomes.

Splicing reactions using cleared extracts should contain only one large complex, the spliceosome formed on exogenously added pre-mRNA. A splicing reaction with a cleared extract analyzed by density gradient sedimentation reveals that the snRNA species remains at the top of gradients if pre-mRNA is not added. However, if the reaction is performed with the addition of pre-mRNA, the pre-mRNA and snRNAs now cosediment into the gradient. This shift of the snRNAs would never be visible with standard extracts, because lower-gradient fractions have too much background. Using this approach, I have begun to analyze the proteins that shift into lower-gradient fractions in response to the addition of pre-mRNA. The use of RNA mutants with defective 5′ and 3′ splice sites will hopefully allow the correlation of *cis*- and *trans*-acting elements. This approach to spliceosome isolation should also be of value in the ultrastructural characterization of the spliceosome.

Alternative Splicing of the Adenovirus E1A Gene

J. Harper, S. Miceli

We have continued our experiments to identify factors that may affect alternative splicing patterns of pre-mRNAs from complex transcription units. Our work has focused on the adenovirus E1A transcription unit, which has three 5′ splice sites and two 3′ splice sites that are used to generate at least five different mRNAs in infected cells. We have previously reported that although the splicing of E1A pre-mRNA appears to be regulated temporally during the course of adenovirus infection, all of these RNAs can be produced during in vitro splicing reactions using extracts prepared from uninfected HeLa cells, indicating loss of regulation under these conditions. This may be due to our inability to extract active regulatory factors by our current methods or may indicate that E1A splicing is regulated in vivo by alteration of the substrate pre-mRNA, rather than solely by changes in *trans*-acting factors.

Despite this disappointing result, we have successfully used E1A pre-mRNA splicing as a model for 5′ splice site selection in vitro to identify an activity that affects competition between alternative 5′ splice sites but has no effect on splicing in the absence of competition. We consistently observe that the ratio of different E1A products varies dramatically between different in vitro reactions, depending on the particular extract used. The reasons for these extract-dependent differences are not yet clear, but we have ruled out such obvious possibilities as differences in salt concentration during extraction or dialysis or differences in total protein concentration. The most useful comparison for evaluating

extract differences is the ratio of 13S to 12S RNA. These RNAs are generated by splicing between two different 5′ splice sites and a common 3′ splice site. In some extracts, splicing wild-type E1A substrate RNA produces predominantly 13S RNA, whereas splicing the same substrate under identical conditions in other extracts produces large amounts of 12S RNA in addition to 13S RNA. These examples represent the extremes of activity observed in a survey of numerous extracts, of which approximately 30% produce high levels of 12S RNA, 10% produce extremely low levels of 12S RNA, and the remaining 60% produce intermediate levels of 12S RNA from the wild-type E1A substrate.

When a mutant substrate, *dl*1500 RNA, that is missing the 13S 5′ splice site is used, high levels of 12S RNA are produced by all extracts. This demonstrates that all extracts contain the necessary factors for 12S splicing; therefore, the inability of some extracts to produce 12S RNA from the wild-type substrate must result from competition between the 12S and 13S reactions. Inhibition of 12S splicing by this competition is not due to depletion of a common splicing factor required for both 12S and 13S splicing under the conditions used. When a mixture of *dl*1500 RNA and another mutant substrate, pm975 RNA, that carries a mutation at the 12S 5′ splice site are spliced in the same reaction, high levels of 12S RNA and 13S RNA are produced by all extracts. This demonstrates that all extracts have sufficient splicing factors needed to produce 12S and 13S RNAs in the same reaction, as long as the reactions occur on separate molecules, and suggests that the variation between extracts is due to a factor that modulates the competition between the 12S and 13S reactions on the same substrate molecule.

The activity that stimulates 12S splicing from the wild-type substrate can be separated from common splicing factors by fractionation of a nuclear extract on a DEAE-cellulose column. The extract is loaded on the column in buffer containing 0.1 M KCl, and the flowthrough fractions are pooled to make fraction I. After the column is washed, bound material is eluted with 0.6 M KCl to make fraction II. Both fractions are dialyzed and concentrated. Fraction I alone does not contain splicing activity; however, fraction II is quite efficient in splicing 13S RNA from the wild-type substrate and 12S RNA from the *dl*1500 substrate. Fraction II by itself makes very low levels of 12S RNA from the wild-type substrate, but addition of fraction I restores the 12S splicing. The amount of 12S RNA synthesized varies proportionately with the concentration of fraction I in reactions supplemented with dilutions of fraction I, although the efficiency of 13S splicing is unaffected. The result is that fraction I contains a soluble factor that affects 5′ splice site selection. Such a factor may participate in regulating correct 5′ splice site selection during splicing of RNAs that are not normally alternatively spliced, as well as affecting alternative splicing patterns. We are continuing to fractionate extracts to purify this factor further and are examining the effect of this factor on the splicing of a variety of substrate RNAs.

Inhibition of Pre-mRNA Splicing by Antisense RNA

S. Munroe

We have previously demonstrated that antisense RNA specifically and efficiently inhibits pre-mRNA splicing in vitro. Subsequent work, described below, has been directed at determining the mechanism of this inhibition and characterizing factors that mediate inhibition by promoting the rapid annealing of complementary RNA molecules.

Antisense RNAs complementary to either the 5′ or 3′ exons inhibit splicing of a pre-mRNA transcript consisting of the first two exons and first intron of human β-globin pre-mRNA. Particularly interesting is the observation that antisense RNAs annealing to regions of the exon some distance from the nearest splice site also inhibit splicing efficiently. For example, a 70-nucleotide antisense RNA, designated E2-70, that is complementary to sequences in the 3′ exon more than 130 nucleotides from the nearest splice site blocks splicing as efficiently as antisense RNAs annealing closer to the 3′ splice site. These results were unexpected since it has been shown that most of the 5′ and 3′ exons can be deleted from this transcript without severely inhibiting splicing. Our results suggest the presence of direct or indirect interactions between exon and splice site sequences. Such interactions may be important for determining the specificity of splicing.

Antisense RNAs annealed to the 3′ exon block, splicing at an early stage, prior to cleavage at the 5′ splice site. In the presence of antisense RNA, E2-70 pre-mRNA accumulates in a 40S RNP complex. This complex cosediments with the 40S presplicing complex formed in an uninhibited reaction that is

known to contain a single small nuclear ribonucleoprotein (snRNP) complex, U2 snRNP. The snRNP components of complexes formed upon inhibition with antisense RNAs are presently being examined to establish more precisely the stage at which spliceosome assembly is blocked.

In contrast to the high level of inhibition observed with antisense RNAs complementary to globin exons, antisense RNAs complementary to regions immediately adjacent to the 3′ splice site of globin pre-mRNA are poor inhibitors. This appears to reflect the inability of antisense RNAs to anneal efficiently near the 3′ splice site. Analysis of annealing by nuclease protection assay shows that E2-130, which spans the 3′ splice site, anneals inefficiently to pre-mRNA. Splicing factors binding at this site may block or displace antisense RNAs. Annealing of a second antisense RNA (E2-70) to a nonoverlapping downstream site substantially enhances annealing of E2-130. This result suggests that antisense RNAs annealed to the 3′ exon block or destabilize binding of factors at the 3′ splice site.

When antisense RNAs are added to in vitro splicing reactions 5 minutes or more after initiation of pre-mRNA splicing, annealing and inhibition are greatly reduced. However, we have found that preannealing of E2-70 facilitates annealing of antisense RNA E2-80B added 10 minutes later to an adjacent site on the 3′ exon. These results are consistent with a model in which antisense RNA bound to the exon blocks assembly of the functional splicing complex at a stage where pre-mRNA is extended in an "open" configuration, in which it can readily base pair to antisense RNAs. In the absence of antisense RNA, this open complex is rapidly converted to a closed complex in which pre-mRNA is sequestered and unable to anneal to late-added antisense RNAs. The addition of antisense RNA at the beginning of splicing may lock in the open complex and inhibits splicing by blocking the transition to a closed complex during spliceosome assembly. Since the transition from the open to closed complex may be mediated by proteins binding along exons, one possible mechanism for inhibition by antisense RNA bound to the exon is that it may perturb the binding of heterogeneous nuclear RNP (hnRNP) proteins to the pre-mRNA. This hypothesis will be tested by examining the binding of proteins to exon sequences in a series of overlapping pre-mRNAs in the presence and absence of bound antisense RNAs.

Inhibition of splicing by antisense RNAs appears to be mediated by several different activities in a HeLa cell nuclear extract that promote, block, or destabilize the annealing of complementary RNAs. We have further characterized requirements for annealing in vitro and have started to purify factors that facilitate annealing of complementary RNAs. Optimal annealing takes place in 15–20 mM $MgCl_2$, 40–60 mM KCl at 37°C in the absence of ATP. These conditions differ from those required for splicing. Annealing of complementary RNA molecules does not require the presence of splice site sequences. Predigestion of nuclear extracts with protease eliminates annealing activity, suggesting that annealing factors include one or more protein components. Several chromatographic procedures have been used to partially purify annealing factors. Annealing activity is bound to DEAE-cellulose at 50 mM KCl, but elutes in a broad peak between 0.1 and 0.3 M salt. Material collected from the front half of this peak was further fractionated on a phosphocellulose column. Most of the activity elutes in a step between 0.15 and 0.3 M KCl. Further activity elutes in the 0.3–0.5 M step. Material collected from these two steps was subjected to further fractionation by Cibacron blue affinity chromatography. The fraction eluting from phosphocellulose with 0.5 M KCl binds tightly to this affinity column, and the fraction eluting with 0.3 M salt binds loosely. This result suggests that at least two independently eluting factors are capable of promoting RNA/RNA annealing. Additional procedures are being tested to purify these annealing factors further.

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TRANSCRIPTIONAL CONTROL

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Studies of regulation of transcription in mammalian cells have shown that transcriptional control is manifested by a complex network of *cis*-acting promoter elements and transcription factors. We use the small DNA tumor virus SV40 as a model system with which to probe transcriptional control in mammalian cells. This virus contains a relatively small regulatory region of about 400 bp that includes the bidirectional early and late promoters and the origin of replication. The early promoter relies entirely on host-cell *trans*-acting factors to initiate transcription. Transcription of the early region results in expression of the large T antigen protein, which, in turn, initiates DNA replication and late transcription. We have focused our studies on the SV40 early promoter and particularly on the structure and function of the enhancer region. Enhancers were first discovered in SV40 and have the ability to activate transcription over very large distances (up to 10 kb) from a position either upstream or downstream from the transcriptional start site. Our past studies showed that the SV40 enhancer is a composite of many subunits that cooperate with one another at different organizational levels to activate transcription. The individual subunits frequently represent sequence motifs found in other enhancers. We refer to these subunits as "enhansons" for the indivisible units of enhancer structure. Enhansons probably represent individual binding sites for transcription factors, although, in certain cases, such as the *jun* and *fos* proto-oncogene products, the factors may represent homodimers or heterodimers.

We have continued our analysis of *cis*-acting elements within the SV40 enhancer, but we are also shifting our emphasis to molecular analysis of the cellular factors responsible for transcriptional regulation. These latter studies have led us to a detailed analysis of the 8-bp octamer motif ATGCAAAT that is found in the promoter of a number of lymphoid-specific and ubiquitously expressed genes. The ubiquitously expressed genes include those encoding the U1 to U6 small nuclear RNAs (snRNAs) and the histone H2B. In collaboration with the laboratory of N. Hernandez (Molecular Genetics of Eukaryotic Cells Section), we have investigated the activity of the SV40 octamer motif in the context of the β-globin promoter, which represents a prototypical mRNA promoter, and in the U2 snRNA promoter. These studies showed that the octamer motif has different functions (ubiquitous or cell-specific), depending on the promoter context in which it is located. In the U2 snRNA promoter, the octamer motif is active ubiquitously, but in the β-globin promoter, it is lymphoid-specific. Unlike the octamer motif, none of the SV40 enhancer elements that activate the β-globin promoter in HeLa cells can activate the U2 snRNA promoter; thus, the octamer motif defines a new class of enhancer elements that are specific for snRNA genes.

The characterization of the octamer motif has been paralleled by the purification of the ubiquitously expressed octamer-motif-binding protein Oct-1 and isolation of a cDNA clone encoding this protein. The gene encoding the related lymphoid-specific octamer-binding protein Oct-2 has been cloned in three other laboratories (P. Sharp and D. Baltimore, Massachusetts Institute of Technology; P. Matthias and W. Schaffner, University of Zurich; and R. Roeder, Rockefeller University). These two proteins are probably responsible for the ubiquitous and lymphoid-specific activity of the octamer motif in the snRNA and immunoglobulin promoters, respectively. They thus serve as a model system to examine how two transcription factors that recognize the same DNA sequence can differentially activate transcription. One explanation is that these two factors share related DNA-binding domains but differ in the activation domains. The cloning of the genes encoding Oct-1 and Oct-2 go a long way toward answering these questions. For example, these two proteins do indeed share very similar DNA-binding domains. This DNA-binding domain is also shared by a pituitary-specific factor, called Pit-1 (or GHF-1), and a nematode cell lineage gene, called *unc-86*. This new 160-amino-acid domain (called POU, pro-

nounced "pow", for *P*it, *O*ct, *U*nc) contains two subdomains, a homeo domain and POU-specific domain. Future efforts will focus on the structure of this new type of DNA-binding domain and on the characterization of the activation domains that differentiate the Oct-1 and Oct-2 factors.

In a new effort initiated this year with other members of the laboratory, we are studying transcriptional regulation of the human immunodeficiency virus (HIV), the etiological agent of AIDS. In this effort, we are concentrating our efforts on adapting the strategies we have used to study the SV40 enhancer to the HIV regulatory region. This year, these studies have allowed us to identify elements that are shared by these two promoters. In the future, we plan to identify the transcriptional control elements that are responsible for T-cell-specific expression of this virus.

Structure and Function of the SV40 Enhancer

R. Aurora, J. Clarke, W. Herr, B. Ondek, S. Stern, M. Tanaka

Figure 1 shows our current understanding of the structure of the SV40 enhancer, with individual enhanson units shown above the sequence and the three A, B, and C enhancer elements initially identified by viral revertant analysis shown below the sequence. The A, B, and C enhancer elements have the property that simple duplication of these elements can create an enhancer. For this reason, P. Chambon and colleagues (Strassbourg) have suggested they be referred to as proto-enhancers. Last year, we showed that the A and B elements, or proto-enhancers, are composed of subunits that must be located in close proximity to each other in order to be functional. These subunits correlated with protein-binding sites and therefore seemed likely to be the indivisible units of enhancer structure. We therefore coined the term "enhansons" to define these units of enhancer structure. Much of our effort on the structure and function of the SV40 enhancer during the past year has focused on characterizing the properties of enhansons and how they cooperate with one another.

Our studies of the SV40 enhancer in the murine embryonal carcinoma cell line F9 were completed this year. We studied the activity of the SV40 enhancer elements in this cell line by using a polyomavirus enhancer replacement vector and also by testing the activity of multimerized synthetic enhancers. These studies clarified the structure of the A element. In our previous studies, we had not identified the

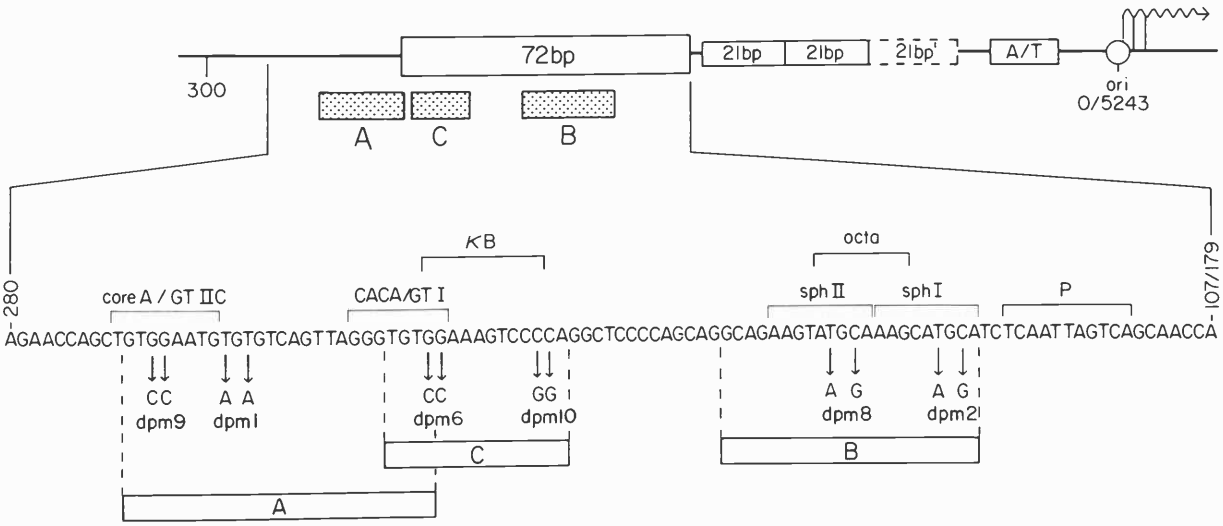

FIGURE 1 Structure of the SV40 enhancer. A diagram of the SV40 early promoter is shown above the nucleotide sequence of the SV40 enhancer region. The diagram shows the early transcriptional start sites to the right (wavy arrow), TATA box region (A/T), perfect (21 bp) and imperfect ("21 bp") 21-bp repeated sequences that contain Sp1-binding sites, and the 72-bp element that is repeated in wild-type SV40. The stippled boxes show the location of the A, B, and C elements originally defined by SV40 revertant analysis in CV-1 cells. Above the sequence of the SV40 enhancer are shown sequence motifs and enhansons discussed in the text. Below the sequence are double point mutations (*dpm*) used in SV40 revertant analyses and the position of the A, B, and C elements. The extended A element results from experiments in murine embryonal carcinoma F9 cells discussed in the text.

second A element enhanson that cooperates with the coreA enhanson (also referred to as GTIIC, see Fig. 1). In undifferentiated F9 cells, the C element is inactive, and this allowed us to determine that the A element extends further downstream than previously recognized and actually overlaps the C element. This became evident because the *dpm*6 mutations, which inactivate the C element, also inactivate the A element. Thus, the second A element enhanson is apparently the CACA/GTI motif. Unlike the coreA enhanson, however, a duplication of the GTI motif that arose in the polyomavirus/SV40 enhancer revertants from F9 cells does not create a proto-enhancer. Thus, some enhansons can cooperate with duplicates of themselves, whereas others apparently do not.

A detailed analysis of the SV40 B element has shown that some enhansons can also function independently as proto-enhancers without an enhanson partner. This conclusion arose from our studies of the SV40 octamer motif that overlaps the sph enhansons I and II. In studies performed in collaboration with N. Hernandez' laboratory (Molecular Genetics of Eukaryotic Cells Section) and described elsewhere in this report, we showed that the octamer motif itself functions as a lymphoid-specific proto-enhancer for activation of the β-globin promoter. Multiple mutations within sequences flanking both sides of the octamer motif did not affect the enhancer activity of the multimerized SV40 octamer motif, but all mutations within the motif inactivated function. This result contrasts with the activity of the sph enhansons in which neither enhanson is sufficient for activity. Therefore, enhansons can be classified into different groups. Similar conclusions have been described by Fromental et al. (*Cell 54*: 943 [1988]), in which the enhansons were classified as class-A enhansons, which cannot cooperate with another enhanson of the same class (e.g., CACA/GTI motif), class-B enhansons, which cooperate with themselves or with class-A enhansons to create proto-enhancers (e.g., coreA or sphII motifs), and class-C enhansons, which are proto-enhancers themselves (e.g., the octamer motif).

This classification of enhansons can explain the unexpected results we obtained from a new series of SV40 revertant isolations. We initially identified enhansons by the structure of revertants of the triple mutant *dpm*1.2.6, in which each of the A, B, and C elements was mutated (see Fig. 1). Some of these revertants contained 9-bp duplications of the coreA or sphI enhansons. To identify new enhansons, we isolated revertants from a different triple enhancer mutant called *dpm*8.9.10, in which the opposite "half" of each element was mutated (see Fig. 1). As tested with synthetic enhancers, these three sets of double point mutations, *dpm*8, *dpm*9, and *dpm*10, inactivate the B, A, and C elements, respectively. Surprisingly, in the *dpm*8.9.10 revertants, no new combination of enhanson duplications was observed. Instead, most revertants contained duplications of the region upstream within which W. Schaffner and colleagues (University of Zurich) have identified other proto-enhancers. This result can now be explained if the GTI motif in the A element and the sphI motif in the B element are class-A enhansons that cannot cooperate with themselves to create a proto-enhancer. Because mutations in neither "half" of the C element resulted in identification of C element subunits, we suspect it is a class-C enhanson or indivisible proto-enhancer. Consistent with this hypothesis, the C element is recognized by a nuclear factor called NF-κB that may act like the lymphoid octamer-motif-binding factor Oct-2 and not require a flanking factor to activate transcription.

To study the protein-protein interactions involved in enhanson function, we have concentrated on two motifs: the coreA enhanson, which cooperates with itself to generate a very active proto-enhancer in HeLa cells, and the AP-1 motif, which binds to the known transcription factors and proto-oncogenes *jun* and *fos*. Screening of a HeLa cell nuclear extract fractionated by heparin agarose chromatography with a coreA motif probe identified a coreA-motif-binding factor. When a probe containing the duplication of the coreA enhanson that is very active in vivo is used in a gel-retardation assay, two different DNA/protein complexes are observed: a more abundant slow-migrating complex II and a less abundant fast-migrating complex I. When one or the other coreA motif is mutated by a single point mutation, only complex I is evident, and both complexes disappear when both coreA motifs are mutated. We interpret these findings to suggest that complex I contains a single site bound by the coreA factor, whereas in the slower-migrating complex II, two coreA motifs are occupied. The overabundance of the slower-migrating complex II suggests a cooperative interaction between bound coreA factors. Consistent with such a cooperation, when the spacing between coreA motifs is altered, the relative abundance of complex II decreases in

approximate proportion to the decrease in enhancer function in vivo. These results suggest that some of the cooperativity between class B enhansons is the result of cooperative DNA binding. This is unlikely to be the whole story, however, because isolated coreA motifs can still bind to this coreA motif factor, but they are nevertheless inactive in vivo.

The analysis of the AP-1 (or P) motif (see Fig. 1) has the advantage that both genes encoding proteins known to bind to this motif, *jun* and *fos*, are cloned and have been extensively characterized. The SV40 AP-1 motif does not have the properties of a proto-enhancer in CV-1 cells, because in neither the *dpm*1.2.6 nor the *dpm*8.9.10 triple mutants was the AP-1 motif duplicated to restore enhancer function. In one revertant of the mutant *dpm*2, isolated a number of years ago, we observed a 9-bp duplication of the AP-1 motif. Such 9-bp duplications are a hallmark of class-B enhansons that can cooperate with themselves. We therefore tested the enhancer potential of the duplicated AP-1 motif and found that it was indeed considerably more active than when one or the other of the two motifs is inactivated by point mutation or when they are separated by 5 bp. We now plan to study the interaction of *fos* and *jun* with the duplicated AP-1 enhansons to understand how the enhansons cooperate to enhance transcription.

We have also taken a foray into yeast to test its suitability as a host to study the SV40 enhancer. Studies of yeast transcription factors during the past few years have shown that some are very similar to mammalian factors. For example, two factors isolated from yeast, GCN4 and yAP-1, are very similar in structure and DNA-binding properties to the mammalian AP-1 family of factors such as *fos* and *jun*. Therefore, we tested the activity of different wild-type and mutant multimerized SV40 enhancer element constructs in the budding yeast *Saccharomyces cerevisiae*. Restriction fragments containing the multimerized enhancers were cloned into a yeast expression vector containing the TATA box from the yeast His4 promoter fused to β-galactosidase. After transformation of yeast, cells were grown and tested for β-galactosidase activity. We expected two kinds of potentially useful results. In the first, certain families of wild-type and mutant multimerized enhancers could exhibit the same pattern of activity in yeast as in mammalian cells. This result would suggest a yeast homolog of a mammalian transcription factor. Alternatively, a motif may be inactive in yeast; in which case, yeast may prove to be an ideal system in which to study the activity of the mammalian factor. The results of the experiment failed to identify SV40 enhancer elements that fit in the first class of active elements that faithfully reproduce the activity found in mammalian cells. For example, certain C element constructs were active in *S. cerevisiae*, but this did not apparently reflect the activity of the κB site (see Fig. 1) because other constructs that still contained a wild-type κB site were inactive. There were members of the second class, however, because none of the B element constructs were active, suggesting that there is no octamer-motif-binding factors like Oct-1 or Oct-2 in yeast. We may therefore be able in the future to study Oct-1 and Oct-2 function in yeast.

Oct-1 (OBP100), a Homeo Domain Protein, Binds to Remarkably Degenerate Octamer Motifs through Specific Interactions with Flanking Sequences

T. Baumruker, R. Sturm, W. Herr

During the past 3 years, we have pursued the characterization of the ubiquitous octamer-motif-binding factor from HeLa cells. This factor binds to two sites, I and II (see Fig. 2), within the SV40 enhancer: Site I contains a 7/8 match to the canonical octamer motif ATGCAAAT, whereas the downstream site II contains two sequences with only six (Octa2) or five (Octa3) matches to the octamer motif. We originally referred to this protein as OBP100 because it is a 100-kD octamer-binding protein. In agreement with D. Baltimore, P. Sharp, and their colleagues (Massachusetts Institute of Technology), we now refer to the ubiquitous octamer-binding protein as Oct-1 and the lymphoid-specific factor as Oct-2. Oct-1 is probably the same as OTF-1, which R. Roeder and colleagues (Rockefeller University) showed activates histone H2B gene expression in vitro, and NFIII, a factor that activates adenovirus DNA replication (P. van der Vliet [University of Utrecht] and T. Kelly [Johns Hopkins] and their colleagues). The pattern of Oct-1 binding to the SV40 enhancer has led us to use the Oct-1 protein as a model system to understand how sequence-specific DNA-binding proteins can bind to families of very degenerate sequences.

The studies described last year showed that Oct-1

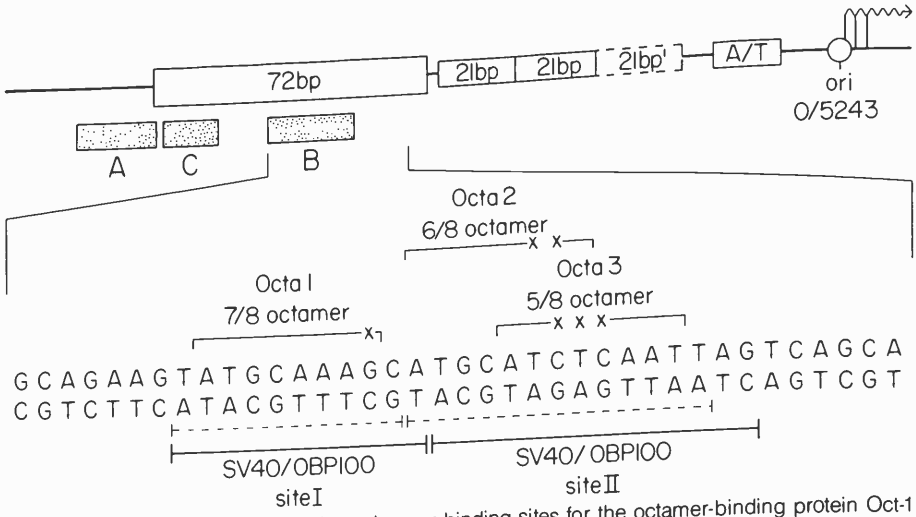

FIGURE 2 Structure of the two SV40 enhancer binding sites for the octamer-binding protein Oct-1 (referred to as OBP100 in the figure). The SV40 early promoter is as described in Fig. 1. The sequence shows the region encompassing the two Oct-1-binding sites. Octamer-related motifs are identified above the sequence, and the limits of the binding sites are shown by the solid lines below the sequence. The dashed lines show our previous delineation of the limits of the two binding sites by chemical modification interference studies.

can bind to two adjacent sites within the SV40 enhancer, of which only site I contains the previously recognized octamer motif (see dashed lines in Fig. 2). The second site was shown by chemical modification interference to extend over a 13-bp sequence containing the overlapping Octa2 and Octa3 sequences. To extend these earlier studies, we combined the use of mutagenesis and chemical modification interference with electrophoretic mobility-shift analysis to examine the precise sequence requirements for Oct-1 binding to sites I and II. These studies showed that the size of these two binding sites differ: Binding site I is 11 bp long, whereas binding site II is 14 bp long (shown by solid bars in Fig. 2). Furthermore, the octamer-related sequence recognized by Oct-1 in site II is not the 6/8 Octa2 sequence but the 5/8 Octa3 sequence. The mutational studies showed that Oct-1 can recognize the very degenerate Octa3 octamer motif because it makes important *sequence-specific* contacts with the DNA flanking the degenerate octamer motif. Thus, sequences that are not normally conserved because they are not essential for binding to a perfect octamer motif become essential for binding to a degenerate motif. These results have important ramifications because they stress the context dependence of any mutational analysis of a *cis*-acting element; thus, although a particular point mutation may have a drastic deleterious effect in a particular promoter element, the same mutant sequence may be functional in a different context.

Once the exact nature of the interaction between Oct-1 and SV40 binding site II became evident, we realized that there is considerable sequence similarity (7 out of 9 match) between binding site II and the TAATGARAT (R = purine) motif found in the immediate-early (IE) promoters of herpes simplex virus (HSV). The TAATGARAT motif is the target for transcriptional activation of the HSV IE genes by the late HSV gene product VP16 (also called Vmw65). VP16 is a structural component of the HSV virion, and upon infection, it is released into the cell where it binds to the DNA indirectly through interactions with one or more cellular factors present in uninfected cells. The HSV TAATGARAT motifs can be divided into two different classes that differ from one another by the sequences flanking the motif. In the first class, the flanking sequences ATGCTAATGARAT create a 7/8 octamer motif (ATGCTAAT) that overlaps the TAATGARAT motif. In the second class, there is no such overlapping motif. Therefore, to test the ability of Oct-1 to bind directly to the TAATGARAT motif, we selected a class II motif from the HSV ICP4 promoter. Gel-retardation studies, chemical modification interference, and DNA affinity precipitation studies showed that indeed the Oct-1 protein can bind directly to the TAATGARAT motif, thereby extending the range of Oct-1 binding sites to include sites that have at best a 4 out of 8 match to the octamer motif.

Figure 3A shows a compilation of different Oct-1

binding sites that we have studied, ordered to generate the maximum sequence relationship between any two adjacent sequences in the figure. This organization shows that a progression of DNA-binding sites from the SV40 site I motif to the TAATGARAT motif can be made in which any two sites are at least 60% similar (9 out of 14 positions). Nevertheless, when SV40 site I and the TAATGARAT motif are directly compared, they bear little resemblance (4 out of 14 matches, Fig. 3B). Thus, the relationship between two very dissimilar binding sites can be established by the progression. These results offer an explanation for how a DNA-binding protein can bind to very dissimilar sequences: Flexible DNA sequence recognition arises because there are few, if any, obligatory contact sites for DNA binding, but, rather, specific binding reflects the sum of many independent interactions.

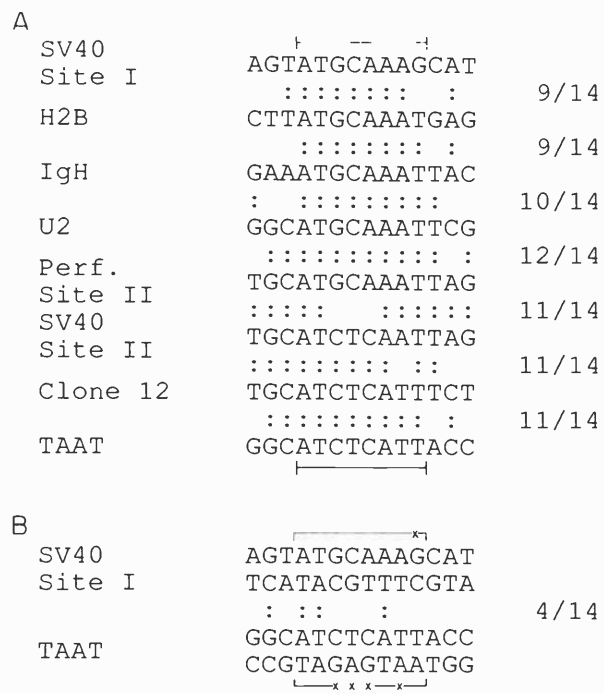

FIGURE 3 Sequence comparison of different Oct-1-binding sites. In *A*, the sequences are ordered to achieve the highest degree of similarity between sites. The numbers to the right between sequences indicate the number of sequence matches (out of 14) between each pair of sites. (H2B) Histone H2B promoter; (IgH) immunoglobulin heavy-chain enhancer; (U2) U2 small nuclear RNA enhancer; (Perf. site II) SV40 site II converted to a perfect octamer motif; (Clone 12) an artificial SV40 site II mutant that binds Oct-1; TAAT, TAATGARAT motif. (*B*) Direct comparison of the SV40 site I and TAATGARAT motif.

The Oct-1 Protein Contains a Bipartite POU DNA-binding Domain with a Homeo Subdomain

R. Sturm, W. Herr, G. Das, M. Cleary

To understand how the Oct-1 protein binds to consensus and degenerate octamer motifs and how it displays a different *trans*-activation potential from the lymphoid-specific Oct-2 protein, it became imperative to obtain a cDNA clone of the gene encoding Oct-1. We were successful in this endeavor, and it led us to the discovery of a new conserved domain called POU (pronounced "pow") within transcription factors.

The first cDNA clone for the gene encoding Oct-1, referred to as *oct-1*, was identified by screening a λgt11 recombinant phage library in situ with an octamer motif DNA probe. We screened a human λgt11 cDNA library prepared from the teratocarcinoma cell line NTERA2D1 (P. Andrews, Wistar) constructed by J. Skowronski (Molecular Genetics of Eukaryotic Cells Section). Of 4×10^5 phage screened, we obtained one positive phage that produced a 190-kD β-galactosidase fusion protein that bound specifically to the octamer motif. To test stringently the DNA-binding specificity of this recombinant protein compared to HeLa-cell-derived Oct-1 protein, we compared their DNA-binding affinity for SV40 site I and the TAATGARAT motif, which differ greatly in sequence (see Fig. 3B). We were encouraged to find that these two proteins showed the same relative affinity for these two very different sites. The authenticity of the cloned *oct-1* gene was further established by showing that, as expected for a ubiquitously expressed gene, *oct-1* mRNA could be detected in all seven cell lines tested with the cDNA clone. Furthermore, rabbit antibodies raised against the recombinant β-galactosidase fusion protein reacted with the HeLa-cell-derived Oct-1 protein.

The Oct-1 antibodies and the RNA hybridizations also showed that there is a relationship between the Oct-1 and Oct-2 proteins, because Oct-1 probes cross-reacted with the Oct-2 protein and mRNA from lymphoid cells. The similarity between the Oct-1 and Oct-2 proteins was established by comparing the amino acid sequences predicted for the two proteins from DNA sequence analysis. The nucleotide sequence of the Oct-1 cDNA clone revealed an open reading frame fused to β-galactosidase extending for 760 amino acid residues. The amino-terminal third of this reading frame is very rich in glutamine (25%),

the central portion contains a high concentration of acidic and basic residues, and the carboxy-terminal region is rich in serines and threonines. When the Oct-1 sequence is compared to the sequence determined for Oct-2 by R. Clerc, L. Corcoran, D. Baltimore, and P.A. Sharp (Massachusetts Institute of Technology), the central 160-amino-acid charged region shows a striking 90% similarity between the two proteins. The carboxy-terminal 60 amino acids within this region are distantly related to the homeo domain first identified in proteins involved in *Drosophila* development. The homeo domain contains an α-helix-turn-α-helix motif that is similar to the DNA-binding domains of prokaryotic DNA-binding proteins. Consistent with this similarity, deletion analysis of the Oct-1 protein showed that the homeo domain is essential for DNA binding.

Twenty-five residues amino-terminal to the homeo domain begins a stretch of 75 residues that is identical at 74 positions in Oct-1 and Oct-2. Surprisingly, comparison of the sequences of the Oct proteins and two other homeo domain proteins, which were identified concurrently with the Oct-1 and Oct-2 proteins, showed that the homeo domains of these four proteins are much more related to one another than to other homeo domain proteins. Furthermore, these new proteins are also very similar in the amino-terminal segment that is nearly identical in the two Oct proteins. Figure 4 shows a schematic representation of the four proteins. The two non-Oct proteins are the pituitary-specific factor Pit-1, characterized by H. Ingraham, M.G. Rosenfeld, and colleagues (University of California, San Diego) (also referred to as GHF-1 by M. Karin and colleagues [UCSD]), and the product of a cell-lineage gene in the nematode *Caenorhabditis elegans* called *unc-86* (M. Finney, G. Ruvkun, and H.R. Horvitz, MIT and Harvard). The extended region of similarity has been called the POU domain, and it contains two subdomains, the POU-specific domain and the POU homeo domain. These two subdomains are linked by a short 15–27-amino-acid-long nonconserved region of dissimilarity between the two proteins.

These sequence comparisons drew together a new class of homeo-domain-containing proteins. None of these four proteins were originally isolated by virtue of being a homeo-domain-containing protein, and they thus form a diverse group of functionally characterized proteins. Three of them are mammalian transcription factors, two of which bind to identical sequences, and the fourth is a developmental gene in worms. Although the other three are expressed only in specific cells, the Oct-1 protein is unusual because it is the first clearly ubiquitously expressed homeo domain protein.

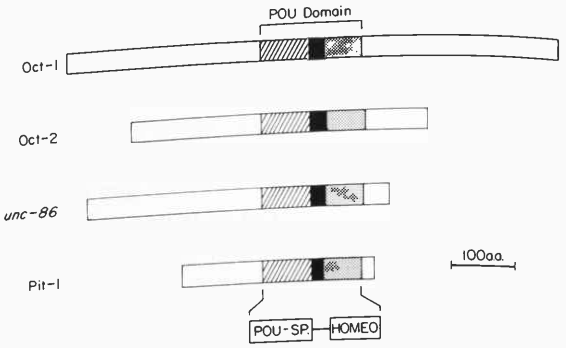

FIGURE 4 Position of the bipartite POU domain within the four POU proteins Oct-1, Oct-2, *unc-86*, and Pit-1/GHF-1 ordered according to size from largest to smallest (*top* to *bottom*). They are aligned according to the position of the POU domain. The POU-specific subdomain is hatched, the POU homeo domain is stippled, and the nonconserved sequences in between are blackened as indicated by the expanded and labeled POU domain shown below.

Why should the POU proteins, unlike the large majority of homeo domain proteins, contain another highly conserved subdomain within the POU domain? Deletion analysis of the Oct-1 protein showed that the POU domain is sufficient for sequence-specific binding to DNA. Figure 5 shows a schematic of the Oct-1 POU domain. The sequence comparison between the POU proteins revealed that the POU-specific subdomain can be subdivided into two highly conserved regions called the A and B boxes. The POU homeo domain contains the helix-turn-helix (H-T-H) motif that contacts DNA. This particular region is very closely related among the four POU proteins and may suggest that they contact DNA in a similar manner. Because the two Oct proteins bind to identical sequences, it seemed plausible that the nearly identical Oct-1 and Oct-2 POU-specific domains might be involved in binding to DNA.

To test whether the POU-specific domain is required for DNA binding, we created amino-terminal deletions of Oct-1 extending into the POU-specific sequences and showed that deletion of the POU A box already results in loss of binding in a gel-retardation assay. To make more subtle mutations, we created three amino acid substitutions within the POU A, B, and homeo domains in sequences that are conserved between all four POU proteins. In addition, to test the flexibility of the "linker" region between the two POU subdomains, we in-

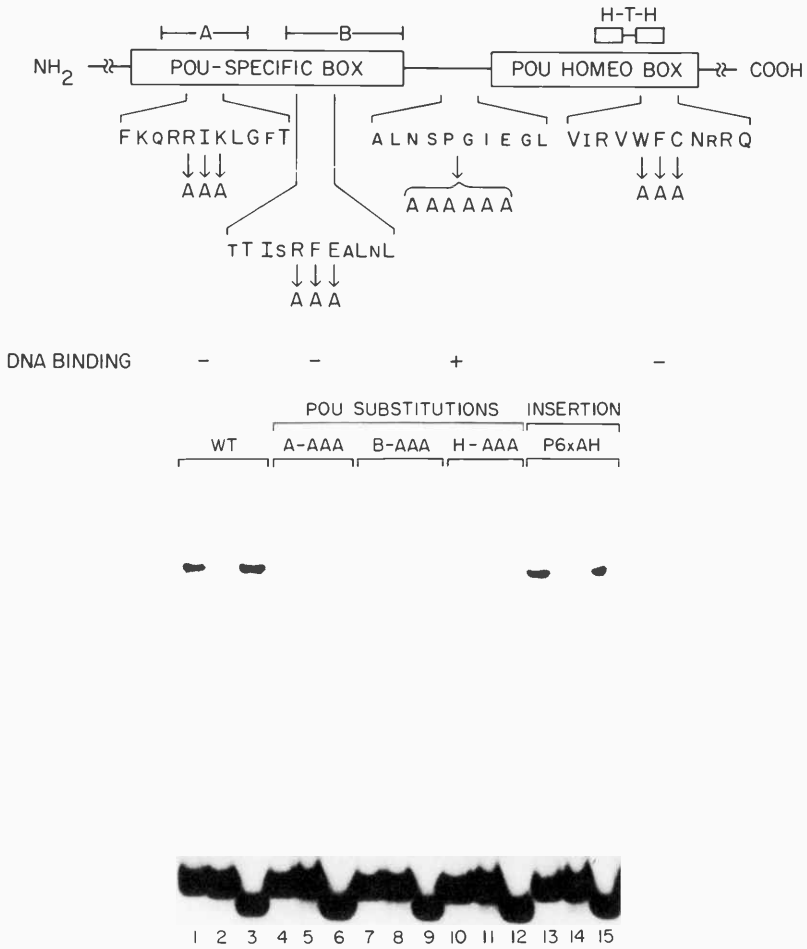

FIGURE 5 Mutations within the POU-specific and POU-homeo domains are deleterious to DNA binding, but an insertion between the two domains does not affect binding. (*Top*) Position of the triple alanine substitutions within the POU A, B, and homeo regions and the six alanine insertion between the two POU subdomains. The amino acid sequence surrounding each set of mutations is shown; the amino acids shown by large letters are identical among all four POU proteins. The relative DNA-binding activity of each mutant is shown at the bottom. (*Bottom*) Gel-retardation analysis of the POU domain point mutants. Three probes, SV40 site I (lanes *1, 4, 7, 10, 13*), nonbinding SV40 site I mutant *dpm*8 (lanes *2, 5, 8, 11, 14*), and the TAATGARAT motif (lanes *3, 6, 9, 12, 15*) (see Figs. 1 and 3), were tested with proteins of wild-type Oct-1 (WT), the POU A (A-AAA), B (B-AAA), and homeo (H-AAA) substitution mutants, and the six alanine insertion (P6XAH) mutant as indicated. The free probe migrates at the bottom of the gel and the complex with Oct-1 migrates near the top.

serted six alanine residues in this region (see Fig. 5). As shown in the gel-retardation assay in Figure 5, the amino acid substitutions all had a severe effect on DNA binding to the very different SV40 site I octamer motif and TAATGARAT motif. The insertion of six alanines, however, had no obvious effect on DNA binding. These results suggest that the POU domain is a new type of bipartite DNA-binding structure in which the homeo subdomain is insufficient for effective DNA binding. We are currently extending the structural studies of the POU domain to determine whether the POU-specific sequences actually contact the DNA or are instead involved in stabilizing the homeo domain.

HIV Enhancer: Common Elements between HIV and SV40 Promoters

W. Phares, J. Clarke, W. Herr

Figure 6 shows a comparison of the SV40 early promoter and the HIV promoter. There are a number of striking similarities between these two promoters: They both contain TATA box motifs (A/T), multiple binding sites for the transcription factor Sp1 (hatched boxes), and either one or two copies of a 10-bp sequence motif, GGAAAGTCCC (in SV40) or GGGACTTTCC (in HIV). This latter motif is recognized by the nuclear factor NF-κB. It was first shown to be functionally important by its central location in the SV40 enhancer C element. In the HIV promoter, this motif is referred to as the enhancer core (EC).

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. In our first efforts, we have asked whether HIV enhancer elements can functionally replace the SV40 enhancer and, if so, whether we could identify those elements by SV40 revertant analysis. Of particular interest was determining whether the enhancer core of HIV, which by sequence is very similar to the SV40 C element, can functionally replace the SV40 enhancer in the SV40 permissive African green monkey kidney cell line CV-1.

We have used the SV40 enhancer replacement vector pSVER, in which the SV40 enhancer is deleted and can be replaced by heterologous enhancers, as a functional assay to identify HIV enhancer elements. Three *Hae*III-resistant fragments of the HIV-1 long terminal repeat (Fig. 6) were individually cloned into the pSVER vector, and DNAs were transfected into CV-1 cells to recover viable virus. All the SV40-HIV recombinant viruses were unable to form plaques on CV-1 cells upon direct transfection of cells, indicating a defective function in the initial recovered viruses. With continued propagation, however, growth revertants arose from recombinants containing the 91-bp *Hae*III fragment that spans the enhancer cores but not from recombinants carrying the upstream 76-bp and 117-bp *Hae*III fragments.

Sequence analysis of the enhancer region of revertants from virus carrying the 91-bp fragment in either orientation revealed tandem duplications that always spanned at least one of the HIV enhancer

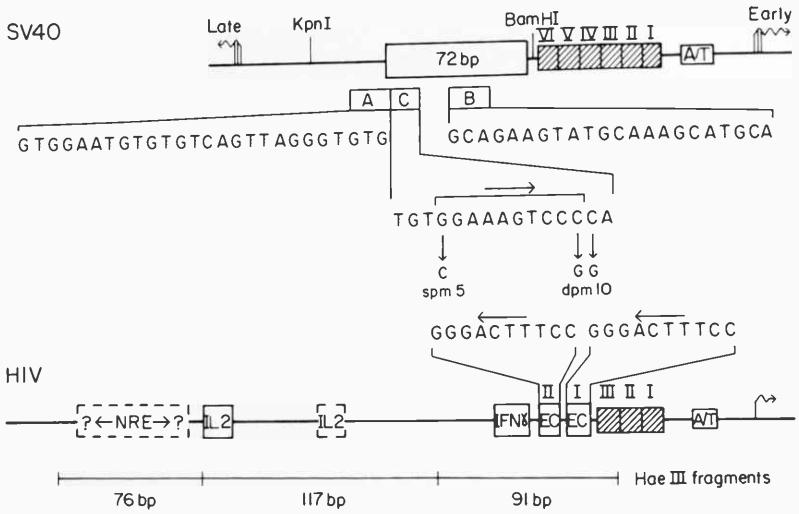

FIGURE 6 Comparison between the SV40 early promoter and HIV promoter. Shown from right to left are transcription start sites (wavy arrow), TATA boxes (A/T), and Sp1-binding sites (hatched boxes). Upstream of the regions are shown the 72-bp element with the position and sequence of the A, B, and C elements for SV40 and the enhancer core elements (EC), homologies to the interferon-γ (IFN-γ) and IL-2 (IL-2) genes, and the approximate position of the negative regulatory element (NRE) for the HIV promoter. The sequence of the 10-bp NF-κB-binding site similarity is shown above the enhancer cores and is bracketed in the C element sequence. The base changes in mutants *spm*5 and *dpm*10 are as indicated.

core sequences that are similar to the SV40 enhancer C element. This analysis therefore indicates that duplication of the HIV enhancer core sequences is sufficient to restore SV40 replication in CV-1 cells. To extend this finding further, we have compared the activities of reiterated copies of the SV40 C element and the HIV ECI and ECII elements, in transient expression assays of transfected DNA. The results show that multiple copies of each of these elements can independently enhance transcription in CV-1 cells to equivalent levels, whereas the point mutants *spm*5 and *dpm*10 (see Fig. 6) abolish activity. Taken together with the SV40 revertant analysis, these results indicate that in CV-1 cells, a nonlymphoid cell line, 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 C element, its activity is unlikely to be lymphoid-specific.

Future studies will extend the viral revertant analysis of the HIV promoter to T cells, the replication permissive host for HIV, and macrophages, a restrictive host, by utilizing a polyomavirus vector that will grow in mouse cells. In addition, we will examine the promoter sequences in different isolates of HIV from AIDS patients. These future studies may indicate which promoter elements are most relevant to pathogenicity of the virus.

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MOLECULAR GENETICS OF EUKARYOTIC CELLS

This section encompasses a very broad range of biological problems, utilizing a wide range of methodological approaches. The two distinct but interweaving themes that repeatedly arise in the individual efforts are the control of gene expression and the molecular basis of normal and abnormal physiology, particularly relating to cancer. The sections led by Drs. Hanahan, Field, and Skowronski utilize the powerful methods of germ line gene transfer to create transgenic mice that model diseases such as cancer, diabetes, hypertension and AIDS, the acquired immune deficiency disease associated with infection by the human immunodeficiency virus type 1 (HIV-1). Dr. Wigler's lab focuses on the function of oncogenes, particularly the *RAS*, *ROS*, and *MAS* oncogenes. Dr. Bar-Sagi's lab also studies the function of the *RAS* oncogenes, and the role of phospholipid metabolism in signal transduction. Dr. Gilman's group studies the mechanisms of signal transduction that result in changes in nuclear transcription. Dr. Hernandez's lab studies the mechanisms of gene transcription in two systems: the genes encoding small nuclear RNAs (snRNAs), and the HIV-1 genome. Dr. Spector's group utilizes high-resolution electron microscopy and image analysis techniques to study the structural and functional organization of the cell nucleus. Dr. Franza's lab studies cellular proteins involved in the control of transcription. He has identified the products of two cellular proto-oncogenes that interact with specific transcription control elements. He also studies cellular proteins that control transcription of the HIV provirus. Dr. Helfman's lab studies the genes encoding the various isoforms of tropomyosins and how different oncogenes act to alter expression of the tropomyosin genes. Dr. Welch's lab continues its studies of the proteins associated with cellular responses to stress. The 2D-Quest facility of Dr. Garrels and co-workers continues to build up its various protein databases and to improve upon methodology for processing two-dimensional gel information.

TRANSGENIC MICE

D. Hanahan
J. Skowronski
L. Field

J. Alexander
J. Almeida
S. Alpert
V. Bautch
S. Efrat
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S. Teplin
L. Usher
P. Weinberg

Gene transfer into the mouse germ line provides an ideal model system in which to study complex biological problems at the organismal level. In the past, a major focus of the Transgenic Mouse Group at Cold Spring Harbor Laboratory has been models of targeted oncogenesis. More recently, our experiments have addressed a broader spectrum of issues, as, for example, tumor angiogenesis, autoimmunity, human immunodeficiency virus long terminal repeat (HIV LTR) tissue specificity, cardiocyte regeneration, and hypertension. These studies illustrate the strengths of the transgenic approach, especially with regard to the ability to characterize genetic and epigenetic events that profoundly influence normal development as well as complex disease processes.

Induction of Angiogenesis during Tumorigenesis

D. Hanahan, J. Alexander [in collaboration with J. Folkman, K. Watson, and D. Ingber, Childrens Hospital Medical Center, Harvard Medical School, Boston]

During the last several years, we have extensively

characterized several lines of transgenic mice that develop tumors of the pancreatic β cells as a result of inheriting an oncogene. Although tumor development is an inevitable consequence of carrying this particular oncogene, there is considerable evidence that the oncogene itself is necessary but not sufficient for the process and that other changes are required as well. This conclusion is based on studies on oncogene expression and its relationship to β-cell proliferation and tumor development, which have been described in previous annual reports (see especially the 1987 Annual Report).

The insulin-producing β cells are localized in approximately 400 focal nodules of cells that are called the islets of Langerhans. The islets are scattered throughout the exocrine pancreas and themselves comprise the endocrine pancreas. Their transformation has been accomplished with a hybrid oncogene composed of the rat insulin gene promoter/enhancer region aligned to transcribe the protein-coding region for SV40 large T antigen. In several lines of mice, large T is expressed in virtually all of the β cells in every pancreatic islet. This expression ensues in the developing endocrine pancreas (and the nervous system) beginning at embryonic day 10 in the 19-day gestation of a mouse and persists in the insulin-producing β cells thereafter. This potent oncoprotein, which binds both the retinoblastoma tumor supressor protein (see E. Harlow, Tumor Viruses Section) and the p53 oncoprotein (itself a putative tumor suppressor), does not immediately elicit a tumor or even abnormal proliferation. Rather, one can discern a progression from normality to hyperplasia to neoplasia.

In one particularly well-characterized line (RIP-Tag2), the islets are histologically normal at birth, but a few begin to evidence hyperplasia by 4–6 weeks (both histologically and by β-cell proliferation). By 9.5 weeks, 50% of the islets are hyperplastic, and by 12 weeks, 70% are hyperplastic. However, only about 2% of the islets progress into histologically distinct solid tumors, which are evident by 12 weeks of age, and kill the animal by 13–14 weeks. Thus, following activation of oncogene expression (the first step), we can discern two additional steps: the development of hyperplasia (the second step) and the progression to neoplasia (the third step). The reproducibility of this multistep tumorigenesis is providing a new approach to study mechanisms of conversion of a normal cell into a cancer cell.

With regard to the progression from hyperplasia to neoplasia, we now have evidence that implicates the induction of angiogenesis as an important event in this process. It is clear from the work of Judah Folkman and his colleagues that virtually every established tumor has the capability to elicit neovascularization, i.e., the formation of new blood vessels. Moreover, the ability to induce capillary ingrowth to a tumor following transplantation can be seen to be crucial to its expansion, since if neovascularization is inhibited, so too is tumor growth. However, it is not clear from this previous work when the ability to induce capillary ingrowth appears during the primary development of a tumor.

Histological analyses of the pancreases of RIP-Tag2 mice at different stages of tumorigenesis have revealed that normal islets are quiescent with regard to the vasculature, whereas every solid tumor (and a small subset of the hyperplastic islets) shows evidence of neovascularization, as assessed by [^{3}H]thymidine autoradiography to detect proliferating capillary endothelial cells, and immunostaining with laminin, which visualizes new capillary sprouts.

It is possible that every islet composed of proliferating cells expressing the large T oncogene is inherently angiogenic, but that its surrounding environment suppresses neovascularization, or that upon reaching a size threshold, an islet automatically becomes angiogenic. Alternatively, individual hyperplastic islets could switch on angiogenic activity in a manner unrelated to size or surrounding environment. To distinguish between these possible mechanisms, an in vitro bioassay for angiogenesis was developed. Normal and hyperplastic islets were physically separated from the pancreas, as were solid tumors. Individual islets or tumors were placed in a microwell containing a collection of capillary endothelial cells dispersed in a collagen gel. The islets from normal mice or from young RIP-Tag2 mice did not influence the endothelial cells. In contrast, every solid tumor elicited a dramatic response. The endothelial cells radially align, begin migrating toward the tumor, form capillary sprouts and then tubes, and finally degrade the basement membrane surrounding the tumor. This recapitulates the known stages of neovascularization of tumors in vivo. Thus, this bioassay distinguishes between normal islets and the tumors that arise out of them.

Hyperplastic islets were then analyzed throughout the reproducible time course of tumor development in the RIP-Tag2 family. In 4–5-week-old mice, none of the islets were angiogenic in vitro. At 6–7 weeks, 0.57% of the islets were angiogenic in vitro; this frequency increased to 2.8% during the 8–10-

week period and was 3.8% at 12 weeks, when 2% of the islets were represented as solid tumors, which were all angiogenic as well.

This statistical analysis of islets throughout the preneoplastic period has revealed that a small fraction are angiogenic in vitro. (Over 1200 isolated islets have been individually isolated and analyzed, which allows this conclusion.) Since more than 50% of the islets are hyperplastic by 9.5 weeks, one can conclude that hyperplasia does not obligate angiogenic activity, since only 2.8% of the islets are angiogenic in vitro. Nor does oncogene expression, since 100% express the large T oncoprotein. Moreover, the in vitro bioassay also demonstrated no strict correlation between islet size and angiogenic activity. Thus, the data support the conclusion that angiogenic activity is induced in a small fraction (~3%) of the islets during the hyperplastic stage. The fraction of angiogenic islets correlates very well with the incidence of solid tumors (2%), whereas neither correlates with the frequency of hyperplasia (50–70%). This suggests that the induction of angiogenesis is a localized, secondary event necessary for the progression from hyperplasia to neoplasia. Future studies will address the mechanism of the switch to an angiogenic state and the causality of that change in mediating tumor progression.

Influences of Nerve Growth Factor on the Development of the Peripheral Nervous System in Transgenic Mice

D. Hanahan, J. Alexander [in collaboration with R.H. Edwards and W.J. Rutter, Hormone Research Institute and Departments of Neurology and Biochemistry and Biophysics, University of California, San Francisco]

Nerve growth factor (NGF) is expressed in tissues that become innervated during neuronal development. In vivo, NGF is known to support the survival and differentiation of both sympathetic and sensory neurons. This conclusion comes from studies in which antibodies to NGF are administered during development, which causes whole populations of neurons to die, and by the complementary approach, addition of exogenous NGF, which increases the number of these same neurons by two- to fourfold.

These observations suggest that NGF is involved in directing the ingrowth of neurites to cells or tissues destined to become innervated and that it does so by being expressed in the target tissue. However, this possibility has been difficult to directly address experimentally. Transgenic mice present a new approach to questions on the roles of growth and differentiation factors in development, and we have now applied this system to studies on the role of NGF in the development of innervation in a peripheral tissue, the endocrine pancreas.

We chose to overexpress NGF in the islet cells of the pancreas for three reasons: (1) The pancreas is innervated by sympathetic, parasympathetic, and sensory neurons, so the response of a variety of neurons can be observed; (2) there is no indication that the pancreatic innervations are so profoundly influential that perturbations of it would be lethal; and (3) we have established that gene expression can be reliably targeted to the pancreatic β cells in transgenic mice through the use of the insulin gene regulatory region.

A hybrid gene composed of the insulin gene promoter/enhancer linked to the protein-coding information for mouse NGF was established in two lines of transgenic mice (RIP-NGF1 and RIP-NGF2). Both express NGF in their pancreatic β cells, as evidenced by immunostaining with antibodies specific for NGF. Nontransgenic mice show no expression. Each transgenic line has a distinct pattern of NGF expression. Line 1 shows heterogeneous, high-level expression, in that only a fraction of the islets express NGF, and only a fraction of the β cells within an islet show expression, albeit at a high level. In contrast, mice in line 2 show evidence of uniform, low-level expression of NGF in virtually every β cell in every islet.

The expression of NGF in the β cells of the islets results in the selective hyperinnervation of those islets by sympathetic neurons. Neither the sensory nor parasympathetic innervation is influenced by overexpression of NGF. There are two major subclasses of sympathic neurons, which are visualized by their expression of somatostatin or neuropeptide Y. Synthesis of NGF in β cells only affects the neuropeptide Y subset, which normally innervates the pancreas, and not the somatostatin-positive class, which projects to different targets.

The innervation of the islets induced by NGF is highly selective not only for the type of neuron it affects, but also for the density and location of the nerve processes. In both lines, the mice show selective hyperinnervation of the islets and not of the surrounding exocrine pancreas. In line 2, which is uniformly expressing NGF in the islets, every islet is

similarly hyperinnervated. In line 1, which is characterized by sporadic islets expressing high levels of NGF, the pattern of hyperinnervation is similarly sporadic, and those islets that are affected have very dense neural processes. Thus, NGF acts in a localized fashion to influence the innervation of the collection of cells expressing it, in a manner that appears semiquantitative.

These studies show that NGF can directly influence the extent of innervation of a target tissue by the levels of its expression in that tissue. NGF can apparently only influence neurons that normally project to that tissue, since the neuropeptide Y-positive neurons do respond, whereas the somatostatin-positive neurons do not. The former innervate the pancreas and the latter do not. A surprise, however, is that the sensory neurons that project into the pancreas are not affected. Sensory neurons are known to be responsive to NGF in vitro and can be affected by anti-NGF antibodies during their development. It is possible that NGF requires a cofactor in order to influence sensory neuron development, and this cofactor may not be available in the islet cells. Alternatively, the sympathetic neurons may compete more effectively for the available NGF. The current studies motivate both further investigation into these possibilities and similar approaches that seek to assess the influence of targeted expression of NGF on central nervous system development in transgenic mice.

Diabetes Induced in Transgenic Mice by Expression of Human Ha-*ras* in Pancreatic β Cells

S. Efrat, C. Jolicoeur, D. Hanahan

Our work has focused in recent years on the study of targeted oncogenesis in β cells in transgenic mice, by introducing hybrid insulin-promoted oncogenes into the mouse germ line. As part of this study, we have generated transgenic mice expressing the activated human Ha-*ras* oncoprotein in β cells. As with several other hybrid insulin oncogenes expressed in the β cells, expression of *ras* does not cause any abnormal cell proliferation. However, unlike mice harboring other hybrid oncogenes, the insulin-*ras* mice develop diabetes several months after the onset of oncogene expression in β cells. The disease results from dysfunction and degeneration of β cells, without an obvious autoimmune response.

The onset of transgene expression occurs in β cells during embryonic development, with no discernible consequences. Young adult mice also do not show any abnormalities. Beginning at 5 months of age, male mice develop hyperglycemia (>400 mg/dl) and glucosuria and die prematurely within a short time thereafter. Histological analysis reveals holes in the majority of the large islets (Fig. 1). In the insulin-*ras* females, no hyperglycemia or premature death has been observed (the oldest female analyzed so far was 13 months old), but holes do occur in a small number of the islets, beginning at about 10 months of age.

The destruction of the islets is not associated with an obvious inflammatory activity. Leukocytes have not been observed to infiltrate the islets of the insulin-*ras* mice, as judged from histological analysis and by immunostaining for surface markers of lymphocytes and macrophages. Immunostaining for the MHC class I and class II antigens does not reveal any abnormal expression on the surfaces of the β cells. In addition, sections of pancreas from diabetic animals have been stained with autologous sera, which did not reveal autoantibodies directed against the islets. These results suggest that the death of β cells in the insulin-*ras* mice does not involve an autoimmune response, although this should be confirmed by classic immunosuppression experiments.

Immunohistochemical analysis with monoclonal antibodies directed against the human *ras* protein (Y13-238 and -259) detects high levels of *ras* in the β cells of both male and female transgenic mice. Control C57BL/6 mouse islets do not stain for *ras*, in contrast to normal human islets (Furth et al., *Oncogene 1:* 47 [1987]). This difference is probably not due to lack of cross-reactivity between the human and the mouse proteins (human and mouse *ras* proteins are identical in the first 94 amino acids, where Y13-259 binds). RNA analysis of mouse β cells, obtained from tumors arising in insulin-SV40 T antigen transgenic mice, also fails to detect any *ras* transcripts (S. Efrat, unpubl.; M. Perucho, pers. comm.).

In human type I diabetes, hyperglycemia appears only when the number of β cells falls below 10% of their normal value. In the insulin-*ras* diabetic males, although the islets are damaged, they contain β cells staining for insulin in numbers well above the critical level (Fig. 1). This suggests that insulin processing or secretion may be impaired in the remaining β cells. Insulin radioimmunoassay reveals very low levels of circulating hormone in the serum of the diabetic

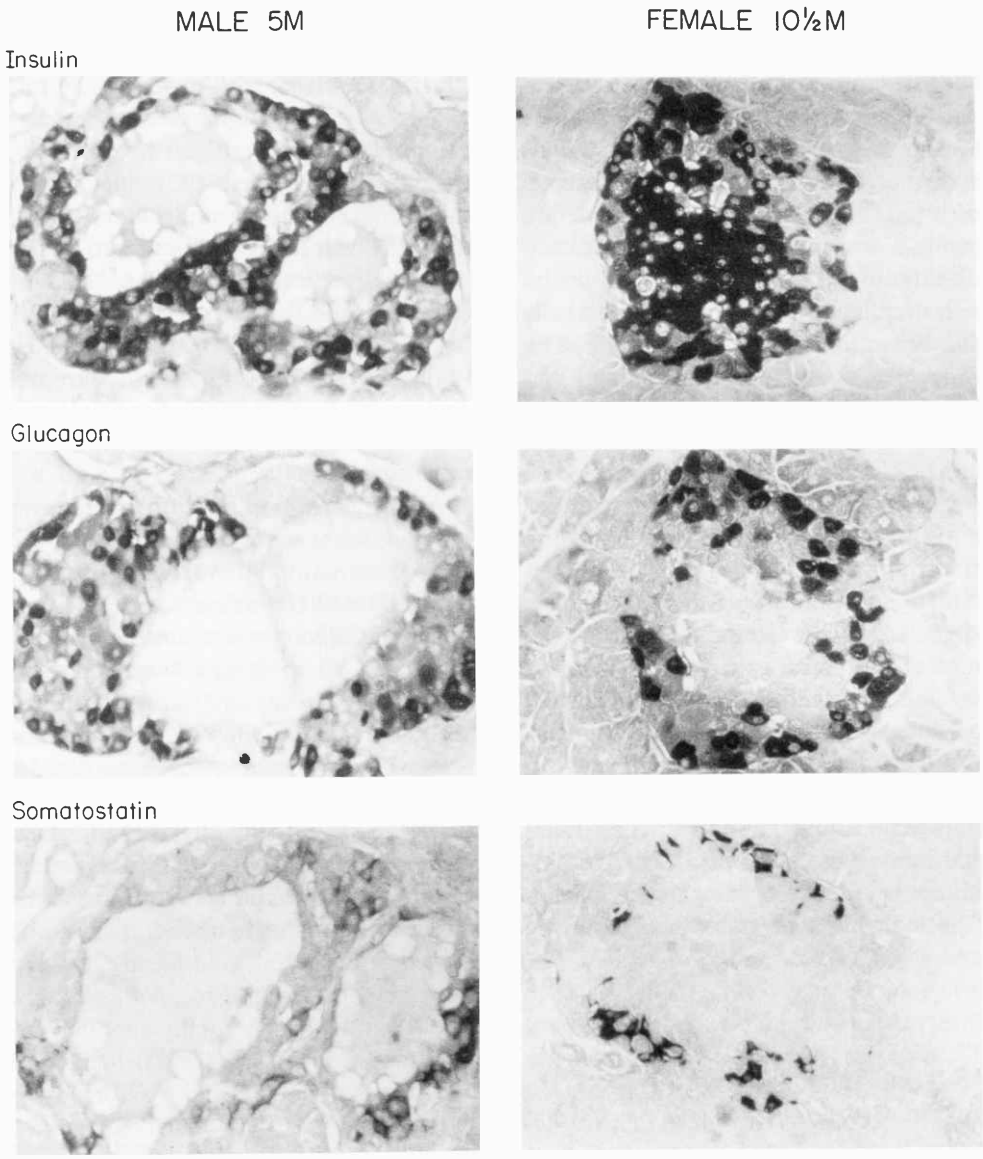

FIGURE 1 Immunohistochemical analysis of islet hormones in the pancreas of insulin-*ras* mice. Thin sections of paraffin-embedded pancreas were immunostained with antisera specific for the indicated hormones and visualized with horseradish-peroxidase-conjugated second antibody. M indicates month. Magnification, 194×.

mice, suggesting that the impairment is at the level of secretion. Immunostaining for the other islet hormones does not reveal any abnormality (Fig. 1). Electron microscopy analysis of islets isolated from diabetic males is in progress to identify any cell lesions that may precede the stage of cell death.

It therefore appears that high levels of activated *ras* protein are toxic to β cells, possibly by causing an impairment in the secretion pathway from the Golgi to the cell membrane. It is possible that *ras* itself, or another G protein, is involved in the regulation of secretory granule traffic in the β cells and that overexpression of *ras* interferes with the normal regulation. The long latency period between the appearance of *ras* in the cells and the observed cell death remains difficult to explain. Interestingly, the same activated human *ras* protein has been overexpressed in several other secretory cell types in transgenic mice, such as the pancreatic acinar cells (Quaife et al., *Cell 48:* 1023 [1987]), where it causes development of neonatal hyperplasia, and in mammary epithelial cells (Andres et al., *Proc. Natl. Acad.*

Sci. 84: 1299 [1987]), where dedifferentiation, tumor formation, and reduced synthesis of milk proteins have been observed in rare cases. However, expression of *ras* in these secretory cells does not lead to cell degeneration, as is observed in β cells.

A second unresolved issue is the strong susceptibility of males to the effect of *ras*. Male mice are known to be more susceptible to diabetes induced either by obesity mutations, such as *db*, or by treatment with streptozotocin, a drug toxic to β cells (Leiter et al., *Immunogenetics 26:* 6 [1987]). The reasons for these gender differences are not understood, although sexual dimorphism in glucose metabolism has been suggested to be involved. Estrogens have been shown to act synergistically with insulin to increase glucose uptake, whereas androgens reduce it, possibly by indirectly antagonizing the action of insulin on cells (Bailey et al. *Diabetologia 19:* 475 [1980]). In the case of the *db/db* mice, it has been suggested that sex steroids can determine the penetrance of the diabetogenic stress imposed by the obesity mutation (Leiter, *Metabolism 37:* 689 [1988]). Experiments are in progress to address these questions in the insulin-*ras* lineage described here, as well as in additional lineages harboring hybrid insulin-*ras* transgenes, employing either the activated or the normal human Ha-*ras* genes. These studies may contribute to our understanding of β-cell function and of possible involvement of sex hormones in β-cell physiology.

Studies of Transgene Integration Using Plasmid Rescue

S. Grant, J. Alexander, D. Hanahan [in collaboration with J. Jesse and F. Bloom, Bethesda Research Laboratories]

For the generation of transgenic mice, linear DNA molecules are microinjected into the pronuclei of fertilized mouse embryos, whereupon the DNA becomes integrated into the mouse chromosome. The typical structure of the integration includes multiple copies of the injected DNA in a head-to-tail tandem array. This integration frequently results in rearrangements and deletions of the injected DNA as well as the chromosomal DNA at the locus of integration. Disruption of the chromosomal DNA may result in an insertional mutation, most frequently manifesting as a recessive embryonic-lethal phenotype. To understand further the process of integration of microinjected DNA and to analyze the transgene-flanking cellular sequences, we have utilized the plasmid-rescue procedure (Hanahan et al., *Cell 21:* 127 [1980]).

We have examined transgenic mice carrying insulin promoter–SV40 T antigen hybrid gene constructs within a pBR-based plasmid. To "rescue" the transgenes, the high-molecular-weight transgenic mouse spleen DNA is digested to completion with a restriction enzyme that cuts once within the construct and in the flanking cellular DNA. This linearized DNA is then ligated at low concentration, to favor circularization, and then transformed into competent *Escherichia coli* and selected for antibiotic resistance encoded by the rescued plasmid sequences.

Although the method of plasmid rescue is highly efficient for yeast, it has been quantitatively inefficient from mammalian DNA (including transgenic mouse DNA) (Hanahan, in E. coli *and* S. typhimurium: *Cellular and molecular biology*, American Society of Microbiology, Washington D.C. [1986]). These observations and reconstruction experiments suggest that plasmid DNA grown in mammalian cells is modified and subsequently restricted by the common *E. coli* strains used for transformation, such as DH5α. In-vitro-methylated plasmid DNAs are restricted and have been used to select strains of *E. coli* that contain mutations in site-specific methylation-sensitive restriction systems, including the McrA and McrB (*m*odified *c*ytosine *r*estriction) (Raleigh and Wilson, *Proc. Natl. Acad. Sci. 83:* 9070 [1986]) and Mrr (N^6methyladenine modification) (Heitman and Model [1987]) loci. We have made derivatives of DH5α and MC1061, by introducing mutations in the Mcr and Mrr loci, and tested these mutant strains for the restriction-like phenotype observed with plasmid rescue from transgenic mouse DNA.

We have used four lineages of transgenic mice carrying insulin promoter–SV40 T antigen hybrid gene constructs, of which two lineages (RIP-Tag2 and RIR-Tag2) express T antigen in β cells from embryonic day-e10 and develop β-cell tumors "*fast*" at 16–24 weeks of age, and two "*slow*" lineages (RIP-Tag3 and RIP-Tag4) that first express T antigen at 10 weeks of age and develop tumors at 30–60 weeks. The fast and slow tumor phenotypes are heritable upon continuous backcrossing and thus represent transgene position effects. Our first observation using the *E. coli* strains with Mcr and Mrr mutations was an increase in efficiency of plasmid rescue compared to the parental strains, suggesting that

these mutations released the inhibition to transformation seen in the parental *E. coli* strains. Interestingly, the number of plasmids rescued in different mutant strains of *E. coli* correlated with the phenotype of tumor formation of these transgenic mice, in that *fast* lineage DNA rescued well in *E. coli* McrA$^-$ McrB$^-$ Mrr$^+$, whereas *slow* lineage DNA rescued only in *E. coli* strains McrA$^-$ Δ(McrB$^-$ Mrr$^-$). This requirement of a deletion encompassing McrB to Mrr for plasmid rescue from slow lineages implies that these deleted loci detect and restrict a modification that distinguishes the slow and fast lineages and therefore may play a role in the different timing of transgene expression.

These methylation-restriction mutant strains have facilitated the recovery of large numbers of plasmids from the four lineages of transgenic mice. We have systematically identified clones that represent junctions between adjacent transgenes in the head-to-tail tandem array, between transgenes and flanking cellular DNA, as well as rearranged and deleted transgenes. We are currently analyzing the structure of these plasmids in detail. In conclusion, our results demonstrate that plasmid rescue using *E. coli* strains deficient in methylation-restriction loci is a rapid and efficient method for retrieving integrated transgenes and their flanking cellular sequences. The requirement for mutations in these *E. coli* restriction systems may, in addition, enable us to investigate the nature of stable position effects that influence tumor formation.

Genetic Control of Autoimmunity toward the Insulin-producing β Cells in Transgenic Mice

J. Skowronski, C. Jolicoeur, S. Alpert, D. Hanahan

Transgenic mice expressing new proteins provide an approach to study self-tolerance and autoimmunity, a disease characterized by a failure to establish or maintain recognition of self. Directing expression of novel antigens to specific cell types, which are known to be susceptible to autoimmune phenomena, such as the pancreatic-insulin-producing β cells, may perturb their recognition as self. If so, the precise knowledge of the transgenic antigen would allow examination of initial events in which an autoantigen and the cell that expresses it become targets for an autoimmune response.

One example of such a system is provided in transgenic mice harboring hybrid genes composed of the rat insulin gene transcription control region fused to the coding information of the SV40 early region (RIP-TAG mice) (Hanahan, *Nature 325:* 155 [1985]). Mice of all lines harboring the hybrid insulin–T antigen genes express large T antigen in the insulin-producing β cells of the pancreatic islets of Langerhans, which, as a consequence, eventually undergo neoplastic transformation. Individual lines of insulin–T antigen transgenic mice reproducibly show distinct patterns of developmental expression of the transgene, which could be classified into one of the two groups, early and late. In lines characterized by the early onset of expression, T antigen is detectable immunohistochemically in the developing pancreas through the second half of prenatal life (Alpert et al., *Cell 53:* 295 [1988]). Expression continues thereafter in most, if not all, of the β cells. In lines showing the late onset, T antigen is not detectable prenatally; it can be first visualized immunohistochemically in a subset of insulin-producing cells of adult mice at 10–12 weeks of age. The immunological response to T antigen appears to depend on the developmental timing of its presentation. Mice of RIP-TAG lines showing early onset of expression of the transgene (RIP-1 Tag2 and RIR Tag2) are immunologically tolerant to the large T antigen, whereas those characterized by the developmentally delayed expression (RIP-1 Tag3 and RIP-1 Tag4 lines) are not (Adams et al., *Nature 325:* 223 [1982]). Interestingly, mice of nontolerant lines spontaneously develop serum antibodies that react with the SV40 large T antigen. The humoral response to T antigen is accompanied by lymphocyte infiltrations of the islets of Langerhans (insulitis) and their destruction. This suggests that the spontaneous immune response is targeted against the T-antigen-expressing β cells. Interestingly, the penetrance of this phenotype is not complete; it was observed to be 60% and 35% when mice of transgenic RIP-1 Tag3 and RIP-1 Tag4 lines, respectively, were randomly sampled from the breeding colony. Thus, delayed expression of the SV40 T antigen is necessary for the heritable predisposition to develop autoimmunity, but it is not sufficient to elicit the autoimmune phenotype.

All the transgenic founder mice generated with the insulin promoter–SV40 early region hybrid gene were constructed in the F_2 hybrids between C57BL/6J (B6) and DBA/2J (D2) inbred strains of mice. The transgenic founder mice were propagated by backcrosses to B6 mice or by intercrosses among trans-

genic mice. Thus, individual mice of the RIP-1 Tag3 and Tag4 mice are not genetically identical, as they have different genetic contributions from the two parental strains. It is therefore possible that there is a genetic component to the autoimmune response.

To test this hypothesis, RIP-1 Tag3 male mice were crossed to females of several different inbred strains, including the parental B6, and D2, strains and B6D2/F_1 hybrid mice. The development of autoimmune phenotype was followed in transgenic progeny until 30 weeks of age. Differences were observed in both the incidence and the dynamics of the development of serum IgG antibodies reacting with SV40 large T antigen. Virtually all of the RIP-1 Tag3 progeny derived from the D2 backcross developed high levels of circulating anti-T-antigen antibodies by 20–25 weeks of age. In contrast, only a minor fraction (17%) of RIP-1 Tag3/B6 mice developed humoral responses against T antigen by 30 weeks. RIP-1 Tag3 progeny derived from the B6D2/F_1 displayed an intermediate frequency of the autoimmune phenotype (55%). Interestingly, in these two last crosses, the dynamics was different from that observed in RIP Tag3/D2 mice, since the incidence of autoimmune mice was increasing more slowly and gradually up to 30 weeks. These observations are consistent with the assumption that a single locus present on the DBA/2J background acts dominantly to promote autoimmune responses. However, altered dynamics of the phenotype suggests that additional loci are involved in genetic control (or modulation) of autoimmunity in RIP-1 Tag3 mice.

To test whether the immunoregulatory loci of the MHC (H-2 in mice) are controlling the autoimmune phenotype in RIP-1 Tag3 mice, the H-2 haplotypes of transgenic progeny derived from the B6D2/F_1 cross were examined. The H-2 haplotypes were distinguished by a restriction-fragment-length polymorphism (RFLP) between the H-2^b (B6) and H-2^d (D2), which is detected by an Aβ cDNA probe. By 30 weeks of age, 90% of mice carrying the hybrid H-2^b/H-2^d haplotypes had high titers of circulating anti-T-antigen antibodies. In contrast, only 30% of the H-2^b/H-2^b mice had high titers. Thus, loci linked to the MHC are associated with the development of autoimmunity in RIP-1 Tag3 mice, and it is tempting to speculate that MHC itself might be involved. The MHC-linked locus (loci) is not strictly required for the development of T-antigen-primed autoimmunity, since a fraction of H-2^b/H-2^b mice also develops spontaneous autoimmunity.

RIP-1 Tag3 transgenic mice are heritably predisposed to develop autoimmune response targeting pancreatic β cells by the virtue of the developmentally delayed expression of the transgene. This predisposition results in overt autoimmune phenotype when combined with additional, proper genetic elements. There are several components to the autoimmune phenotype. Developmentally delayed expression of the SV40 large T protein, which precludes establishment of the self-tolerance to this novel β-cell antigen, is the crucial event, since neither predisposition to develop autoimmunity (specifically targeting β cells) nor immunoregulatory defects have ever been observed in the mouse strains used in our transgenic experiments. Penetrance of the autoimmune phenotype is under additional genetic control to which both the H-2 and non-H-2 loci contribute. H-2 seems to be the major determinant of susceptibility (or resistance). However, our results suggest that effects of H-2 can be modulated by other loci. Complex genetic control of autoimmune phenotype in RIP-1 Tag3 mice and, in particular, its association with the loci of the MHC are reminiscent of human autoimmune diseases and, specifically, human-insulin-dependent diabetes mellitus and its animal models, where β-cell autoimmunity has been found correlated with particular class II loci of the MHC. It is not clear whether class I or class II loci, or both, are the susceptibility loci in RIP-1 Tag3 mice, and further studies are necessary to define them.

Latency and Activation of the HIV-1 Transcription Regulatory Region in Transgenic Mice

J. Skowronski, L. Usher

Human immunodeficiency virus (HIV) is the etiological agent of AIDS, the acquired immunodeficiency syndrome. The major target in HIV infections is the CD4 subset of T lymphocytes, where the CD4 antigen acts as a receptor for the virus. Cell types other than CD4 T lymphocytes can also be productively infected with HIV. These additional cell types include endothelial cells, monocytes, glial cells, and possibly others.

Infections of humans with HIV are characterized by a prolonged absence of detectable virus production, as well as an absence of any clinical manifestations of the disease after the primary infection. The latency of viral infection in humans has been mimicked in experiments with in-vitro-infected human

T cell lines. Here, the dormant state of the provirus has been found to correlate with the transcriptional inactivity of the viral promoter, which, subsequently, can be activated by a variety of stimuli that also activate T cells. In addition, the *trans*-acting proteins of several viruses, including herpesviruses, adenoviruses, and some lymphotropic viruses, are capable of activating transcription of the HIV promoter. The implications of these results are twofold. First, they indicate that transcriptional activation of the HIV promoter elements is critical for transition of HIV to the active state. Second, they implicate antigenic stimulation and opportunistic infections as potential in vivo routes to activate the transcriptionally dormant HIV promoter and consequently elicit virus production in latently infected individuals.

We have recently initiated experiments directed toward the development of transgenic mouse models to study the transcriptional regulation of the HIV-1 promoter in vivo in various tissues and cell types, under a variety of physiological and experimental conditions. This approach, where the introduced gene is heritably present in every cell of the transgenic animal, should allow us to overcome the limitations of experiments performed directly on human subjects and provide small animal models that are amenable to genetic and biochemical studies. To define tissue specificity of the HIV transcriptional control elements, two reporter genes were placed under the transcriptional control of the HIV-1 long terminal repeat (LTR) and introduced into the germ line of the inbred C3HeB/FeJ mice. *E. coli* β-galactosidase (β-Gal) and SV40 large T antigen (TAG) have been used as the two reporter proteins. A common advantage to both of these reporter proteins is that immunological and histochemical reagents exist, which allow sensitive detection of these proteins in situ. In addition, T antigen is a potent oncoprotein capable of altering growth characteristics of a broad variety of different cell types, including lymphoid cells, when expressed in transgenic mice. Advantages of constructing transgenics in inbred lines of mice are twofold. First, all of the progeny originated from the same transgenic founder could be considered genetically identical; thus, any alterations of the phenotype in individual mice of a transgenic line, if observed, will result from epigenetic factors, rather than from genetic factors. Second, inbred background should allow transfers of selected populations of cells between transgenic and wild-type animals of the same inbred strain (e.g., adoptive transfers of the immune system), which may facilitate our analyses.

Multiple lines of transgenic mice were established with each of the two hybrid genes (HIV LTR/β-Gal lines 1 through 6 and HIV LTR/TAG lines 1 and 2) and we have initiated their analysis. Autopsies performed on a limited number of the HIV LTR/TAG transgenic animals at different ages revealed that HIV LTR/Tag1 mice reproducibly develop hyperplasias of the thymus. Thymuses are overtly enlarged at 2 months of age (two- to fivefold) and by 6–9 months, occupy the major part of thoracic cavity, which probably results in respiratory and circulatory insufficiency leading to premature death of animals. In contrast, mice of the HIV LTR/Tag2 line do not exibit any overt phenotype. Expression of the LTR T-antigen transgenes was analyzed by RNase protection of an antisense probe after hybridization to RNAs isolated from numerous tissues and organs of these mice. Two transcripts of sizes predicted for the correctly initiated (within the HIV LTR) and spliced mRNAs encoding the SV40 large T and small T-antigens were detected in RNA isolated from all major lymphoid organs, including thymus, spleen, and lymph nodes of mice of both transgenic lines. Transgenes were also expressed in skin and small intestines both in transgenic lines and in the bone marrow of HIV LTR/Tag2 mice. Transcripts from the transgenes have not been detected in several other tissues such as brain, kidney, testis, muscle, or heart in either of the transgenic lines. Such patterns of expression of the transgenes, observed reproducibly in mice of two independently derived transgenic lines, indicate that the HIV promoter is lymphoid-specific. This conclusion is not contradicted by the relatively high level of expression of the transgenes in skin and intestine, because both of these tissues perform immune functions and contain major populations of T and/or B lymphocytes, respectively. We have also initiated analysis of all six lines of the HIV LTR/β-Gal mice using an extremely sensitive fluorescent assay of the β-galactosidases in live cells (in collaboration with S. Fiering and L.A. Herzenberg, Stanford University). Suprisingly, no expression of β-Gal transgenes has been detected in the lymphoid compartment of mice of any of the transgenic lines. There are many possible explanations of so drastically different behavior of T antigen and β-Gal reporter genes. One possibility is that SV40 large T and small T antigens, once expressed, may freeze the target cell in a condition permissive for transcriptional activity of the HIV LTR. Alternatively, the LacZ protein-coding region may contain dominant, *cis*-acting signals that silence the HIV LTR upon

transmission through the mouse germ line. These and other possibilities are currently under investigation.

We are now defining the exact specificity of the HIV promoter for different cell types within the lymphoid compartment of the HIV LTR/TAG mice, which is composed of different functionally distinct populations of B and T lymphocytes. We have developed an assay using the polymerase chain reaction (PCR) that allows detection of specific transcripts in a small number of cells. When combined with cell-sorting techniques, this procedure will allow us to analyze expression of the transgenes in functional subsets of transgenic lymphocytes. Solid tissues, such as skin and intestine, are also amenable to this type of analysis. The identity of cells residing in skin and capable of supporting expression of the HIV LTR is of a special interest, since these cells have been implicated in pathogenesis of Kaposi's sarcoma, a proliferative disorder of skin frequently associated with AIDS.

In summary, through these transgenic experiments, we hope to identify cell types and in vivo conditions that are permissive for expression of the HIV promoter. This knowledge should provide a stage to define tissue-specific *trans*-acting factors critical for transcriptional activation of HIV from the latent state.

Transgenic Models of Myocardiocyte Proliferation

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The regulation of cell division has been a major focus of research at Cold Spring Harbor Laboratory for many years. We are specifically interested in the control of myocardiocyte proliferation. Cardiac tissue has long been viewed as incapable of regeneration. Unlike their skeletal muscle counterpart, cardiac muscle lacks precursor stem-cell populations (the so-called satellite cells). Thus, physical or chemical trauma, which induces skeletal muscle regeneration via satellite cell proliferation, fails to elicit a similar response in the heart. The unfortunate consequence of this is that cardiac tissue cannot be replaced after destructive trauma, as, for example, damage resulting from an infarct. Previously, we generated transgenic mice that carry fusion genes composed of the atrial natriuretic factor (ANF) promoter linked to sequences encoding the SV40 large tumor antigen (TAG). These animals, designated ANF-TAG, develop unilateral atrial tumors; although both atria express T antigen, only the right atrium exhibits a hyperplastic response to the oncoprotein. Given the pronounced hyperplasia observed in ANF-TAG atria, much of our efforts have involved further characterization of these animals with the hope of identifying molecular controls that regulate myocardiocyte proliferation. Furthermore, we have initiated studies to determine if ventricular myocytes can be induced to proliferate in a manner analogous to that observed in atrial cells. The results that we have obtained with respect to these issues are considered separately below.

GENETIC LOCI AFFECTING ATRIAL HYPERPLASIA IN ANF-TAG MICE

The ANF-TAG transgenic mice were produced in (C57BL/6J $\times$ DBA/2J)/F_2 embryos (abbreviated (B6 $\times$ 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 that 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. After four generations of D2 backcrossing, right atrial hyperplasia is apparent in neonatal pups by 6 days of age. Moreover, these mice die on average at 8 weeks of age, with severe right atrial hyperplasia. In contrast, mice derived from four successive backcrosses to the B6 progenitor exhibit no atrial tumorigenesis prior to 1 year of age, and a significant number of these mice die of presumed old age without displaying any overt cardiac pathology at necropsy.

More intriguing are the distinct differences in atrial tumor morphology observed in the two sublines. Mice from the D2 subline show a gross hyperplasia involving essentially all cells of the right atrium. Moreover, unilateral left atrial involvement is never observed. In contrast, tumors developing in atria of the B6 subline appear (based on histological examination) to arise from a focal event (i.e., only a portion of the atrial cells are involved). The latency and focal nature of pathology in the C57BL/6J subline invite consideration of a cooperative genetic event. 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. It should be noted that the influence of genetic background on atrial tumorigenesis has been observed in two independent ANF-TAG lineages, which essentially rules out trivial explanations related to the effect of transgene integration site on the phenotype.

Crossing mice from the ANF-TAG D2 subline with B6D2/F_1 mice results in a segregating pattern of cardiac pathology and death (40–60% D2-like), supporting the involvement of one or possibly two genetic loci. Interestingly, the steady-state level of T antigen also appears to segregate into two discrete classes among transgenic siblings generated in these crosses. The D2 and B6 sublines have been established as a true breeding genetic resource. Using these animals, we will attempt to map genetically the loci responsible for the segregating phenotype by utilizing standard linkage analyses as well as recombinant inbred lines of mice. In so doing, we hope to determine the number of loci that influence the observed differences in atrial tumorigenesis as well as the position of these genes on the mouse linkage map. Moreover, it will be of interest to determine if segregation of T antigen steady-state levels is related (or causal) to the pattern of atrial tumorigenesis.

Last year, we initiated a series of experiments in which the ANF-TAG transgene was introduced into the *i*nverted *v*iscera (iv) background. iv influences the spatial development of the internal viscera; animals that are +/+ or +/iv have normal viscera, whereas animals that are iv/iv have viscera that develop as a mirror image compared to that of unaffected mice. Our goal was to cross the ANF-TAG transgene into this background and assess the sidedness of the ensuing atrial tumorigenesis. Animals that exhibited the iv phenotype were identified by simple ECG analyses. Characterization of several (iv/iv, +/ANF-TAG) animals has indicated that atrial pathology follows the viscera; i.e., hyperplasia develops in the functional right atria. However, in performing control analyses on ANF-TAG animals that failed to show the iv phenotype (genetic intermediates in this experiment), we discovered several mice that exhibit bilateral atrial hyperplasia. This change in atrial pathology may be attributable to several causes. For example, the genetic background of the iv mice may be sufficiently different from that of the ANF-TAG progenitors so as to cause bilateral hyperplasia. Indeed, the results above clearly indicate that genetic background can exert profound influence on atrial tumorigenesis. Alternatively, a mutation capable of altering the pathology may have arisen during generation of the iv/ANF-TAG hybrids. The new phenotype may have evolved as a consequence of changes in steady-state oncogene expression, induction of requisite progression factors, or repression of anti-oncogenes. To address directly the nature of the bilateral phenotype will first necessitate the generation of true breeding populations of mice. Once these animals are available, we will initiate linkage analyses to map genetically the locus (loci) responsible for the phenotype.

In conclusion, traditional genetic backcross experiments have revealed at least one (and more likely two) genetic locus that regulates both the temporal pattern and eventual end-stage morphology of T-antigen-induced cardiac pathology. We hope to identify these loci genetically over the next year by utilizing traditional linkage studies as well as recombinant inbred lines of mice. In addition, the iv experiments have identified an additional trait that bestows a bilateral hyperplastic phenotype.

PROPAGATION OF SUBCUTANEOUS TUMOR TRANSPLANTS DERIVED FROM ANF-TAG ATRIA

The unilateral atrial hyperplasia in ANF-TAG mice may arise as a consequence of physiological differences between the left and right portions of the heart. For example, differential chamber pressure, oxygenation, or sympathetic innervation could exert a profound influence, which is ultimately manifested as unilateral atrial tumorigenesis. To address this issue directly, we evaluated the growth potential of left and right transgenic atria at a physiologically neutral site. Nude mice, which lack the capacity to mount an immune response against foreign tissues, received both left and right transgenic atria from neonatal mice. Transplantation of neonatal right atria resulted in the proliferation of myocytes (four out of four transplants), which continued to express the ANF-TAG transgene. In contrast, transplanted left atria did not proliferate to any detectable extent in four attempts. These results imply that an intrinsic differential capacity to respond to an intracellular mitogenic signal (i.e., SV40 T antigen) exists between right and left atrial cells. Moreover, these results rule

out the possibility that unilateral hyperplasia is a sole consequence of physiological differences within the context of the heart.

The tumorigenic capacity of hyperplastic right atria was also assessed in syngeneic animals. Injection of minced hyperplastic ANF-TAG atria subcutaneously into numerous mice gave rise to a single tumor (TSP.A) palpable after a 9-month latency. Subsequent passage of the TSP.A tumor resulted in enhanced growth typified by an increase in the frequency of transplants yielding tumors (>90%) and by a markedly decreased average latency (2.5 months) upon passage. The TSP tumors were encapsulated by a thin layer of connective tissue and were nourished by several prominent blood vessels arising predominantly from the dorsal scapular fat pad of the host mouse. The ANF-TAG fusion gene and T antigen were detected in TSP tumor tissue by Southern and Western blots, respectively. These results confirm an atrial origin of the TSP tumor. ECG leads placed across the TSP tumors revealed an abnormal arrhythmic electrical activity independent of the host's cardiac activity; that this electrical activity originated from the TSP tumor was confirmed by ECG analysis of explanted tumors in vitro.

Both the TSP tumors and the original hyperplastic ANF-TAG atria have surprisingly retained a phenotype characteristic of highly differentiated cardiac tissue, at the level of gross structural organization and at the level of molecular expression. This observation is striking in view of the fact that cardiocytes from the TSP tumors have been propagating outside of the context of a heart for a period greater than 18 months. It is of interest to note that there is no detectable α-actin isoform switching in either the TSP tumors or the hyperplastic atria, suggesting that these myocytes retain a differentiated state similar to that of normal adult tissue. In this regard, T-antigen-induced proliferation differs markedly from other forms of myocyte growth, where α-actin switching has been shown to be a biochemical hallmark. The absence of induction of contractile protein fetal isoforms in the presence of cell proliferation is an important consideration with regard to the potential of cardiocyte regeneration. Finally, prominent vascularization accompanies cardiocyte proliferation in the TSP tumors. Moreover, the ectopic cardiac tissue is no longer a "vital" host organ, and the subcutaneous localization of the transplant renders it experimentally accessible. These properties suggest that the TSP transplants will provide an attractive model system for the study of cardiac-specific angiogenesis.

TRANSGENIC MODELS OF VENTRICULAR MYOCYTE PROLIFERATION

The highly differentiated state displayed by the TSP tumors, and in particular by the hyperplastic atria, generates renewed interest in the potential of inducing cardiocyte regeneration in vivo. Clearly, T antigen is capable of reversing the events that arrest atrial myocardiocyte division in transgenic model systems. Indeed, recent observations that document an association between viral oncogenes and the retinoblastoma protein, a putative tumor suppressor protein (see E. Harlow and E. Moran, Tumor Viruses Section), suggests a possible molecular mechanism for this reversal. The ability to effect a controlled proliferation of differentiated cardiocytes, particularly ventricular myocytes, by transient exposure to T antigen (or a T-antigen derivative) may be of considerable therapeutic value.

Given this, it is imperative to establish the responsiveness of ventricular myocytes to T antigen. In generating the ANF-TAG mice, three transgenic animals were obtained that died at 1–3 weeks of age. Histological analysis of the hearts from these animals indicated gross atrial hyperplasia. In addition, there was significant hyperplasia of the ventricular septum, as well as the right and left ventricle walls. Immunohistological analyses revealed that the hyperplastic ventricular myocytes were expressing T antigen. Ventricular expression of the endogenous ANF gene is observed during late embryological development. We postulate that the ANF-TAG animals exhibiting ventricular oncoprotein expression carried transgenes that integrated in a chromosomal site compatible with normal developmental expression. In support of this notion, none of the established ANF-TAG lineages exhibit T-antigen expression during embryological development, in either the atrium or ventricle. Thus, it would appear that ventricular myocytes are responsive to T antigen, at least at late stages of embryological development. To address the issue of ventricular responsiveness further, we have generated fusion genes comprising ventricle-specific promoters fused to T-antigen sequences. The promoters were isolated from the human cardiac actin gene and the rat α-myosin heavy chain. We have initiated microinjections with these fusion genes and hope to have

transgenic animals shortly. Such animals will provide a powerful model system with which to assess the proliferative potential of ventricular myocytes.

Electrophysiology of Hyperplastic ANF-TAG Atria

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

Atrial hyperplasia in the ANF-TAG mice is accompanied by a progressive increase in both the frequency and severity of cardiac conduction abnormalities, as assessed by electrocardiographic analysis. These conduction abnormalities are manifest as ventricular contractile arrhythmias and can eventually be lethal to the animal. Generally, disturbances in cardiac conduction reflect changes in cellular electrophysiological parameters, such as resting membrane potential, action potential waveform, and automaticity, and in the patterns of intercellular conduction. Reductions in the transmembrane potential would result in action potentials with slower upstroke depolarization rates, as well as depressing atrial impulse conduction leading to conduction block. The combination of slower conduction, conduction block, and depressed action potentials are factors that contribute to reentrant arrythmias (which accompany a significant number of human myocardial infarctions) and may explain the rapid and fractionated ECG of transgenic mice with atrial hyperplasia. Studies were therefore initiated to determine if the ANF-TAG mice might serve as a useful model for conduction abnormalities.

In the initial experiments, intracellular recordings were performed on myocytes from three nontransgenic control atria and four ANF-TAG transgenic atria. The preliminary results indicate that expression of T antigen in cardiac tissue elicits several perturbations of cellular electrophysiology. Spontaneous and stimulated muscular contractions were evident in normal atria; however, most regions of hyperplastic atria did not contract spontaneously and were paced ineffectively by a stimulating bipolar electrode. The most striking difference between hyperplastic and normal atrial tissue was the diminished mean diastolic potential (MDP), which decreased from a normal value of -78 ± 4 mV ($n=17$) to -63 ± 1 mV ($n=46$) in the hyperplastic atria ($p<0.0001$). To assess action potential waveforms, atria were paced by a 50-V square-wave pulse lasting 2–5 msec. Normal atrial cells had action potentials characterized by a rapid initial upstroke, a brief or negligible plateau phase, and a gradual repolarization smoothly returning to the MDP. In contrast, hyperplastic atrial cells had action potential waveforms with markedly reduced upstroke amplitudes and velocities. Action potential durations were not altered when cells fired spontaneously with near-normal upstrokes. Several cells in the hyperplastic tissue spontaneously fired rapid bursts of rhythmic and arrhythmic activities, whereas other cells were quiescent. Quiescent cells were generally unresponsive to stimulation either by neighboring cells firing spontaneously or by a bipolar electrode. A nodule at the expected site of the sinoatrial node was found in one hyperplastic atrium. Cells in this region exhibited spontaneous rates of 11–13 beats/second, which is similar to the normal heart rate in mice.

We have identified several cellular electrophysiological perturbations resulting from the expression of T-antigen oncoprotein in cardiac atria. It is of interest to note that the hyperplastic atria have regions that are electrically quiescent. Similarly, histochemical studies have shown a heterogeneous pattern of TCA cycle activity, as evidenced by a succinate dehydrogenase assay. Given the fact that both TCA cycle and electrical activity are dependent on adequate perfusion, it will be of interest to determine the spatial relationship between these two physiological markers. Moreover, further characterization of these electrophysiological perturbations during the development of atrial hyperplasia should enhance our understanding of the molecular mechanisms by which cardiac arrhythmias arise and develop.

Transgenic Models That Aberrantly Express Peptide Hormones Regulating Cardiovascular Homeostasis

M. Steinhelper, P. Weinberg, S. Teplin, L. Field [in collaboration with K.L. Cochrane, University of Virginia]

The maintenance of blood pressure and electrolyte homeostasis in mammals is regulated in large measure by the action of peptide hormone systems operating both in the peripheral circulation and in the central nervous system. Disruption of the regulatory systems comprising these hormones often results in profound pathophysiological consequences.

The acute role of vasoactive compounds may be readily assessed by bolus administration. However, the chronic effects of these compounds are less amenable to study largely because of limitations in existing delivery systems. We are using the transgenic approach to introduce into the mouse germ line fusion genes that may circumvent these limitations and provide lineages of mice with heritable defects in cardiovascular regulation. Our initial experiments will focus on atrial natriuretic factor (ANF), a peptide hormone that is synthesized in the cardiac atria. ANF is secreted from the atrium in response to increases in blood pressure. Once in the circulation, ANF elicits a natriuretic and diuretic effect by altering glomerular filtration rate and renal collecting duct transport.

To study the effects of chronically elevated levels of ANF, we have designed fusion genes that should lead to peripheral overexpression of the hormone. Thus, we have combined the sequences coding for ANF with the 5′ regulatory sequences of the mouse albumin gene, the mouse transthyretin gene, or the rat cytosolic phosphoenolpyruvate carboxykinase gene. Expression of these fusion genes should result in secretion of ANF from hepatocytes (in addition, the transthyretin construct should be active in the choroid plexus). Multiple founder mice carrying albumin-ANF (ALB-ANF, seven mice), transthyretin-ANF (TTR-ANF, ten mice), and phosphoenolpyruvate carboxykinase-ANF (PEPCK-ANF, seven mice) have been generated. For the ALB-ANF mice, transgenic lineages have been established and fusion gene expression has been examined. One lineage has been shown to have approximately two- to threefold elevated levels of serum immunoreactive ANF. Although this represents a clear elevation in circulating hormone, the net increase in steady-state levels is somewhat disappointing. Nevertheless, this lineage will be used to assess the consequences of moderately elevated ANF levels. The TTR-ANF and PEPCK-ANF founders are presently establishing lineages, and fusion gene expression will be evaluated as the animals become available. Given the number of independent transgenic animals in hand, as well as the different promoters that were used, we anticipate obtaining animals expressing a broad spectrum of circulating ANF concentrations. Such a distribution of animals would provide a formidable model system in which to assess the consequences of chronic hormone overexpression.

Finally, investigation of hormones that regulate blood pressure requires a quantitative means of assessing changes in this parameter. Blood pressure measurements in small rodents such as mice may be obtained using pneumatic pulse transducers and occluding cuffs that are quite similar to those familiar instruments used by our own physicians. However, indirect blood-pressure measurements of rodents are less reliable compared with those of humans, owing to the requirement for either gentle restraint of conscious rodents or the use of anesthesia. Highly reliable blood-pressure measurements in rodents thus require a more experimentally complex method. To this end, we have established methodology to obtain direct blood-pressure measurements on conscious mice. A cannula fashioned from PE-10 tubing, inserted via the femoral artery into the inferior aorta, was positioned a few millimeters distal to the renal artery. Mice were conscious and freely moving about their cage within 1 hour after surgery, and pulsatile arterial pressure was detected using a pressure transducer coupled to a physiograph. One-hour recordings of pulsatile arterial pressure with pulse pressures of 20–50 mm Hg have been obtained from nontransgenic mice as long as 1 week after surgery, demonstrating the amenability of the murine cardiovascular system to rigorous analysis. This type of analysis will thus provide a reliable means with which to assess the physiological perturbations resulting from transgene expression.

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GENETICS OF CELL PROLIFERATION

Michael Wigler

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Our laboratory studies the relationship among oncogenes, signal transduction, and malignant transformation, with particular emphasis on *RAS* genes. Three mammalian *RAS* genes, Ha-*ras*, Ki-*ras*, and N-*ras*, are capable of the malignant transformation of cultured animal cells. Mutations in these genes have been linked to a large number of human cancers. Perhaps as much as 25% of human tumors contain mutant *RAS* genes, pointing to a common metabolic defect in a large fraction of human malignancy. It is therefore imperative that we understand the biochemical function of *RAS* proteins.

The *RAS* genes encode closely related proteins that bind guanine nucleotides (Scolnick et al., *Proc. Natl. Acad. Sci. 76:* 5355 [1979]; Shih et al., *Nature 287:* 686 [1980]); Ellis et al., *Nature 292:* 506 [1981]) and are localized to the inner surface of the plasma membrane (Willingham et al., *Cell 19:* 1005 [1980]; Papageorge et al., *J. Virol. 44:* 509 [1982]). Normal *RAS* proteins also slowly hydrolyze GTP (Gibbs et al., *Proc. Natl. Acad. Sci. 81:* 5704 [1984]; McGrath et al., *Nature 310:* 644 [1984]; Sweet et al., *Nature 311:* 273 [1984]). These properties are similar to those of the G proteins, which has led to the widespread

expectation that *RAS* proteins, like G proteins, are involved in the transduction of membrane signals that are linked to cellular proliferation or differentiation. Many of the mutations that activate the *RAS* genes result in the production of proteins with impaired GTP hydrolysis (Gibbs et al., *Proc. Natl. Acad. Sci. 81:* 5704 [1984]; McGrath et al., *Nature 310:* 644 [1984]; Sweet et al., *Nature 311:* 273 [1984]). This has suggested that, like G proteins, *RAS* proteins are active when bound to GTP but inactive when bound to GDP. The biochemical function of the mammalian *ras* proteins is unknown.

We have been studying the function of the yeast *RAS* genes in the expectation that such study will lead to insights into the functioning of the mammalian *RAS* genes. *Saccharomyces cerevisiae* have two genes, *RAS1* and *RAS2*, that are structurally homologous to the mammalian *RAS* genes (DeFeo-Jones et al., *Nature 306:* 707 [1983]; Dhar et al., *Nucleic Acids Res. 12:* 3611 [1984]; Powers et al., *Cell 36:* 607 [1984]). The yeast and mammalian *RAS* genes are functionally related as well, since mammalian *RAS* genes can complement yeast lacking their endogenous *RAS* genes (Kataoka et al., *Cell 40:* 19 [1985]), and yeast *RAS* genes can malignantly transform cultured animal cells (DeFeo-Jones et al., *Science 228:* 179 [1985]). In the yeast *S. cerevisiae, RAS* proteins appear to control events related to growth arrest. The *RAS2* gene can be activated by a point mutation analogous to the point mutation of Ha-*ras*, which activates its oncogenic potential (Kataoka et al., *Cell 37:* 437 [1984]). Cells carrying the activated $RAS2^{val19}$ gene fail to arrest in G_1 when starved, remain heat-shock-sensitive when they reach stationary phase, and fail to accumulate storage carbohydrates (Kataoka et al., *Cell 37:* 437 [1984]; Sass et al., *Proc. Natl. Acad. Sci. 83:* 9303 [1986]). These same sets of phenotypes are observed when the adenylyl cyclase pathway is activated (Uno et al., *J. Biol. Chem. 257:* 14110 [1982]) and first led us to suspect an interaction between *RAS* proteins and adenylyl cyclase.

Our laboratory is also continuing investigations into two mammalian oncogenes. The *MAS* oncogene encodes a potential hormone or neurotransmitter receptor with seven transmembrane domains. The overexpression of *MAS* leads to a minimally transformed phenotype. The *ROS* oncogene encodes a large transmembrane tyrosine kinase, which also is a potential receptor for an unknown ligand. The *ROS* gene is expressed and rearranged in some glioblastomas.

Interaction between *RAS* Proteins and Yeast Adenylyl Cyclase

J. Field, J. Colicelli, R. Ballester, T. Michaeli

In yeast, *RAS* proteins are required for the proper functioning of adenylyl cyclase (Toda et al., *Cell 40:* 27 [1985]). This is readily seen both from studies in vivo with mutant yeast strains (Toda et al., *Cell 40:* 27 [1985]; Nikawa et al., *Genes Dev. 1:* 931 [1987]) and from studies in vitro (Broek et al., *Cell 41:* 763 [1985]; Field et al., *Mol. Cell. Biol. 72:* 2128 [1987]; Field et al., *Mol. Cell. Biol. 8:* 2159 [1988]). Our in vitro systems use *RAS* proteins purified from an *Escherichia coli* expression system (Broek et al., *Cell 41:* 763 [1985]; Gross et al., *Mol. Cell. Biol. 5:* 1015 [1985]) and an adenylyl cyclase complex purified from *S. cerevisiae* (Field et al., *Mol. Cell. Biol. 8:* 2159 [1988]). Our method of purification of adenylyl cyclase is novel. It involves making in yeast a fusion protein of adenylyl cyclase with a small amino-terminal peptide epitope. Extracts of yeast are passed over an affinity column containing monoclonal antibodies directed against the peptide epitope. Adenylyl cyclase is then eluted with synthetic peptide, resulting in a greater than 100-fold purification. The resulting complex contains a 70-kD component that copurifies with adenylyl cyclase activity in glycerol sedimentation gradients.

We conclude from our in vitro studies that *RAS* proteins interact directly with the adenylyl cyclase complex. The addition of either yeast *RAS2* protein or mammalian Ha-*ras* protein can result in a greater than 20-fold stimulation of activity. We can also conclude from in vitro work that *RAS* proteins bound to GTP stimulate adenylyl cyclase, but *RAS* proteins bound to GDP do not (Field et al., *Mol. Cell. Biol. 8:* 2159 [1988]). Thus, the activity of *RAS* proteins is controlled by the guanine nucleotide they bind, consistent with the model of oncogenesis proposed for mutant, activated *RAS*. We have also concluded from our work that the stimulation of adenylyl cyclase requires the continued presence of *RAS* proteins.

We cannot conclude from our work that *RAS* proteins act directly on adenylyl cyclase itself, since the adenylyl cyclase complex copurifies with a 70-kD protein (Field et al., *Mol. Cell. Biol. 8:* 2159 [1988]). We are currently investigating the role of the 70-kD protein in the *RAS* responsiveness of adenylyl cyclase (1) by attempting the purification of the 70-kD protein and cloning the gene that encodes it and

(2) by performing deletion analysis of adenylyl cyclase.

Control of *RAS* Protein Activity

S. Powers, D. Broek, S. Cameron, K. Ferguson, K. O'Neill

In *S. cerevisiae*, the *CDC25* protein appears to control *RAS* protein activity. *cdc25*^{ts} alleles were first discovered as cell-cycle G_1 arrest mutants (Hartwell et al., *Genetics 74:* 267 [1973]; Hartwell, *Bacteriol. Rev. 38:* 164 [1974]). Cells lacking *cdc25* are deficient in cAMP and have aberrant adenylyl cyclase activity (Martegani et al., *EMBO J. 5:* 375 [1986]; Broek et al., *Cell 48:* 789 [1987]; Camonis et al., *EMBO J. 5:* 375 [1986]; Nikawa et al., *Genes Dev. 1:* 931 [1987]). Cells that contain the activated, mutant RAS2^{val19} gene do not require the *CDC25* product (Broek et al., *Cell 48:* 789 [1987]; Robinson et al., *Science 235:* 1218 [1987]). These data are consistent with a model in which the *CDC25* product acts upstream of *RAS* and causes its activation, possibly by catalyzing nucleotide exchange.

Further evidence in favor of this model has come from our discovery of mutant *RAS* proteins that behave as though they interfere with *CDC25* activity (Powers et al., *Mol. Cell. Biol. 9:* 390 [1989]). These mutant *RAS* genes were found in the course of a genetic screen for temperature-sensitive *RAS* mutants. We found in this screen dominant temperature-sensitive lethal *RAS2* alleles. Significantly, lethality can be overcome by the presence of the *CDC25* gene on a high-copy plasmid but only if a wild-type *RAS2* or *RAS1* gene is also present. Lethality can also be overcome if cells contain the mutationally activated *RAS2*^{val19} gene. Thus, the mutant *RAS* proteins appear to interfere with the activation of wild-type *RAS* proteins, perhaps by forming a complex with *CDC25* proteins. The mutations in interfering *RAS* genes localize to the region that encodes part of a consensus nucleotide-binding site common to many GTP-binding proteins (Powers et al., *Mol. Cell. Biol. 9:* 390 [1989]).

To explain our results with *CDC25* and *RAS*, we propose that *RAS* proteins and *CDC25* proteins normally undergo a transient and direct interaction, similar to models that have been proposed to explain the interaction of receptors with G proteins (Gilman, *Ann. Rev. Biochem. 56:* 615 [1987]; Stryer, *Ann. Rev. Neurosci. 9:* 87 [1986]). As in those models, *CDC25* proteins interact with the GDP-bound form of *RAS* proteins and, by virtue of stabilizing the transitional state of nucleotide-free *RAS* protein, catalyze nucleotide exchange. We propose that the dominant temperature-sensitive *RAS* proteins remain bound to *CDC25* protein because alterations in the consensus nucleotide-binding site alter nucleotide affinity and stabilize a nucleotide-free *RAS-CDC25* protein complex.

Interfering Mutants in Signal Transduction Pathways

S. Powers, T. Michaeli, J. Field, J. Colicelli, R. Ballester

The discovery that there exist mutant forms of *RAS* that interfere with activation of normal *RAS* led us to think about interfering mutants in a more general sense. In the broadest possible terms, if there is a signal transduction pathway wherein protein X interacts with protein Y, which then interacts with protein Z in a cascade of information flow, one can expect at least four types of dominant interfering mutant proteins: mutants of X that complex ineffectively with Y; mutants of Y that complex ineffectively with X; mutants of Y that complex ineffectively with Z; and mutants of Z that complex ineffectively with Y. Genetic screens can be designed to search for mutations that produce these kinds of proteins, and such mutants may be valuable tools in the analysis of complex signaling pathways.

We have applied this approach to the *RAS*/adenylyl cyclase pathway of *S. cerevisiae*. We randomly mutagenized Ha-*ras* genes by passage of plasmids carrying Ha-*ras* through a mutator strain of *E. coli* (Silhavy et al., in *Experiments with gene fusions*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York [1984]), and screened the mutagenized plasmids for their ability to suppress the heat-shock sensitivity of strains of yeast carrying the *RAS2*^{val19} gene. One such mutant was found, and sequence analysis revealed that it contained an arginine for cysteine substitution at codon 186 (T. Michaeli, submitted). This disrupts the Cys-*A*-*A*-*X* (where *A* is any aliphatic amino acid and *X* is the terminal amino acid) consensus sequence of *RAS* proteins (Taparowsky et al., *Cell 34:* 581 [1983]; Powers et al., *Cell 36:* 607 [1984]) that functions as a target for the fatty acid addition which causes membrane localization

of *RAS* (Willumsen et al., *Nature 310:* 583 [1984]; Powers et al., *Cell 47:* 413 [1986]). We found that other mutations in this region which destroy the consensus sequence also result in Ha-*ras* genes that interfere with the phenotype of $RAS2^{val19}$.

Our analysis of the mutant Ha-*ras* proteins with a disrupted Cys-*A-A-X* consensus sequence indicates an unexpected complexity of *RAS* interactions. The mutant proteins remain cytosolic, in keeping with the findings of other investigators that the Cys-*A-A-X* sequence is required for membrane localization (Willumsen et al., *Nature 310:* 583 [1984]). The Ha-*ras* mutants do not block $RAS2^{val19}$ protein from localizing to the membrane. Their effect is therefore not likely to be due to dominant effects on *RAS* protein processing. Competition experiments indicate that the effects of the Ha-*ras* mutants are competed by overexpression of $RAS2^{val19}$ but not by overexpression of *CYR1*, the gene that encodes adenylyl cyclase. From this, we conclude that these Ha-*ras* mutant proteins interfere with a cytosolic factor which may facilitate the interaction of *RAS2* with adenylyl cyclase. Alternatively, the mutant Ha-*ras* may interfere with a second function of *RAS*. Evidence for multiple functions of *RAS* in yeast is given below.

We have also found mutant *CYR1* genes that interfere with the phenotypes of $RAS2^{val19}$. We used a strategy similar to the one described above. We passaged a plasmid carrying *CYR1* through a mutator strain of *E. coli* and readily found clones of *CYR1* that blocked the heat-shock sensitivity of $RAS2^{val19}$ strains. The ease with which this screen yielded interfering mutations in *CYR1* led us to suspect that virtually any mutation that disrupted the enzymatic function of adenylyl cyclase could result in an interfering protein. Direct tests proved this hypothesis to be correct. The region encoding the catalytic portion of the adenylyl cyclase is located at the 3′ end of *CYR1* (Kataoka et al., *Cell 43:* 493 [1985]). Frameshift or deletion mutations in this region result in the production of interfering forms of the *CYR1* product. Competition assays suggest that the effects of defective *CYR1* genes can be suppressed by overexpression of *RAS* proteins. It is likely, we think, that the mutant *CYR1* genes encode proteins that form ineffective complexes with *RAS* proteins. More generally, it may be true that proteins which are the targets of *RAS* action can interfere with *RAS* function when they are functionally incompetent.

Feedback Regulation of RAS Activity

S. Cameron

In the course of screens for genes that, when overexpressed, can suppress the phenotypes induced by $RAS2^{val19}$, we cloned two genes of *S. cerevisiae* that encode cAMP phosphodiesterases *PDE1* and *PDE2* (Sass et al., *Proc. Natl. Acad. Sci. 83:* 9303 [1986]; Nikawa et al., *Mol. Cell. Biol. 7:* 3629 [1987]). Together, these genes appear to encode the totality of cAMP phosphodiesterase activity measurable in yeast cell extracts (Nikawa et al., *Mol. Cell. Biol. 7:* 3629 [1987]). Surprisingly, we found that cells which lacked these genes, but that were otherwise normal, did not accumulate enormous levels of cAMP (Nikawa et al., *Genes Dev. 1:* 931 [1987]). One explanation for this result is that elevated levels of cAMP directly or indirectly feedback to turn off the further production of cAMP. Confirmation of this theory comes from examining cAMP levels in cells that lack the *PDE* genes but contain the $RAS2^{val19}$ gene (Nikawa et al., *Genes Dev. 1:* 931 [1987]). Such cells have enormously elevated levels (over 1000-fold!) of cAMP. In addition to confirming the existence of feedback, these studies indicate that the $RAS2^{val19}$ protein is unresponsive to feedback controls.

Feedback requires the activity of the cAMP-dependent protein kinases (cAPK). The catalytic subunits of these genes are named *TPK1*, *TPK2*, and *TPK3* (Toda et al., *Cell 50:* 277 [1987]). Cells with attenuated *TPK* genes have enormously elevated cAMP levels when they are grown in rich medium containing glucose (Nikawa et al., *Genes Dev. 1:* 931 [1987]). This result is consistent with the idea that, through homeostasis, the activity of the cAPK regulates cAMP levels. A dramatic demonstration of this is seen upon feeding glucose to cells with an attenuated cAPK system. Wild-type cells show a biphasic response to glucose when fed: A tenfold elevation of cAMP ensues within minutes and diminishes to basal levels within 10 minutes (Beullens et al., *Eur. J. Biochem. 172:* 227 [1988]). In cells with an attenuated cAPK system, glucose feeding does not induce a biphasic response in cAMP levels. Rather, there is a sharp, perhaps 100-fold, elevation of cAMP levels that remain elevated as long as glucose is present (Wigler et al., *Cold Spring Harbor Symp. Quant. Biol. 53:* 649 [1988]). This result indicates that (1) the

cAPK system participates in the physiologic feedback regulation of the cAMP response to glucose and (2) glucose is a stimulant of the system, and the system remains stimulated as long as glucose is present. These results on feedback of cAMP production also suggest the possibility that the levels of cAMP in a cell may oscillate in response to cycles of stimulation and feedback.

RAS-independent Pathways for Growth Control

S. Cameron, J. Colicelli, A. Vojtek, K. Ferguson, H. Xu

The yeast *S. cerevisiae* undergoes marked changes in response to nutrient limitation. Diploid cells will sporulate under appropriate starvation conditions. Haploid cells respond by becoming heat-shock-resistant and by accumulating storage carbohydrates. Since these responses can be mimicked by mutations that lower the activity of the cAMP-dependent protein kinase (cAPK) and can be blocked by mutations that raise the activity of the cAPK, it is natural to assume that physiologic modulation of cAMP activity by cAMP regulates these responses. However, the cAPK system may not be the only signaling system that generally regulates growth and responses to nutrition. We have used the genetics available in yeast to examine this question more directly.

The yeast *S. cerevisiae* contains a cAMP-responsive kinase activity. Genes encoding a regulatory subunit, *BCY1* (Toda et al., *Mol. Cell. Biol. 7:* 1371 [1987]), and three catalytic subunits, *TPK1*, *TPK2*, and *TPK3* (Toda et al., *Cell 50:* 277 [1987]), have been isolated. Disruption of the *BCY1* gene results in a very severe phenotype (Toda et al., *Mol. Cell. Biol. 7:* 1371 [1987]). We have characterized the role of the cAPK catalytic subunit genes in producing the *bcy1*$^-$ phenotype, and in the process, we have generated mutant cAPK catalytic subunit genes (*TPK*) that suppress the *bcy1*$^-$ defects (Cameron et al., *Cell 53:* 555 [1988]). The mutant *TPK* genes appear to encode functionally attenuated catalytic subunits of the cAPK. *bcy1*$^-$ yeast strains containing the mutant *TPK* genes respond appropriately to nutrient conditions, even in the absence of *CDC25*, both *RAS* genes, or *CYR1*. Together, these latter genes encode the known components of the cAMP-generating machinery. The results indicate that cAMP-independent mechanisms must exist for regulating glycogen accumulation, sporulation, and the acquisition of thermotolerance in *S. cerevisiae*. In particular, *RAS*-independent signaling systems must exist. Indeed, we have isolated many genes that can modulate the phenotype of the activated *RAS*/cAMP signaling system but that do not appear to belong to that signaling system.

Evidence for Additional Functions of *RAS*

S. Powers, T. Michaeli, A. Vojtek

Most of the effects of *RAS* on yeast cells can be explained by their action on adenylyl cyclase. The phenotype of cells containing *RAS2*^{val19} can readily be understood as a consequence of the perturbation of cAMP production: cAMP levels are elevated in cells containing *RAS2*^{val19}; activation of the cAMP-dependent protein kinases leads to a phenotype that closely resembles that due to *RAS2*^{val19}; and elevated expression of cAMP phosphodiesterases reverses the *RAS2*^{val19} phenotype (Sass et al., *Proc. Natl. Acad. Sci. 83:* 9303 [1986]; Nikawa et al., *Mol. Cell. Biol. 7:* 3629 [1987]). Moreover, the lethality that otherwise results from disruption of both *RAS* genes can be overcome by disruption of the gene, *BCY1*, that encodes the cAMP-dependent protein kinase regulatory subunit (Toda et al., *Cell 50:* 277 [1987]). The resulting unbridled protein kinase activity is sufficient to complement the loss of *RAS* function. However, there are subtle effects of disruption of both the *RAS1* and *RAS2* genes that do not appear to be identical to the effects of disrupting the adenylyl cyclase gene, and mutations in *RAS* act in ways that cannot be explained readily by effects upon adenylyl cyclase.

There are two major differences between cells lacking *RAS* and cells lacking *CYR1*. First, haploid spores that lack the *CYR1* gene are often viable, although they give rise to very slow growing colonies, whereas haploid spores that lack both *RAS1* and *RAS2* genes are almost never viable (Toda et al., in *Oncogenes and cancer*, Japan Sci. Soc. Press, Tokyo/VNU Sci Press [1987]). Second, overexpressing the *TPK* genes can readily suppress the growth defect resulting from lack of *CYR1*, but cannot so readily suppress the growth defects resulting from lack of *RAS* genes (Wigler et al., *Cold Spring Harbor Symp. Quant. Biol. 53:* 649 [1988]). Indeed, such

RAS-deficient strains suppressed by *TPK* genes are often temperature-sensitive. Thus, it appears that *RAS* may have additional functions besides the stimulation of adenylyl cyclase.

The results described above can each be explained in many ways. For example, one may propose that there is a second gene encoding adenylyl cyclase. We have rigorously eliminated this possibility. If such a second adenylyl cyclase existed, it would have to produce 1000-fold lower cAMP levels than the *CYR1* gene. Alternatively, one can propose that it is better to have no cAMP (*cyr1*$^-$) than a little (*ras1*$^-$ *ras2*$^-$). However, there is one telling piece of evidence that rules out this possibility. We have shown that many strains that lack both *CYR1* and *RAS*, but that are viable because *TPK* genes are highly expressed, are temperature-sensitive. This temperature sensitivity is cured by expressing *RAS* in such cells. Thus, *RAS* can act even in the absence of adenylyl cyclase. Lack of *CDC25* function produces the same defects as lack of *RAS* function even in the absence of *CYR1*. Hence, we conclude that *RAS* has additional functions besides stimulating adenylyl cyclase and that these functions are also shared by *CDC25* (Wigler et al., *Cold Spring Harbor Symp. Quant. Biol. 53:* 649 [1988]).

Comparison of Mammalian and Yeast RAS

S. Powers, J. Field, K. Ferguson, R. Ballester, T. Michaeli, J. Colicelli

The similarities of the mammalian and yeast *RAS* genes are striking. The mammalian Ha-*ras* can complement yeast lacking their own *RAS* genes. Purified Ha-*ras* protein can stimulate purified yeast adenylyl cyclase (Broek et al., *Cell 41:* 763 [1985]). Genetic experiments demonstrate that Ha-*ras* can provide the additional functions of *RAS* in yeast, complementing the loss of *RAS* even in strains that lack adenylyl cyclase (Wigler et al., *Cold Spring Harbor Symp. Quant. Biol. 53:* 649 [1988]).

There are other similarities between mammalian and yeast *RAS*. Mammalian Ha-*ras* protein, like yeast *RAS*, is probably subject to feedback inhibition (Bar-Sagi and Feramisco, *Science 233:* 1061 [1986]). We have evidence, too, that Ha-*ras* can interact with *CDC25*. The analogous mutations can be introduced into Ha-*ras*, which cause the dominant interfering mutants of *RAS2*. When these mutant Ha-*ras* genes are expressed in yeast, they also appear to block *CDC25* activity (Powers et al., *Mol. Cell.Biol. 9:* 390 [1988]). Similar mutants are also interfering in animal cells (Feig and Cooper, *Mol. Cell. Biol. 8:* 3235 [1988]), suggesting that there is a mammalian protein that catalyzes nucleotide exchange in mammalian *RAS* proteins. Perhaps there is a *CDC25* homolog in mammals.

Two other questions of similarity are raised by our studies of yeast. First, in yeast, *RAS* absolutely controls its effector pathway. It is not clear if this is so in mammals, although we suspect it is so. Second, in yeast, it is likely that *RAS* has more than one function. It is quite possible that *RAS* proteins also have more than one function in mammalian cells. This might explain some of the difficulty of assigning a function to mammalian *RAS*.

There are obvious differences between mammalian and yeast *RAS* proteins. The most glaring difference appears to be in the immediate biochemical function of the *RAS* proteins in their respective hosts. It is unlikely that mammalian *RAS* functions to stimulate adenylyl cyclase in vertebrates (Birchmeier et al., *Cell 43:* 615 [1985]). Indeed, a fundamentally different model of *RAS* action has been proposed in mammalian cells. Our model of *RAS* action is rather like the model of action for G proteins and transducin (Gilman, *Ann. Rev. Biochem. 56:* 615 [1987]). This model is strongly supported by experiments in yeast. A radically different model, emerging from the discovery of a *G*TPase-*a*ctivating *p*rotein (GAP), and speculative analogies between *RAS* proteins and bacterial elongation factor EF-Tu, has been proposed (Adari et al., *Science 240:* 518 [1988]; Cales et al., *Nature 332:* 548 [1988]). Our own opinion is that the latter model is wrong. There are too many similarities between yeast and mammalian *RAS* to cause us to abandon the yeast model. Our own studies point to the complexity of *RAS* interactions with its effectors, and although the identities of the individual effectors may have evolved during speciation, we feel that the patterns of *RAS* interactions may have changed little in evolution.

The Search for the Mammalian *RAS* Target

J. Collicelli, T. Michaeli, C. Birchmeier, J. Field

Our discoveries of interfering mutants in the *RAS* signaling pathway have led to a strategy for searching for the target of *RAS* protein action in mammalian cells. We have postulated that the function of *RAS*

in yeast would be inhibited by the expression, in yeast, of the mammalian target for *RAS* action. We have therefore constructed rat brain cDNA libraries in yeast expression vectors to test this idea. In a pilot experiment, we transformed yeast containing the activated $RAS2^{val19}$ gene and screened for heat-shock-resistant colonies among the transformants. One colony was identified that contained a vector that reproducibly induced heat-shock resistance in yeast strains carrying $RAS2^{val19}$. Analysis revealed that this vector contained a cDNA insert with the potential to encode a protein highly homologous to the *Drosophila dunce* product (Chen et al., *Proc. Natl. Acad. Sci. 83:* 9313 [1986]), and we tentatively call our gene *DNC* (Colicelli et al., *Proc. Natl. Acad. Sci.* [1989] in press).

The *dunce* locus was identified in mutant flies with defects in learning (Chen et al., *Proc. Natl. Acad. Sci. 83:* 9313 [1986]). *dunce* encodes a cAMP phosphodiesterase, leading us to suspect that the mammalian gene we cloned also encoded a cAMP phosphodiesterase. To test this idea, we transformed a yeast strain that lacks the two genes which encode cAMP phosphodiesterases, *PDE1* (Nikawa et al., *Mol. Cell. Biol. 7:* 3629 [1987]) and *PDE2* (Sass et al., *Proc. Natl. Acad. Sci. 83:* 9303 [1986]). $pde1^{-}$ $pde2^{-}$ yeast carrying the rat *DNC* express a high-affinity cAMP phosphodiesterase (Colicelli et al., *Proc. Natl. Acad. Sci.* [1988] in press). We do not believe *DNC* encodes the mammalian *RAS* target. Rather, it is likely that *DNC* suppresses $RAS2^{val19}$ by lowering cAMP levels. As precedent, overexpression of either *PDE* gene suppresses $RAS2^{val19}$ (Sass et al., *Proc. Natl. Acad. Sci. 83:* 9303 [1986]; Nikawa et al., *Mol. Cell. Biol. 7:* 3629 [1987]).

These results have import for the study of cAMP phosphodiesterase activity in mammals. First, we have now identified a strategy useful for cloning mammalian cAMP phosphodiesterases. This may lead to the discovery of a wide family of enzymes of great physiological importance. Second, once cloned, the biochemical and pharmacological properties of mammalian phosphodiesterases may be studied after their expression in yeast lacking their own endogenous phosphodiesterases.

The *ROS* Oncogene

C. Birchmeier, S. Sharma, K. O'Neill, L. Rodgers

As reported previously, we have identified oncogenic forms of the human *ROS* gene (Fasano et al., *Mol. Cell. Biol. 4:* 1695 [1984]; Birchmeier et al., *Mol. Cell. Biol. 6:* 3109 [1986]). This gene has the potential to encode a transmembrane tyrosine kinase (Neckameyer and Wang, *J. Virol. 53:* 879 [1985]; Birchmeier et al., *Mol. Cell. Biol. 6:* 3109 [1986]). Like other oncogenes in this family, *ROS* probably encodes a membrane receptor/kinase. We have noted a high frequency of expression of *ROS* in malignant glioblastomas (Birchmeier et al., *Proc. Natl. Acad. Sci. 84:* 9270 [1987]), and as such, *ROS* may encode a surface antigen useful for diagnosis and possibly therapeutic intervention.

We have continued our study of *ROS* in three veins: First, we have developed polyclonal antibodies to the *ROS* product. These recognize a 270-kD glycoprotein in most cells that express *ROS*. As such, *ROS* would encode one of the largest known membrane receptor/kinases. In collaboration with Tom Jessel at Columbia University, these antibodies have been used to study the expression of *ROS* in developing rat embryos. Our data suggest that *ROS* is normally expressed on radial glial cells in the developing spinal cord.

Second, we have cloned most and perhaps all of the cDNA of the large 8.3-kb *ROS* transcript found in glioblastoma cells. DNA sequencing is not yet complete, but it is apparent that *ROS* encodes a protein that is most similar to the *Drosophila sevenless* product (Hafen et al., *Science 236:* 55 [1987]; Basler and Hafen, *Cell 54:* 299 [1988]; Bowtell et al., *Genes Dev. 2:* 260 [1988]). *sevenless* encodes a protein required for the proper development of fly retinal cells. One interesting possibility is that *ROS* encodes a membrane receptor important in cell-cell recognition.

Third, we have begun to characterize abnormal *ROS* expression in one glioblastoma cell line, U-118 MG. In this cell, the *ROS* locus has fused with a second locus, and a chimeric transcript and protein result. Analysis of the cDNA for this transcript indicates that the loss of the extracellular and transmembrane domains of *ROS* has occurred, leaving the tyrosine kinase domain intact. Just such a rearrangement has been seen in the *MET* oncogene (Dean et al., *Nature 318:* 385 [1985]) and suggests that the rearrangement has activated the oncogenic potential of *ROS*. One other peculiar feature of the rearranged *ROS* in U-118 MG is that the normal *ROS* allele is absent. This and other data suggest that the *ROS* rearrangement has resulted from an intrachromosomal deletion of chromosome 6 and a loss of the normal chromosome 6. We will further characterize the nature of this event.

The *MAS* Oncogene

D. Young, L. Rodgers

The human *MAS* oncogene was discovered by us using the cotransfection and tumorigenicity assay (Young et al., *Cell 45:* 711 [1985]). It encodes a protein with seven transmembrane domains and probably belongs to the family of genes encoding hormone receptors that couple to G proteins. Cells transformed by *MAS* grow to high cell density and form tumors in nude mice but do not appear morphologically transformed and do not grow in suspension in soft agar. Recently, we cloned the rat homolog of *MAS* (Young et al., *Proc. Natl. Acad. Sci. 85:* 5339 [1988]). It encodes a protein very similar to the human protein. In rats, the expression of *MAS* appears to be restricted to the brain, particularly the cerebral cortex and hippocampus, suggesting that *MAS* encodes a receptor for a neurotransmitter (Young et al., *Proc. Natl. Acad. Sci. 85:* 5339 [1988]). Most recently, Hanley and co-workers in England have reported evidence that angiotensin II is a ligand for the *MAS* protein (Jackson et al., *Nature 335:* 437 [1988]). In collaboration with Richard Axel and Tom Jessel at Columbia University, we are attempting to confirm these results (so far unsuccessfully).

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RAS ONCOGENES AND SIGNAL TRANSDUCTION

D. Bar-Sagi N. Gale A. Samatar
L. Graziadei J. Suhan
S. Kaplan

During the past year, we have continued to investigate the role of *ras* proteins in growth control. Our goal is to define the mechanisms by which *ras* oncogenes disrupt the normal proliferative program of eukaryotic cells. A critical step toward understanding the effects of *ras* proteins on cell growth is the identification of biochemical targets of these proteins. On the basis of their structural and biochemical characteristics, the *ras* proteins are considered to have function(s) analogous to those of the G proteins involved in intracellular signal transduction systems. Recent studies from several laboratories, including

our own, have indicated that *ras* proteins may function as regulatory components of the phospholipid metabolism signaling pathway. Therefore, the focus of our work has been the analysis of the biochemical link between *ras* proteins and phospholipid metabolism. In addition, we have continued our studies on the effects of *ras* proteins on membrane turnover and the functional significance of these effects for the growth-promoting activity of *ras* proteins.

RAS Proteins Can Activate Phospholipase C in HL-60 Membranes

D. Bar-Sagi [in collaboration with S. Cockcroft, University College, London]

We have established a cell-free membrane system to investigate whether *ras* proteins may affect the activity of the enzyme phospholipase C (PLC). The plasma membranes were isolated from the human leukemic (HL-60) cells prelabeled with [^{3}H]inositol. Purified preparations of proto-oncogenic and oncogenic forms of the human Ha-*ras* proteins were obtained from the *Escherichia coli* expression system (Fig. 1). Incubation of membranes with various amounts of purified Ha-*ras* proteins had no effect on PLC activity as measured by the formation of the hydrolytic products [^{3}H]inositol bis (IP_2) and tris (IP_3) phosphates. However, when coupled to the nonhydrolyzable analogs of GTP (GTPγS or Gpp[NH]p), both the proto-oncogene and oncogene *ras* proteins stimulated the formation of IP_2 and IP_3 in a time- and dose-dependent manner. It has been shown previously that GTPγS by itself (as well as other nonhydrolyzable GTP-analogs) can stimulate IP_2 and IP_3 production in a dose-dependent manner. The possibility that the stimulatory effect of Ha-*ras* proteins on PLC activity resulted from the dissociation of GTPγS from the Ha-*ras* proteins was controlled by measuring the amount of GTPγS that dissociates from the *ras* protein during the incubation period. We have found that the amount of free GTPγS present in the incubation mixture could not account quantitatively for the effects observed with *ras* proteins coupled to GTPγS. The specificity of the effect of Ha-*ras* proteins on PLC activity was established by testing the effects of other purified preparations of G proteins (α-subunits of G_s, G_i, and G_o) on PLC. Even when coupled to GTPγS, these proteins had no effect on IP_2 and IP_3 production at all concentrations tested. We conclude that purified Ha-*ras* proteins can exert a stimulatory effect on PLC activity in a cell-free system. Using various mutant *ras* proteins (in particular "effector domain" mutants), we are currently investigating the mechanisms underlying the effects of *ras* proteins on PLC activity.

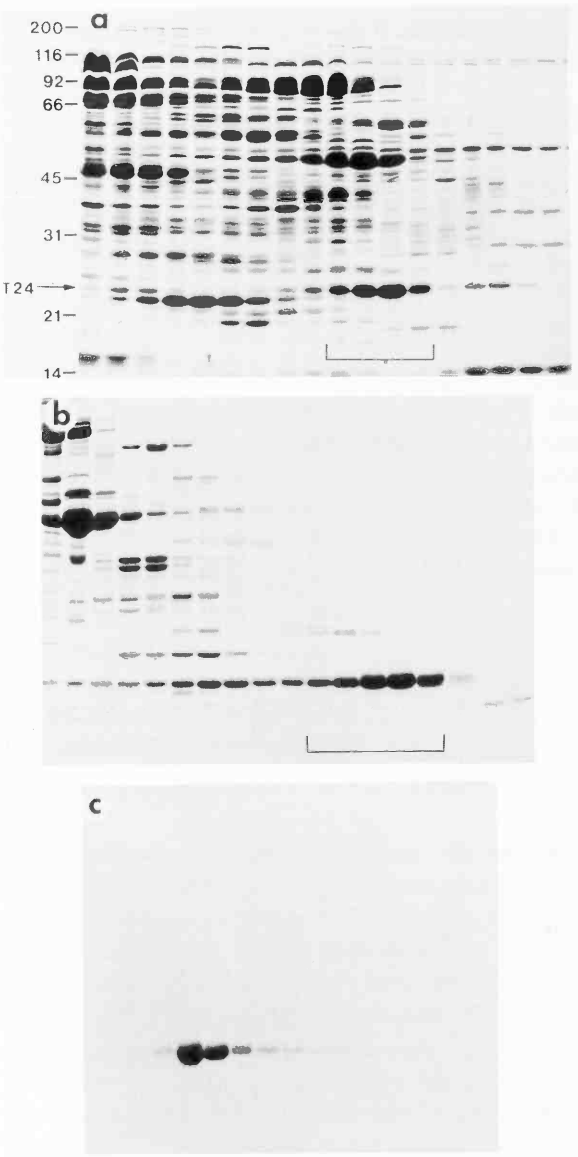

FIGURE 1 SDS-PAGE patterns at different stages of purification of the oncogenic (T24) Ha-*ras* protein. The DE-52 fractions containing T24 (panel *a*) were precipitated by 60% ammonium sulfate. The precipitate was dissolved and applied to a Sephadex G-75 column. The T24-containing fractions from the Sephadex G-75 column (panel *b*) contained few detectable impurities that could be totally eliminated by hydrophobic chromatography on phenyl-Sepharose column (panel *c*).

Phospholipase A_2 in *ras*-transformed Cells

D. Bar-Sagi, J. Suhan, S. Kaplan

We have made several observations consistent with the possibility that the activation of phospholipase A_2 (PLA_2) is associated with the acquisition and/or maintenance of the transformed phenotype. First, we have found that normal rat kidney (NRK) cells transformed by the v-Ki-*ras* (KNRK) show approximately twofold enhancement of PLA_2 activity compared to normal NRK cells (Fig. 2b,c). Moreover, we have observed that the addition of serum to membrane preparations of NRK cells resulted in a fourfold stimulation of PLA_2 activity, whereas in KNRK cells, serum had no apparent effect on PLA_2 activity (Fig. 2d). Second, in a recent study, we have found that microinjection of the *ras* oncogene protein into quiescent fibroblasts induced a rapid (~30 min after injection) and time-dependent stimulation of PLA_2 activity. Third, analysis of the spatial relationship between PLA_2 and the Ki-*ras* oncogene protein, carried out at a high level of res-

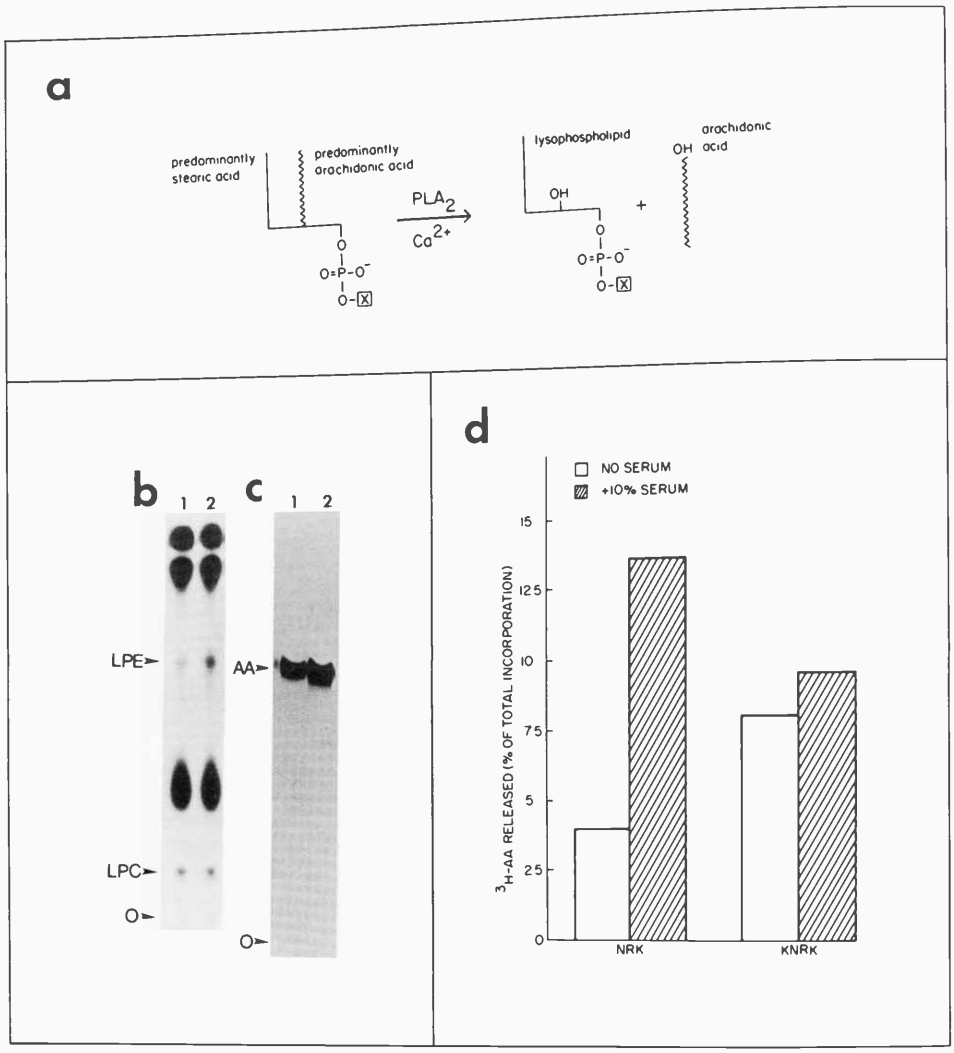

FIGURE 2 Effect of *ras* proteins on PLA_2 activity. (*a*) Scheme for phospholipid hydrolysis by PLA_2; (*b,c*) PLA_2 activity in normal (NRK) and *ras*-transformed (KNRK) cells. Cellular phospholipids were labeled with $^{32}PO_4$ or with [^{3}H]arachidonic acid. (*b*) ^{32}P-labeled phospholipids were extracted and analyzed by TLC. (c) [^{3}H]Arachidonic acid released to the medium was extracted and analyzed by TLC. (*1*) NRK cells; (*2*) KNRK cells. (O) Origin of TLC plate; (AA) arachidonic acid; (LPE) lysophosphatidylethanolamine; (LPC) lysophosphatidylcholine. (*d*) Activation of PLA_2 by serum in membrane preparations of NRK and KNRK cells.

olution by the use of the double immunogold labeling method, revealed a close proximity (~100–500 Å) between PLA_2 and the *ras* oncogene protein in the ruffles of Ki-*ras*-transformed cells. Similar observations were made in normal cells. To investigate the specificity of the double-immunogold-staining pattern of *ras* protein and PLA_2, we have used the same labeling technique to examine the relationship between the distribution of the *ras* oncogene protein and another membrane protein, Na-K ATPase. We have found no spatial proximity between the Na-K ATPase staining and *ras* staining. Therefore, the coincident subcellular location of the *ras* protein and PLA_2 could not be attributed solely to the occurrence of the two proteins in the same subcellular compartment (i.e., the cell membrane). Finally, we have found that microinjection of an inhibitory anti-PLA_2 antibody into *ras*-transformed cells resulted in the transient reversion of the transformed phenotype. Taken together, our findings to date may indicate some functional relationship between *ras* protein and PLA_2.

Molecular Cloning of Phospholipase A_2

N. Gale, L. Graziadei, A. Samatar, D. Bar-Sagi

To clone cDNAs encoding cellular form(s) of PLA_2, we have used two approaches: First, using the oligonucleotide hybridization procedure, we screened a rat pancreatic λgt11 cDNA library. The sequence of the oligonucleotide was derived from the published cDNA sequence of the rat pancreatic PLA_2. In our primary screening, we have identified 15 positive plaques. These plaques were isolated, purified, and rescreened with two oligonucleotide probes corresponding to the amino- and carboxy-terminal regions of the pancreatic PLA_2. The phage DNAs from the clones that were positive for both probes were isolated, and the size of the cDNA inserts was found to be approximately 500–600 bp. Preliminary characterization of the clones by restriction analysis and immunological screening strongly suggests that they are the full-length cDNAs that encode the rat pancreatic PLA_2. Studies are now under way to determine the sequence of these cDNAs and to establish whether these clones were derived from the same template mRNA. We will utilize these cDNAs to determine the expression of rat pancreatic PLA_2 in other rat tissues and as probes for the screening of other expression libraries to identify homologous sequences corresponding to other form(s) of PLA_2.

In the second approach, we used rabbit polyclonal anti-PLA_2 antibody and a monoclonal anti-PLA_2 antibody to screen rat pancreatic and rat brain λgt11 cDNA libraries. We generated the antibodies against porcine pancreatic PLA_2 and showed that they recognize cellular form(s) of PLA_2 in rat cells. To date, the immunological screening of the cDNA expression libraries yielded two independent clones: (1) a rat pancreatic cDNA clone that appears to be different from the pancreatic clones identified by the oligonucleotide screening method and (2) a rat brain cDNA clone that expresses an immunoreactive polypeptide and is a probable candidate for a cDNA encoding some form of cellular PLA_2. The cDNA inserts from these clones are currently being characterized.

Effect of *ras* Proteins on Phospholipid Methylation

S. Kaplan, D. Bar-Sagi

Phospholipid methylation involves the stepwise methylation of phosphatidylethanolamine (PE) to form phosphatidylcholine (PC) by apparently two methyltransferases. On the basis of earlier studies, phospholipid methylation has been postulated to play an important role in receptor-mediated events in several systems, including oocyte maturation, NGF-induced PC12 differentiation, and histamine secretion in mast cells. Because all of these cellular responses can be triggered by *ras* proteins, we considered the possibility that phospholipid methylation may be the common determinant mediating the diverse effect of *ras* proteins. We have begun to examine this possibility by studying methylation of phospholipids in membrane preparations. Phospholipid methylation can be assayed by measuring the incorporation of radioactive methyl group of S-adenosyl-L-[*methyl*-^{3}H]methionine (^{3}H-SAM), a methyl donor, into a lipid fraction. The identity and relative abundance of the methylated phospholipids can then be determined by thin-layer chromatography (TLC) (Fig. 3). For the purpose of characterizing the effect of *ras* proteins on phospholipid methylation, we have initially investigated the effect of guanine nucleotide on phospholipid methylation. We have observed that the addition of GTPγS (10 μM) to the incubation

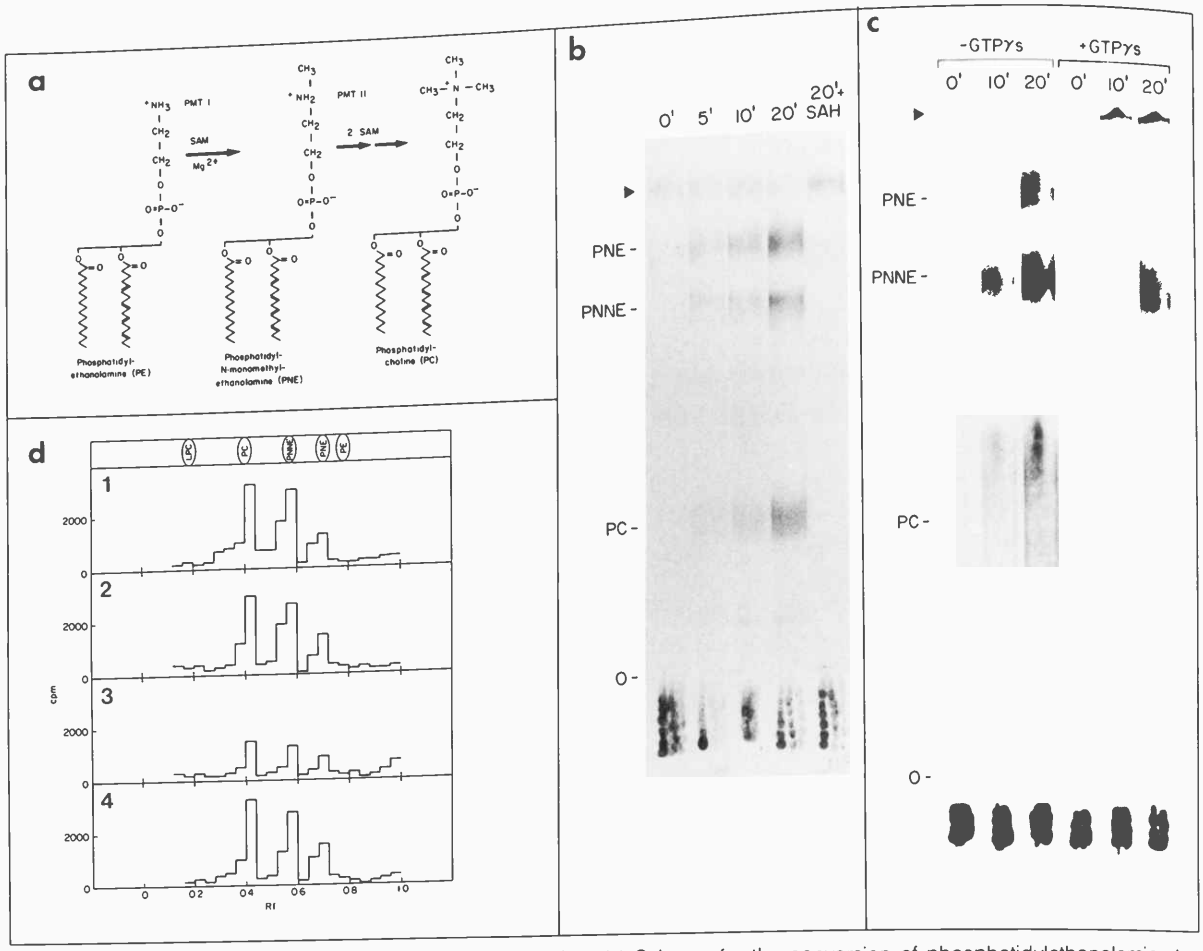

FIGURE 3 Effect of *ras* proteins on phospholipid methylation. (*a*) Scheme for the conversion of phosphatidylethanolamine to phosphatidylcholine. (PMT I) Phospholipid methyltransferase I; (PMT II) phospholipid methyltransferase II; (SAM) S-adenosylmethionine. (*b*) Time-dependent formation of methylated species in HL-60 membrane preparation. Chromatographic separation was carried out on TLC plates with the solvent system of chloroform:methanol:acetic acid:water (75:45:12:3). ^{3}H-labeled phospholipids were visualized by autoradiography and identified by cochromatography with standards detected with iodine vapor. (O) Origin; (arrowhead) unidentified band; (SAH) S-adenosylhomocystein (5 mg/ml). (*c*) Inhibitory effect of GTPγS (10 μM) on phospholipid methylation. HL-60 membranes were incubated in the presence of [^{3}H]SAM with or without GTPγS for the indicated intervals, and ^{3}H-methylated phospholipids were analyzed by TLC. (*d*) Chromatographic pattern of the ^{3}H-methylated phospholipids. (*1*) 20-min incubation; (*2*) 20-min incubation in the presence of Ha-*ras*^{val12} (1 μM); (*3*) 20-min incubation + GTPγS (10 μM); (*4*) 20-min incubation + GTPγS (10 μM) + Ha-*ras*^{val12} (1 μM). Quantitation of ^{3}H-methyl-incorporated phospholipids was determined by scraping the labeled phospholipids off the plates and liquid scintillation counting.

mixture in the presence of Mg^{++} (5 mM) resulted in significant inhibition of the methylation response. These observations may suggest the involvement of an inhibitory guanine-nucleotide-binding protein in the regulation of phospholipid methylation. More recently, we have examined the effects of *ras* proteins on phospholipid methylation. We have found that in the absence of GTPγS, addition of *ras* proteins (both proto-oncogenic and oncogenic forms, 1 μM) had no effect on phospholipid methylation. However, in the presence of GTPγS, the Ha-*ras* oncogene protein prevented the inhibitory effect induced by GTPγS.

Regulation of Membrane Turnover by *ras* Proteins

D. Bar-Sagi [in collaboration with B. Gomperts, University College, London]

We have previously demonstrated that microinjection of the Ha-*ras* oncogene protein into quiescent

fibroblasts results in a rapid and dose-dependent stimulation of membrane ruffling and pinocytosis. Furthermore, microinjection of anti-*ras* antibodies inhibited these membrane activities in both normal and v-Ki-*ras*-transformed cells. These findings suggest that *ras* proteins participate in the regulation of membrane turnover. To identify the function of *ras* proteins in membrane turnover, we have used rat peritoneal mast cells that, upon exposure to appropriate ligands, undergo exocytotic degranulation. Rat mast cells were found to express low levels of the normal *ras* protein, as detected by immunoblotting. Microinjection of the oncogenic Ha-*ras* protein induced exocytotic degranulation. This effect was produced in the absence of external ligands and was dependent on extracellular calcium. In contrast, microinjection of the same amount of the proto-oncogenic Ha-*ras* protein had no apparent effect. The ultrastructural features of exocytotic degranulation in mast cells injected with the *ras* oncogene protein are similar to those seen when mast cells are activated by soluble ligand (Fig. 4). Taken together with the stimulatory effect of the *ras* proteins on pinocytotic activity previously shown in fibroblasts,

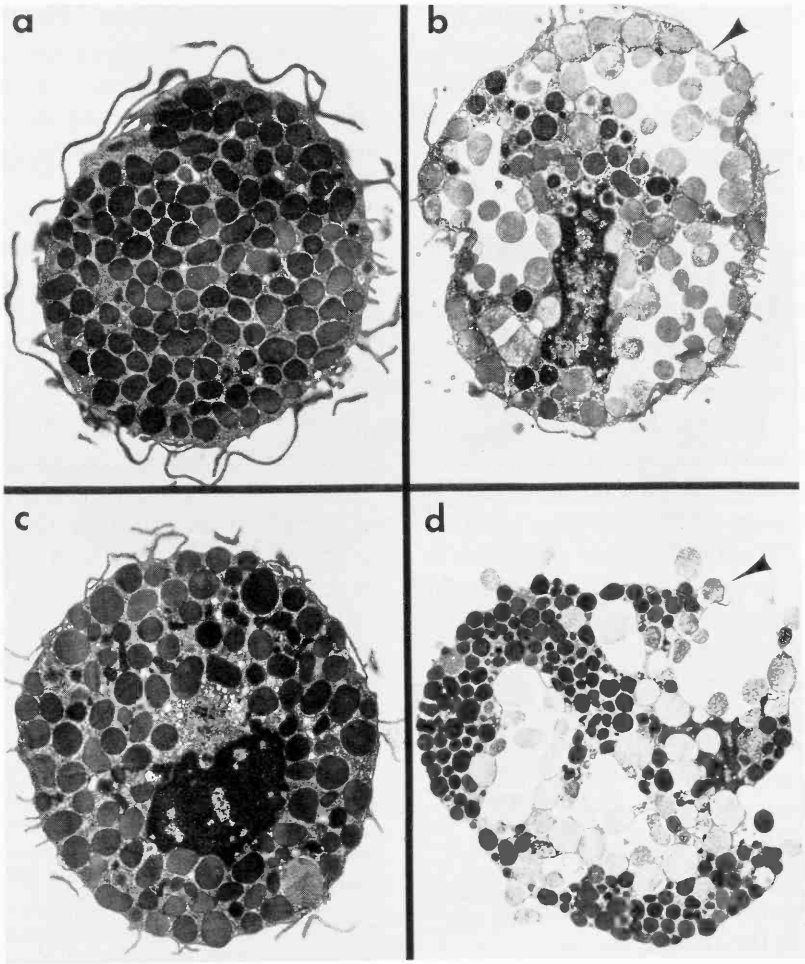

FIGURE 4 Ultrastructural analysis of mast cells. (*a*) Resting mast cell; (*b*) mast cell 2 min after stimulation with compound 48/80 (2 μg/ml); (*c*) mast cell injected with buffer alone; (*d*) mast cell injected with the *ras* oncogene protein. The injected cells were fixed and processed for transmission electron microscopy at 4 hr postinjection. In the resting cell (*a*) or cell injected with buffer alone (*c*), the granules are dense, and there is no evidence of exocytosis. The cells stimulated with compound 48/80 (*b*) or injected with the *ras* oncogene protein (*d*) show an exocytotic response as indicated by the conversion of most of the granules to less-dense granules and the occurrence of exocytotic pits.

our present findings suggest a role for *ras* proteins in the signaling process that controls membrane recycling via an exocytotic-endocytotic shuttle.

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NUCLEAR ONCOGENES AND SIGNAL TRANSDUCTION

M. Gilman L. Berkowitz K. Riabowol
R. Graham W. Ryan

Development, differentiation, and cellular proliferation are largely coordinated by extracellular signals. These signals, usually in the form of polypeptide growth factors and hormones, carry complex instructions to cells that specify dramatic changes in cellular behavior. These changes in behavior are effected by stable changes in the pattern of gene expression in the cell. How are these signals transmitted to the nucleus to generate changes in the pattern of cellular gene expression? Recent evidence suggests that this is essentially a two-step process.

First, the immediate signals triggered at the cell surface in the first few seconds and minutes are transmitted directly to a group of immediate-early genes, some of which are activated within 5 minutes of exposure to the signal.

Second, products of these genes act to activate or repress a second class of genes that includes genes directly involved in the cellular response.

Our work concerns the c-*fos* proto-oncogene, which occupies a critical focus for this signaling cascade. The c-*fos* gene is an immediate-early gene that is a direct target for the first events triggered by extracellular signals. Transcription of the c-*fos* gene is activated within 5 minutes of exposure to signals and is subsequently shut down approximately 30 minutes later. Activation of transcription is independent of new protein synthesis in the cells. Our primary effort is aimed at understanding how signaling information travels from the cell surface to the c-*fos* gene and how c-*fos* transcription is activated in response to this information. Our strategy is to work backward from the gene toward the cell surface by (1) identifying the DNA sequences within the c-*fos* gene that are the targets for different intracellular signaling pathways, (2) identifying cellular proteins that interact with these sequences, and (3) using these proteins to identify the next agents up the signaling cascade. Figure 1 summarizes our current picture of the signaling network.

A second focus for our efforts is to understand how the products of the c-*fos* gene and related genes function to control the expression of the second wave of genes involved in the cellular response to signals. In particular, we want to understand the biological specificity of these signals—how different signals elicit different responses in cells. Here, our strategy is to examine at a molecular level the proteins induced in cells in response to distinct signals to understand (1) the basis for the specificity of induction and (2) the functional differences among proteins induced by different signals.

c-*fos* Sequences in Communication with Distinct Intracellular Signal Transduction Pathways

M. Gilman, R. Graham

The c-*fos* gene is activated by several distinct intracellular signal transduction pathways. We wish to determine which sequences within the c-*fos* gene are in communication with each of these pathways. Previous work by ourselves and several other groups has established that a 20-bp sequence element, the serum response element (SRE), located 300 bp upstream of the start site for c-*fos* transcription, is required for the response of transfected c-*fos* genes to whole serum. Serum, however, is a complex mixture of growth factors that activate multiple signal transduction pathways. Therefore, we have asked which individual pathways act on c-*fos* via the SRE and which pathways act through other sequences.

Our results show that at least two distinct intracellular signal transduction pathways converge on the SRE (see Fig. 1). One pathway acts through the cellular enzyme protein kinase C. Induction of transfected c-*fos* genes in mouse fibroblasts by activators of protein kinase C is completely dependent on the SRE, and an oligonucleotide comprising the SRE at least partially restores response to these agents to a c-*fos* promoter deleted for sequences upstream of −151. Growth factors also trigger signals independent of protein kinase C, although their precise biochemical nature is not understood. These signals, too, are dependent on the SRE, and response to protein-kinase-C-independent signals is also restored to deleted c-*fos* promoters by an SRE oligonucleotide. Thus, at least two (and perhaps more) intracellular signaling pathways act on the c-*fos* gene via the SRE.

In contrast, at least two other pathways are independent of the SRE. One pathway, involving the intracellular second messenger cAMP, acts through several other sequence elements in the gene (see below). In addition, another pathway that uses intracellular calcium as a second messenger is also independent of the SRE. Like cAMP, calcium induction of the c-*fos* gene is not impaired by SRE mutations; however, the calcium-responsive sequences in the gene have not yet been indentified.

Although most of our work in this area has involved fibroblasts, we are now beginning to study c-*fos* induction in T cells. In the body, most T cells

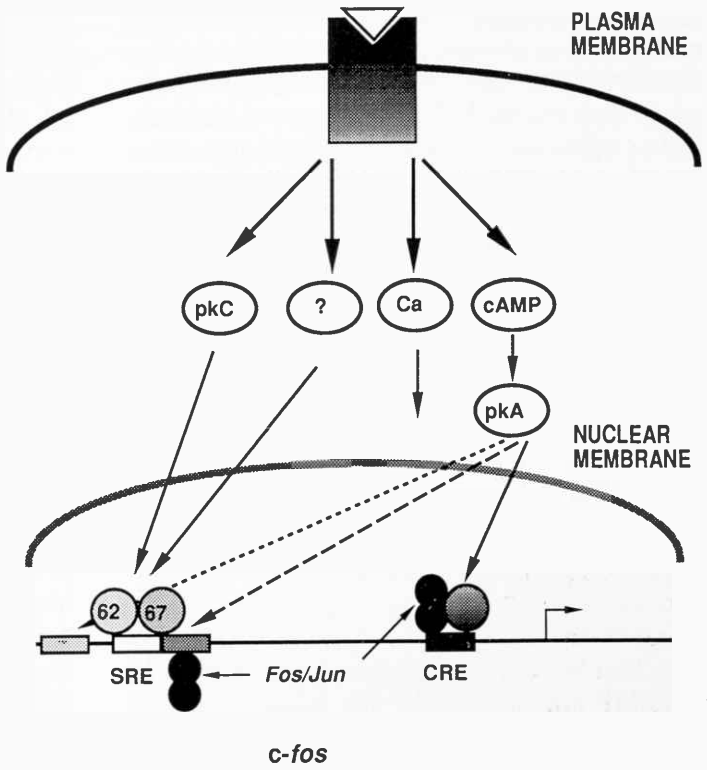

FIGURE 1 Summary of signaling pathways and intracellular mediators involved in the induction of c-*fos* transcription. Shown are the several distinct signal transduction pathways known to trigger c-*fos* transcription (see text). The ovals represent known or potential cytoplasmic mediators of these signals. Arrows from these ovals connect the pathways to the c-*fos* *cis*-acting elements required for their action on c-*fos* transcription. For the cAMP pathway, arrows with solid lines represent the strongest element, whereas arrows with dashed lines represent weaker elements. Circles represent nuclear DNA-binding proteins identified or inferred from DNA-binding studies discussed in the text. Rectangles along the representation of the c-*fos* gene are identified regulatory elements.

remain in a resting state until they encounter their cognate antigen, at which time they initiate a defined program of gene expression that results in proliferation and secretion of a variety of lymphokines. The c-*fos* gene is induced as part of this cascade. Because signal transduction in T cells is not as well understood as in fibroblasts, we are using our bank of c-*fos* promoter mutants to study the different pathways that communicate T-cell activation signals to the nucleus.

Finally, we are investigating the role played by the SRE in the transcriptional shutoff of the c-*fos* gene that follows induction. Shutoff requires new protein synthesis, suggesting that c-*fos* transcription is actively repressed by a protein synthesized in response to the primary signal. An obvious candidate for such a protein is the Fos protein itself. This became an especially attractive hypothesis with the demonstration that complexes of the Fos and Jun proteins bind to specific DNA sequences. We have shown that a sequence located immediately adjacent to the SRE is a binding site for the Fos/Jun complex in vitro. However, mutations in this site that abolish binding of the complex do not affect transcriptional shutoff, nor do mutations in the cAMP response elements (CRE) in the c-*fos* gene, which also bind the Fos/Jun complex in vitro. Therefore, we are now testing whether it is the SRE itself that controls both activation and repression. We have used site-directed mutagenesis to generate a large collection of mutations in the SRE, and we have begun to test whether any of these mutant SREs are defective in induction and/or repression. Our preliminary data suggest that in fact certain SRE mutations result in an element that confers stable, rather than transient, serum induction on a deleted c-*fos* promoter. Thus, it is likely that the SRE is a target for both positive and negative signals.

c-*fos* DNA Sequences Responsive to cAMP

L. Berkowitz, K. Riabowol, M. Gilman

Expression of the c-*fos* gene is rapidly and transiently induced in BALB/c 3T3 fibroblasts by agents that elevate the intracellular concentration of cAMP. Like induction by serum, induction of c-*fos* expression by cAMP does not require new protein synthesis, suggesting that the c-*fos* gene is a direct target of this signaling pathway. Unlike induction by serum, response to cAMP does not require the SRE. Using gene transfer, DNA binding, and microinjection assays, we have localized the sequences in the mouse c-*fos* promoter that mediate the response to cAMP.

We find that there are several elements within the promoter that mediate the response to cAMP (see Fig. 1). All elements share a match to the consensus core sequence established for CREs: TGACG. The major CRE in the c-*fos* promoter as established by transient expression assays is an element located at position -65 that was previously identified as a basal promoter element and a protein-binding site. We have shown that this sequence acts as a CRE in its natural context in the c-*fos* promoter. However, it is not the only CRE in the promoter, because mutation of this site, although it abolished cAMP response of a -71 deletion, did not abolish response of a c-*fos* promoter carrying sequences through -356. Using site-directed mutagenesis, we localized these additional CREs to positions -290 and -340 in the c-*fos* promoter. The results of the transient expression assays permitted us to establish the order of potency of these sites as $-65 > -290 > -340$. This heirarchy corresponded precisely with the avidity with which these elements bind cellular CRE-binding factors in vitro.

To determine whether the elements we have mapped using the transient expression assay actually function as CREs for the endogenous c-*fos* gene in its natural chromatin configuration, we developed a microinjection competition assay. In this assay, we microinjected double-strand oligonucleotides corresponding to the c-*fos* CRE at -65 into fibroblasts, treated the injected cells with agents that elevate cAMP concentrations, and assayed for expression of the endogenous c-*fos* gene by indirect immunofluorescence using affinity-purified antibodies against Fos. The CRE oligonucleotide constitutes an in vitro binding site for cellular CRE-binding proteins. If interaction of these proteins with CREs in the endogenous c-*fos* gene is required for response to cAMP, we predicted that microinjection of this oligonucleotide would block c-*fos* induction. We observed (Fig. 2) that the CRE oligonucleotide blocked c-*fos* induction by cAMP but not by serum. In contrast, an oligonucleotide encoding the SRE blocked induction by serum, but not by cAMP. A mutant CRE oligonucleotide had no effect on c-*fos* expression under any conditions. Thus, sequence elements related to the CREs mapped using the transient expression assay are functionally required for induction of the endogenous c-*fos* gene by cAMP.

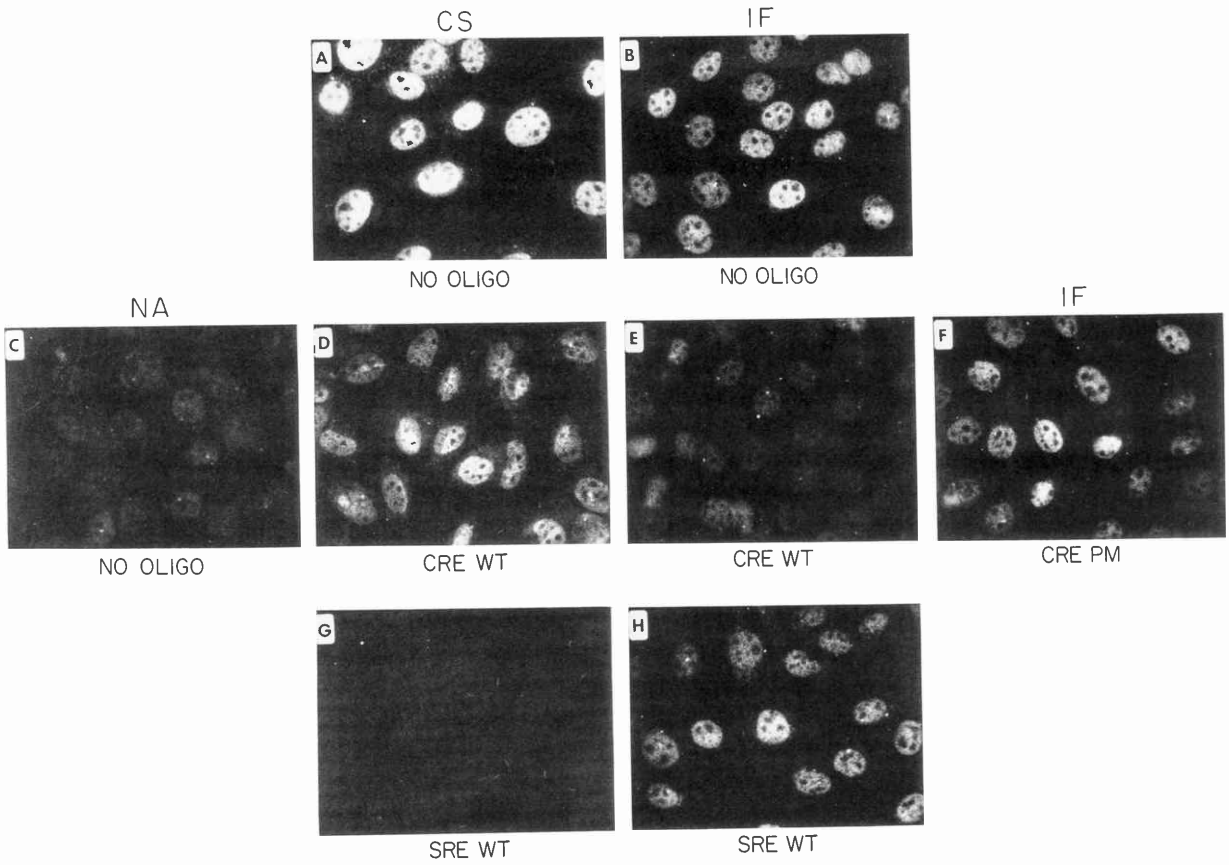

FIGURE 2 Injection of CRE oligonucleotides blocks cAMP-induced endogenous c-*fos* expression. Quiescent BALB/c 3T3 fibroblasts (*C*) or cells stimulated for 90 min with 10% calf serum (*A,D,F,G*) or with IBMX and forskolin (*B,E,H*) were processed to detect c-*fos* immunofluorescence. One hour prior to stimulation, cells were microinjected with double-strand oligonucleotides as described in text. (*A,B,C*) Cells received no oligonucleotide; (*D,E*) cells received the wild-type CRE oligonucleotide; (*F*) cells received the mutant (pm3) CRE oligonucleotide; (*G,H*) cells received the wild-type SRE oligonucleotide. Fields of cells were photographed and processed identically to allow direct comparison between panels.

These experiments also establish that these elements are positive activators of transcription and that there are no unrelated elements in the c-*fos* gene that are also capable of mediating response to cAMP.

Currently, our efforts are aimed at studying the cellular proteins that interact with the c-*fos* CREs to determine how transcription is activated in response to cAMP. Using the polymerase chain reaction technique, we have isolated cDNAs encoding full-length CREB proteins. We plan to raise antibodies to these proteins for in vitro transcription and microinjection assays. In addition, given the role for the catalytic subunit of cAMP-dependent protein kinase in c-*fos* induction by cAMP (see below), we will study the effect of phosphorylation on the function of these proteins.

The Catalytic Subunit of cAMP-dependent Protein Kinase Induces Expression of Genes Containing cAMP-responsive Enhancer Elements

K. Riabowol, M. Gilman [in collaboration with J.S. Fink, D.A. Walsh, R.H. Goodman, and J.R. Feramisco]

The mechanism by which cAMP exerts its cellular effects is largely through the cAMP-dependent protein kinase (A-kinase). However, the way that cAMP affects eukaryotic gene expression is less clear. In *Escherichia coli*, cAMP regulates the expression of genes involved in sugar catabolism by binding to catabolite gene activator protein (CAP). Subsequent

binding of the cAMP-CAP complex to specific DNA sequences directly activates transcription of cAMP-regulated genes, including those involved in sugar catabolism. The holoenzyme of A-kinase exists as an enzymatically inactive tetramer, composed of two catalytic (C) and two regulatory (R) subunits. When levels of cAMP are elevated, cAMP binds to the R subunits and the holoenzyme dissociates, yielding an R subunit dimer and two active C subunits. Both the C and R subunits of A-kinase have been suggested to mediate the transcriptional regulation of cAMP-responsive genes. The R subunits could function in a manner similar to that of bacterial CAP and, upon binding cAMP, act directly upon a target DNA site in cAMP-responsive genes. Alternatively, the C subunit of A-kinase could phosphorylate chromosomal or other nuclear proteins, which could then induce gene expression.

To distinguish between these two models, we microinjected purified C and R subunits, separately and together, and determined their effects on the expression of cAMP-regulated genes. Two different assays were used to measure cAMP-responsive gene expression. First, a C6 glioma cell line containing a stably integrated fusion gene consisting of the human vasoactive intestinal peptide (VIP) gene promoter/enhancer region joined to the *E. coli lacZ* gene was tested. Expression of the fusion gene in individual cells was detected by staining using the chromogenic β-galactosidase substrate X-gal. Treatment with 5 μM forskolin and 0.5 mM isobutyl methylxanthine (IBMX) for 6 hours, which increases intracellular levels of cAMP, induced β-galactosidase expression as detected by the appearance of cells that stained blue with X-gal. We found that injection of purified C subunit also resulted in the expression of β-galactosidase in the injected cells, whereas injection of R_I or R_{II} subunits, or of C subunit plus varying amounts of R_{II} subunit, did not induce expression of the fusion gene. As a second assay, we examined the regulation of an endogenous gene within the context of its native chromatin configuration. Thus, we measured the appearance of *fos* protein in the nuclei of cells microinjected with the different subunits of A-kinase. Expression of *fos* protein was monitored by indirect immunofluorescence using affinity-purified antibodies raised against v-*fos* protein. Figure 3 shows that by 90 minutes, expression of *fos* protein was markedly induced in cells injected with C subunit (panel A), and by 3 hours, very high levels of nuclear *fos* staining were observed (panel B). Panel D shows that a mixture of equal amounts of C and R_{II} subunits failed to induce *fos* expression in these cells. Injection of *ras* protein, which is also known to induce *fos* expression in fibroblasts, was included as a positive control and is shown in panel C.

These experiments show that the C subunit of A-kinase is sufficient to induce the expression of at least two genes that are regulated by cAMP and suggest that protein phosphorylation plays a role in this process. The experiments in which a mixture of C and R subunits were injected indicate that R_{II} can inactivate the C subunit, most likely by forming a catalytically inactive tetramer. Furthermore, the fact that R_I or R_{II} were unable to induce transcription by themselves implies that these R subunits do not play a crucial role in the expression of these genes. Since these microinjection experiments were carried out in the absence of agents that increase intracellular levels of cAMP, they also suggest that activation of cellular proteins by binding cAMP is not required for expression of the genes tested.

Cellular Proteins That Interact with the c-*fos* SRE

W. Ryan, R. Graham, M. Gilman [in collaboration with R. Franza, Cold Spring Harbor Laboratory]

The c-*fos* SRE is clearly a multifunctional element. It is a target for activation by at least two distinct intracellular signaling pathways, and it confers constitutive activity on the c-*fos* promoter. In addition, preliminary data suggest that it is also a target for repression of c-*fos* transcription following serum stimulation. Despite these multiple activities, until recently only a single cellular protein that binds to this element has been identified. This protein, serum response factor (SRF), is a 67-kD phosphoprotein that binds in a symmetrical fashion to the SRE. Its DNA-binding activity does not appear to be modified in response to signals that induce c-*fos* transcription, so its precise role in the control of c-*fos* transcription in response to extracellular signals is not clear; it is reasonable to expect that other cellular proteins may be involved in the complex regulatory events that occur at the SRE.

We have recently identified two new cellular proteins that interact with the SRE. One binds directly to the SRE, and the second appears to recognize the SRE-SRF complex. To identify any additional cellular proteins that bind to the SRE, we

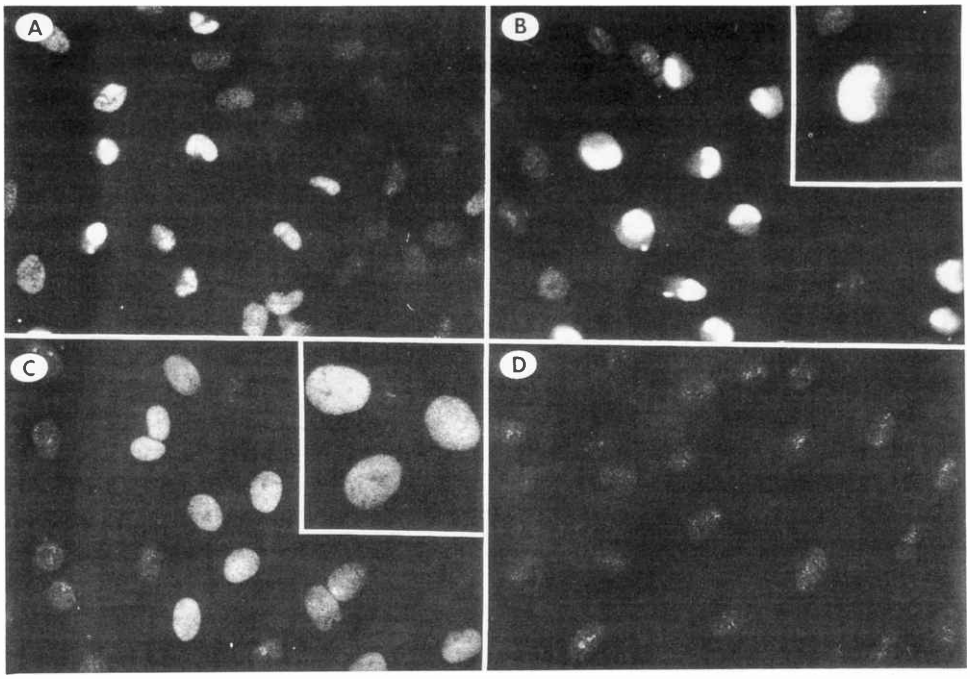

FIGURE 3 Microinjection of the C subunit of cAMP-dependent protein kinase induces expression of *c-fos*. Logarithmically growing rat 208F fibroblasts were made quiescent by incubation in medium containing 0.1% FCS for 24 hr prior to injection. Purified C subunit of A-kinase was injected, and the cells were incubated for 90 min (*A*) or 3 hr (*B*) prior to fixation and staining for the *fos* protein. *Ras* protein (*C*) and a mixture of equal amounts of C and R_{II} subunits (*D*) were also injected, and the cells were fixed and processed for immunocytochemistry with *fos* antibody following 3 hr (*ras*) or 6 hr (C + R_{II}) of incubation at 37°C. No nuclear fluorescence was observed in cells injected with the mixture of C and R_{II} when cells were fixed and processed for immunofluorescence 1, 2, or 4 hr following injection (not shown). Insets in panels *B* and *C* show higher magnification micrographs of the nuclei of C subunit and *ras* injected cells, respectively.

fractionated a nuclear extract prepared from human T cells by heparin-agarose chromatography (Fig. 4). We assayed column fractions using two assays for sequence-specific DNA-binding proteins. One assay, the commonly employed mobility-shift assay, originally detected only a single, specific SRE-binding protein, which turned out to be SRF. The second assay, a DNA-affinity precipitation assay, detected a second specific SRE-binding protein. This protein was distinct from SRF in several features, including its size (62 kD), its biochemical and chromatographic properties, and its DNA-binding specificity. In particular, it makes quite distinct contacts with SRE DNA. Unlike SRF, which binds symmetrically to both sides of the SRE dyad, the 62-kD protein binds only to the 5′ half of the SRE, a surprising observation in view of the similarity of the two halves of the SRE dyad.

The second new protein has the unusual property that it does not bind directly to the SRE as do SRF and the new 62-kD protein, but instead binds to the complex of SRF and SRE DNA. We detected this activity following fractionation of nuclear extracts by heparin-agarose chromatography, when we observed that the mobility of the SRF-SRE complex in mobility-shift gels increased following chromatography. We found that by adding flowthrough fractions from the heparin-agarose column to heparin-agarose fractions containing partially purified SRF, we could reconstitute the lower mobility complex observed in crude nuclear extracts. These observations suggested that the complex formed in crude extracts was in fact a ternary complex consisting of SRE DNA, SRF, and an unknown additional protein separable from SRF by heparin-agarose chromatography. We have partially purified this activity and identified it as a polypeptide of 62 kD. Although it is fully separable on heparin-agarose from the 62-kD protein described above that directly binds the SRE, it is possible that these activities represent alternate forms of the same polypeptide.

Formation of the ternary complex requires more

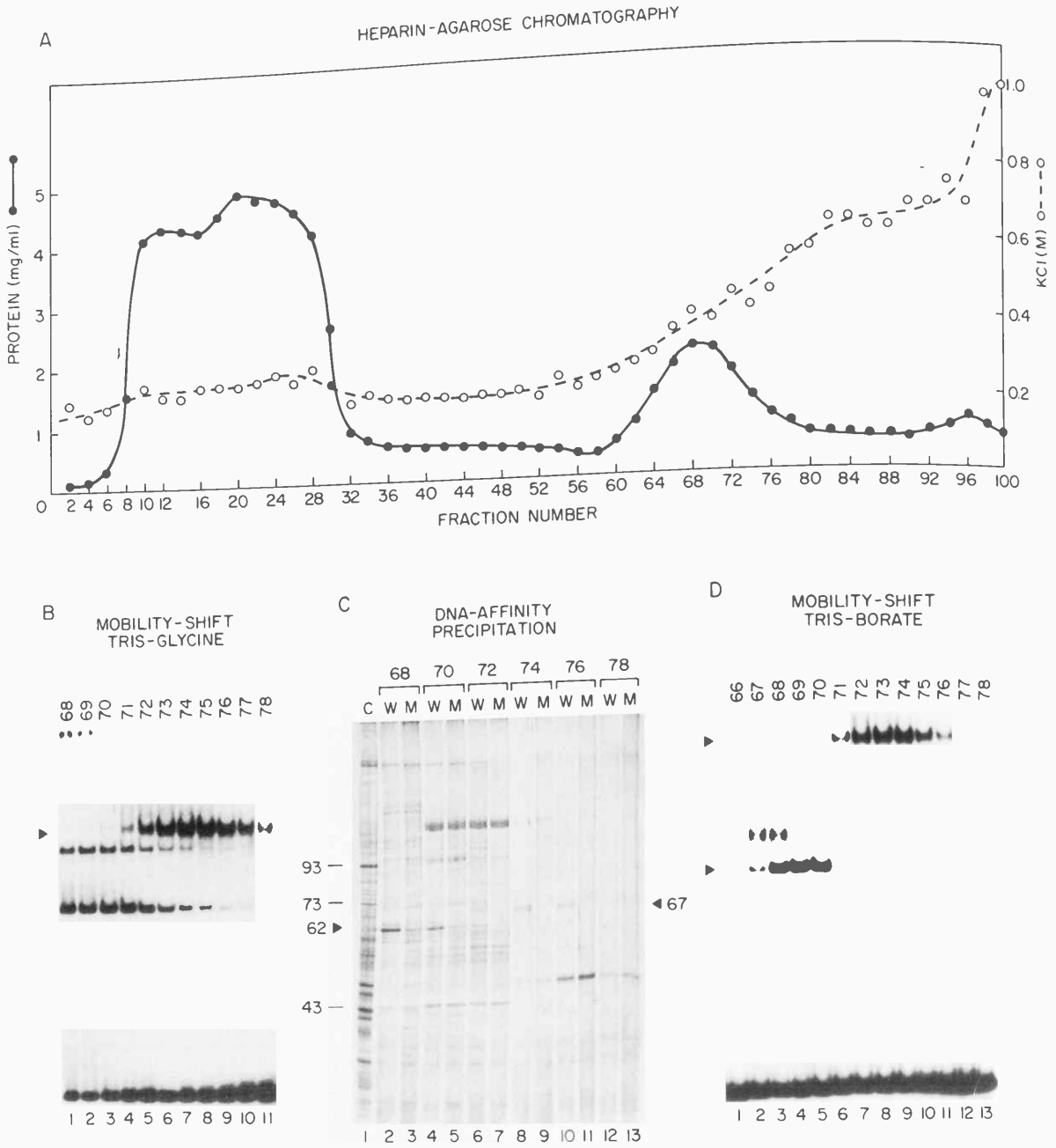

FIGURE 4 Heparin-agarose chromatography of H9 cell nuclear extract. (*A*) Column profile. (●) Protein concentrations in the corresponding fractions; (O) estimated KCl concentrations in the fractions as determined by conductivity measurements. (*B*) Mobility-shift assay of peak fractions. The assay was carried out without a preincubation step, using Tris-glycine electrophoresis buffer. The probe was the wild-type SRE. The arrowhead indicates the specific complex that formed in these assays. The other bands formed equally well in a parallel assay using a mutant SRE probe (data not shown). (*C*) DNA-affinity precipitation assay of selected fractions from the heparin-agarose column. The indicated fractions were incubated with either ligated wild-type SRE oligonucleotide (W, lanes *2,4,6,8,10,12*) or mutant SRE (M, lanes *3,5,7,9,11,13*). The arrowheads indicate proteins specifically recovered with the wild-type oligonucleotide. Lane *1* contains H9 whole-cell extract. The indicated markers are hsp90 (93 kD), hsp70 (73 kD), and actin (43 kD). Protein bands were visualized by silver staining. (*D*) Mobility-shift assay performed using the preincubation protocol and Tris-borate electrophoresis buffer. The probe was the wild-type SRE. The arrowheads indicate specific complexes that did not form in a parallel assay using the mutant SRE probe (data not shown).

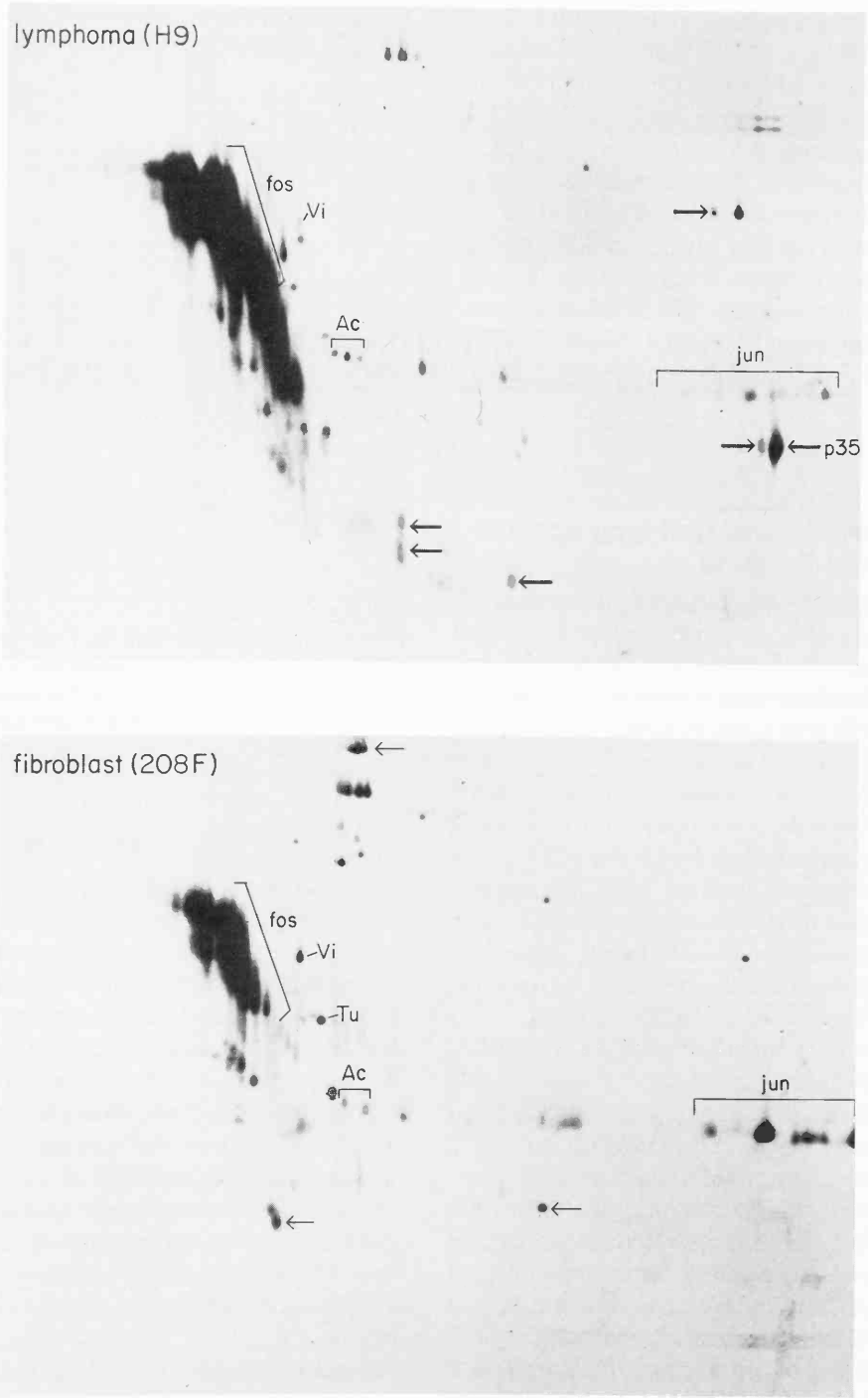

FIGURE 5 High-resolution two-dimensional gels of c-*fos* immunoprecipitation products. Lymphoid (*top* panel, human H9) or fibroblast (*bottom* panel, rat 208F) cells previously labeled with [^{35}S]methionine were harvested under nondenaturing conditions. Cell lysates were immunoprecipitated using affinity-purified antibodies raised against bacterially produced *fos* protein. Washed pellets were boiled in Laemmli sample buffer and subjected to two-dimensional gel electrophoresis and fluorography. Light arrows indicate polypeptides unique to fibroblasts. Bold arrows indicate H9-specific immunoprecipitation products. Actin is labeled Ac on both autoradiograms.

SRE DNA sequence than does binding of SRF alone, suggesting that this new activity may recognize DNA. This idea is supported by two observations. First, using methylation interference assays, we find that methylation of guanine residues that do not interfere with SRF binding block formation of the ternary complex. Furthermore, we have identified single point mutations in the SRE that bind SRF but fail to form the ternary complex, suggesting that this new factor specifically recognizes SRE DNA sequences. Preliminary analysis of these SRE mutants in vivo suggests that this new complex acts to repress c-*fos* transcription.

Stimulus-specific and Cell-type-specific *fos*-associated Proteins

K. Riabowol, M. Gilman [in collaboration with R. Franza, Cold Spring Harbor Laboratory]

Several lines of evidence suggest that the c-*fos* protein may play a role in gene transcription. c-*fos* is known to bind specific DNA sequences implicated in transcriptional control, in a synergistic manner with the product of the c-*jun* proto-oncogene. Overexpression of c-*fos* can also induce expression of cotransfected reporter plasmids whose transcription is directed by several heterologous promoter/enhancer sequences. Immunoprecipitation of c-*fos* protein under nondenaturing conditions reveals the presence of several *fos*-associated and *fos*-related proteins. Some of these proteins have been identified as the products of the c-*jun* proto-oncogene (Fos-associated) and of the FRA1 (Fos-related antigen 1) gene. In addition to these associated and related proteins, we have observed a variety of other proteins that are specifically immunoprecipitated using our polyclonal rabbit affinity-purified *fos* antibodies. Several of the immunoprecipitated polypeptides that are cell-type-specific are indicated in Figure 5. Thus, it appears that subsets of proteins associate with c-*fos*, only some of which are common to markedly different cell types. In addition, we have observed the association of c-*fos* with proteins of distinct relative molecular weights when quiescent fibroblasts are stimulated with different agents. Figure 6 shows immunoprecipitations from rat 208F fibroblasts that were stimulated with serum or with agents that increase intracellular levels of cAMP (IBMX and forskolin). Although most protein bands are found in

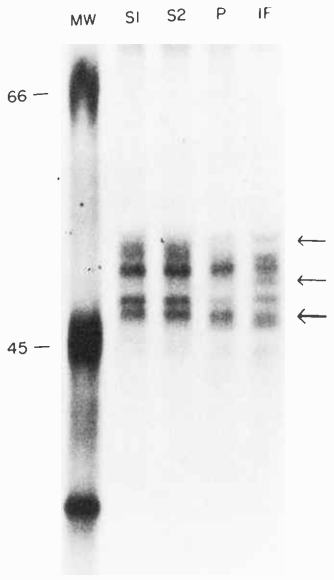

FIGURE 6 Stimulus-specific *fos*-associated proteins. Quiescent rat 208F fibroblasts were stimulated with 10% fetal calf serum (lanes S1 and S2), the phorbol ester PMA (lane P), or with IBMX plus forskolin (lane IF) for 90 min in the presence of 250 μCi/ml of [^{35}S]methionine. Cell lysates were prepared and immunoprecipitated as described in Fig. 1. Immunoprecipitates were analyzed by one-dimensional SDS-PAGE and fluorography. The bold arrow identifies the *fos*-associated c-*jun* protein and the light arrows indicate serum-specific or IF-specific *fos*-associated proteins.

immunoprecipitations of cells stimulated with both agents, some bands are unique. To better characterize and identify such cell-type-specific and stimulus-specific proteins associated with c-*fos*, we are now beginning to raise polyclonal and monoclonal antibodies specific for individual proteins present in the c-*fos* immunoprecipitation complex.

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TRANSCRIPTION INITIATION AND TERMINATION IN SnRNA GENES AND HUMAN IMMUNODEFICIENCY VIRUS

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We are interested in the mechanisms that govern initiation and termination of transcription, and we are studying these mechanisms in two types of transcription units: the human small nuclear RNA (snRNA) genes U1, U2, and U6 and the human immunodeficiency virus type 1 (HIV-1).

SMALL NUCLEAR RNA GENES

The snRNA genes U1 to U7 encode snRNAs that are packaged with a set of proteins to form small nuclear ribonucleoprotein particles (snRNPs). The snRNPs U1, U2, U4, U5, and U6 are all involved in the splicing of pre-mRNAs, whereas the U7 snRNP is involved in the 3′ processing of histone pre-mRNAs. All of the snRNA genes characterized to date are transcribed by RNA polymerase II (pol II) except for the U6 gene, which is transcribed by RNA polymerase III (pol III). However, the pol II and pol III snRNA genes have very similar promoters and seem to be more related to each other than to mRNA pol II transcription units or to the well-characterized pol III transcription units, such as the 5S and tRNA genes.

The promoters of the human pol II snRNA genes are essentially bipartite and consist of an "snRNA proximal element" (snPE) located around position −50 upstream of the cap site, and an enhancer located around −220. The snPE is unique to snRNA genes and is functionally equivalent to the TATA box of mRNA promoters; it is essential for efficient transcription and localizes the start site of transcription. The enhancer consists of two adjacent motifs: the octamer motif ATGCAAAT, also found in the immunoglobulin promoters and enhancers, and an Sp1-binding site. The enhancer stimulates transcription from the promoter relatively independently of distance and orientation.

The formation of the pol II snRNA 3′ ends occurs by an unusual mechanism, which most probably involves termination of transcription. The *cis*-acting elements required include a short sequence located downstream from the last coding nucleotide of the genes called the "3′ box," as well as sequences in the 5′-flanking region of the genes. Indeed, when the U1 or U2 promoters are replaced by mRNA promoters, the 3′ box is ignored and RNAs extend to a polyadenylation site inserted downstream. We have recently completed a detailed mutational analysis of the human U2 5′-flanking region, which showed that the 5′ elements required for 3′-end formation coincide with promoter elements. In this analysis, replacement of the U2 enhancer by SV40-derived synthetic enhancers inhibited RNA 3′-end formation at the 3′ box. Moreover, the synthetic enhancers did not stimulate transcription from the U2 promoter, although they efficiently activate mRNA promoters. This suggested that the pol II snRNA genes are transcribed by a specialized transcription complex that recognizes the 3′ box as a termination signal and does not contain the same type of enhancer-binding factors as mRNA transcription complexes.

The U6 gene is transcribed by pol III and transcription ends in a run of T residues, the 3′ signal characteristic for termination of transcription by pol III. However, the structure of the U6 promoter

suggests that the gene belongs to a new family of pol III transcription units, of which the human 7SK gene is another member. Indeed, the U6- and 7SK-coding regions are not required for transcription, even though they contain a homology with the A box, a promoter element required for transcription of several pol III genes. Instead, the 5'-flanking sequences of the genes are sufficient to direct transcription and contain sequences that are very similar to promoter elements of the pol II snRNA genes. The U6 and 7SK promoters contain an enhancer region with one or more octamer motifs, and the U6 and U2 enhancers can functionally replace each other. The U6 and 7SK promoters also contain an snPE around position −55, as well as a motif not found in the pol II snRNA promoters: a T/A-rich region located around −25 and reminiscent of the TATA box of mRNA genes. Because the U2 and U6 promoters are very similar but yet are recognized by different RNA polymerases, they constitute an ideal system to study the determinants of RNA polymerase specificity.

HUMAN IMMUNODEFICIENCY VIRUS

HIV-1 causes AIDS. Infection of T cells by the virus leads to a latency period of variable length. The transition to productive infection is thought to be triggered by immunological activation of the infected T cells. Initially, immunological activation may induce cellular *trans*-acting factors, which in turn stimulate transcription from the promoter contained within the viral long terminal repeat (LTR). The virus is then able to synthesize its own *trans*-activator, *tat*-1, which further stimulates transcription from the HIV LTR by several hundredfold, leading to virus replication and cell death. Interestingly, the target sequence (TAR) for *trans*-activation by the *tat* protein lies downstream from the transcriptional start site. The mechanisms of *tat trans*-activation are poorly understood and may include stimulation of transcription initiation, stimulation of transcription elongation (antitermination), and posttranscriptional effects.

That *tat* may act as an antiterminator is suggested by the observation that in the absence of *tat*, short transcripts ending just downstream from the TAR region are generated. In the presence of *tat*, these short transcripts disappear, and transcripts reading through the putative premature termination site are detected. Thus, *tat* may act as a specific antiterminator that relieves termination downstream from the TAR region.

The pol II snRNA Promoters Are Activated by a New Class of Enhancer Elements

U. Grossniklaus, N. Hernandez [in collaboration with M. Tanaka and W. Herr, Cold Spring Harbor Laboratory]

Recent experiments with yeast and mammalian *trans*-activators, such as GAL4, GCN4, and steroid receptors, have revealed a general mechanism for activation of pol II transcription. These *trans*-activators consist of a DNA-binding domain and an activation domain characterized by a high concentration of negative charges. Surprisingly, the activation domains of different *trans*-activators are interchangeable. For example, the activating domain of GAL4, when fused to the DNA-binding domain of the human estrogen receptor, can stimulate transcription from the vitellogenin A2 promoter in HeLa cells. Moreover, hybrid mammalian promoters in which DNA-binding sites for GAL4 have been inserted upstream of the TATA box can be stimulated by GAL4 supplied in *trans*. From these observations, a model emerges in which the activating domains of different transcription factors are equivalent, and the specificity of transcription activation is solely conferred by the specificity of the DNA-protein interactions.

In this context, the octamer motif presents a paradox. This motif is found in the promoter region of heavy- and light-chain immunoglobulin genes, in the heavy-chain (IgH) enhancer, and in the SV40 enhancer. In these contexts, and when inserted upstream or downstream from other mRNA promoters, the octamer motif displays lymphoid-specific activity. But the octamer motif also plays a role in non-B cells as an enhancer of snRNA transcription and as a cell-cycle-regulated proximal element in the histone H2B gene. These different patterns of lymphoid-specific and ubiquitous transcriptional regulation parallel the expression of two proteins that bind indistinguishably to the octamer motif. The protein Oct-2 is expressed only in B cells, whereas Oct-1 is ubiquitously expressed (see R. Sturm and W. Herr, Tumor Virus Section). This observation, however, is not sufficient to explain the different patterns of expression of snRNA and immunoglobulin genes. Indeed, if the activation domains of different transcription factors are interchangeable and the specificity of activation is conferred solely by the specificity of DNA binding, why are immunoglobulin genes not expressed in HeLa cells?

To resolve the paradox, we have studied the abilities of different versions of the SV40 B element to enhance expression of the human β-globin and U2 snRNA promoters in HeLa cells and in B cells. As shown in Figure 1, the B element contains a tandemly repeated Sph motif (indicated by arrows) and a seven out of eight match to the octamer consensus ATGCAAAT (bracketed). In HeLa cells, the ability of the B element to enhance β-globin expression is dependent on the Sph motifs, whereas the octamer motif is inactive. We introduced a set of double point mutations into the B element, as indicated in Figure 1. *dpm*2 modifies the second Sph motif and obliterates Sph motif activity. *dpm*7 modifies the octamer motif and strongly reduces its activity, but it has no effect on Sph motif activity. *dpm*8 destroys both Sph and octamer motif activities. These different versions of the B element were polymerized to generate synthetic enhancers containing six copies of the element, and the ability of these synthetic enhancers to stimulate the β-globin and the U2 promoters was tested in B cells and in HeLa cells. The results are summarized in Figure 2.

In the context of the β-globin promoter, enhancer activity in B cells was dependent on a functional octamer motif, whereas in HeLa cells, enhancer activity was dependent on functional Sph motifs. The enhancement pattern of read-through transcripts derived from cryptic promoters within the U2 vector (labeled "read-through" in Fig. 2) was identical to that observed with the β-globin promoter. These read-through transcripts are of the mRNA type as opposed to the snRNA type, because they are not terminated at the 3′ box but extend to a polyadenylation further downstream. In contrast to the β-globin promoter and the cryptic promoters within the U2 vector, activation of transcription from the U2 promoter was always dependent on a functional octamer motif. These results suggested that (1) the Sph-motif-binding proteins that can activate transcription from

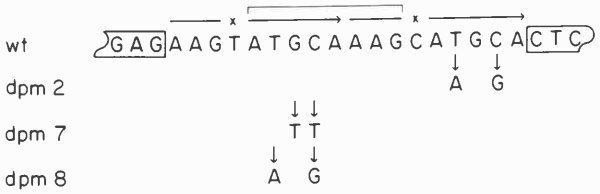

FIGURE 1 Sequence of the wild-type and mutant B20 synthetic enhancer repeats. The Sph motifs are underlined and the octamer motif at the junction of the two Sph motifs is bracketed. The changes introduced by the double point mutations *dpm*2, *dpm*7, and *dpm*8 are indicated.

	β-globin		U2	
	B cells	HeLa	HeLa	
			Read-through	U2 5′end
wt	+	+	+	+
dpm2 (sph⁻)	+	–	–	+
dpm7 (oct⁻)	–	+	+	–
dpm8 (sph⁻/oct⁻)	–	–	–	–

FIGURE 2 Summary of the activities of the B20 wild-type and mutant synthetic enhancers in the context of the β-globin and U2 promoter in B cells and HeLa cells.

the β-globin promoter in HeLa cells cannot activate transcription from the U2 promoter and (2) the ubiquitous octamer-binding protein present in HeLa cells is an enhancer-binding protein that stimulates only snRNA promoters. This, in turn, suggests that the ubiquitous octamer-binding protein is not a transcriptional activator of the GAL4 class. To test this hypothesis directly, we inserted GAL4-binding sites in the U2 and β-globin promoters and determined the ability of each hybrid promoter to be *trans*-activated by GAL4. Although the β-globin promoter was efficiently stimulated by GAL4, the U2 promoter was not, confirming that the U2 promoter is stimulated by a special class of *trans*-activator.

Although the 6XB20 *dpm*2 β-globin promoter was inactive in HeLa cells, it could be activated by coexpression of the herpes simplex virus *trans*-activator VP16 (VMW65). This protein normally *trans*-activates the viral immediate-early genes, although it does not bind DNA. VP16 seems, then, to convert the ubiquitous octamer-binding protein into a *trans*-activator of the GAL4 class.

Figure 3 shows a series of cartoons depicting hypothetical transcription complexes that form in B cells or HeLa cells, on either the β-globin or U2 promoter carrying the SV40 B element. For simplicity, only one copy of the B element is shown, and pol II is not represented. Figure 3A shows the transcription complex formed on the β-globin promoter in B cells. A TATA box complex is bound close to the cap site, and the B-cell-specific octamer-binding protein Oct-2 is bound to the octamer motif of the B element. This factor activates transcription from mRNA promoters and is therefore depicted as a protein of the GAL4 class containing an acidic

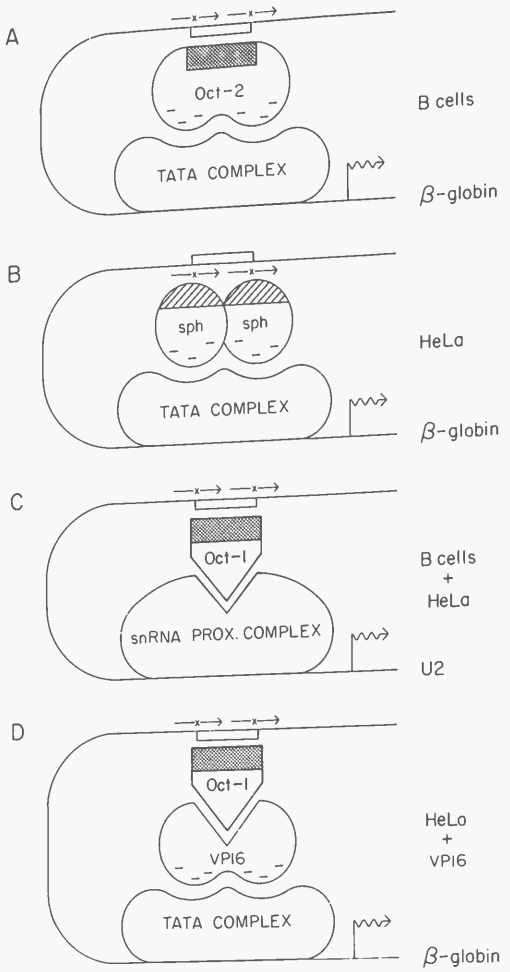

FIGURE 3 Model for activation of the β-globin and U2 promoters by the octamer and Sph motifs. Hypothetical complexes formed on the β-globin and U2 promoters in B cells and in HeLa cells are shown. The DNA is represented by a thin line, the Sph motifs are indicated by arrows, and the octamer motif is indicated by an open box. For simplicity, only one wild-type B20 repeat of the synthetic enhancer is shown. (In *D*, B20 wild type is replaced by B20/*dpm2*, i.e., there are no functional Sph motifs.) The start site of transcription is depicted by a wavy arrow. The DNA is looped and shown binding a proximal promoter complex (TATA complex or snRNA proximal complex) and a distal *trans*-activator. Successful protein-protein interactions between the *trans*-activator and the proximal complex result in an increased rate of transcription initiation. The octamer motif DNA-binding domains are stippled and the Sph motif DNA-binding domain is shown striped. The *trans*-activation domains of the GAL4-type *trans*-activators are shown as acidic domains. Oct-1 and Oct-2 are likely candidates for the *trans*-activation factors binding to the octamer motif (for further details, see text).

activation domain. This domain can interact productively with the TATA box complex, and this results in efficient initiation of transcription. Figure 3B shows the transcription complex found on the β-globin promoter in HeLa cells. In this case, Oct-2 is replaced by the Sph-binding factors, which can also productively interact with the TATA box complex through acidic domains, consistent with the idea that the activation domains of the GAL4 type of *trans*-activators are interchangeable. In the U2 promoter, however, the TATA complex is replaced by an snPE complex, which interacts productively only with Oct-1 (Fig. 3C). Because Oct-1 does not stimulate transcription from mRNA promoters, it does not belong to the GAL4 class of *trans*-activators and therefore is not represented with an acidic activation domain. The activation domain is of another nature and is specific for the snPE. In Figure 3D, VP16 is shown serving as an adaptor between the Oct-1 protein and a TATA box complex, essentially providing Oct-1 with the necessary acidic domain.

This model is consistent with all of our results and explains why the ubiquitous octamer-binding protein, although able to bind the octamer motif, will not stimulate transcription from an immunoglobulin promoter. It also emphasizes that not all *trans*-activation domains are interchangeable and that the specificity of *trans*-activation is conferred not only by DNA-binding specificity, but also by protein-protein interactions between proximal transcription complexes and distal enhancer-binding proteins.

cis-Acting Elements Involved in U2 and U6 Transcription

S. Lobo, N. Hernandez

A U6 promoter truncated at position −70 and thus missing the enhancer region is still recognized by pol III in vitro. In the pol II U2 promoter, the corresponding region contains only one element essential for efficient transcription, the proximal element. To determine which *cis*-acting elements in the U6 promoter define it as a pol III promoter, we introduced a series of clustered point mutations between positions −70 and +1 and analyzed their effect in vivo and in vitro. Mutations that modified the snPE strongly reduced transcription both in vivo and in vitro. Mutations that affected the T/A-rich region reduced transcription in vitro severely and generated novel start sites in vivo. α-Amanitin experiments in whole cells demonstrated that these new start sites were used by pol II. Thus, mutation of the T/A-rich region converts the U6 promoter into a pol II promoter, which, like the U2 promoter, is

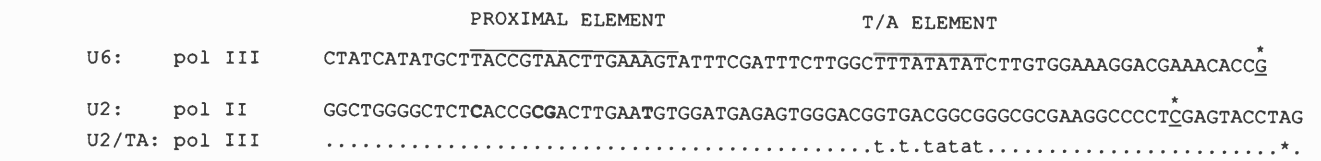

FIGURE 4 Clustered point mutations that convert the U2 promoter into an RNA pol III promoter. (Line *1*) U6 5′-flanking sequences from −76 to +1. The proximal element and the T/A-rich element are overlined. The start site of transcription is indicated by a star. (Line *2*) 5′-flanking sequences of the pU2/-247/RA.2 construct, which are identical to natural U2 5′-flanking sequences from −71 to −4. The region around the U2 cap site has thus been replaced by other sequences, and transcription from the U2 promoter starts mainly at the nucleotide indicated by a star. Four mismatches between the U2 and U6 proximal elements are indicated in bold letters. (Line *3*) Mutations that convert the U2 promoter into an RNA pol III promoter. The location of the start site used by RNA pol III is indicated by a star.

inactive in vitro. We showed that, indeed, this pol II activity was dependent on the snPE, like in the U2 promoter.

Interestingly, the site of transcription termination changed depending on which RNA polymerase was transcribing the gene. pol III transcription from the U6 promoter read through a 3′ box inserted downstream and terminated at a run of Ts. When the T/A-rich region was mutated, however, transcription ended at the 3′ box. This suggests that in pol II snRNA genes, the polymerase itself or an associated factor is involved in recognition of the 3′ box. Since transcription from mRNA promoters ignores the 3′ box, it also suggests that the U1–U5 snRNA genes are transcribed by pol II transcription complexes different from those that transcribe mRNA genes.

The observation that mutation of the T/A-rich region converted the U6 promoter into a pol II promoter suggested that this region constitutes a dominant signal for pol III specificity. To test this directly, we created a T/A-rich element in the U2 promoter by mutating 7 bp as shown in Figure 4. Indeed, this mutation converted the U2 promoter into a pol III promoter, active in vitro. Thus, in the human U6 snRNA gene, pol III specificity is conferred by a single, short *cis*-acting element, which may be binding a single *trans*-acting factor. We are now focusing on the identification and characterization of this factor as well as the factors that bind to the snPE.

Function of Mammalian RNA 3′-end Formation Signals in the Fission Yeast *Schizosaccharomyces pombe*

P. Reinagel, N. Hernandez [in collaboration with D. Frendewey, Cold Spring Harbor Laboratory]

snRNA transcripts end at a position determined by a 3′-flanking signal, the 3′ box. This signal can only be recognized by RNA pol II transcription complexes that initiate at snRNA promoters. Little is known about the mechanism by which the 3′ box is recognized, so it is of interest to determine whether this signal can function in fission yeast where genetic dissection of function is possible. Weak homology with the vertebrate 3′-box consensus can be found at variable distances downstream from the cloned *Schizosaccharomyces pombe* snRNA genes.

We have tested the ability of *S. pombe* to recognize a human snRNA promoter and 3′ box. For this purpose, a construct containing the human U2 promoter followed by (1) spacer sequences, (2) a 3′ box, and (3) the Ad2 L3 polyadenylation site was introduced into *S. pombe*, and transcription from the human U2 promoter was monitored by RNase T1 protection analysis. No correctly initiated RNA could be detected, suggesting that the human U2 promoter was not utilized by the *S. pombe* transcription machinery. To determine whether the human 3′ box could be utilized, we replaced the human U2 promoter by the 5′-flanking sequence of the *S. pombe* U4 snRNA gene (obtained from D. Tollervey). This chimeric gene is expressed in *S. pombe* and gives rise to transcripts that ignore the 3′ box signal. Thus, if snRNA 3′-end formation in fission yeast occurs by a mechanism analogous to that in vertebrates, the 3′-flanking signal must be significantly diverged from the 3′ box of the human genes. We are now determining whether the L3 polyadenylation signal is functional in *S. pombe*.

Premature Termination in the HIV-1 LTR in Soluble Extracts from HeLa Cells

M. Sheldon, S. Ifill, N. Hernandez

When a plasmid carrying the HIV-1 LTR is transfected into COS cells, short transcripts terminated

50–60 nucleotides downstream from the cap site are observed. Cotransfection of an expression vector carrying the viral *trans*-activator *tat* suppresses these short transcripts and activates HIV expression several hundredfold.

To determine whether the short transcripts are generated by premature termination of transcription and whether *tat* acts as an antiterminator, we set out to reproduce HIV premature termination of transcription in vitro. In some extracts from HeLa cells, we observe short transcripts of the expected length when a plasmid carrying the HIV-1 LTR is used as a template. These short transcripts are not observed with a template carrying the Ad2 E4 promoter and are sensitive to low concentrations of α-amanitin, indicating that they are synthesized by RNA pol II. We are now determining whether these extracts also produce short transcripts when programmed by the HIV-2 LTR. Our goal is to obtain antitermination by *tat* or by extracts derived from *tat*-expressing cells in vitro so that we can analyze the reaction biochemically.

PUBLICATIONS

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CELL BIOLOGY OF THE NUCLEUS

D.L. Spector W.-K. Chan J.P. Suhan
M. R. Delannoy

During the past year, our research program has continued to focus on two areas: (1) biochemical studies on the *myc* oncoprotein and (2) the structural and functional organization of the cell nucleus. Our structural studies have been greatly facilitated by the use of image analysis and three-dimensional reconstruction techniques at the electron microscopic level. In addition, 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 Joe Suhan.

Three-dimensional Organization of Small Nuclear Ribonucleoproteins

D.L. Spector, M.R. Delannoy

Using antibodies directed against small nuclear ribonucleoproteins (snRNPs), we have shown that these particles are concentrated within particular regions of the cell nucleus. These regions have been referred to as "nuclear speckles." Recently, our studies on this nuclear region have been aided by the use of image analysis and three-dimensional reconstruction techniques. Images of cell sections observed in the electron microscope (Fig. 1a) can be directly transmitted to the image analysis system, where a variety of operations can be performed on the images. One such operation includes the formation of a binary image of immunoreaction product (Fig. 1b). From such an image, measurements and statistical analyses can be performed. Using this technique, we have determined that cell sections immunostained with anti-Sm monoclonal antibodies contain approximately 20–30 snRNP clusters that occupy approximately 14% of the nuclear area of interphase cell sections. We are currently evaluating cells at various stages of the cell cycle in order to determine if the number and/or nuclear area containing snRNPs is constant or changes through the cell cycle.

Our previous studies on the distribution of snRNPs in the cell nucleus have used immunofluorescence microscopy or immunoelectron microscopy. Both of these techniques resulted in the acquisition

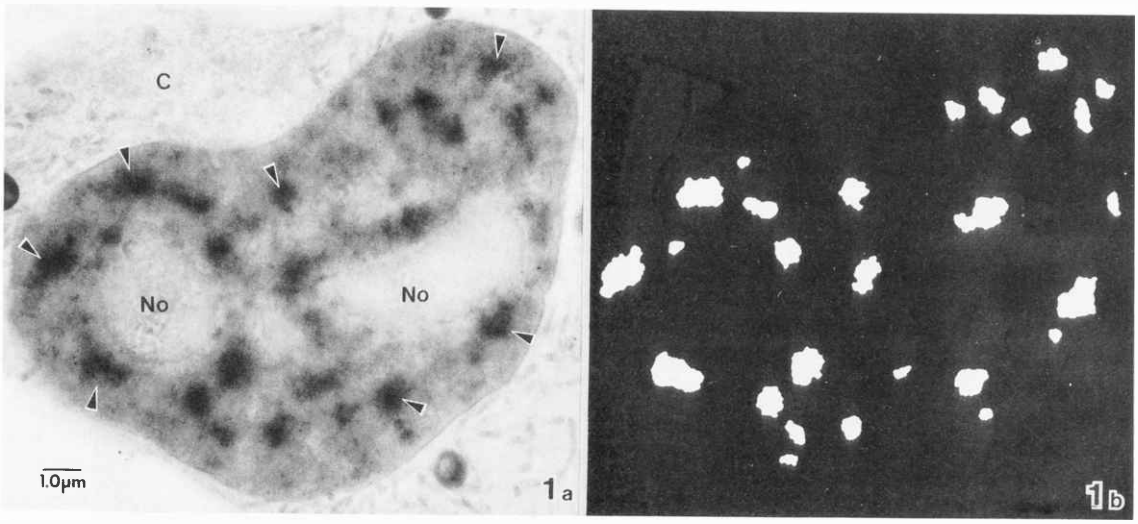

FIGURE 1 Distribution and quantitation of snRNP clusters. (*A*) 0.5 μm section of a CHOC 400 cell showing the distribution of snRNPs via immunoperoxidase staining. Arrowheads point to the snRNP clusters. The nucleoli (No) and cytoplasm (C) do not stain. This section has not been poststained. (*B*) Computer-generated binary image of the immunoreactive regions of *A* showing the snRNP clusters in white. Using such a binary image, it has been determined that there are 27 snRNP clusters in this cell section and that these clusters occupy approximately 14% of the nuclear area in this section.

of two-dimensional images that represent a small sample of the distribution of snRNPs within the whole nucleus. To gain a more complete understanding of the distribution of snRNPs within the cell nucleus, we have begun to evaluate three-dimensional reconstructions of serial sections through complete nuclei. CHOC 400 cells were grown in monolayer culture and were incubated for 16 hours with 3-μm mean diameter lectin-coated polystyrene spheres. These spheres are taken up by the cells and serve as fiduciary markers in aligning serial sections. Figure 2 (A–D) is a three-dimensional model of a reconstruction from 17 100-nm serial sections. The snRNPs are shown in white, nucleoli are shown in grey, and the nuclear envelope is shown as a line surrounding each section. Stereopair A and B provides a view from the top surface of the cell looking down into the nucleus. Stereopair C and D provides a view from the bottom surface of the cell, which was attached to the petri dish, looking up toward the cell surface. The distribution of snRNPs appears to extend between the nucleolar surface and the nuclear envelope, forming a reticular network. The nucleoli appear to lie closer to the upper surface of the nucleus. Using the three-dimensional reconstruction program, we can rotate or tilt this model and cut in the X, Y, and Z axes. Using these criteria, we are in the process of determining whether a reproducible three-dimensional pattern of snRNPs exists and whether this pattern is cell-cycle-regulated.

Breakup and Morphogenesis of snRNP-enriched Nuclear Speckles

D. L. Spector, J. P. Suhan

As a means of trying to establish the functional significance of the nuclear regions enriched in snRNPs, we have evaluated several approaches that have altered cellular metabolism in order to determine their effects on the snRNP clusters. We have previously shown that these clusters break up during mitosis and alter their organization after the inhibition of heterogeneous nuclear RNA (hnRNA) synthesis by the adenosine analog 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB). In addition, we have shown that the in situ sites of DNA replication, as measured by [^{3}H]thymidine incorporation, and the in situ sites of transcription, as measured by [^{3}H]uridine incorporation, are not coincident with the nuclear regions enriched in snRNPs. These data raise the possibility that functions other than DNA replication and transcription are associated with this nuclear region; one such function may be pre-mRNA processing.

In light of the findings of Yost and Lindquist (*Cell 45:* 185 [1986]), who demonstrated that the splicing of pre-mRNA precursors (which do not code for heat-shock proteins in *Drosophila* cells) were blocked by heat shock, we were interested in determining if

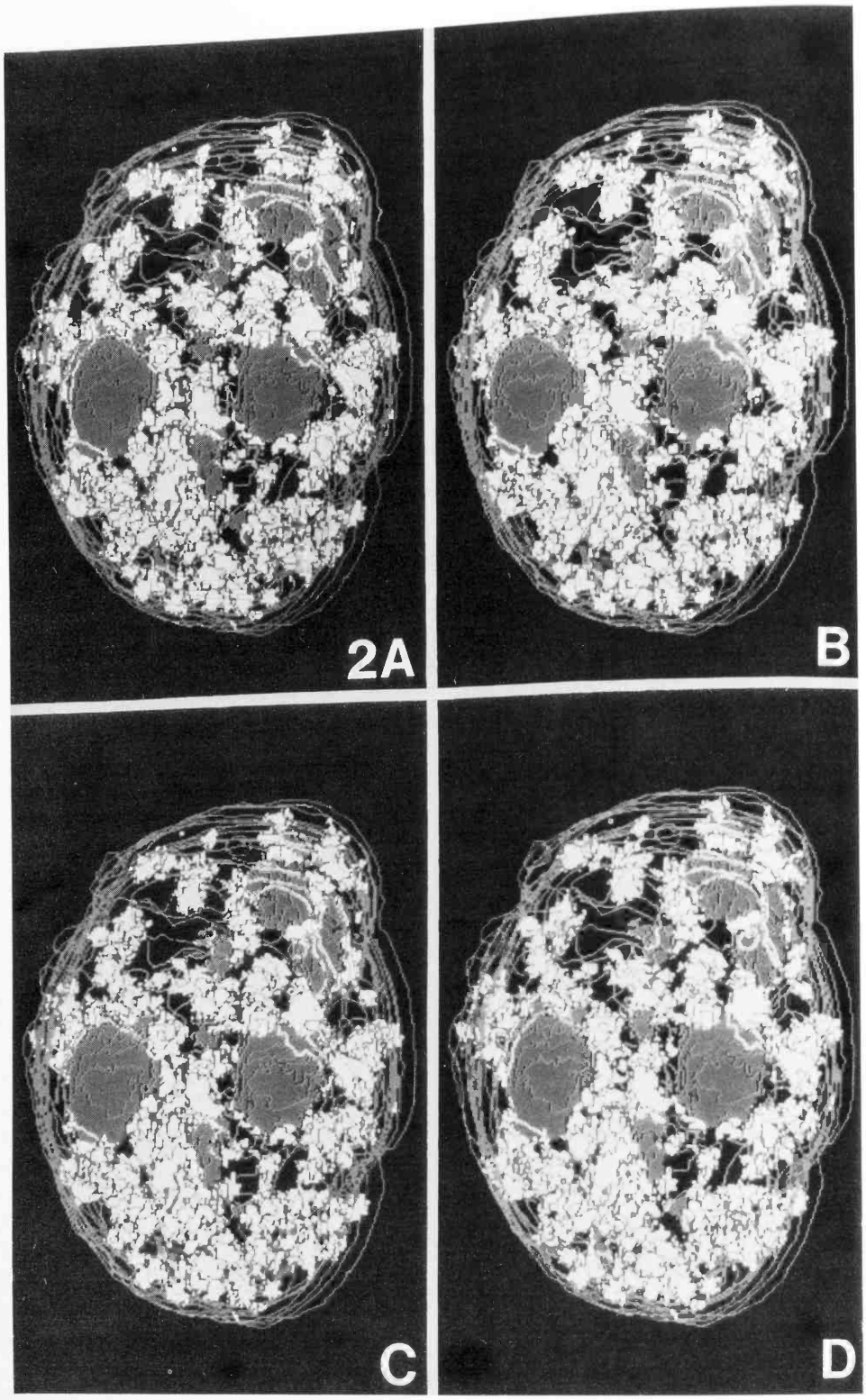

FIGURE 2 Model of a three-dimensional reconstruction of a CHOC 400 cell nucleus, showing the distribution of snRNPs (region in white), nucleoli (region in grey), and the nuclear envelope (grey line around each section). *A-B* and *C-D* represent stereopairs. Stereopair *A-B* provides a view from the top surface of the cell looking down into the nucleus. Stereopair *C-D* provides a view from the bottom surface of the cell looking up toward the cell surface.

heat shock altered the organization of the snRNP-enriched nuclear speckles. To address this question, we heat-shocked CHOC 400 cells for 15 minutes at 45°C and fixed them for immunofluorescence microscopy or allowed them to recover at 37°C for various periods of time prior to fixation and immunolabeling. The snRNPs were detected by FITC-labeled monoclonal anti-Sm antibodies, and *hsp70* was detected by rhodamine-conjugated anti-*hsp70* polyclonal antibodies. Control cells grown at 37°C showed a typical speckled snRNP staining pattern (Fig. 3b) and no detectable *hsp70* (Fig. 3c). After a 15-minute heat shock at 45°C, the speckled staining pattern was disrupted, and snRNPs appeared to be diffusely distributed throughout the nucleoplasm, excluding the nucleoli (Fig. 3e). After a 15-minute recovery at 37°C, two to three speckles began to reappear (Fig. 3h). Initial speckle formation was almost always adjacent to the nucleoli. Several exciting possibilities come to mind as to why the initial speckles appear to be adjacent to nucleoli. Perhaps the speckled pattern is anchored in the nucleoplasm to the surface of the nucleoli. Alternatively, the nucleolar organizer region (NOR) may more broadly serve as a nuclear organizer, or perhaps the nucleus contains other organizing centers that have yet to be identified. Since heat shock causes a change and not a loss of the snRNP immunofluorescence pattern, it is unlikely that these initial snRNP clusters represent the sites of snRNA transcription. Furthermore, the snRNP proteins that are recognized by anti-Sm antibodies are complexed with newly synthesized snRNAs in the cytoplasm before they return to the nucleus. These interesting observations are currently being pursued by immunoelectron microscopy. After 30 minutes (Fig. 3k) and 60 minutes (Fig. 3n) of recovery, the number of speckles continued to increase for 2 hours, when the typical speckled staining pattern was reformed (Fig. 3q). By 2 hours of recovery, *hsp70* appeared in the nucleoli (Fig. 3r), indicating that we had stimulated the heat-shock response. In summary, we have shown a breakup of snRNP-enriched nuclear speckles after heat shock and a time-dependent recovery of the speckled staining pattern after cells are returned to their normal growing temperature. Experiments using Northern blot analysis are currently under way to determine if the morphological changes of the speckled staining pattern can be directly correlated with the levels of dihydrofolate reductase (DHFR) pre-mRNA processing. Since the DHFR message in CHOC 400 cells represents approximately 10% of the cellular message, we may be able to identify differences in the amounts of precursor and spliced product over time.

Structural and Functional Domains of the c-*myc* Oncoprotein

W.-K. Chan, D.L. Spector [in collaboration with E. Chang and J. Anderson, Cold Spring Harbor Laboratory]

Our studies are aimed at dissecting the structural and functional domains of the c-*myc* oncoprotein as a means of understanding its role in the cell nucleus. We have expressed the recombinant human c-*myc* protein using the PL-AR expression system, followed by purification using a scheme developed by Watt et al. (*Mol. Cell. Biol. 5:* 448 [1985]). Several problems were encountered during purification due to the cross-linking of the c-*myc* protein with disulfide bonds forming insoluble inclusion bodies in *Escherichia coli*. To circumvent these problems, we expressed the c-*myc* protein in the T_7 polymerase system and developed a new purification protocol that resulted in at least 95% pure c-*myc* protein (Fig. 4, lane 1) devoid of cross-linking by disulfide bonds (Fig. 4, lane 2).

We next attempted to illustrate the structural domains of the c-*myc* protein by limited proteolysis. When purified protein was digested with trypsin, four protease-resistant fragments of 44K, 34K(triplet), 22K, and 18K were observed (Fig. 5, lanes 3–9). These fragments were also seen when proteinase K or V8 protease was used (data not shown). This result suggested that there might be several domains in the c-*myc* protein molecule that fold in a manner that renders them relatively insensitive to protease digestion. To verify whether the authentic c-*myc* protein possesses similar domains, we performed similar experiments on in-vitro-translated protein and on the c-*myc* protein from Colo 320 cells. Similar protease-resistant bands were observed when the protein was labeled with [^{35}S]cysteine. Interestingly enough, when the protein was labeled with [^{35}S]methionine, only the 44K and 34K bands were observed. Since the methionine residues of the protein cluster in the amino terminus of the protein (positions 1, 101, 120, and 134) and the ten cysteine residues are widely scattered in the molecule (positions 25, 70, 116, 133, 171, 188, 208, 299, 342, and 438), the result suggested

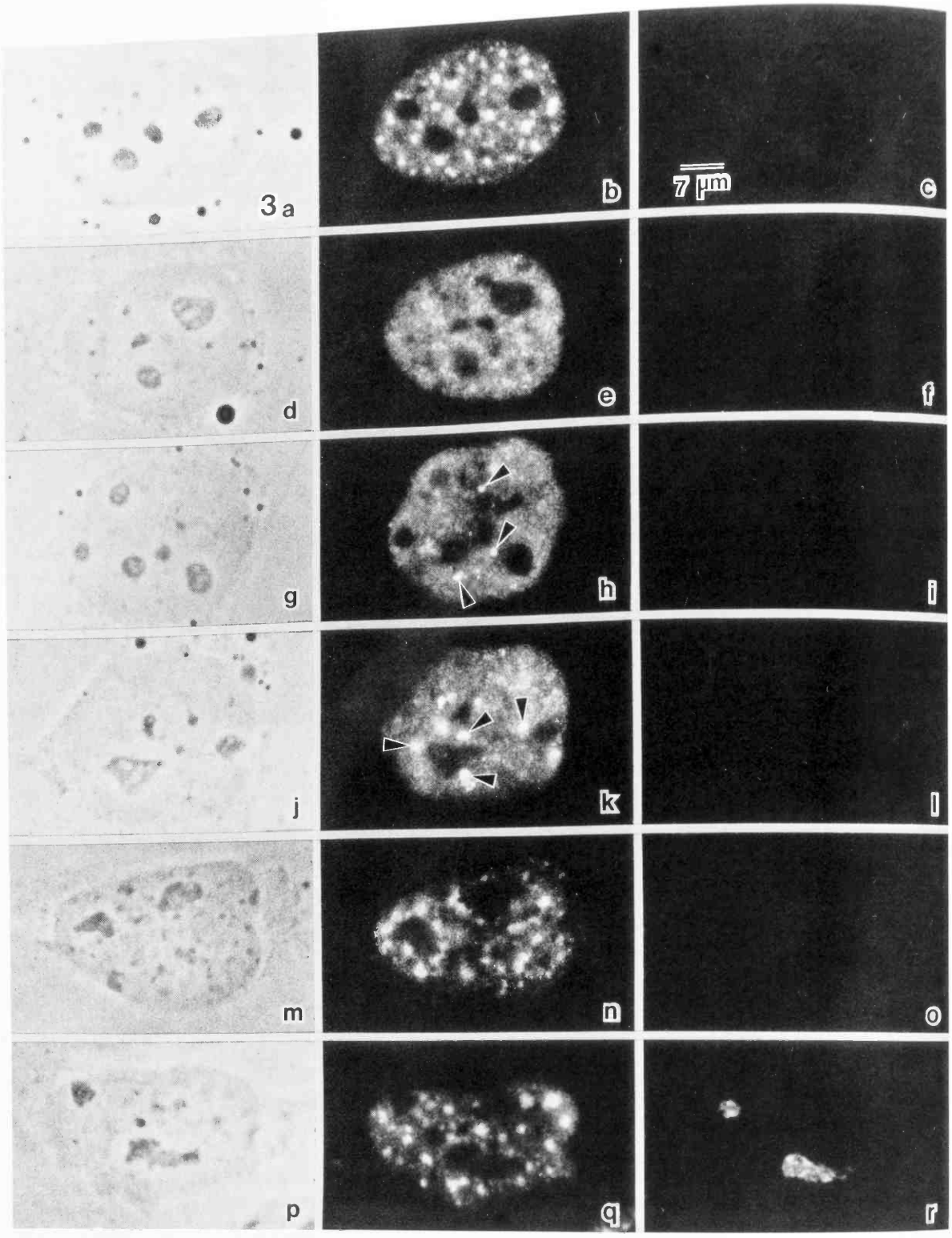

FIGURE 3 Breakup and morphogenesis of snRNP-enriched nuclear speckles. See text for a detailed description.

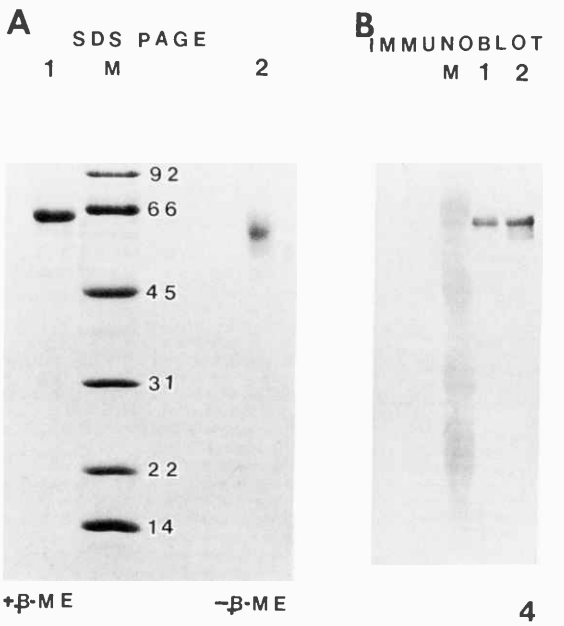

FIGURE 4 Characterization of the c-*myc* oncoprotein expressed in the T_7 polymerase system. (*A*) SDS-PAGE of purified c-*myc* oncoprotein. Purified protein runs around 65 kD (lane *1*), although it contains only 439 amino acids. This protein is devoid of nonspecific cross-linking by disulfide bonds as it enters the gel even without the addition of β-mercaptoethanol (lane *2*). (*B*) Immunoblot of the purified c-*myc* protein. To show the correct identity of the purified protein, a well-characterized monoclonal antibody, B3 (from R. Watt), was used to probe the protein and the same single protein band reacted with the antibodies (lanes *1* and *2*). Lane *2* is loaded with five times more protein than lane *1*. Lane *M* contains the molecular-weight markers.

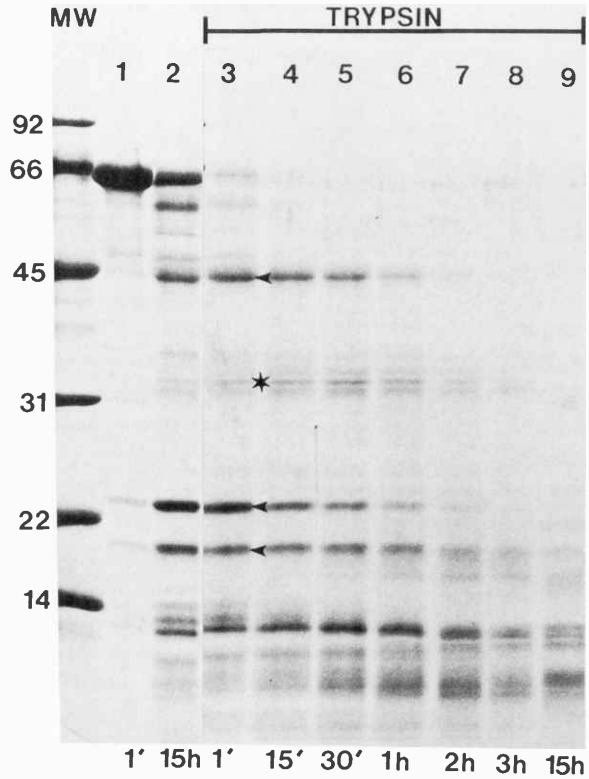

FIGURE 5 Partial proteolysis of the recombinant c-*myc* oncoprotein. The purified protein was digested with trypsin at a substrate-to-enzyme ratio equal to 2000:1 (w/w) at room temperature. After different times, an aliquot of the protein was taken out and analyzed in SDS-PAGE. (*1,2*) Without protease digestion; (*3–9*) Digested with trypsin. The time shown at the bottom of each lane is the time of incubation of the mixture at room temperature. The highlighted bands are the 44K, 34K, 22K, and 18K proteolytic fragments.

that the 44K and 34K bands may be from the amino terminus of the protein, whereas the 22K and 18K bands may be from the carboxyl terminus of the protein. To confirm this, we are in the process of purifying and determining both the amino- and carboxy-terminal sequences of the proteolytic fragments (in collaboration with Dan Marshak, Tumor Virus Section). Nevertheless, the above data suggest that the *E. coli*-synthesized c-*myc* protein is folded in a fashion similar to that of the in-vitro-translated and cellular c-*myc* proteins.

After the establishment of an efficient purification scheme for each individual fragment, we will determine the domain that is important for oligomerization and/or nucleic acid binding. The purified intact c-*myc* protein and its proteolytic fragments will be used for crystallization to determine the three-dimensional structure of the protein. It is hoped that an understanding of the structure of the protein will help to elucidate its precise function in the cell nucleus.

Nuclear Association of the c-*myc* Oncoprotein

W.-K. Chan, D.L. Spector

As an approach to understand the function of the c-*myc* oncoprotein in the cell nucleus, we would like to identify nuclear proteins that interact with c-*myc* in the nucleus by coimmunoprecipitation experiments. During the past year, we have generated several polyclonal antibodies against the purified recombinant c-*myc* protein from *E. coli* (Fig. 6A, lane 2), and we recently generated a battery of monoclonal antibodies (assisted by Ed Harlow's group [Tumor Virus Section]; Fig. 6A, lanes 3, 5, and 6) that recognize different epitopes on the c-*myc* protein (Fig. 6B, contrast lanes 3 and 5). These

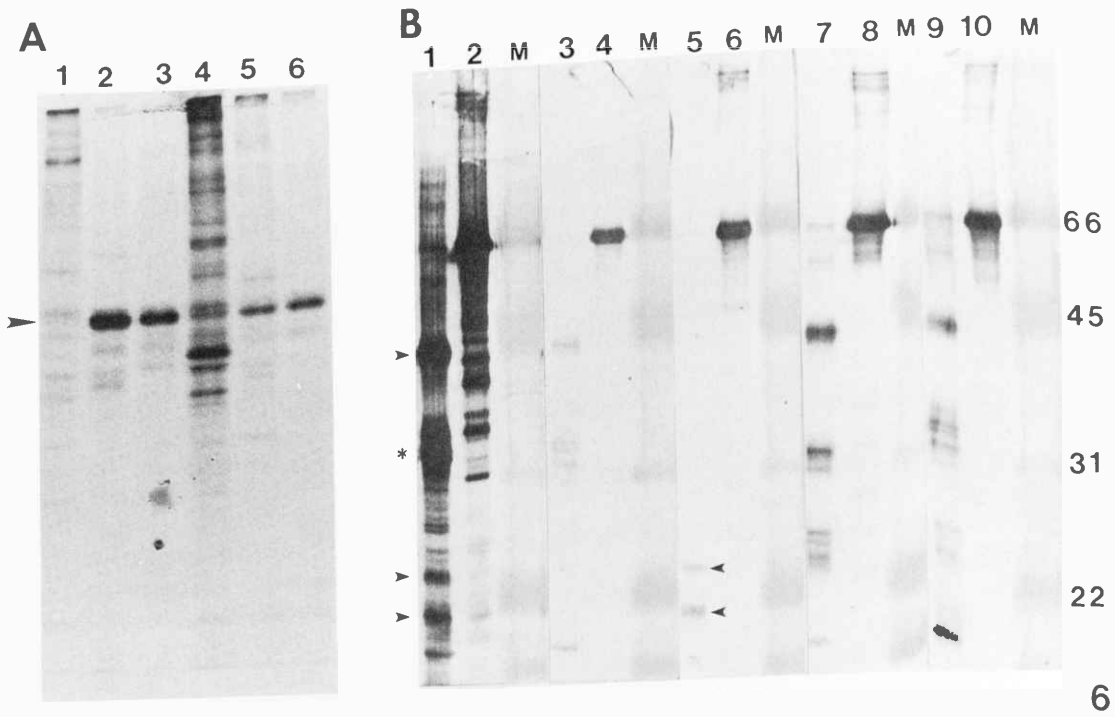

FIGURE 6 Characterization of the polyclonal and monoclonal antibodies (MAbs) against the c-*myc* oncoprotein. (*A*) Immunoprecipitation of the c-*myc* protein from Colo 320 cells using polyclonal or monoclonal antibodies. Colo 320 cells were labeled with [^{35}S]methionine, lysed, and immunoprecipitated with polyclonal or monoclonal antibodies. The immunoprecipitates were analyzed in SDS-PAGE. (*1*) Immunoprecipitate from normal rabbit serum; (*2*) from rabbit 138, which was immunized with recombinant c-*myc* protein; (*3*) from MAb KC8; (*4*) from MAb KC14; (*5*) from MAb KC26; (*6*) from MAb KC33. (*B*) Characterization of the reactivities of some of the MAbs against c-*myc* protein by immunoblotting. The purified c-*myc* protein was either digested with trypsin (substrate to enzyme = 2000:1) or was undigested and run in SDS-PAGE and transferred onto nitrocellulose. Lanes *1,3,5,7,* and *9* were loaded with trypsinized c-*myc*, whereas lanes *2,4,6,8,* and *10* were loaded with intact c-*myc*. Lanes *1* and *2* were probed with serum from rabbit 138; lanes *3,4* with KC8; lanes *5,6* with KC26; lanes *7,8* with KC33; and lanes *9,10* with B3. Note that KC26 recognizes different proteolytic fragments as compared with other MAbs. Lane *M* represents the molecular-weight markers.

antibodies will be useful reagents for coimmunoprecipitation experiments to investigate the possible c-*myc*-associated and/or related protein(s). Different conditions of immunoprecipitation including the use of different detergents, ionic strength, and pH of the buffer will be tested to reveal the possible c-*myc* complex in the nucleus. The identification and characterization of the c-*myc*-associated protein(s) may shed some light on the function of the c-*myc* protein in the nucleus.

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CELLULAR *TRANS*-ACTIVATORS OF GENE EXPRESSION

B.R. Franza J. Ross

We study the cellular proteins involved in the control of growth of mammalian cells. A focus for these studies has been the identification and characterization of cellular proteins that interact either directly or indirectly with specific nucleic acid structures. For some time, it has been known that certain DNA sequence elements direct the expression of genes by modulating transcription. We have identified several proteins that interact with different sequence structures and have learned some interesting things about them: (1) The protein products of several cellular proto-oncogenes cooperatively interact with at least two specific sequence elements. (2) Certain combinations of these proteins interact with single-stranded DNA containing specific sequences. (3) Several cellular proteins interact with the direct repeat sequence elements in the human immunodeficiency virus type-1 long terminal repeat (HIV-1 LTR). (4) These same proteins interact with control elements in T-cell-specific genes, including the interleukin-2 receptor α gene (IL-2Rα). (5) Induction of binding of certain of these proteins is independent of de novo protein synthesis. (6) Expression of the *trans*-activator gene of the human T-lymphotrophic virus type I (HTLV-I) results in activation of binding of at least one of these proteins. Some details of these studies are discussed below.

FOS-ASSOCIATED PROTEIN P39 IS THE PRODUCT OF THE *JUN* PROTO-ONCOGENE

Proto-oncogenes, the cellular homologs of retroviral oncogenes, play key roles in the regulation of normal cell growth and development. An understanding of the role of the products of proto-oncogenes in normal cellular processes is a necessary prelude to determining the molecular basis of oncogenesis. Several proto-oncogene products have been shown to function in one or another aspect of intra- or intercellular communication (signal transduction) as extracellular growth factors, cell-surface receptors, G proteins, kinases, or transcription factors. I have pursued studies for several years (in collaboration with Tom Curran and his colleagues at the Roche Institute of Molecular Biology) aimed at elucidating the function of the *fos* proto-oncogene and related genes. This has led to the characterization of Fos as a member of a set of cellular proteins that recognize specific DNA elements involved in transcriptional control. Included in this set is another proto-oncogene product (Jun). We have suggested that the proteins encoded by the *fos* and *jun* gene families function as transcriptional regulators in coupling short-term signals elicited by cell-surface stimulation to long-term responses by regulating patterns of gene expression through control elements such as AP-1 (activator protein-1 binding site) and CRE (cAMP-response element binding site). We are attempting to determine the mechanism(s) whereby Fos and Jun bring about such effects. Eventually, we hope to correlate these observations with studies of the physiological variables that alter the function of Fos and Jun (as well as Fra-1 and JunB) during normal cell growth, during the cell-division cycle, and in transformed cells.

The *fos* oncogene is encoded by two murine (FBJ-MSV, FBR-MSV) retroviruses and one chicken (NK24) retrovirus that cause bone tumors. The *fos* proto-oncogene, c-*fos*, is expressed at low levels in the majority of cell types; however, it is induced rapidly and transiently by a bewildering array of extracellular stimuli. These stimuli may be associated with mitogenesis, differentiation, or neuronal excitation. These observations led to the classification of c-*fos* as a cellular immediate-early gene that might participate in a signaling process in several cell types. It is our contention that the protein product of c-*fos* regulates expression of specific target genes containing AP-1 and/or CRE sites in these many situations.

The protein product of c-*fos* (Fos) is a 62-kD nuclear phosphoprotein that participates in protein complexes with a 39-kD cellular protein (p39). Antibodies raised against a synthetic peptide corresponding to Fos amino acids 127–152 precipitate a complex array of proteins from serum-stimulated cells. The collaboration between the Franza and Curran laboratories was first initiated to analyze the many proteins present in these immunoprecipitates using the high-resolution two-dimensional gel system and computer-accessible protein database developed by Garrels and Franza. These studies revealed several important features: (1)

Fos undergoes complex posttranslational modification, resulting in more than 20 distinct forms that can be resolved on two-dimensional gels. (2) Fos associates with at least three distinct proteins, one of which exhibited cell-type specificity (it was identified in PC12 cells treated with nerve growth factor [NGF] but not in serum-stimulated fibroblasts). These proteins were referred to as Fos-associated proteins (Fap). (3) Several proteins, antigenically related to Fos, were induced by serum in fibroblasts and by NGF in PC12 cells. Thus, extracellular stimuli led to the appearance of several protein complexes containing Fos and the synthesis of several Fos-related proteins.

Previous studies demonstrated that the Fos protein complex and related proteins exhibited nonspecific DNA-binding activity and were associated with chromatin in isolated nuclei. These data suggested that Fos might function as a DNA-binding transcriptional regulator. A clue to a specific DNA-binding activity was obtained from studies performed by Bruce Spigelman and colleagues at Harvard, in which a gel-retardation complex composed of DNA and protein was inhibited by anti-Fos antibodies. We noted that the DNA (derived from a control region of an adipocyte differentiation-sensitive gene [aP2]) contained a sequence very similar to the AP-1 site present in the $hMTII_A$ and SV40 enhancer elements. To study this interaction, we employed the microscale DNA-affinity precipitation (DNAP) assay (see the 1987 Annual Report) in which several oligonucleotides containing normal and mutated AP-1-binding sites were evaluated. We found that many control regions containing AP-1 sites interacted specifically with the Fos complex, several Fos-related antigens (FRAs), and other cellular proteins. It was clear from these studies that a number of cellular proteins interacted with AP-1 sites, although previously, other investigators had identified a single 47-kD protein and designated it the activator protein-1 (AP-1). Pursuing these studies led to the identification of the Fos-associated protein, p39, as the product of the c-*jun* proto-oncogene (see Fig. 1).

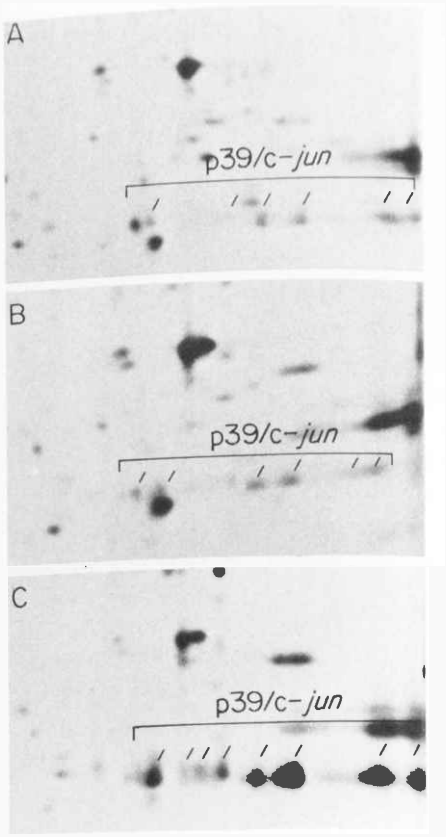

FIGURE 1 Comigration of proteins immunoprecipated by anti-Jun and p39 isolated in DNAP assays with oligonucleotides containing AP-1 binding sites. H9 cells (1×10^8) were stimulated by the addition of Ca^{++} ionophore A23187 to a final concentration of 10^{-6} M for 20 min, and [^{35}S]methionine (2.0 mCi) was then added. Cells were harvested 30 min later; extracts were prepared and examined by immunoprecipitation (*A*) or by DNAP assays (*B,C*). Recovered proteins were separated on two-dimensional gels. Only the portion of the gel in which p39 migrates is present. (*A*) H9 cells were treated with A23187 labeled with [^{35}S]methionine as described above, and the cells were then harvested and prepared for immunoprecipitation. Cell extracts were incubated with anti-PEP-1. DNAP analysis with the HIV (−357 to −316) oligonucleotide. DNAP analysis was carried out with biotinylated DFSE2 oligonucleotide. Both oligonucleotides contain AP-1-binding sites, and in DNAP assays, they bind Fos, Fos-related antigens, and p39. The hash marks on each panel indicate the number of forms of p39 that were resolved on the two-dimensional gels.

COOPERATIVE INTERACTION OF NUCLEAR PROTO-ONCOGENE PRODUCTS WITH SPECIFIC NUCLEIC ACID ELEMENTS

To investigate further the nature of the Fos and Jun interaction with the AP-1 and CRE sites and to determine the role of protein complex formation in DNA binding, we reconstructed the protein-protein and protein-DNA interactions in vitro using Fos and Jun synthesized in reticulocyte lysates. The Fos-Jun complex formed extremely rapidly in vitro and possessed similar, although not identical, hydrodynamic properties to the complex present in cell extracts. Although Jun exhibited some AP-1 binding, Fos acted with Jun to give enhanced, specific DNA-binding activity. The increased affinity of the Fos-

Jun complex for DNA resulted from a stabilization of the protein-DNA complex. These experiments demonstrated a cooperative interaction of the protein products of two proto-oncogenes with DNA elements (AP-1 and CRE) involved in transcriptional regulation. In addition, we have demonstrated cooperative interactions at the AP-1 and CRE sites using in-vitro-translated Fra-1 and Jun. Similarly, we have shown that the product of the *jun*-related gene, JunB (first identified by Daniel Nathans and colleagues at the Johns Hopkins Medical School) associates with either Fos or Fra-1 at AP-1 and CRE sites using the DNAP assay and DNA-mobility shift assay. Therefore, the products of several distinct nuclear proto-oncogenes contribute to stable, specific interactions with nucleic acid structures that mediate transcriptional control.

SEQUENCE-SPECIFIC SINGLE-STRANDED DNA RECOGNITION BY THE FOS/JUN PROTEIN COMPLEX

We have demonstrated that in-vitro-translated Fos and Jun proteins interact with single-strand sequence structures containing the AP-1-binding site (see Fig. 2). Additionally, we have demonstrated that the Fra-1

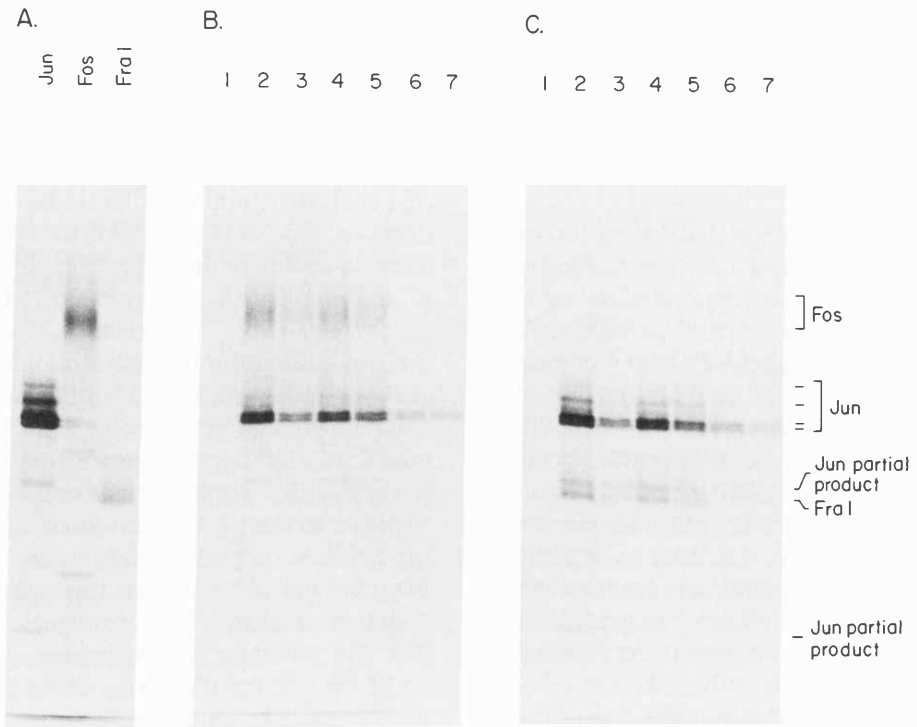

FIGURE 2 Fos + Jun and Fra1 + Jun interact with the AP-1 structure in single-stranded DNA. The DNA sequences of oligonucleotides used in these studies are

DAP-1ss 5′TCGACG**T**GACTC**A**GCGCGCATCG**T**GACTC**A**GCGCGC3′
αDAP-1ss 5′GCGCGC**T**GAGTC**A**CGATGCGCGC**T**GAGTC**A**CGTCGA3′

The bold letters designate the two bases in each AP-1 motif that were altered to generate mutant oligonucleotides (T to A and A to T, respectively, for each strand). The annealed duplexed oligonucleotide is referred to as DAP-1. Each oligonucleotide is a two-time iterated sequence of the AP-1 structure in the human metallothionein II_A gene. (*A*) Each lane represents the translation products of mRNA for c-*jun*, c-*fos*, and c-*fra1*. Visualization of protein is the result of incorporation of [^{35}S]methionine during the in-vitro-translation reactions. (*B*) Mixture of Fos and Jun and resultant DNAP assays: (*1*) Reaction mixture containing all components except for biotinylated (b-) oligonucleotide; (*2*) reaction containing b-DAP-1 double-strand probe; (*3*) reaction containing b-mutant DAP-1 double-strand probe; (*4*) reaction containing b-DAP-1ss probe; (*5*) reaction containing b-α DAP-1ss probe; (*6*) reaction containing b-mutant DAP-1ss probe; (*7*) reaction containing b-mutant αDAP-1ss probe. (*C*) Mixture of Fra1 and Jun and resultant DNAP assays. All lanes are the same as the Fos + Jun DNAP assays.

gene product also cooperatively associates with the same single-strand nucleic acid structures (see Fig. 2). The product of the Jun-related gene, JunB, also associates with Fos or Fra-1 at AP-1 single-strand sites. Little is known of specific interactions of mammalian cellular proteins with single-stranded DNA. It is tempting to speculate that a functional role of these various complexes might involve either the induction of deformation of duplexed DNA or stabilization of a nonduplexed structure. In any event, models for eukaryotic transcription, and possibly replication, can now be considered in which the appearance of an AP-1 motif within DNA could either positively or negatively affect such syntheses of nucleic acid. It is unlikely that the AP-1 single-strand sequence structure will remain unique in this regard.

MULTIPLE CELLULAR PROTEINS INTERACT WITH THE HIV-1 ENHANCER ELEMENT

The strategy of combining the microscale DNA-affinity precipitation assay with high-resolution two-dimensional gels has proven itself useful to the study of the control of human retroviral gene expression. Viruses like the HIV-1 require cellular proteins for their replication. Specifically, cellular proteins that control the expression (transcription) of cellular genes are also required to generate mRNA from the integrated retroviral DNA. HIV-1 actually contains DNA sequences that are identical to regulatory sequences attached to normal cellular genes. Cellular proteins recognize these sequences just as they do when they are linked to cellular genes. In this way, the virus utilizes cellular systems to activate and maintain transcription. mRNA is the genetic material of these types of viruses and therefore the cell actually provides the means for the virus to propagate itself. Any hope of understanding how the AIDS virus remains dormant in the cell and then is activated to express its mRNA rests on understanding how these normal cellular proteins function. The process of latency itself is interesting because its maintenance may involve cellular proteins that silence specific cellular genes as a normal consequence of differentiation.

Since HIV-1 is a small genetic unit compared to the entire human cellular genome, it actually provides us with a very powerful tool for understanding both its regulation and the regulation of cellular genes. Through evolutionary time, such retroviruses have condensed a set of regulatory elements into a small piece of genetic material. It is clear that these elements provide the virus with the ability to be silenced (i.e., nonexpressed, latent state), to be activatable when the growth status of the cell in which they reside changes, and to be highly expressed when the intracellular setting is appropriate. We have learned to take advantage of the virus in that we use its small control region to detect the cellular proteins needed to regulate its expression, and we have used this information to probe the regulation of complicated human cellular genes.

One result of these investigations has been the identification of several cellular proteins that interact with the HIV-1 enhancer and with a control element essential for inducible expression of IL-2Rα. Investigations of the IL-2Rα promoter have been done in collaboration with Warner Greene and colleagues at Duke University Medical School. The IL-2Rα element is structurally similar to the 10-bp sequence that is repeated in the HIV-1 enhancer. Figure 3 shows a region of a two-dimensional gel in which several of the proteins that interact with either of these control elements are represented. The cells used in this study are a human T-lymphoblast line called Jurkat. In Figure 3A, the proteins expressed in unstimulated Jurkat cells that interact specifically with the 10-bp sequence are shown. Stimulation of the cells with the tumor promoter, phorbol 12-myristate 13-acetate (PMA), results in quantitative and qualitative changes in the interacting proteins. Proteins designated KP are members of the set of proteins that we have previously designated HIVEN86A. The alphanumeric system that we now use to map and keep track of these complicated interactions is consistent with the QUEST database system. Each spot has a unique designator, and in all subsequent investigations, we will use this designator to link the protein spots from different images in which they appear. Our suspicion, several years ago, that more than one protein would be found to interact with transcription control elements has proven to be the case. It is fortunate that we have the database system to monitor these numerous interactions. It should also be evident that even when the precise biochemical function of each of these proteins is known, we will still require the database system to discern how the cell regulates them through processes such as differentiation or the cell division cycle.

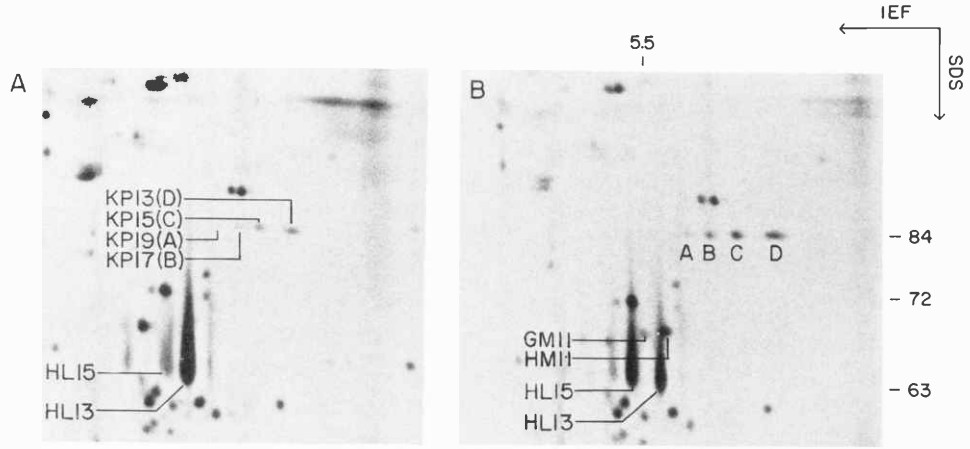

FIGURE 3 Multiple T-cell proteins specifically interact at either the HIV-1 enhancer or the IL-2Rα promoter. Jurkat T cells were prelabeled with [^{35}S]methionine for 3.5 hr, washed, and then incubated in the presence (*B*) or absence (*A*) of PMA for 30 min. ^{35}S-labeled extracts were mixed with biotinylated dimers of the wild-type (*A,B*) IL-2Rα (or HIV-1 enhancer) oligonucleotide probes. Nucleoprotein complexes were precipitated with avidin agarose and associated proteins were subjected to two-dimensional gel electrophoresis.

PROTEIN SYNTHESIS IS NOT REQUIRED FOR THE MODULATION OF INTERACTIVE EVENTS AT THE HIV-1 ENHANCER

One issue in determining the mechanisms controlling the transcription status of a gene is determining if the proteins required to effect the change must themselves be synthesized as part of the modulatory event. A convention that has been adopted by numerous investigators is the use of protein synthesis inhibitors that act on living cells to prevent de novo synthesis consequent to the addition of an activating agent. PMA, mitogenic lectins, serum, and growth factors comprise some of the agents that activate transcription of specific cellular genes. To discern whether any of the proteins we already had identified to interact with the HIV-1 enhancer would show a quantitative change even in the presence of a protein synthesis inhibitor, we devised the following approach. The Jurkat cells (or any other growing cell) are first metabolically labeled with a radioactive amino acid. The cells are then removed from the labeling environment, and a protein synthesis inhibitor is added to their growth media. Then, either nothing else is added or different activating agents are added to the growth media. After specified intervals, the cells are harvested and lysed, and DNAP assays are performed. Subsequent two-dimensional gel analysis of the DNA-protein complexes provides results such as those shown in Figure 4.

It is clear that the protein synthesis inhibitor cycloheximide is capable of inducing quantitative and qualitative alterations in the proteins that interact with the HIV-1 enhancer even in the absence of an activating agent. This is not unprecedented. The time of exposure critically affects the results. In this experiment, the cycloheximide and PMA were incubated with the cells for 90 minutes prior to harvesting the cells. If the interval were 30 minutes, one would see much less of a cycloheximide effect and much more of a PMA effect. This suggests that the one thing cycloheximide is doing is preventing the production of sufficient protease to digest these proteins and therefore they accumulate. It also suggests that PMA may not only recruit already synthesized proteins, but induce their production as well when an inhibitor of protein synthesis is not present in the growth media. The proteins represented in these panels are not the only ones we have identified whose interactions with the HIV-1 enhancer can be induced by cycloheximide. It is therefore likely that the cell at any time is prepared to alter the expression of at least some subset of genes independent of de novo synthesis of protein. Irrespective of how many biological reasons there may be for such a mechanism, it implies that evolutionary selection has required some cellular responses to be very quick.

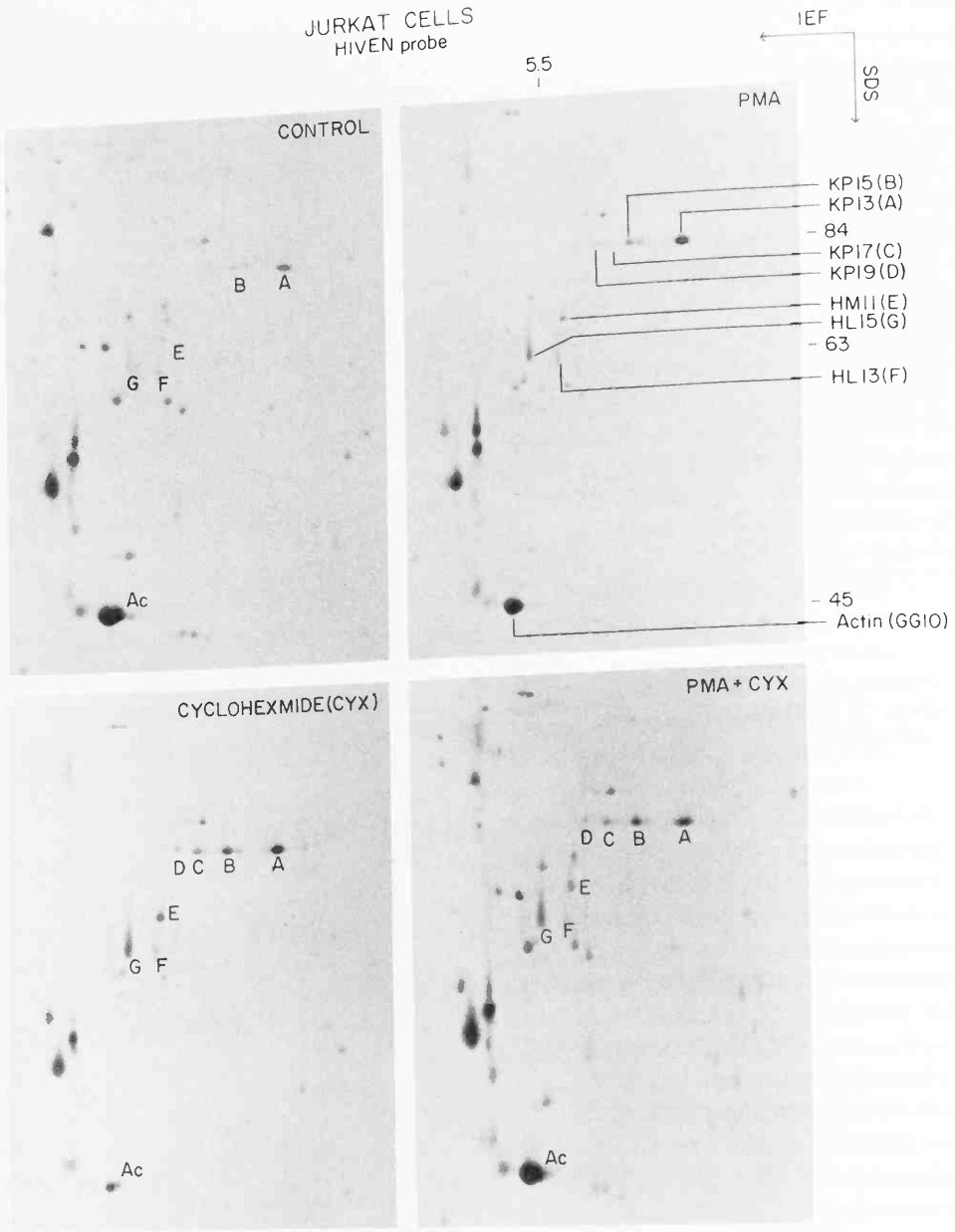

FIGURE 4 Induction of specific DNA-protein interactions by protein synthesis inhibitors. Jurkat T lymphoblasts were incubated with [^{35}S]methionine for 3.5 hr. The cells were then placed in normal growth media to which either nothing (*A*), PMA (*B*), cycloheximide (*C*), or PMA + cycloheximide (*D*) was added. After 90 min, the cells were harvested and lysed, and DNAP assays were performed. The oligonucleotide probe used for these studies represents the region in the HIV-1 LTR between −106 and −80 (direct repeat region).

A HUMAN RETROVIRAL *TRANS*-ACTIVATOR ALTERS THE INTERACTION OF HIVEN86A (KP SERIES) WITH DIFFERENT CONTROL ELEMENTS

In collaboration with Warner Greene and colleagues at Duke University Medical School, we have investigated the effect constitutive production of HTLV-I Tax protein has on gene expression. Using Jurkat T cell lines that express the 40-kD Tax protein, Greene et al. demonstrated deregulated expression of the IL-2Rα receptor gene. The sequence element required for this effect was demonstrated to be structurally similar to the 10-bp direct repeat element in the HIV-1 enhancer. As shown in Figure 5, the binding of HIVEN86A to this region of the IL-2Rα

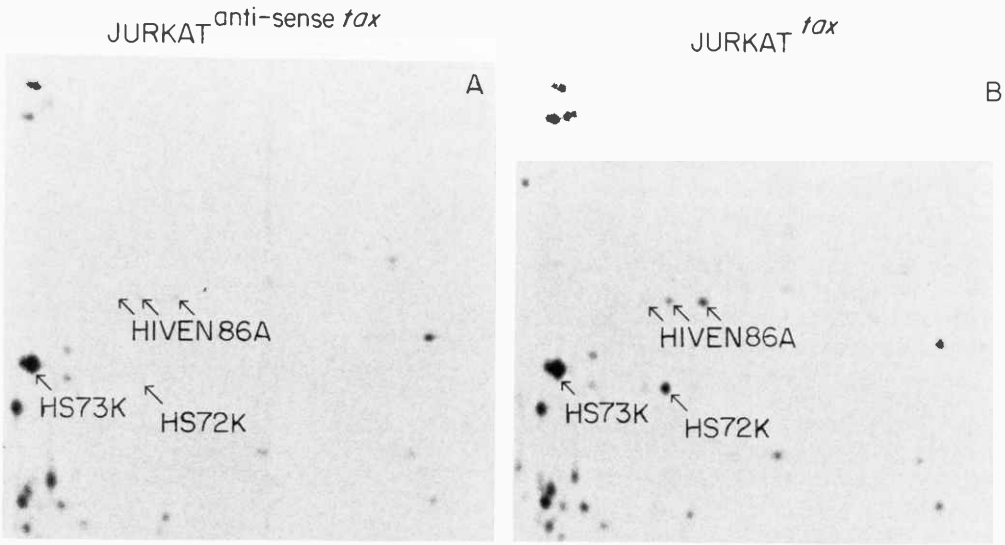

FIGURE 5 Microscale DNA-affinity precipitation assay of Tax-induced cellular DNA-binding proteins that interact with the promoter. Biotinylated IL-2Rα oligonucleotide DNA representing bases +291 to +245 of the IL-2Rα promoter was incubated with [^{35}S]methionine-labeled nuclear proteins isolated from J-anti-*tax*-10 (*A*) or J-*tax*-9 (*B*), followed by DNA-protein complex precipitation with avidin-agarose and two-dimensional gel electrophoresis. The different isoelectric forms of the HIVEN86A protein and two heat-shock proteins (HS72K and HS73K) are identified by arrows. HS73K is coprecipitated in these assays independent of Tax expression and DNA probe sequence; HS72K represents a Tax-inducible protein with apparently nonspecific DNA-binding properties.

promoter is quantitatively greater in the Tax-expressing Jurkat cells compared to similar numbers of Jurkat cells that contain an antisense *tax* gene construct. A similar increase is detected when an oligonucleotide representing the HIV-1 enhancer is used as the probe in the DNAP assay. It may be that the deregulated expression of IL-2Rα gene expression encountered in HTLV-I leukemias is to some extent the result of Tax alteration in the availability of proteins like HIVEN86A for interaction with control elements in certain genes.

The systematic application of the microscale DNA-affinity precipitation assay and immunoprecipitation assays in conjunction with high-resolution two-dimensional gels has permitted rapid, comprehensive assessment of protein-DNA interactions and the identification of specific members of the protein complexes that associate with transcriptional control elements. It is expected that sustained application of this strategy will expand our knowledge of the proteins involved in the control of cellular gene expression.

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MOLECULAR BIOLOGY OF THE CYTOSKELETON

D.M. Helfman S. Cheley, S. Erster, K.I. Galactionov, L. Goodwin, J.P. Lees-Miller, L.A. Finn, W.M. Ricci, R.F. Roscigno, K.S. Weber, A. Yan

Our research has continued to focus on the structure, expression, regulation, and function of the genes encoding tropomyosin in muscle and nonmuscle cells. Tropomyosins are important elements of the contractile systems of skeletal, cardiac, and smooth muscle cells and nonmuscle cells. Although they are expressed in all cells, different isoforms of the protein are characteristic of specific cell types. The generation of tropomyosin isoform diversity involves the expression of multiple genes, some of which encode multiple isoforms via tissue-specific alternative RNA processing. During the past year, we have completed much of the structural analysis of the genes that express muscle and nonmuscle tropomyosins. This work has demonstrated that the rat genome contains three functional genes that express at least 12 distinct tropomyosin isoforms. One gene encodes rat fibroblast tropomyosin-1 (TM-1) and skeletal muscle

β-tropomyosin. A second gene encodes skeletal muscle and smooth muscle α-tropomyosins and at least seven other distinct tropomyosin isoforms, including four isoforms expressed in fibroblasts (TM-2, TM-3, TM-5a, and TM-5b) and three isoforms unique to brain tissue. Interestingly, this gene was also found to contain two alternative promoters that contribute to the multiplicity of isoforms expressed from this gene. The third gene that we have characterized appears to be somewhat unique in that it expresses only a single isoform, namely, rat fibroblast TM-4. The molecular basis for the generation of tropomyosin isoform diversity by alternative RNA processing is not known and constitutes one major area of our research. Another interest of our laboratory has been to study the function and expression of tropomyosin in normal and transformed fibroblasts. Below is a summary of our present studies.

Studies of Alternative Splicing of Tropomyosin Pre-mRNAs

D.M. Helfman, L.A. Finn, W.M. Ricci,
R.F. Roscigno, K.S. Weber

The generation of protein isoform diversity by alternative RNA splicing is a fundamental mechanism of eukaryotic gene expression, which contributes to tissue-specific and developmentally regulated patterns of gene expression. Alternative RNA splicing pathways have also been demonstrated for a number of viral genes. At present, little is known about the mechanisms that determine alternative RNA splicing of complex transcription units. In particular, it is not known how alternative splice sites are selected and whether the splicing signals in complex transcription units differ from those in simple transcription units. The identified sequence elements required for pre-mRNA splicing include the consensus sequences found at the 5′ and 3′ splice sites and lariat branchpoints. Sequence comparisons between splice junctions of alternative and constitutive exons have failed to identify any significant differences, suggesting that these sequences alone do not account for the choice between alternative splice sites. It seems likely that regulation of splice-site selection in transcripts containing alternative 5′ or 3′ splice sites will involve other *cis*-acting elements. In addition, a number of studies have suggested the existence of *trans*-acting factors that interact with sequence elements in the pre-mRNA to promote differential splice-site selection. However, the identity of the *trans*-acting factors and the signals they recognize remain unknown.

We have been using the rat TM-1 gene as a model to investigate the mechanism of a type of developmental and tissue-specific alternative splicing. This gene is composed of 11 exons (Fig. 1). Exons 1 through 5 and 8 and 9 are common to all 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 the present studies, we have focused on the mutually exclusive internal alternative splice choice involving exon 6 (fibroblast-type splice) and exon 7 (skeletal-muscle-type splice). Our previous studies of tropomyosin pre-mRNA splicing revealed an ordered pathway of splicing in which either internal alternatively spliced exon must first be joined to the downstream common exon before it can be spliced to the upstream common exon (Helfman et al., *Genes and Dev. 2:* 1627 [1988]). These studies demonstrated that splicing of exon 5 to exon 6 (fibroblast-type splice) and exon 5 to exon 7 (skeletal-muscle-type splice) was dependent on precursors in which exon 6 or exon 7 was first joined to exon 8.

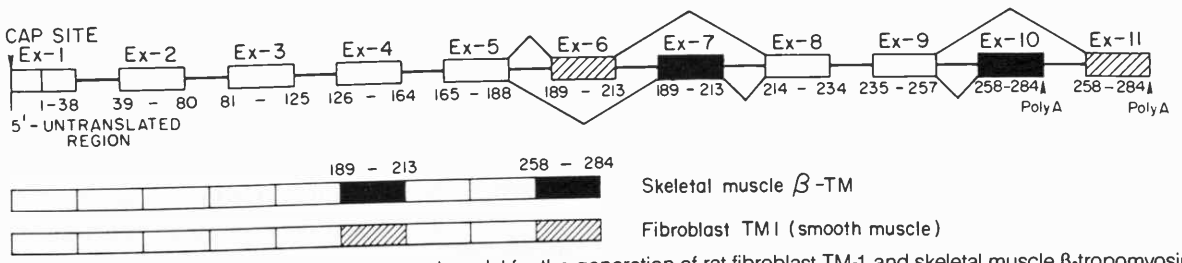

FIGURE 1 Schematic diagram of the gene and model for the generation of rat fibroblast TM-1 and skeletal muscle β-tropomyosin mRNAs by alternative RNA splicing. Open boxes represent common exons, hatched boxes represent fibroblast or smooth muscle exons, closed boxes represent skeletal muscle exons, and horizontal lines represent introns; they are not drawn to scale. The amino acids encoded by each exon are indicated. The cap site and polyadenylation signal AATAAA are also indicated.

The data are consistent with a model in which the critical event in alternative splicing occurs during the joining of exon 6 to exon 8 (fibroblast-type splice) or exon 7 to exon 8 (skeletal-muscle-type splice).

To study further the mechanism and regulation of alternative splice-site selection, we have characterized the branchpoints used in processing tropomyosin pre-mRNAs in vitro using nuclear extracts obtained from HeLa cells. Splicing of exon 5 to exon 6 (fibroblast-type splice) involves the use of three branchpoints located 25, 29, and 36 nucleotides upstream of the 3′ splice site of exon 6. Splicing of exon 6 (fibroblast-type splice) or exon 7 (skeletal-muscle-type splice) to exon 8 involves the use of the same branchpoint located 24 nucleotides upstream of this shared 3′ splice site. In contrast, the splicing of exon 5 to exon 7 (skeletal-muscle-type splice) involves the use of three branchpoints located 144, 147, and 153 nucleotides upstream of the 3′ splice site of exon 7. These results are in contrast to the majority of introns that have been studied in which only a single adenosine residue, located within 18–40 nucleotides from the 3′ splice site, is utilized during lariat formation. These studies raise the possibility that the use of branchpoints located a long distance from a 3′ splice site may be an essential feature of some alternatively spliced exons.

We have begun to study the role of these unusual branchpoints located upstream of exon 7. Deletion analysis of sequences between these branchpoints and the 3′ splice site of exon 7 revealed that these intron sequences were not necessary for utilization of this 3′ splice site in vitro. However, these sequences were found to play an important role in alternative splice-site selection. Transfection of a wild-type minigene into HeLa cells containing exons 5 through 9 results in spliced RNA containing only exons 5+6+8+9 (fibroblast-type splice). Interestingly, deletion of sequences between the 3′ splice site of exon 7 and the upstream branchpoints resulted predominantly in spliced RNA containing exons 5+7+8+9 (skeletal-muscle-type splice). Thus, intron sequences upstream of exon 7 contain an important *cis*-acting element involved in alternative splice-site selection. Computer analysis of these regions revealed that intron sequences upstream and downstream from exon 7 can form stable secondary structures that would sequester this exon and prevent its utilization. Thus, a simple mechanism may account for the fact that exon 7 is not used in nonmuscle cells (e.g., HeLa and rat fibroblasts), because it is sequestered and unable to interact with the splicing machinery due to RNA folding. Furthermore, specific sequences within introns could interact with tissue-specific factors that stabilize or destabilize (e.g., an RNA helicase) the formation of RNA secondary structures, leading to alternative splice-site selection. In addition, alternative splicing may not be the result of tissue-specific splicing factors that recognize specific 5′ and/or 3′ splice sites directly, but rather through tissue-specific factors that control the interaction of a general splicing factor(s) with the alternative splice sites. It is interesting to note that we have demonstrated efficient splicing of exon 5 to exon 7 and exon 7 to exon 8 (skeletal-muscle-type splices) in HeLa cell nuclear extracts using precursors that lacked downstream and upstream intron sequences, respectively. Thus, utilization of the 5′ and 3′ splice sites of exon 7 does not have an absolute requirement for skeletal-muscle-specific factors. In addition, the mechanism by which splicing of exon 5 to exon 6 or exon 7 is dependent on these exons being joined to exon 8 is not yet known. One possibility is that joining together two exons and thereby removing a flanking intron may prevent the formation of RNA structures that interfere with the interaction of a given 5′ or 3′ splice site with the splicing machinery. Work is currently under way to explore further the role of specific intron sequences in alternative splice-site selection and to identify tissue-specific factors that may interact with specific regions of the pre-mRNA to generate alternative RNA splicing.

Sequence Analysis of the Rat TM-1

S. Erster, L.A. Finn, D.M. Helfman

We have determined the sequence of the entire rat TM-1 gene. Our sequence data extend from approximately 600 bp upstream of the cap site to approximately 600 bp downstream from the polyadenylation signal of exon 11. Characterization of the fibroblast TM-1 and skeletal muscle β-tropomyosin cDNAs derived from this gene, as well as the promoter region and the intron/exon borders, has been described previously (Helfman et al., *Mol. Cell. Biol. 6:* 3582 [1986]). The sequence analysis confirmed our previous observations, based on nuclease protection and Northern analyses, that only two mRNAs are expressed from the TM-1 gene. This gene is far less complex than the gene that encodes the

rat skeletal muscle α-tropomyosin, as the latter gene contains two alternate exons encoding amino acids 39–80 and four alternate exons encoding the carboxy-terminal region (compare Figs. 1 and 2). By comparison, it has recently been determined that the chicken gene that encodes chick fibroblast TM-1 and skeletal muscle β-tropomyosin also encodes a low-molecular-weight tropomyosin isoform via an alternate internal promoter. Thus far, we have found no evidence of such a promoter in the rat TM-1 gene. Our current hypothesis is that in rat, unlike chicken, the low-molecular-weight β-type tropomyosin is the product of a separate gene, which encodes rat fibroblast TM-4 (see below).

Another feature of the rat TM-1 gene is the presence of long AC dinucleotide repeats in introns 1 and 9. Repeats consisting of alternating purines and pyrimidines $(RY)_n$ are abundant in eukaryotic genomes. These sequences have the potential to assume a left-handed Z-DNA conformation and have been shown to have positive and negative effects on transcription. The presence of these repeats is also associated with increased recombination frequencies. Sequence analysis of several gene families, such as globin and the immunoglobulins, has demonstrated that these repeats are often the endpoints of substitution and insertion/deletion events, which may play a role in gene conversion. This observation is of note because the alternating purine and pyrimidine repeats that are located in introns 1 and 9 in the TM-1 gene correspond to positions in the rat α-tropomyosin gene, where there are additional promoters and exons that are missing from the TM-1 gene (compare Figs. 1 and 2).

The DNA sequence information is being used to search for potential regulatory elements involved in tissue-specific RNA processing. For example, using computer programs, we are searching for possible RNA secondary structures in defined regions of the pre-mRNA that may be involved in splice-site selection. We have examined the nucleotide sequences in the region of the alternative 3′ splice sites and polyadenylation sites and have determined that the more distal fibroblast-specific exon, which is utilized in all smooth muscle and nonmuscle cells, is more homologous to the consensus splice-site sequences than

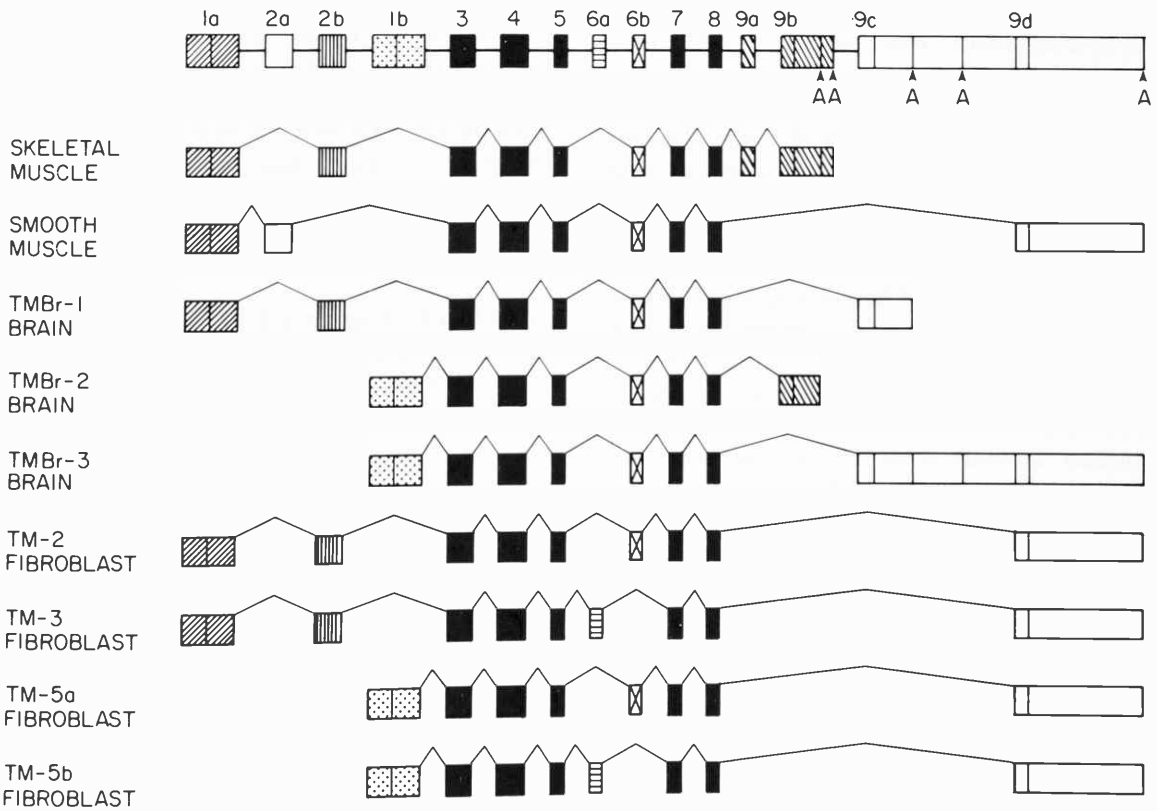

FIGURE 2 Schematic diagram of the α-tropomyosin gene and model for the generation of muscle (skeletal and smooth) and nonmuscle isoforms. Boxes represent exons and horizontal lines represent introns; they are not drawn to scale. The different polyadenylation signals are also indicated (*A*). For an explanation, see text.

those derived for the skeletal muscle 3′ splice site. We have also detected purine-rich and pyrimidine-rich regions immediately upstream of the skeletal-muscle-specific exon, which may interfere with utilization of this exon by the formation of stable secondary structures (see below).

In Vivo Analysis of Tropomyosin Pre-mRNA 3′-end Processing

S. Erster, L.A. Finn, D.M. Helfman

Tropomyosin pre-mRNA 3′-end processing requires alternative exon selection and utilization of the appropriate poly(A) site (Fig. 1). At present, it is not known if the 3′-end processing involves tissue-specific splice-site selection and/or tissue-specific polyadenylation. We have studied these events by transient expression of 3′-end minigenes in cultured cells, followed by nuclease protection and primer extension analyses. In COS cells, we detect splicing of the constitutive exon 9 to both alternate exons 10 and 11, along with cleavage at both poly(A) sites, with a preference for the fibroblast-specific pathway. Mouse 3T3 fibroblasts appear to utilize the fibroblast-specific exon 11, and mouse C2 myotubes use the skeletal-muscle-specific exon 10, suggesting that these minigenes are processed in a tissue-specific fashion in these cell lines. To identify *cis*-acting elements involved in splice-site selection, we have constructed a number of minigenes containing mutations in defined regions. We have found a region in intron 9 that when deleted results in the use of the skeletal-muscle-specific exon 10 in 3T3 fibroblasts. Thus, intron sequences upstream of exon 10 appear to contain an important *cis*-acting element involved in splice-site selection. It is possible that in fibroblasts, the tropomyosin pre-mRNA assumes a secondary structure in which the skeletal-muscle-specific exon is inaccessible to the splicing machinery and that our deletions have disrupted this structure such that the skeletal-muscle-specific exon can now be utilized. As mentioned above, the region just upstream of the skeletal-muscle-specific exon contains a purine-rich region followed by a pyrimidine-rich region. Interestingly, this region is extremely homologous in sequence and putative secondary structure to the same region of the chicken TM-1 gene. Deletions and mutations in this and other regions will hopefully allow us to define further the *cis*-acting sequences involved in the regulation of tropomyosin pre-mRNA alternative processing.

Structural Analysis of the Gene Encoding Rat Fibroblast TM-4

J.P. Lees-Miller, A. Yan, D.M. Helfman

We have determined the complete nucleotide sequence of the gene encoding rat fibroblast TM-4. The 18-kb sequence included 1600 bp of the 5′-untranslated region and 200 bp 3′ to the poly(A) cleavage site. The gene was found to contain eight exons. Thus far, we have been unable to obtain any evidence that this gene expresses more than a single form of tropomyosin. For example, we did not find any sequences within the introns of the TM-4 gene encoding potential novel exons that would indicate a potential alternative splice. Further experiments using labeled intron sequences as Northern probes, or RNase protection analysis with a full-length TM-4 antisense message, were also negative with RNAs from a wide variety of tissue sources, including slow-twitch and fast-twitch skeletal muscle and cardiac muscle. It is therefore unlikely that the rat TM-4 gene is alternatively spliced. Preliminary primer-extension experiments aimed at localizing the TM-4 gene transcription initiation site indicate that it is 120 bp upstream of the translation initiation site. This result will be confirmed with RNase protection. Future work on the TM-4 gene will be focused on determining what promoter and enhancer elements are responsible for its transcriptional regulation.

Structural Analysis of the α-Tropomyosin Gene and the mRNAs Expressed from It in the Brain

J.P. Lees-Miller, L. Goodwin, D.M. Helfman

The α-tropomyosin gene consists of 15 exons, 5 of which are common to all known mRNAs that are expressed from it (Fig. 2). The remaining exons include two exons (exons 1a and 1b) that are transcribed from separate promoters, two alternatively spliced internal cassettes (exons 2a or 2b and exons 6a or 6b), and four exons encoding different carboxy-terminal amino acids (exons 9a, 9b, 9c, and 9d). The various splice choices allow for the possible production of 24 proteins from the α-tropomyosin gene. At present, we are reasonably certain that at least nine of the possible isoforms are made (Fig.

2). These isoforms are distributed in a specific manner among tissues such as striated muscle, smooth muscle, kidney, liver, and brain. In addition, four fibroblast isoforms are expressed from this gene (see below).

We are particularly interested in three products that are expressed in brain. Three α-tropomyosin cDNAs (Ok4, 1.2 kb; Ok15, 1.1 kb; and Ok10, 2.8 kb) were isolated from a rat brain library. Ok4 and Ok10 possess a novel carboxy-terminal coding region that is specific to brain and correspond to TMBr-1 mRNA and TMBr-3 mRNA, respectively (Fig. 2). Multiple polyadenylation sites are associated with the use of this exon (exon 9c), resulting in the length difference of Ok4 and Ok10. The third cDNA clone we isolated (Ok15) contains a novel 5′ end that is encoded by a second α-tropomyosin gene promoter and a novel 3′ end, resulting from exon skipping of the striated muscle carboxy-terminal coding region, corresponding to TMBr-2 mRNA (Fig. 2). The exon skip causes a frameshift that bypasses the normal stop codon and radically alters the amino acid sequence, so that it is in no way similar to the known tropomyosin repeat pattern. Making use of a number of restriction sites, we have engineered Ok154, which contains the 5′-coding sequence of Ok15 (TMBr-2) and the 3′-coding sequence of Ok4 (TMBr-1). RNase protection experiments with antisense RNAs derived from each of Ok4, 15, and 154 demonstrated that they are all expressed specifically in brain.

We have also made full-length sense RNAs for each brain-specific cDNA and translated them in vitro with a rabbit reticulocyte lysate. We are in the process of characterizing the mobility of the resulting proteins by two-dimensional gel electrophoresis. We have also mapped and sequenced the exon/intron borders in the α-tropomyosin gene corresponding to the novel 5′- and 3′-coding regions that are found in Ok15 and Ok4, respectively (Fig. 2). This work includes the sequencing of 1400 bp upstream of the novel promoter and 2000 bp downstream from the 3′-coding region of Ok4 and Ok10. RNase protection and S1 analyses of the novel promoter indicate the presence of multiple transcription initiation sites from 140 to 230 bases upstream of the translation initiation site. This work will be confirmed with primer-extension analysis. In the future, we hope to determine the cell-type specificity of each tropomyosin isoform in the developing rat brain and in cultured nervous system cell lines. We also hope to elucidate some of the factors required for brain-cell-specific expression of the α-tropomyosin gene.

Four Rat Fibroblast Tropomyosins Are Expressed from a Single Gene via Alternative Splicing and Utilization of Two Promoters

L. Goodwin, S. Cheley, D.M. Helfman

Multiple forms of tropomyosins have been detected in many cultures of nonmuscle cells. For example, on the basis of two-dimensional gel analysis, it was reported previously that rat embryonic fibroblasts contain at least five tropomyosin isoforms: three major tropomyosins termed TM-1, TM-2, and TM-4, with apparent molecular weights of 40,000, 36,500, and 32,400, respectively, and two relatively minor tropomyosins, termed TM-3 and TM-5, with apparent molecular weights of 35,000 and 32,000, respectively (Matsumura et al., *J. Biol. Chem. 258:* 954 [1983]). Each isoform can be identified in cell-free translation products of fibroblast mRNA, indicating the existence of multiple mRNAs. We have previously described the isolation and characterization of full-length cDNA clones encoding rat fibroblast TM-1 and TM-4 (Yamawaki-Kataoka and Helfman, *J. Biol. Chem. 269:* 14,440 [1985]; Yamawaki-Kataoka and Helfman, *J. Biol. Chem. 262:* 10,791 [1986]). These studies revealed that these two isoforms are the products of two separate genes. The derived amino acid sequence of these cDNA clones revealed TM-1 contains 284 amino acids, whereas TM-4 contains 248 amino acids. In addition, we demonstrated that the same gene that encodes rat fibroblast TM-1 also encodes skeletal muscle β-tropomyosin via alternative RNA splicing and polyadenylation (Helfman et al., *Mol. Cell. Biol. 6:* 3582 [1986]).

As described above, we have characterized the gene encoding rat fibroblast TM-4 and have not found any evidence for the generation of additional isoforms from this gene via alternative RNA processing. During the past year, we have isolated and characterized full-length cDNA clones to four additional tropomyosin isoforms expressed in rat fibroblasts, termed TM-2, TM-3, TM-5a, and TM-5b. We have determined that these four isoforms are expressed from a single gene by alternative splicing and utilization of two alternate promoters (Fig. 2). TM-2 and TM-3 are expressed from the upstream promoter, and TM-5a and TM-5b are transcribed from an internal promoter. TM-2 and TM-3 both contain 284 amino acids and differ at an internal region of the protein from amino acid 189 to 213,

due to alternative splicing of exons 6a and 6b (Fig. 2). TM-5a and TM-5b both contain 248 amino acids and differ at an internal region due to alternative splicing of exons 6a and 6b (Fig. 2). The functional significance of these four related isoforms is unknown and is currently under study (see below). The generation of these four isoforms from a single gene raises a number of interesting questions concerning the expression of these isoforms. For example, two-dimensional gel analysis of the tropomyosins expressed in cell lines transformed with Kirsten virus (NRK 1569 cells) and Rous sarcoma virus (NRK 4/435) demonstrated that these cells do not express TM-2 and TM-3 but do express TM-5a and TM-5b. Using probes derived from the full-length cDNA clones, we have established that the absence of TM-2 and TM-3 proteins in these transformed cells correlates with a corresponding lack of mRNA for these isoforms. At present, we do not know if the lack of detectable mRNA encoding TM-2 and TM-3 in these transformed cells is due to inhibition of transcription from the upstream promoter of this gene or by another mechanism, e.g., changes in nuclear transport, splicing, or mRNA stability. In the future, we will examine the effect of various nuclear and cytoplasmic oncogenes on tropomyosin expression to determine if there are alterations in transcription, processing, and translation of tropomyosin mRNAs in transformed cells.

Expression and Function of Tropomyosin in Normal and Transformed Cells

L. Goodwin, D.M. Helfman

Comparative two-dimensional protein gel analysis of normal and transformed cells has revealed that, of the numerous cytoskeletal proteins, tropomyosin expression is selectively altered in transformed cells. In general, these studies demonstrate that in transformed cells, the levels of one or more of the major tropomyosin isoforms of higher molecular weight are decreased or missing, whereas the levels of one or more of the lower-molecular-weight tropomyosin isoforms are increased. The alterations in tropomyosin synthesis have been reported to occur in cells transformed by a variety of agents, including chemical carcinogens, UV radiation, and DNA and RNA tumor viruses. Moreover, the changes in tropomyosin expression following transformation occur in cells of all species examined, including chicken, rodents (mouse and rat), and humans. Collectively, these results indicate that alterations in tropomyosin expression are common features of the transformed phenotype and that tropomyosin genes may represent a target for oncogene action.

As described above, rat fibroblasts transformed by Rous sarcoma virus or Kirsten virus (analyzed by two-dimensional protein gel) show a complete loss of expression of TM-2 and TM-3 concomitant with an increase in the levels of TM-5a and TM-5b expression. In addition, these alterations in tropomyosin expression appear to correlate well with the rearrangement of microfilament bundles and the morphological alterations observed in transformed cells. To determine the functional significance of the altered pattern of tropomyosin isoform expression in transformed cells, tropomyosin isoforms (i.e., TM-2 and TM-3) will be introduced into living cells by microinjection of RNA or protein and via expression of cloned cDNAs using eukaryotic expression vectors. In this manner, we hope to address several questions. For example, will forced expression of specific tropomyosin isoforms in transformed cells result in the reorganization of the microfilament system and changes in morphology?

To synthesize biologically active mRNAs encoding specific tropomyosin isoforms, the full-length cDNAs encoding TM-1, TM-2, TM-3, TM-4, TM-5a, and TM-5b were subcloned into SP6 plasmids. Direct purification of individual tropomyosin isoforms from rat fibroblasts has been difficult using conventional purification methods, which is likely due to the fact that the different protein isoforms have such similar physiochemical properties. To prepare each of the fibroblast tropomyosin isoforms (TM-1, TM-2, TM-3, TM-4, TM-5a, and TM-5b), we have utilized a plasmid-cloning system that allows for the production of the protein in *Escherichia coli*. Using this system, we will prepare homogeneous preparations of each of the isoforms. We have already prepared large quantities of TM-2 from *E. coli* for these studies. We are also interested in examining the localization of the various tropomyosin isoforms in normal cells. To this end, we will inject the individually fluorochrome-labeled protein species into living cells and follow the dynamic subcellular distribution and incorporation into the microfilaments. In conjunction, we plan to prepare isoform- specific antibodies to determine the patterns of localization of each isoform. The distribution of

various tropomyosin isoforms in the normal cell may allow insight into the role of tropomyosin in the cytoskeleton and thus how perturbations in the regulation of tropomyosin isoform synthesis contributes to the transformed phenotype.

Analysis of Tropomyosin Function in Adenovirus-transformed Rat Cells

K.I. Galactionov, D.M.Helfman

We have used an established rat fibroblast cell line (REF52 cells) and its adenovirus-transformed counterpart (Ad5D.4A) to examine the role of a major tropomyosin isoform in cytoskeleton organization and cell shape. Normal rat fibroblasts (e.g., REF52 cells) contain six different isoforms of tropomyosin. In the adenovirus-transformed cells (Ad5D.4A), only TM-1 is absent. It was previously shown that repression of TM-1 synthesis likely occurs at the transcriptional level (Yamawaki-Kataoka and Helfman, *J. Biol. Chem. 262:* 10791 [1987]). Neither the mechanism(s) responsible for repression of TM-1 synthesis in the adenovirus-transformed cells nor the specific function of TM-1 in normal cells is known. The absence of TM-1 protein in the Ad5D.4A cell line has been suggested to be responsible for the absence of microfilament bundles and the accompanying changes in cell morphology observed in these cells. In addition, transformation of REF52 cells with other oncogenic viruses (Kirsten, SV40, etc.) also decreases the level of TM-1 protein, but to still detectable levels. We wish to determine if the absence of the TM-1 isoform in the adenovirus-transformed cells is directly responsible for the changes in actin filament assembly and cell morphology characteristic of these cells.

The first goal of this study was to obtain stable adenovirus-transformed cells expressing TM-1 protein. To express the TM-1 isoform, we have used cDNA and genomic clones encoding this isoform and a number of eukaryotic expression plasmids. For transfection experiments, three types of constructs were made; two of them contain the *Drosophila* and human heat-shock promoters, respectively, and the third contains the SV40 early promoter. Tropomyosin cDNA containing sequences corresponding to the first nine exons was subcloned adjacent to the genomic 3′ end in order to obtain correct processing of the TM-1 transcript. These chimeric cDNA/genomic sequences were subcloned into plasmids containing the inducible (heat-shock) or constitutive (SV40) promoter regions. We have obtained stable transformants using cotransfection of TM-1 constructions and pKO*neo*, followed by selection with G418. Work is now under way to study the phenotype of transformants. We also wish to study the function of TM-1 in normal cells. In this case, transfection of REF52 cells with the TM-1 sequences in the antisense orientation was done using the same inducible and constitutive promoters. Studies are under way to determine the changes in TM-1 expression in these cells and possible changes in the microfilament structures and cell shape. Finally, we plan to study the function of TM-1 in different cell types by microinjection of fluorescently tagged TM-1 molecules and determine its localization and possible rearrangements during the cell cycle and following serum stimulation. For this purpose, we are using a plasmid-cloning system that allows for the production of TM-1 protein in *E. coli*.

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MAMMALIAN STRESS RESPONSE

W.J. Welch A.P. Arrigo R. Beckmann N.J.C. Lamb L.A. Mizzen K.T. Riabowol J.P. Suhan L. Cipp

In addition to our studies on the physiology of the stressed cell and the biochemistry of the individual stress proteins, we are beginning to pursue new areas of research regarding the mammalian stress response. Specifically, it has become quite evident that the stress response is associated with a number of diseases in animals and may prove to have valuable diagnostic potential. For example, it is now well established that a stress response accompanies many viral infections as well as a variety of tissue and organ traumas such as stroke and heart attack. Finally, autoantibodies to certain stress proteins may underlie the severe pathological abnormalities seen in some patients with autoimmune diseases. Moreover, immunologists are now beginning to observe that one or more of the stress proteins may be directly involved in the immune response and that the febrile response, in general, may serve to augment the activities of the immune system.

At the cellular level, new data continue to implicate a role for stress proteins in a number of cellular processes including the regulation of steroid receptors, various protein kinases, and finally mechanisms by which proteins are assembled into larger macromolecular complexes. Considerable excitement continues to grow concerning the role for many of the stress patterns in the pathways by which proteins are translocated across intracellular membranes and properly targeted to their final destination within the cell. Consequently, we have experienced a tremendous increase in the number of laboratories and investigators who are beginning to focus their attention on the physiology of the stressed cell, the biochemistry of the individual stress proteins, and the medical applications of the stress response in terms of both diagnostic and therapeutic routes.

Identification and Characterization of Two Stress Proteins Present within the Mitochondria

L.A. Mizzen, W.J. Welch [in collaboration with W.C. Chang and J. Garrels, Cold Spring Harbor Laboratory]

During the past year, we have identified, characterized, and purified two previously unrecognized stress proteins that are components of the mitochondria. The first, heat-shock protein (hsp) 58-kD, increases in cells subjected to heat shock or a number of other agents, such as heavy metals that similarly induce a heat-shock-like response. The second, glucose-regulated protein (grp) 75-kD, is a member of the so-called glucose-regulated stress protein family. This family of proteins (grp 75-kD, grp 80-kD, and grp 100-kD) exhibits increased synthesis in cells deprived of glucose, calcium, or oxygen or in response to agents that perturb the ability of the cell to maintain homeostatic calcium levels. We have observed that hsp58 and grp75, like most mitochondrial proteins, are synthesized initially as precursors containing an amino-terminal extension that apparently serves as a "signal sequence," directing their import into the mitochondria. Biochemical studies, in particular exposure of purified mitochondria to various proteolytic enzymes, indicate that both hsp58 and grp75 are present within the mitochondria and not exposed to the cytosolic compartment. Both immunological and biochemical data have demonstrated that the grp75 protein represents yet another member of the hsp70 family of stress proteins. The other members of this family appear to be involved in the ATP-dependent and transient interaction with a number of other cellular proteins, presumably facilitating their final three-dimensional confirmation. Consequently, we suspect that grp75 similarly serves a role in the folding and unfolding of target proteins but within its own compartment, the mitochondria.

The hsp58 protein appears to be homologous to the previously described bacterial and plant proteins referred to as "chaperonins." The chaperonins have been shown to facilitate the assembly of monomeric proteins into larger macromolecular complexes. Thus, again by analogy, we are examining whether hsp58 similarly serves in a catalytic manner to assemble proteins with its own compartment, the mitochondria. After induction of the stress response, we suspect that the cell, in the process of replacing mitochondrial proteins that have become denatured, expresses higher levels of these two mitochondri-

al proteins to facilitate the assembly of new protein complexes in the mitochondria and thereby provide for the restoration of proper mitochondrial function.

70-kD Stress Proteins Participate in a Variety of Cellular Processes

W.J. Welch [in collaboration with E. White and D. Spector, Cold Spring Harbor Laboratory; W. Farrar, National Institutes of Health; D. Toft, Mayo Clinic; K. Milarski and R. Morimoto, Northwestern University; and N. Spector, Dana Farber Cancer Institute]

As we examine the structure and function of the hsp70 stress proteins, we are continuing to find new aspects concerning the role of these proteins in a diverse number of different biological systems. As was discussed in last year's Annual Report, many viral infections often result in the increased expression of the 70-kD stress proteins. Moreover, in many cases, a number of virus-encoded proteins involved in the transformation process interact with the hsp70 proteins. In a collaborative study with E. White and D. Spector (see Cell Biology of the Nucleus, this section), we found an increased expression of the 70-kD stress proteins in cells infected with adenovirus. Other laboratories have demonstrated that such increased expression is due to activation of the 70-kD gene by the adenovirus EIA protein. In addition, using a combination of immunological and biochemical methods, we found that the EIA protein and 72-kD stress protein are co-localized in the infected cell. At the present time, the significance of this finding with respect to the activity of the EIA protein remains unclear. Further studies are aimed at determining whether the association with the 72-kD stress protein serves in any way to stabilize and regulate the biochemical activity of the EIA protein (White et al., *J. Virol. 62:* 4153 [1988]).

Recent studies, in collaboration with David Toft at the Mayo Clinic, have demonstrated that many steroid receptors also appear to associate with two of the major stress proteins. Most steroid receptors exist as soluble moities within the cytoplasm and/or nucleus. Steroids diffuse across the plasma membrane, bind to their receptor, and thereby result in a change in receptor confirmation. The receptor, now in its "transformed" or activated state, will bind to its target genes and promote gene transcription. Our studies have shown that the steroid receptor, in its "nontransformed" or inactivated state, forms a complex with both the 90-kD and 70-kD stress proteins. Through its interaction with the 90-kD protein, it is thought that the steroid receptor protein is unable to bind to its target gene. Following binding of the steroid to its receptor, the 90-kD stress protein exits the complex, and the receptor, still in association with the 70-kD stress protein, subsequently binds to DNA and activates transcription. Studies are therefore in progress to dissect the role of both the 90-kD and 70-kD stress proteins in regulating and facilitating the action of the steroid receptor. The current hypothesis is that although the 90-kD protein prevents binding of the receptor to DNA in the absence of hormone, the 70-kD protein somehow facilitates a confirmational change in the activated receptor and thereby promotes or facilitates the binding of its receptor to its target gene (Kost et al., *Mol. Cell Biol.* [1989] submitted).

Yet another emerging area concerning a role for the 70-kD stress proteins involves mitogenesis and the cell cycle. In the case of human cells, one of the 70-kD stress proteins is synthesized at the G_1/S boundary in the normal unstressed cell. After its synthesis, the 70-kD protein again becomes complexed with a number of cellular proteins; the exact identity of such proteins is currently under investigation. Interestingly, the cellular complexes containing the 70-kD protein are observed to vary as a function of the cell cycle. Similar differences in complex formation are seen at different points of the cell cycle following heat shock. Consequently, we are trying to determine the exact identity of those proteins interacting with the 70-kD protein and the relevance of such interactions (Milarski et al. *J. Cell Biol.* [1989] in press).

Finally, in collaboration with a number of laboratories, we are beginning to see a role for the 70-kD stress proteins in immune cell function. Although too extensive to list here, one of our collaborative studies has shown that both mitogen and lymphokine stimulations of T lymphocytes result in the increased synthesis of both the 70-kD and 90-kD stress proteins (Ferris et al., *Proc. Natl. Acad. Sci. 11:* 3850 [1988]). Moreover, these treatments also result in a rapid and significant increased phosphorylation of the low-molecular-mass 28-kD stress protein. The activation of B lymphocytes by bacterial endotoxins similarly results in the increased expression of the 70-kD and 90-kD stress proteins (in collaboration with N. Spector and L. Adler, Dana Farber Cancer Institute). Interestingly, once acti-

vated, the B cells now appear to be more resistant to thermal stress than do their resting counterparts.

Role of the Heat Shock or Stress Response in Human Disease and Medicine

W.J. Welch [in collaboration with J. Winfield, University of North Carolina, Chapel Hill; M. Tytell, Wake Forest University; T. Nowak, National Institutes of Health]

Because the heat shock or stress response appears to serve in the defense of cells to changes in its environmental circumstance, it is not entirely surprising that the response may be implicated in a number of important diseases. Indeed, a number of recent studies have demonstrated that tissue and organ traumas are accompanied by induction of the stress response and that the stress proteins themselves may play a central role in the repair of lesions incurred due to the trauma. For example, we have found that transient ischemic episodes in the brain (the basis of stroke) result in the activation of the stress response in some but not all areas of the brain. More interesting is the fact that those areas within the brain that survive the trauma exhibit the highest induction of the stress proteins, whereas those areas that are unable to survive show little or no induction of the stress response. Therefore, using our battery of antibodies to the stress proteins in immunocytochemical studies, a number of investigators are examining which regions within the brain show an induction of the stress response and whether such induction (or lack thereof) is associated with the survival (nonsurvival) of the cells following trauma (Vass et al., *Acta Neuropathol. 77:* 128 [1988]).

In a collaborative effort with M. Tytell's laboratory at Wake Forest University, a similar study examining light damage in the rat retina has revealed some exciting results concerning the potential therapeutic use of the stress response. During the past 15 years, it has been well established that cells in tissue culture can be made resistant to thermal killing simply by a prior exposure of the cells to a nonlethal heat-shock treatment. This phenomenon, referred to as thermotolerance, is transient with the decay of the tolerant state correlated with the turnover of the major stress-induced protein, 72 kD. Tytell's laboratory has now demonstrated an exciting potential use of thermotolerance in the whole animal. Specifically, their laboratory has been examining photoreceptor degeneration in the retina of rats following exposure to bright light. After exposure to intense light, rats are sacrificed 2 weeks later, and the degree of photoreceptor cell damage is ascertained. Normally, after an intense light exposure, greater than 90% of the photoreceptor cells are observed to die. If, however, the animals are first made thermotolerant (by prior exposure in a warm chamber in which body temperature is increased to 41–42°C), photoreceptor damage due to the same light exposure is significantly reduced. Moreover, by examining the levels of the 70-kD stress protein that accumulate in the retina of animals made thermotolerant, a direct correlation was observed with respect to the levels of the protein and the light protection observed. These studies then indicate that our ability to manipulate the levels of the stress proteins in the animal may prove useful in protecting tissues and organs after physical injury, disease, and/or exposure to environmental insults (Barbe et al., *Science 241:* 1817 [1988]).

Finally, in the area of infectious diseases, a number of reports have demonstrated that certain immunodominant proteins of infectious microorganisms (*Plasmodium falciparum*, trypanosomes, mycobacterium tuberculosis, *Leishmania*) are homologs of the stress proteins of eukaryotes. Often, the individual so infected mounts an immune response against the parasite forms of the stress proteins, and later in life, the individual develops a severe autoimmune disease. Consequently, it has been proposed that the basis of this autoimmunity is due to the production of autoantibodies against the individual's own stress proteins. That this may indeed be the case is indicated by recent studies by John Winfield at the University of North Carolina in collaboration with our research group. Specifically, in individuals with systemic lupus erythematosus (SLE), autoantibodies to both the 70-kD and 90-kD stress proteins have been observed. Similarly, autoantibodies to the 70-kD stress protein have been found in sera from rheumatoid arthritis patients, another autoimmume disease (Minota et al., *J. Exp. Med. 168:* 1475 [1988]). Keeping in mind that such individuals also produce autoantibodies to other self-proteins, we still do not know whether those autoantibodies directed against the stress proteins are in fact the causative agent of the disease. Nor do we know how and why such autoantibodies to the stress protein arise. However, these and other results have generated considerable excitement among many immunologists, and we can

in the future expect new and exciting findings concerning the role of the stress response and stress proteins in the immune response.

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QUEST LABORATORY/TWO-DIMENSIONAL GEL BIOTECHNOLOGY RESOURCE

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B.R. Franza H. Sacco P. Myers S.-U. Din
J. Kos G. Mak T. Husain

The QUEST laboratory has completed a major phase of its development with the publication of the REF52 database as a series of three papers in the *Journal of Biological Chemistry*. The first paper describes in detail our methods of image analysis and provides important studies of the accuracy and completeness of our analysis. The second paper describes our methods of database construction and

presents the "core" of the REF52 database. In the third paper of the series, we present the results of our studies of growth-related protein changes in normal and virally transformed REF52 cells using the full power of the database. Highlights of this work are presented below.

We have entered a transitional phase during which we seek to upgrade our technologies. The limitations of the present system are in the speed of the computer analysis, the large amount of manual work that is still required, and most importantly, in the long-term standardization of the two-dimensional gel patterns. An upgrade to modern workstations will give us a 10- to 15-fold increase in computing speed, and straightforward software enhancements can eliminate several of the manual steps of image analysis.

The problem of two-dimensional gel standardization is an engineering problem beyond our scope at Cold Spring Harbor Laboratory, but several companies are now vigorously working in this area. We hope soon to replace our 10-year-old Gel Laboratory with a new laboratory based on standardized commercial equipment. The patterns obtained using such equipment should be obtainable in many other laboratories, making our databases much more valuable to the scientific community. To further this aim, Jim Garrels is spending a sabbatical period with the Millipore Corporation near Boston. From October 1988 through September 1989, he will be working 2 days per week on average at Millipore helping them to test and evaluate equipment and methods for standardized two-dimensional gel electrophoresis. The remainder of his time during the sabbatical period is devoted to the development of software for Sun workstations, to continuing development of databases, and to the planning of the QUEST facility of 1990.

Methods and Accuracy of Two-dimensional Gel Analysis

J.I. Garrels

The methods of computer analysis currently used in the QUEST facility have now been published in great detail (Garrels, *J. Biol. Chem. 264:* 5269 [1989]). Within this publication, analyses are reported that test the accuracy and reproducibility of the system. The method of fitting two-dimensional Gaussian curves to the spots of two-dimensional gel images has an average error per pixel of only 13%, whereas the integrated spot intensities are in error by less than 4% on average. Since every Gaussian curve that models a spot has a positive error over some pixels and a negative error over others, the errors of the integrated intensity are much less than the average pixel error.

More than 2000 spots were routinely detected in the gels analyzed for the REF52 database. Of these, more than 97% were matched between typical gels, with less than 1% error. The greatest amount of analysis time, and the greatest source of ambiguity, is in making the decisions about how to split misshapen spots and spot clusters. At present, most of this work is done manually, but in the future, we plan to use methods of automatic editing. The spot-splitting decisions for each new gel will be guided by the previous decisions made for gels already in the database.

To construct databases for the quantitative analysis of more than 2000 proteins per cell, we must realize that many important proteins are still missing from the analysis. The intensities of the proteins detected on a typical gel range from 4 parts per million (ppm) of the applied sample radioactivity to more than 20,000 ppm. To examine the numbers of proteins detected versus protein intensity, we constructed the histogram shown in Figure 1A. The distribution has a peak near 16 ppm, with a sharp drop in the number of proteins detected near the limit of sensitivity.

In Figure 1A, the numbers of low-intensity proteins are underestimated because many are obscured beneath larger proteins on the gel. To estimate the number of hidden proteins at each level of protein intensity, test patterns were created within the computer. For each class of protein intensities, 100 spots were added at random locations to a typical gel image, and after reanalysis of the image by the background subtraction and spot detection programs, the number of added spots that were detected was scored. The probability of spot detection for spots of each size class is shown in Figure 1B, and a corrected distribution of protein intensities is shown in Figure 1C. As many as 1400 proteins (with intensities above the limit of sensitivity) might be obscured beneath the 2000 most abundant proteins. Most importantly, the corrected distribution suggests that as we gain the ability to resolve and detect proteins of lower intensity, the number of detected proteins will continue to rise rapidly.

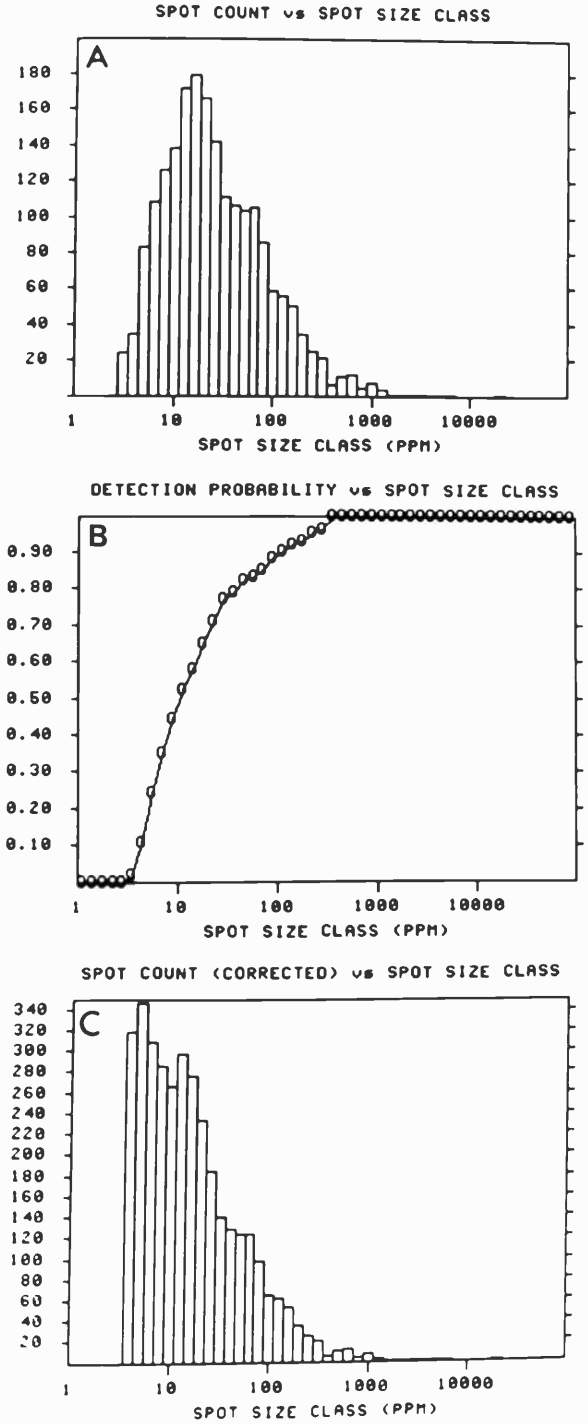

FIGURE 1 Number of protein spots detected versus spot size on a typical two-dimensional gel. (*A*) Number of spots versus spot size, before correction for hidden spots. (*B*) Probability of detection for spots in each size class, determined as described in text. (*C*) Estimated number of spots in each spot size class after correction for hidden spots. The hidden spots would have been visible had they not been obscured beneath other spots and background noise.

The Network Database Structure

J.I. Garrels, B.R. Franza

The methods for relating data from many quantitative two-dimensional gel experiments into a protein database, and the application of these methods to the REF52 protein database, have been fully described (Garrels and Franza, *J. Biol. Chem. 264:* 5283 [1989]). Gels from each experiment are matched as a group called a matchset. Within each matchset, every gel is matched to every other gel. Spots not detected in all gels are "added" to the gels from which they are missing, so that the coordinate positions of all spots are marked in all gels. (After repeated Gaussian fitting, some of the added spots are found to represent minor spots that were missed, and others are scored as undetected spots.) In this scheme, no particular gel need be designated as a standard.

Linker gels are used to connect the various experiments of the database. Any gel can become a linker gel simply by being included in more than one matchset, and the database grows as a network of linked matchsets. The system can find paths of gel-to-gel matches and linkers from one gel (such as one that a user might be viewing at a workstation screen) to any other gel in the database. Using the shortest available paths, and checking alternate pathways if available, the system can retrieve quantitative and annotative data for any gel of the database, relative to the spots of any other gel in the database. Examples of using the network database to recall spot names, standard spot numbers, and other information are shown in Figure 2.

One advantage of the network structure is that it allows each new matchset to be added to the database by linking to whatever previous gel pattern it is most closely related. Such a network structure can support quite large databases (eventually hundreds or thousands of gels) while keeping the average path length between any two gels relatively small. Moreover, the linker concept can be extended to the linking of databases developed for different gel systems or for different biological species. By choosing representative gels as linkers and by carefully matching as many of the spots as possible (given the full experience of the database developer), independent databases can be linked with respect to a substantial number of their proteins.

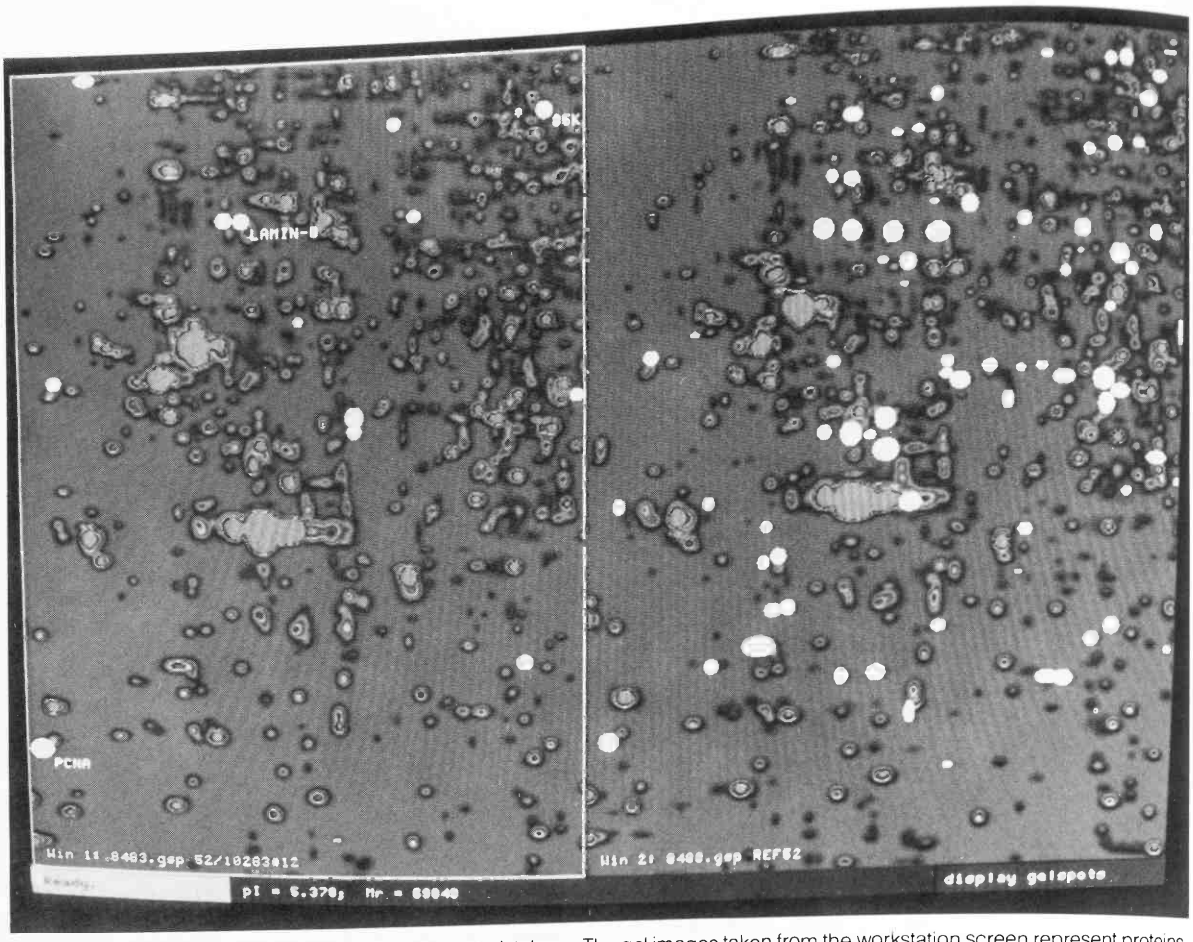

FIGURE 2 Examples of data from the REF52 network database. The gel images taken from the workstation screen represent proteins from REF52 cells at two different stages of growth. (*Right*) Highlighted spots are the nuclear proteins; (*left*) the highlighted spots are the proteins with PCNA-like regulation. It can be seen that many of the latter are nuclear. The names of proteins with PCNA-like regulation are shown at the left. For Lamin-B, the isoelectric point and apparent molecular weight are displayed at the bottom of the screen. Using the network database, quantitative and annotative data (including names, standard spot numbers, isoelectric point, molecular weight, and other annotations) can be entered relative to any gel and retrieved relative to any other gel in the database.

The REF52 Database

J.I. Garrels, B.R. Franza [and the entire QUEST laboratory]

Many results of the analysis of REF52 cells have been reported previously; however, the most important result has come only with the analysis of the complete database of normal and transformed REF52 cells using the latest tools we have developed for database analysis (Garrels and Franza, *J. Biol. Chem. 264:* 5299 [1989]). The proliferation- and transformation-sensitive nuclear protein PCNA was used as a model to search for other proteins that are altered in rate of synthesis both by proliferative stimuli and by transformation. The major properties of PCNA-like regulation, illustrated graphically in Figure 3A, are (1) enhanced rate of synthesis in SV40- and adenovirus-transformed cells at all cell densities, (2) decreased synthesis in normal REF52 cells at confluence, (3) sharply elevated synthesis after confluent REF52 cells are refed, (4) elevated synthesis at 24 hours, but not at 3 hours, after serum-deprived REF52 cells are fed with fresh serum, and (5) no suppression of synthesis in serum-deprived SV40- and adenovirus-transformed cells.

A new program was used to assign a score to each protein according to its degree of PCNA-like regulation, and a set of 26 proteins that meet all criteria were selected. Of these proteins, 14 are nuclear and 1 has been identified as the nuclear matrix protein lamin-B. The high percentage of nuclear proteins is a significant property of this set, since only 10% of all proteins on the REF52 protein map are nuclear. The most important property of this set is that nearly

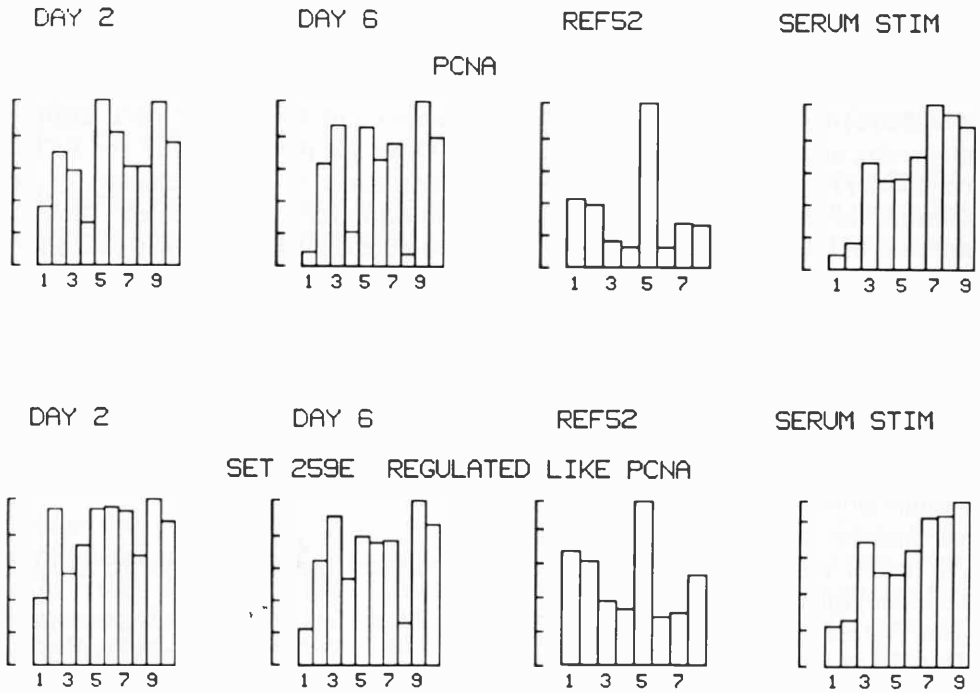

FIGURE 3 Patterns of regulation for PCNA and a set of coregulated proteins. (*Top*) Bar graphs for PCNA; (*bottom*) bar graphs showing the average quantitative behavior for a set of 26 proteins with PCNA-like regulation. Each bar represents data from one gel; the same gels are plotted on the top and bottom rows. (*Graph 1*, Day 2) Cells in log phase growth: REF52, six SV40 transformants, one Ki-MSV transformant, and two adenovirus transformants, respectively. (*Graph 2*, Day 6) Cells at confluence: REF52, six SV40 transformants, one Ki-MSV transformant, and two adenovirus transformants, respectively. (*Graph 3*, REF52) REF52 cells grown to confluence and refed. Cells were labeled at days 2, 3, 4, and 6 after plating (bars 1–4). Another culture of REF52 cells was labeled 15 hr after refeeding on day 6 (bar 5). Finally, three cultures of REF52 cells were grown for 12 days and were labeled with no refeeding (bar 6), at 5 hr after refeeding (bar 7), and at 15 hr after refeeding (bar 8). (*Graph 4*, Serum Stim) Response to feeding in serum-deprived cells. REF52 cells were deprived of serum for 60 hr and labeled without fresh serum (bar 1) or at 3 hr (bar 2) or at 21 hr (bar 3) after refeeding with fresh serum. SV40-transformed REF52 cells (bars 4–6) and adenovirus-transformed cells (bars 7–9) were similarly deprived of serum and refed. PCNA and the coregulated proteins are responsive to serum only in normal REF52 cells.

all members share a sixth property that was not used in their selection. In Kirsten murine sarcoma virus (Ki-MSV)-transformed REF52 cells, PCNA and the set of coregulated proteins are markedly repressed at confluence, just as in normal REF52 cells. This behavior is illustrated in Figure 3B, which depicts the average quantitative behavior for the set as a whole. It therefore appears that PCNA and a set of coregulated proteins are synthesized at high levels, uncoupled from the rate of proliferation, in cells transformed by DNA tumor viruses but that the coupling of synthesis to growth rate is normal in retrovirus-transformed cells. These results show that thorough analysis of a protein database can reveal sets of coordinately regulated proteins that (1) are involved in cell proliferation and (2) may have altered regulation in transformed cells.

The HeLa Database

C. Chang, H. Sacco, G. Mak, P. Myers, J.I. Garrels
[in collaboration with G. Morris and M. Mathews and
L. Mizzen and W. Welch, Cold Spring Harbor Laboratory]

A HeLa cell database has been targeted because it is a popular human cell line in which many biochemical studies have been carried out and because it is in use in several other laboratories at Cold Spring Harbor. The present HeLa cell database contains basic characterizations of protein turnover, phosphorylation and glycosylation, and subcellular fractionation. In particular, HeLa cells have been used for studies of the cell cycle, of heat shock, and of mitochondrial function.

A group of 85 proteins that show elevated synthesis in response to heat shock have been identified using the QUEST system. Another group of proteins have been identified as mitochondrial by the criterion that their processing into mature forms is blocked by the mitochondrial inhibitor nonactin. In pulse-chase experiments, these proteins fail to appear in the first 5 minutes, but they do appear during subsequent chase periods. In nonactin-treated cells, precursor forms with extra basic charge accumulate in the cytoplasm. One protein, hsp58, is both a heat-shock protein and a mitochondrial protein. In collaboration with L. Mizzen and W. Welch (now at the University of California, San Francisco), it was shown that this protein can be localized to mitochondria by immunofluorescence. Antibodies to the hsp58 protein cross-react with a *Tetrahymena* protein, which in turn is homologous to the bacterial heat-shock protein groEL. This information adds relevance to the HeLa database by identifying a mitochondrial protein thought to function as a "molecular chaperone."

The cell-cycle analysis of HeLa cells was performed in collaboration with G. Morris and M. Mathews. Examination of cells sorted by centrifugal elutriation showed remarkably few cell-cycle-specific differences. Of over 1500 proteins examined, only 6 were elevated at G_1 phase, 8 at S phase, and 6 at G_2 phase. PCNA was the most prominent among the proteins induced at S phase.

Experiments with Human HOS Cells

J.I. Garrels, C. Chang [in collaboration with C.C. Kumar, Schering Research]

HOS cells are human osteosarcoma-derived fibroblast cells. They provide a human cell culture that is analogous in several ways to our REF52 cell system. Normal HOS cells are flat and nontumorigenic. Transformed HOS cells have been derived using tumor viruses (Ki-MSV) and chemical carcinogens (MNNG). Revertants of the virus-transformed cells are also available.

The work of Dr. Kumar has shown that a gene for a smooth-muscle-specific myosin light chain is expressed in HOS cells and that the mRNA synthesis of this gene is turned off in transformed HOS cells. On two-dimensional gel patterns, the spots corresponding to myosin light chains have been identified with specific antibodies, and the disappearance of the smooth muscle form has been confirmed. Interestingly, in a revertant of the K-HOS cells, the smooth-muscle-specific form is again synthesized.

Other parallels exist with the REF52 database. Transformation-specific changes of tropomyosins and actins have been detected. The PCNA protein is elevated in the transformed cells and expressed at lower levels in the revertant. In the future, we plan to build a database for HOS cells so that these changes can be more fully explored. In particular, we would like to try to locate in human cells more of the proteins that were found to have PCNA-like regulation in REF52 cells.

Proteins from Transgenic Mouse Cells

J.I. Garrels, C. Chang, P. Myers [in collaboration with D. Hanahan, S. Baekkeskov, Hagedorn Research Laboratory, Gentofte, Denmark, and S. Grant and S. Efrat, Cold Spring Harbor Laboratory]

D. Hanahan's laboratory has used the QUEST facility extensively to analyze pancreatic islet cells from transgenic mice. By putting SV40 T antigen under control of the insulin or glucagon promoters, they have achieved cell-type-specific transformation of cells in vivo. Cell lines have also been derived from some of these tumor lines. We have helped these investigators to analyze their material on two-dimensional gels and to interpret their data in the light of our experience with normal and transformed rat cells.

The samples analyzed have been used to analyze both the developmental progress of pancreatic islet tissue and the progression of transformation in the islets. Of the proteins identified so far, two proteins identified as growth- and transformation-sensitive in rat REF52 cells (PCNA and a coregulated protein at 85 kD) were also found to be regulated by growth and transformation in mouse pancreatic islet cells. We plan to develop the database much further after our upgrade to new equipment is complete. The transgenic mouse database not only allows our collaborators to more fully investigate the effects of specific genes on development and transformation, but also gives us the opportunity to compare many of the changes already observed in SV40-transformed mammalian cell lines with the changes brought on by SV40 transformation in vivo.

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]

As described in our previous report, we aim to make the yeast database into a resource that can complement the genetic map and the DNA and protein sequence databases as a basic tool for the yeast community. We have already shown that we can compare experiments carried out with different laboratory strains, and a matchset containing gels from six common strains has been constructed as a linker. The commercial strains, on the other hand, have been found to be much less related, and it is not clear whether they can be linked in detail into the database of laboratory strains. If so, the results could be useful in the identification and characterization of commercial strains, which are resistant to conventional genetic analysis.

We have in the past year analyzed many of the key yeast experiments on three gel types. Although this entails extra effort (relative to the single gel type used for the REF52 database), it provides additional information (>3300 proteins scored). We have used a broad (pH 3.5–10, LKB ampholytes) pH range on 10% slab gels to resolve most proteins with a molecular weight above 20,000 and a narrow pH range (pH 4–8, BDH ampholytes) on 10% slab gels to resolve acidic proteins better. Additional basic and low-molecular-weight proteins are resolved on nonequilibrium gels run on 15% slab gels.

In an experiment to identify proteins coded from genes with introns, the *rna2* mutant was used at the permissive and nonpermissive temperatures. Of the well-resolved proteins on the gels (>1500 scored), only 45 were missing in *rna2* cells at the elevated temperature. A simultaneous study to score the effects of elevated temperature on normal yeast cells has revealed 62 proteins induced by threefold or more (heat shock) and 97 proteins inhibited by threefold or more (heat stroke). The knowledge of the heat-shock and heat-stroke responses was necessary to score the *rna2* experiment. In another experiment, all detectable proteins were examined by kinetics of labeling for protein processing and turnover rate. These results will be compared to the heat-shock results to determine if any relationship exists between protein processing or turnover and the responses to elevated temperature.

Services and Training Offered by the QUEST Facility

C. Chang, H. Sacco, P. Myers, J.I. Garrels

Two-dimensional gel electrophoresis and analysis have been carried out for a number of other investigators. Small projects are analyzed by the QUEST staff and the results are reported to the investigator. For larger service projects, where we are not directly engaged in the science or in the interpretation of the gels, we train the investigators to perform their own analysis. Cecile Chang has developed a three-part training course that has been given to a total of ten people. In addition, she gives individual training to each investigator who uses a workstation. Users who have had training and who have spent substantial time using the QUEST facility are Steve Briggs and Beth Elliot (CSHL plant group), Jeri Higginbotham (Pioneer Hi-Bred and CSHL plant group), Peter Hornbeck (NIH), and Steinunn Baekkeskov (Hagedorn Research Laboratory, Gentofte, Denmark).

Our future plans include much more extensive training, including a 2-week summer course in the principles and applications of two-dimensional gel protein databases.

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GENETICS

With the ever increasing use of recombinant DNA in biological research, it might have been supposed that classical genetic methods would have become redundant. However, exactly the opposite has proved to be the case, and an increased premium is now placed on study of organisms that are amenable to both classical and molecular genetic approachs. At Cold Spring Harbor Laboratory, we have continued to use the two yeasts, *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*, and maize for genetic studies. An account of the recent work of the Genetics Group follows. During the last year, Amar Klar left to take up a position at The National Cancer Institute in Frederick, Maryland.

EUKARYOTIC CELL-CYCLE CONTROL

D. Beach R. Booher, L. Brizuela, L. Christy, S. Dembski, G. Draetta, B. Ducommun, M. McLeod, L. Molz

Our research on the cell cycle of eukaryotic cells initially focused almost exclusively on the fission yeast. This organism is particularly well suited to genetic and molecular approaches to the problem of cell-cycle regulation. Taking advantage of the insights and reagents the laboratory has developed working with yeast, our research on the cell cycle of higher vertebrates has now matured into a major area of activity that complements the primarily genetic approaches provided by ascomycete genetics. During the last year, we were joined by Paul Young (a visiting scientist from Queens University, Ontario) and a new postdoctoral fellow, Bernard Ducommun.

Cell Cycle of Fission yeast

R. Booher, L. Molz, B. Ducommun, D. Beach

The main focus of our work on the fission yeast cell cycle during the last year has been the *cdc13*$^+$ gene and its relationship to *cdc2*$^+$. *cdc2*$^+$ encodes the catalytic subunit of a protein kinase that acts in the regulation of both DNA synthesis and the initiation of mitosis. We previously identified an allele of *cdc2*$^+$ that is defective in mitosis but not DNA replication. As an extragenic suppressor of this mutation, we isolated a new allele of *cdc13*$^+$, a gene that is only required for the initiation of mitosis. We determined the nucleotide sequence of *cdc13*$^+$ and found that it encodes a protein homologous to mitotic cyclins. The distinguishing feature of this class of proteins is their gradual accumulation during interphase, but abrupt proteolytic degradation at each cell division.

Immunofluorescence and immunoblotting experiments revealed that the *cdc13*$^+$ product is a nuclear protein that behaves in exactly this manner (Fig. 1). Furthermore, we showed that the *cdc13*$^+$-encoded cyclin acts as a regulatory subunit of the *cdc2*$^+$ protein kinase. The cyclin appears to target *cdc2*$^+$ to the cell nucleus and also to control its catalytic activity. We believe that degradation of the cyclin at the metaphase/anaphase transition of mitosis is responsible for inactivating *cdc2*$^+$ and thus allowing cells to return to interphase.

Further studies on the fission yeast cell cycle include investigation of a new class of genes (*mcs*) that were isolated as suppressors of a particular *cdc2* mutant and also the *cdc25*$^+$ gene, which appears to function as a posttranslational activator of *cdc2*$^+$.

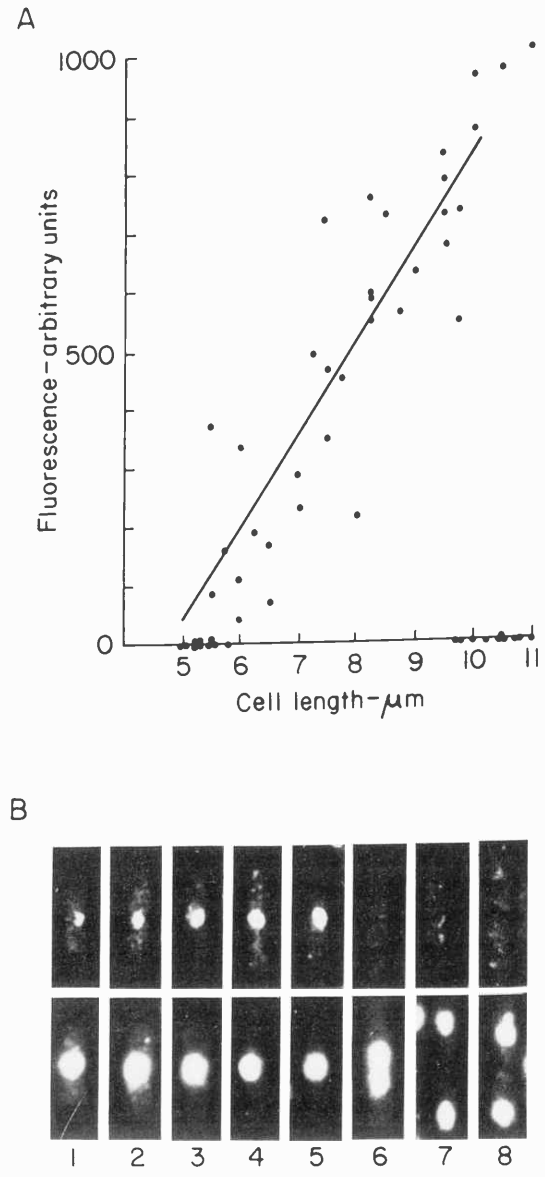

FIGURE 1 Immunofluorescence staining of the *cdc13*$^+$-encoded cyclin of fission yeast. Expressed either quantitatively as a function of cell length (*A*) or as micrographs (*B*). The lower panel of *B* shows cell nuclei stained with DAPI.

Vertebrate Cell-cycle Control

L. Brizuela, L. Christy, G. Draetta, D. Beach

The last year has seen dramatic changes in our understanding of the cell cycle of vertebrate cells, including those of man. Following our identification of *cdc2* homologs in cells of diverse evolutionary origin, we were able to show (in collaboration with W. Dunphy and J. Newport, University of California, San Diego) that the *cdc2* protein kinase is one component of the long elusive inducer of mitosis, known as M-phase-promoting factor (MPF). We further showed, in collaboration with the laboratory of J. Ruderman (Duke University), that the mitotic cyclins of the surf clam are further components of MPF. With the assistance of L. Meijer (Marine Laboratory, Roscoff, France), we found that both *cdc2* and cyclins are components of the M-phase-associated histone H1 kinase, which in its most active form now appears to be the same entity as MPF.

These findings contribute to a remarkable unification of eukaryotic cell-cycle research, as a result of which it is now clear that the *cdc2* protein kinase and also cyclins play a fundamental role in the cell cycle of all eukaryotes. Furthermore, it transpires that geneticists, classic biochemists, and cell biologists have each converged upon these same two molecular entities, but from a quite different original perspective (Fig. 2).

A further wholly unexpected finding, developed in collaboration with the laboratory of T. Roberts (Dana Farber), was that the *cdc2* protein kinase of human HeLa cells is the most heavily tyrosine-phosphorylated protein of the cell. This also turns out to be the case in mouse 3T3 cells and a variety of others (Fig. 3). The exact tyrosine kinase responsible for this phosphorylation is not presently clear, but c-*src* is a good candidate. The biological role of *cdc2* tyrosine phosphorylation is under active investigation.

Regulation of Meiosis

M. McLeod, M. Dembski, D. Beach

Fission yeast is an excellent model system in which to investigate the molecular basis of commitment to meiosis due to the ease of genetic investigation and the ability to obtain large amounts of synchronized meiotic cells. During the last year, considerable progress has been made in identifying and isolating genes regulating this process.

In *Schizosaccharomyces pombe*, entry into meiosis is regulated by both the mating-type genes and environmental signals. Only diploids expressing all four of the mating-type genes are able to undergo meiosis, and full expression of the mating-type genes requires conditions of nutritional limitation. We have

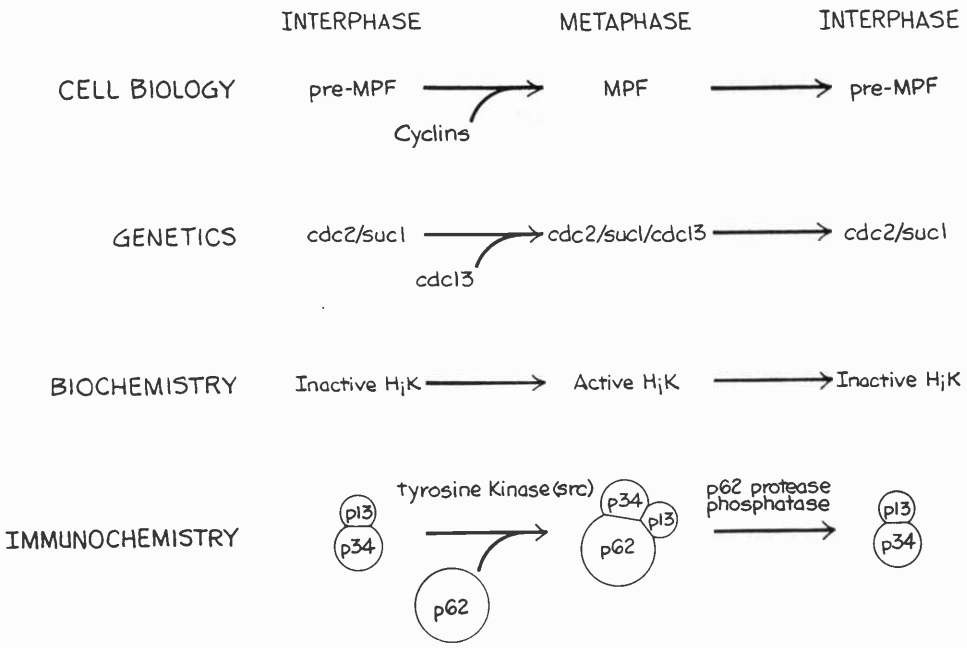

FIGURE 2 Different perpectives of the eukaryotic mitotic oscillator. (MPF) M-phase-promoting factor; (H1K) M-phase-associated histone H1 kinase.

shown that the primary role of the *mat* genes in meiosis is to regulate transcription of the *mei3*$^+$ gene. The product of the *mei3*$^+$ gene is a 21-kD protein that acts as an inhibitor of the *ran1*$^+$ protein kinase. The *ran1*$^+$ protein kinase is required during vegetative growth and acts as a critical negative regulator of meiosis. Thus, loss of *ran1*$^+$ kinase activity, either through mutation or through expression of *mei3*$^+$ causes cells to undergo meiosis.

Mutations at several genetic loci have been identified that are able to suppress the phenotypes associated with loss of *ran1*$^+$. We have characterized one of these, *cgs1*$^+$, and have physically isolated the gene. Cells containing a lesion in *cgs1* rapidly lose viability as they become limited for nutrients and approach stationary phase. Examination of the morphology of these cells reveals that they become aberrently elongated in response to nutrient depletion. In addition, the cells are severely meiotically defective.

Sequence analysis of *cgs1*$^+$ shows that the gene encodes the regulatory subunit of cAMP-dependent

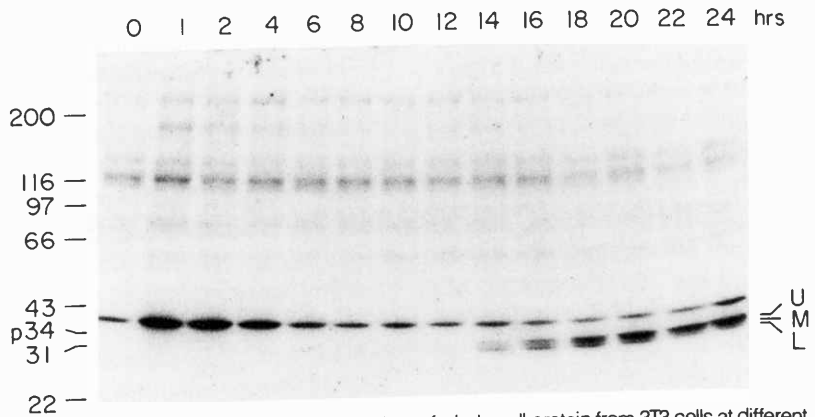

FIGURE 3 Antiphosphotyrosine immunoblot of whole-cell protein from 3T3 cells at different times after mitogenic activation by serum. The triplet bands at 34 kD are the *cdc2* protein kinase. (From A. Morla et al., in press).

protein kinase. The product of the *cgs1*$^+$ gene is a spliced mRNA and the predicted protein product shares 60% homology both with the regulatory protein from *Saccharomyces cerevisiae* and with that from other eukaryotes. cAMP-independent kinase activity is observed in fractionated extracts from cells containing a disrupted allele of *cgs1*. We propose that loss of the regulatory subunit causes unregulated cyclic A kinase activity that prevents cells from entering meiosis. All of the observable phenotypes associated with loss of *cgs1*$^+$ are also seen when cells are forced to express high amounts of *ran1*$^+$ protein kinase. We thus propose that both kinases may share a subset of substrates. Future studies will be directed toward devising genetic screens that will enable us to identify possible substrates of both cAMP-dependent protein kinase and *ran1*$^+$ kinase.

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PRE-mRNA SPLICING IN *SCHIZOSACCHAROMYCES POMBE*

D. Frendewey J. Potashkin R. Li
A. Serrano C. Leptac

We are investigating the posttranscriptional processes that are responsible for producing mature mRNAs from their initial transcript precursors (pre-mRNAs). These pre-mRNA processing reactions include attachment of a 7-methylguanosine "cap" structure to the 5′ end, addition of a polyadenylic acid "tail"

to the 3′ end, and removal of introns. This last process, pre-mRNA splicing, is the primary focus of our research.

The splicing of pre-mRNAs occurs in the nucleus in large ribonucleoprotein (RNP) complexes called spliceosomes. The most conspicuous spliceosomal components are the small nuclear RNAs (snRNAs) U1, U2, U4, U5, and U6, which reside in RNP particles known as snRNPs. Each snRNP consists of a single snRNA (with the exception of the U4/6 particle) and several common and RNA-specific proteins. The spliceosome is an assembly of these snRNPs, bound to the pre-mRNA substrate, and probably ancillary proteins, which either contact the pre-mRNA or are transiently or loosely bound to the snRNPs. To understand how pre-mRNA splicing is achieved, the constituents of the spliceosome must be identified and characterized. Our approach to this problem has been to use the molecular genetic advantages of yeast (in our case *Schizosaccharomyces pombe*) to identify genes that are required for pre-mRNA splicing. We expect that many of these genes will encode products that function in the spliceosome.

Search for New Pre-mRNA Processing Mutants

J. Potashkin, D. Frendewey

Last year, we reported that we had produced a bank of temperature-sensitive (ts^-) mutant strains of *S. pombe* and had isolated three recessive pre-mRNA splicing mutants from a screen of the first 100 members of this collection. Following a convention agreed to by the yeast RNA-processing community, these mutants have been named *prp1, prp2*, and *prp3* to indicate defects in *p*re-*R*NA *p*rocessing. During the past year, we have continued our genetic investigation of mRNA synthesis in *S. pombe* by extending the screen for pre-mRNA splicing mutants to include the complete ts^- bank. Several new candidates for prp^- mutants have been identified.

Three classes of mutant phenotypes that indicate aberrations in mRNA processing were revealed by our search. The first class is represented by *prp1* and *prp2*. These mutants accumulate unspliced pre-mRNA and U6 pre-RNA (see below) and stop making mature β-tubulin mRNA (our test RNA) very soon after being shifted to the nonpermissive temperature (37°C). Thus, the mutants in this class are completely blocked for pre-mRNA splicing. This RNA-processing defect is always associated with a "tight" ts^- phenotype; these strains do not grow at 37°C. The second mutant mRNA processing phenotype is similar to that expressed by *prp1* and *prp2*, except that some mature β-tubulin mRNA is detected at the nonpermissive temperature. The amount of spliced mRNA observed at 37°C in these mutants is usually less than that seen at 23°C but depends on the severity of the defect. The *prp3* mutant is an example of this class. These mutants are "leaky" ts^- strains in that they grow slowly at the nonpermissive temperature. We have also observed a few mutants that exhibit a third phenotype that may not reflect a defect in pre-mRNA splicing. This class of ts^- mutants does not accumulate unspliced β-tubulin pre-mRNA; instead, an RNA is produced that is larger than the unspliced pre-mRNA accumulated by *prp1, prp2*, and *prp3*. The larger RNA could be the result of aberrant 3′-end formation, alternative splicing, or a new transcription initiation site. We have recently begun to analyze the defect in these unusual mutants.

Unspliced U6 RNA Precursor Accumulates in the prp^- Mutants

J. Potashkin, D. Frendewey

One of the analyses that we routinely perform on the prp^- mutants is a visualization of their snRNAs by Northern blotting. The snRNAs are the only known pre-mRNA splicing factors that we can readily investigate in *S. pombe*. The Northern analysis allows us to detect unusual changes in the amount or size of a particular snRNA that might correlate with the mutant phenotype. A check of the snRNAs in *prp1, prp2*, and *prp3* indicated normal amounts and sizes for U1, U2, U4, and U5 RNAs. However, we observed a slight but reproducibly lower level of U6 RNA in the mutants compared to that in the wild type grown under identical conditions. At the time, we did not know the meaning of this observation, but the surprising results of Ohshima and Tani (*Nature 337:* 87 [1989]) soon suggested an explanation. They found that the gene for the U6 RNA of *S. pombe* contains an intron. This is the only example of an intron in an snRNA gene from any source. In addition, the U6 intron has several

structural features—a length of 50 nucleotides and consensus sequences at the 5′ and 3′ splice sites and the presumptive branch point—that are typical of introns found in *S. pombe* pre-mRNAs. Thus, we thought that the reduction in U6 RNA in the *prp*⁻ mutants might be caused by a defect in the splicing of the intron from the U6 RNA precursor.

We wanted to test this hypothesis and, at the same time, use the *prp*⁻ mutants to address two unresolved questions concerning U6 RNA processing. First, Ohshima and Tani were unable to demonstrate the existence of an unspliced U6 RNA precursor, probably because they analyzed RNA from normal, rapidly growing cells, where a high splicing efficiency results in a low steady-state level of pre-RNAs. The block in splicing in the *prp*⁻ mutants might allow easier detection of the U6 precursor. Second, since the U6 intron resembles the introns of pre-mRNAs, an interesting question is whether this intron, which is part of a suspected RNA polymerase III transcript, is spliced by the same apparatus that works on pre-mRNAs, which are transcribed by RNA polymerase II. If this is the case, then the splicing of the U6 RNA precursor would require the same genes that are required for pre-mRNA splicing, and therefore it should behave like a pre-mRNA in the *prp*⁻ mutants.

Our initial experiments aimed at answering these questions were made by Northern blot analysis using an antisense RNA probe complementary to the human U6 RNA sequence, which we had previously shown recognizes an RNA of the expected size in *S. pombe*. At 23°C, the amounts of mature U6 RNA in the *prp*⁻ mutants are not different from that in the wild type (Fig. 1a). After a 2-hour shift to 37°C, the amount of mature U6 transcript is reduced slightly in the mutants compared to the wild type, and there is accumulation of a longer transcript (about 150 nucleotides in length) in both the wild-type and mutant strains. This is the size expected for the unspliced precursor. After 6 hours at 37°C, the accumulation of the longer transcript persists in *prp1*, *prp2*, and *prp3* but not in the wild type.

To determine whether or not the longer RNA was an unspliced precursor, we used an oligonucleotide that specifically recognizes the fission yeast U6 intron as a probe on RNA blots. No precursor signal was detected at 23°C (Fig. 1b). The bands that are less than 80 nucleotides are background signals that are not observed when the blot is probed with another intron-specific probe. After the cells are shifted to 37°C for 2 hours, an intron-containing transcript of

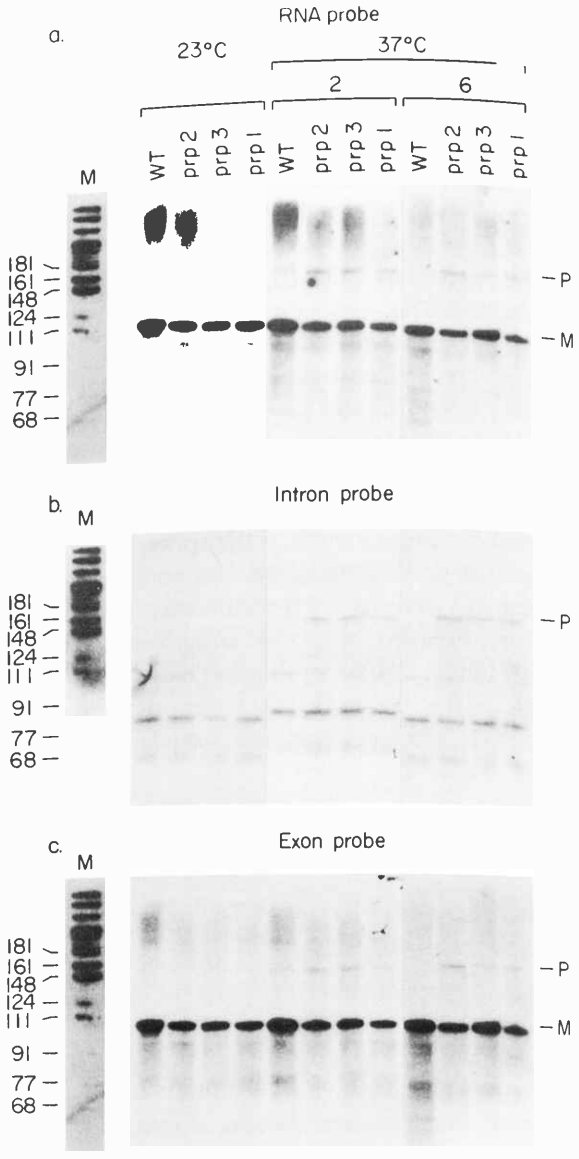

FIGURE 1 Northern blot analysis of U6 RNA in the *prp*⁻ mutants. Total RNA from the wild type or *prp1*, *prp2*, and *prp3* grown to mid-log at 23°C and then shifted to 37°C for either 2 or 6 hr was fractionated by electrophoresis on a polyacrylamide/urea gel. The RNA was then blotted onto a nylon membrane and hybridized to the following probes: (*a*) An antisense RNA derived from the human U6 gene; (*b*) an oligodeoxynucleotide complementary to the intron of the *S. pombe* U6 gene; (*c*) an oligodeoxynucleotide complementary to the second exon of the *S. pombe* U6 RNA. P marks the position of the U6 RNA precursor, and M indicates the position of the mature U6 RNA.

150 nucleotides appears in both the wild-type and mutant strains. This RNA is seen exclusively in the *prp*⁻ mutants after 6 hours at 37°C. When the autoradiograms from the intron oligonucleotide (Fig. 1b) and anti-U6 RNA probings (Fig. 1a) of the same

blot are aligned, the longer transcript detected with the anti-U6 RNA probe corresponds exactly with that of the intron-containing precursor.

The intron-specific oligonucleotide was stripped from the membrane, and the blot was reprobed with an oligonucleotide specific for the second exon of the U6 pre-RNA. The same pattern of precursor and mature transcripts is observed with the exon 2 probe as with the anti-U6 RNA probe (compare Fig. 1, a and c). There is an accumulation of precursor in *prp1, prp2*, and *prp3* after both a 2- and 6-hour shift to 37°C and a concomitant decrease in the mature transcript compared to the wild type. The accumulation of the U6 RNA precursor in the wild-type strain after a 2-hour shift to the elevated temperature may be the result of a transient decrease in the efficiency of splicing associated with a mild heat shock caused by the temperature shift. These observations were confirmed in a series of primer-extension experiments with total RNA prepared from the wild type and the mutants as template.

A comparison of the amounts of precursor and mature U6 RNAs in the mutants at 23°C and 37°C clearly indicated that there is a significant accumulation of unspliced U6 RNA in response to a shift to the nonpermissive temperature. The pattern of precursor accumulation in the mutants is the same as that exhibited by α- and β-tubulin pre-mRNAs; *prp1* and *prp2* accumulate more precursor and produce less spliced product than *prp3* at the nonpermissive temperature. This strongly suggests that some of the same *trans*-acting factors that are required for the processing of pre-mRNAs are also needed for the processing of the U6 precursor and is consistent with the structural identity of the U6 intron with the introns of fission yeast pre-mRNAs.

prp4: A Pre-mRNA Splicing Mutant with Reduced snRNA Content

J. Potashkin, R. Li, A. Serrano, D. Frendewey

Much of our effort during the last year has been devoted to the analysis of an interesting mutant that we have recently named *prp4*. As with the other *prp*⁻ mutants, *prp4* accumulates unspliced pre-mRNAs and U6 pre-RNA at 37°C compared to the wild type grown under identical conditions. This mutant is a member of the phenotypic class represented by *prp3*; it grows slowly at 37°C and produces mature β-tubulin mRNA. However, the pre-mRNA splicing defect in *prp4* is much less severe than in *prp3*. In fact, *prp4* was not identified as a *prp*⁻ mutant in our original Northern blot screening of the *ts*⁻ bank. We discovered the pre-mRNA accumulation in *prp4* only when we used a more sensitive RNase protection assay.

We began studying *prp4* because it appeared to have a defect in U2 RNA synthesis. As we reported last year, this mutant was identified from a screen of the first 70 *ts*⁻ strains in our collection. It was shown to have a reduced amount of U2 RNA compared to the wild type and to produce two aberrant U2 transcripts whose sizes are about 110 nucleotides and 400 nucleotides. (The normal *S. pombe* U2 RNA is 186 nucleotides.) We now know that all of the nucleoplasmic snRNAs that we have been able to analyze are affected in *prp4*.

The *prp4* strain was backcrossed to the wild type several times. Sporulation and tetrad analysis revealed that the mild *ts*⁻ growth phenotype segregated 2:2, with the defect in snRNA synthesis. Figure 2 shows that there is a two- to fivefold reduction in the amounts of U1, U2, U4, and U5 RNAs, as a proportion of whole-cell RNA mass, in the progeny from the *ts*⁻ spores (−) compared to the wild-type spores (+). There is also a slight reduction in U6 RNA levels that is not evident in the exposure of the U6 probing shown in Figure 2. In addition to decreased snRNA content, larger transcripts are produced for U2 (not seen in Fig. 2), U4, and U6 RNAs in *prp4* at the nonpermissive temperature. The larger U6 RNA is unspliced U6 pre-RNA, which accumulates in *prp4* as in the other *prp*⁻ mutants. The longer U2 and U4 transcripts, however, are extended at their 3′ ends. Utilizing a variety of assays, we have not detected the extended U2 and U4 RNAs in the wild type at either 23°C, 30°C, or 37°C. We therefore believe that these RNAs are not normal precursors that are accumulating in the mutant at 37°C but are aberrant transcripts peculiar to *prp4*. Less-abundant larger RNAs are also detected by the U1 and U5 probes in the wild-type RNA samples (Fig. 2). These RNAs, as with the major transcripts, are reduced in the mutant. We are currently investigating whether *S. pombe* expresses multiple forms of the U1 and U5 RNAs.

Figure 2 also shows that K RNA, the RNA subunit of the tRNA-processing enzyme RNase P, is dramatically reduced in *prp4*. K RNA is presumed to be nucleoplasmic and is found in a simple RNP. The reduction in K RNA in *prp4* indicates that it is related

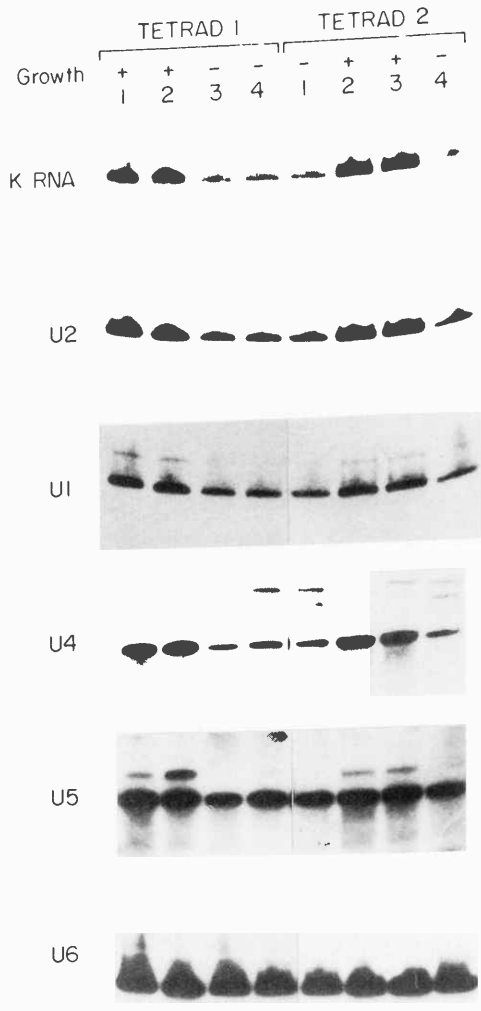

FIGURE 2 Northern blot analysis of snRNA from the progeny of a cross between *prp4* and the wild type. *prp4* was mated to a wild-type strain and allowed to undergo meiosis. Tetrads (containing four haploid spores) were dissected, and the spores were grown at 23°C and then shifted to 37°C for 5 hr. Total RNA was prepared from the progeny of each of the four spores (1, 2, 3, and 4), fractionated, and blotted as in Fig. 1. The blot was sequentially probed with antisense RNAs or oligodeoxynucleotides that recognize K RNA and U1, U2, U4, U5, and U6 RNAs. A plus (+) indicates a wild type, and a minus (−) indicates a *ts*⁻ spore. The results of two tetrads are shown.

to the other snRNAs with respect to its synthesis or maintenance. Consistent with its considerably lower levels of K RNA, *prp4* accumulates pre-tRNAs at 37°C that are not processed at their 5′ ends. Thus, *prp4* exhibits defects in pre-mRNA, U6 pre-RNA, and pre-tRNA processing.

The one snRNA that we have tested whose quantity in *prp4* is similar to the wild type is U3 RNA. This snRNA is thought to reside in the nucleolus and be required for rRNA processing. We also compared the amounts of 7SL RNA (a small cytoplasmic RNA found in an RNP), the large and small rRNAs, and tRNA between *prp4* and the wild type. As with U3 RNA, no differences were observed. All of the results we have described here were obtained from experiments in which equal mass amounts of wild-type and *prp4* RNAs were compared. However, essentially identical results are obtained when the snRNA content is analyzed on the basis of cell equivalents. We have also examined the mRNA transcribed from the alcohol dehydrogenase (*adh*) gene, which contains no introns. The results indicate that the mass proportion of the *adh* mRNA in *prp4* is identical to that of the wild type. Therefore, RNAs that reside in the cytoplasm are not affected in *prp4*. We have investigated the steady-state mass proportions of all classes of cellular RNAs encoded by the nuclear genome and have found that only RNAs that are presumed to reside as snRNPs in the nucleoplasm (as opposed to the nucleolus) are reduced in *prp4* compared to the wild type.

In summary, the *prp4* mutant exhibits an interesting but somewhat perplexing molecular phenotype. Compared to the wild type, *prp4* maintains a significantly lower level of spliceosomal snRNAs, yet it is not severely impaired for pre-mRNA splicing. The best explanation for this result might be that the snRNA requirement for splicing is dependent on growth rate, and *prp4* produces sufficient quantities of snRNA to achieve a near-normal pre-mRNA splicing efficiency at its low growth rate. In contrast to *ts*⁻ stains, the wild type increases its growth rate when shifted from 23°C to 37°C. We have evidence that along with the increase in growth rate, the steady-state level of the snRNAs increases. In fact, we were able to detect the difference in snRNA content between the wild type and *prp4* primarily because *prp4* fails to increase its snRNA accumulation in response to temperature shift. A decreased snRNA level is not an obligatory consequence of a low *ts*⁻ growth rate at 37°C, since all of the other *ts*⁻ mutants that we have investigated, including the pre-mRNA splicing mutants *prp1*, *prp2*, and *prp3*, appear to have normal quantities of snRNA. The *ts*⁻ growth defect in *prp4* may be caused by an inability to increase snRNA synthesis in response to the higher growth temperature. Alternatively, the low snRNA concentration in *prp4* could be a secondary symptom of a defect in another cellular process. We are currently

attempting to determine if the cause of the *prp4* phenotype is a defect in transcription initiation or termination, 3′-end processing, or instability of the snRNPs.

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In Press, Submitted, and In Preparation

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Potashkin, J. and D. Frendewey. 1989. *prp4*: A fission yeast pre-mRNA splicing mutant that maintains a reduced snRNA content. (In preparation.)

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PLANT GENETICS

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Characterization of Extrachromosomal Forms of *Mu*

V. Sundaresan, M. Papazian

During the past year, we have continued our investigations into the mechanism and regulation of transposition of the maize transposable element system, 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.

An extrachromosomal circular form of the *Mu* transposons has been described previously (Sundaresan and Freeling, *Proc. Natl. Acad. Sci. 84:* 4924 [1987]). These extrachromosomal *Mu* elements are correlated with *Mu* transposition activity and are presumptive real or abortive transposition intermediates. Initially, only the *Mu1* and *Mu1.7* elements were identified as extrachromosomal circles. During the past year, screening of libraries constructed from fractions of purified extrachromosomal DNA revealed that several other *Mu* elements also generate this extrachromosomal DNA species. Some of these were found to be homologous to elements *Mu4*, *Mu6*, and *Mu7* cloned independently by V. Chandler (University of Oregon) by utilizing their homology with *Mu1* termini (Fig. 1). Our results suggest that these elements are also transposable, confirming the idea that the 200-bp *Mu* inverted repeat termini are sufficient for an element to transpose in an active *Mu* background. We have been attempting to define the nature of the junctions between these termini by studying the cloned *Mu* circles. However, these junctions appear to be extremely unstable, presumably because of their ability to form cruciform structures. As a result, all of our clones to date have deletions of variable lengths that include the junction sequences. Other approaches including the polymerase chain reaction (PCR) are being attempted to circumvent this problem.

Maize Nuclear Proteins That Bind to the Terminal Inverted Repeat of the *Mu-1 Transposable Element*

Z.-Y. Zhao, V. Sundaresan

At present, little is known about the protein factors that catalyze *Mu* transposition or the genes encoding them. The search for the transposase of the *Mu* elements is of the utmost importance if we are to understand the mechanism of *Mu* transposition. We initiated our search for the *Mu* transposase by using the *Mu-1* terminal inverted repeat (TIR) sequence as a probe, for two reasons: (1) All the members of the *Mu* family share the same TIRs as *Mu-1*, but with

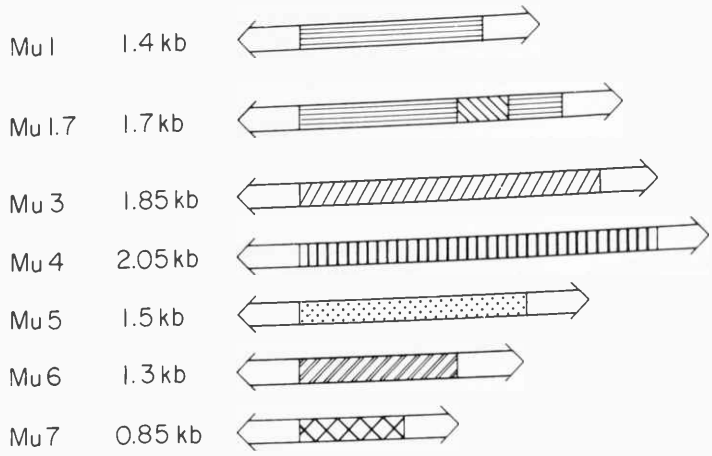

FIGURE 1 Structure of the *Mu* elements that have been cloned, showing that they share the same 200-bp termini but nonhomologous internal regions, with the exception of *Mu1* and *Mu1.7*, which are closely related.

different internal sequences. Therefore, we assume that TIRs play an important role if the *Mu* elements share the same mechanism of transposition. (2) For many transposons found in bacteria and yeast, the ends of these transposons play an important role in transposition. For some transposons found in bacteria, such as Tn*3* (Ichikawa et al., *Proc. Natl. Acad. Sci. 84*: 8220 [1987]) and bacteriophage Mu (Craigie et al., *Cell 39:* 387 [1984]), the transposase binds specifically to their TIR sequences.

Crude nuclear proteins were isolated from *Mu*-active, *Mu*-inactive, and normal maize plants at the seven-leaf stage. The right TIR DNA sequence (200 bp) of the *Mu-1* element was 3′-end-labeled and used as the probe in mobility-shift ("gel-retardation") experiments. The mobility of the labeled fragment, after incubation with or without the nuclear proteins, on a polyacrylamide gel showed that nuclear protein(s) from either *Mu*-active or *Mu*-inactive plants did bind to the *Mu-1* TIR sequence (Fig. 2) The binding is specific for the *Mu-1* TIR sequence because it can be competed out by the unlabeled *Mu-1* TIR fragment (Fig. 2, lanes 6 and 11), but not by linear pUC119 DNA fragments (pUC119 DNA cut

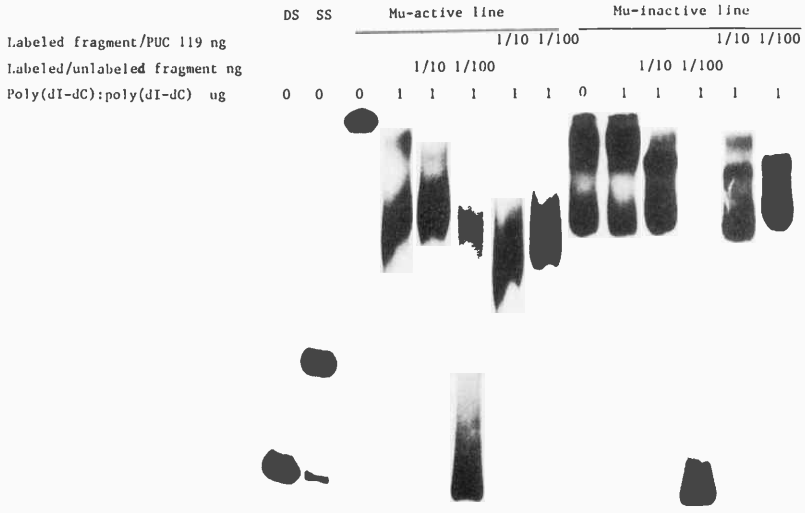

FIGURE 2 Gel retardation assays with *Mu-1* TIR probe. (Lanes *1* and *2*) Free double-stranded and single-stranded probes, respectively. (Lanes *3–7*) Probes incubated with proteins from the *Mu*-active line and poly(dI-dC) (no poly[dI-dC] in lane *3* and 1 μg in other lanes); specific competition with unlabeled *Mu* TIR (lanes *5* and *6*) and nonspecific competition with pUC119 (lanes *7* and *8*). (Lanes *9–14*) Probes incubated with proteins from the *Mu*-inactive line and the other factors; same as the *Mu*-active line (specific competition, lanes *11* and *12*, and nonspecific competition, lanes *13* and *14*).

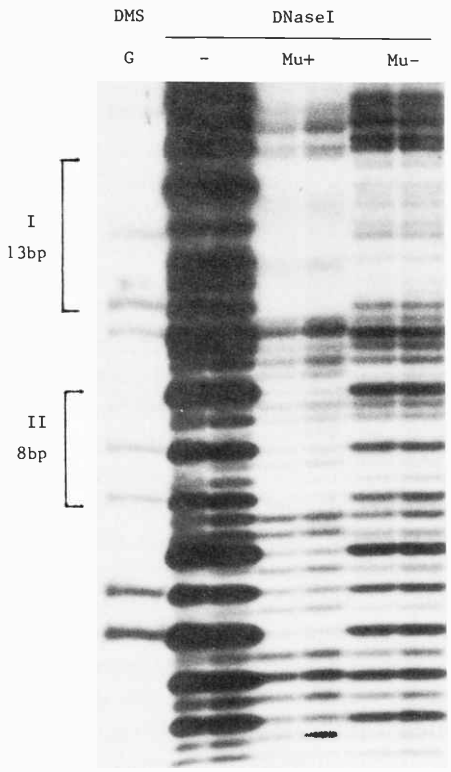

FIGURE 3 DNase I footprinting of the *Mu-1* TIR fragment. (Lane *1*) DMS-"G" track; (lanes *2* and *3*) no protein; (lanes *4* and *5*) incubation with nuclear protein from the *Mu*-active line; (lanes *6* and *7*) incubation with nuclear protein from the *Mu*-inactive line.

with *Hae*III to generate linear DNA fragments as a nonspecific competitor; Fig. 2, lanes 8 and 14). When the 200-bp TIR was cut into four smaller fragments (i.e., 22, 62, 43, and 95 bp), the binding assay indicated that the nuclear protein(s) binds only to the 62-bp fragment but not to the other fragments (data not shown).

To determine whether the binding proteins from *Mu*-active and *Mu*-inactive lines are the same or different, and to localize the protein binding site(s) on the *Mu-1* TIR DNA sequence, DNase I footprinting was performed. The footprinting revealed that the proteins from *Mu*-active lines bound to the TIR at two different sites such that a 13-bp fragment and another 8-bp fragment were protected. The two sites are 5 bp apart (Fig. 3, lanes 4 and 5). However, the protein from *Mu*-inactive lines only revealed one of these binding sites (the 13-bp site) on the TIR (Fig. 3, lanes 6 and 7). The two binding sites (13 bp and 8 bp) were located within the 62-bp region of the TIR (Fig. 4). These data are consistent with the gel-retardation results. In addition, there appears to be a third factor that binds specifically to the outside end of the *Mu* inverted repeat; however, we have not yet characterized this site in detail. The nuclear protein(s) from normal maize plants, B73 and W22, showed the same binding activity for the *Mu-1* TIR as the *Mu*-inactive lines.

Our current working model is as follows: The transposition process involves two or more protein factors, which may be encoded by the host genome or by as yet undiscovered *Mu* elements. At least one of the factors is present only in active *Mu* stocks and is a good candidate for a transposase. The other two factors appear to be ubiquitous and may also be necessary for transposition. In this regard, it is useful to note that analogous situations exist in bacteria; e.g., transposition of the bacterial transposon Tn*10* requires IHF and HU5 in addition to IS*10* transposase (Morisato and Kleckner, *Cell 51:* 101 [1987]). Furthermore, in the case of the *P* element of *Drosophila melanogaster*, a protein that binds specifically to the 31-bp inverted repeat of the *P* element has been found in both *P* and *M* strains (Rio and Rubin, *Proc. Natl. Acad. Sci. 85:* 8929 [1988]). Since the *P*-element-encoded transposase does not show a specific affinity for this sequence, it is assumed

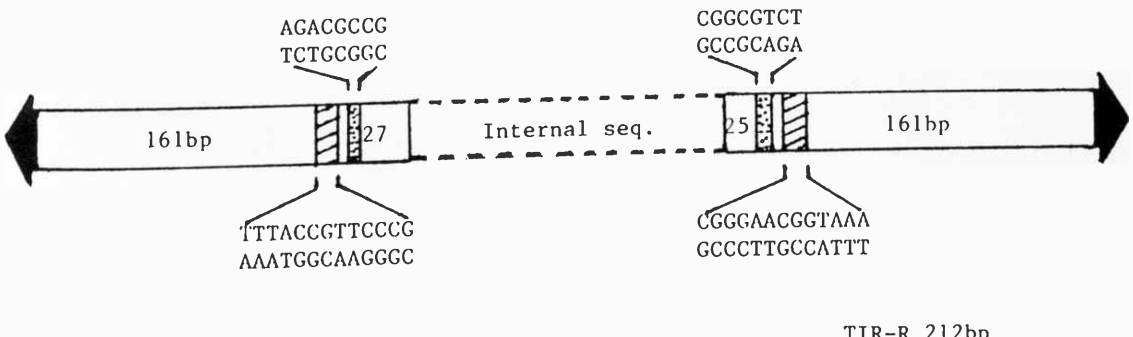

FIGURE 4 Localization of the two protein-binding sites I and II on the *Mu-1* TIR sequence.

that both proteins (one host-encoded and one *P*-element-encoded) are required for transposition.

Our future work on *Mu* will involve purification of the proteins and cloning the genes encoding them, in order to elucidate their roles in catalyzing or regulating *Mu* transposition.

Regulation of *Mu* Activity

J. Brown, V. Sundaresan

Mu transposons are present in multiple copies in the maize genome and can be either actively transposing ("on") or inactive ("off"). We have been studying the regulation of *Mu* activity, using *bzMum-9* as a reporter. The *bzMum-9* allele resulted from an insertion of *Mu* at the *Bz* gene. In plants that contain inactive *Mu* transposons (*Mu*-off), *bzMum-9* acts as a null, giving a bronze color to the kernel, whereas in plants that contain active *Mu* (*Mu*-on), *bzMum-9* becomes mutable, giving bronze kernels with purple spots.

In a low percentage of the progeny of self-pollinated and outcrossed plants, all or most of the *Mu* transposons in a genome change from active to inactive. It has been reported that the *Mu*-off state is irreversible in the case of self-pollinated plants, but reversible in outcrossed plants, by plants crossing "off" to "on" plants (Bennetzen, *Mol. Gen. Genet. 208:* 45 [1987]). In these crosses, the inactive state was found to be "female-dominant"—the state of activity of the female parent determined the outcome (Walbot, *Genetics 114:* 1293 [1986]). In contrast, our recent results indicate that *Mu*-off plants from self-pollinators (bronze) can readily be reactivated (purple-spotted), regardless of sex, by crossing to or by *Mu*-on plants (see Fig. 5). The terminal inverted repeats of *Mu* transposons contain *Hin*fI sites. In *Mu*-on plants, *Mu* sequences are digested to completion by *Hin*fI, but in *Mu*-off plants, they are resistant to *Hin*fI digestion. Since *Hin*fI activity is inhibited by methylation of DNA, it is thought that *Mu* sequences are methylated in the *Mu*-off state. Genomic Southern analyses of *Hin*fI-digested DNAs from our reactivation crosses, when probed with *Mu*, show that in each case *Mu* methylation is associated with inactivation and loss of methylation is associated with reactivation. That inactive *Mu*-offs derived from self-pollinators can be reactivated suggests that inactive *Mu* derived by selfing does not

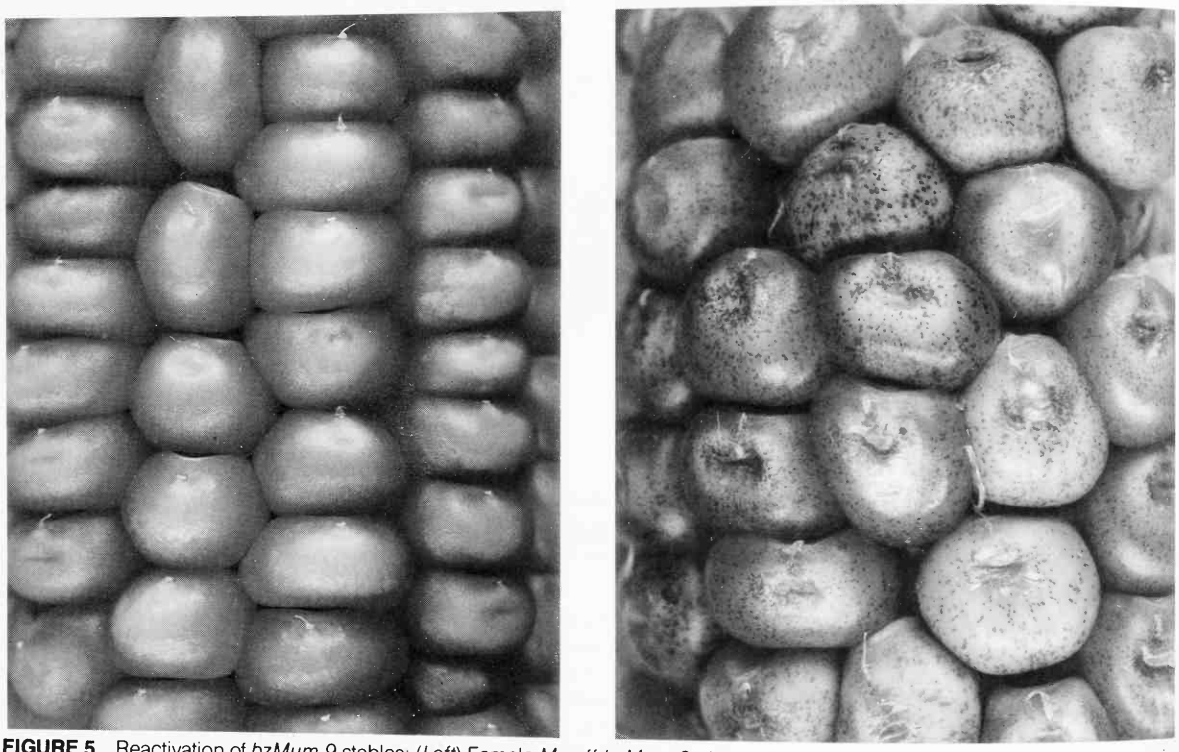

FIGURE 5 Reactivation of *bzMum-9* stables: (*Left*) Female *Mu*-off *bzMum-9* plants crossed to standard (non-*Mu*) *bz* males remain *Mu*-off (bronze). (*Right*) Female *Mu*-off *bzMum-9* crossed to *Mu*-on *bz* males reactivate to the *Mu*-on state (spotted).

behave differently from inactive *Mu* derived by outcrossing. In addition, the female dominance of the *Mu* state previously reported is not a general property of *Mu*.

Our observation can be reconciled with the earlier reports as follows: In separate studies using active *bzMum-9* stocks, we have found that *Mu* activity is lost at a higher rate through the male than through the female. Furthermore, in a plant in which *Mu* activity is turning off, there appears to be a gradient of inactivation going up the plant so that the pollen gives more inactive *Mu* progeny than the ear (R. Martienssen, pers. comm.). This behavior has been observed in cycling *Spm* stocks as well (Federoff, *Cell 56:* 181 [1989]). Therefore, the outcome of a reactivation cross would depend on the active parent used. In our crosses, the active *Mu* stock used for reactivation exhibits a very low rate of loss of activity (<2%) both through the pollen and through the ear. If the active *Mu* stock used is "cycling" to an inactive state of *Mu*, then the loss would be significantly higher through the pollen. In that case, it would fail to reactivate efficiently when crossed to an inactive *Mu* female, which would lead to the apparent "female dominance" reported by the workers cited earlier.

Intragenic Recombination at *A1*

J. Brown

Since *A1* function is required for production of red and purple anthocyanin pigments in the maize plant and kernel, intragenic recombination events between mutant alleles of *A1* restoring *A1* expression can be scored easily. *a1Mum2*, a *Mu*-induced mutable allele that contains a *Mu1* transposon at nucleotide −100 (relative to the *A1* transcription start site) (O'Reilly et al., *EMBO J. 4:* 877 [1985]), and *a1*, *aDt*-induced mutable allele that contains an *rDt* transposon at +1077 (Brown et al., *Mol. Gen. Genet. 215:* 239 [1989]), were tested for recombination. Neither *a1Mum2* nor *a1* give *A1* progeny when either allele is homozygous. From crosses of *a1Mum2/a1* plants with *a1/a1* plants, 11 of 23,477 kernels that had restored *A1* were found. The ratio of genetic to molecular distance is 0.1 cM/kb in this case. McClintock had previously reported a similar genetic result using *a1-m2*, which contains an *Spm* insertion at −100 (Masson et al., *Genetics 117:* 117 [1987]), and also *a1*. Of 70,039 kernels from test crossed *a1m-2/a1* plants, 17 were restored for *A1* (McClintock, *Maize Genet. Coop. Newsl. 39:* 42 [1965]). Since the distance between insertions is 1.2 kb, this gives a recombinational distance of 0.05 cM/kb.

When 0.05–0.1 cM/kb at *A1* is compared to the overall genome correlation of 3.0×10^{-4} cM/kb (1200 cM per genome, Coe et al., in *Genetic maps*, Cold Spring Harbor Laboratory [1984], and 3×10^9 bp, Galbraith et al. *Science 220:* 1049 [1983]), it seems that recombination rates at *A1* can be high. This supports the idea that the larger genomes contain highly recombinogenic regions (perhaps the structural genes) interspersed with nonrecombinogenic regions (Thuriaux, *Nature 268:* 460 [1977]).

Genetic Imprinting of the *R*-locus in Maize

J. Colasanti, A. Klar, V. Sundaresan

Epigenetic signals are differentially inherited by cells of identical genetic background during the development of multicellular organisms. In this way, gene expression required for the development of specific structures and organs is possible while genetic totipotency is maintained. Imprinting of genetic information in plants is believed to play a major role in development of the whole organism. We are studying a case of genetic imprinting in maize where a gene is expressed differentially depending on whether it is transmitted through the male or female gametes.

The *R* locus of maize is involved in the regulation of anthocyanin pigment production in the seed and plant. A functional *R* allele allows the expression of purple pigmentation in the outer seed tissue (aleurone). In the *R*-mottling phenomenon, the expression of *R* in seeds of *R/r* heterozygotes depends on the direction of the cross. Certain alleles of *R* (such as *R-r*) exhibit normal seed pigmentation when transmitted through the female gametophyte; however, when transmitted through the male gametophyte, *R* is expressed incompletely and the aleurone pigmentation is mottled (Fig. 6). Kermicle (*Genetics 66:* 69 [1970]) has shown, using B-A chromosome translocations, that the absence of mottling when *R* is transmitted through the female is not due to an extra dose of the *R* allele in the triploid endosperm of the kernel. This suggests that during the development of the male gametophyte, the *R*-locus is imprinted or altered in an epigenetic fashion such that its expression is suppressed in the next generation.

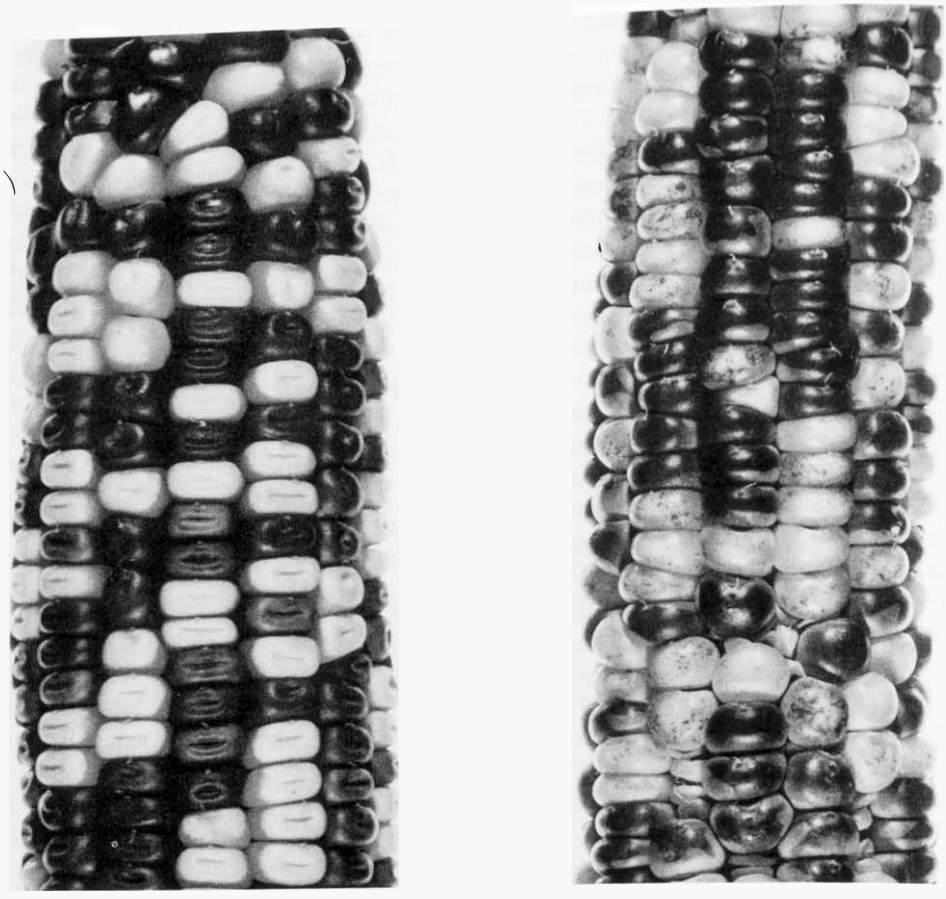

FIGURE 6 Normal ears of corn segregating the *R-r* and *r-g* alleles. Full-colored kernels on the left ear and the mottled kernels on the right ear are both heterozygous for the *R* allele. Full-colored kernels have received the *R-r* allele from the female gametophyte, whereas the same allele was transmitted through the male gametophyte in the mottled kernels.

We have used a developmental mutant of maize (*Ts6*) to determine whether *R*-allele imprinting in the pollen occurs as a result of the position of the tassel in the plant or whether imprinting is gametophyte-specific. *Ts6* is a dominant mutation that results in the formation of a female flower at the position where the male flower would normally form. The tassel seed ear is a large, multispike tassel-like structure with extensive silks; the ear produces no pollen, but it can be cross-pollinated to yield mature kernels capable of expressing full color.

The question we asked was, Do kernels of a tassel seed ear carrying the *R-r* allele exhibit the *R*-mottling phenotype? That is, will *R-r* be imprinted in the female gametophytes derived from what is normally the cell lineage leading to male gametophytes? A *Ts6* stock carrying the recessive *r-g* allele was crossed to an *R-r* stock, and the progeny that inherited *Ts6* were crossed to pollen from *r-g* and *R-g* testers. Both the tassel seed ear and the normal ear of the *Ts6* plants were pollinated. From several such crosses, it was apparent that the tassel seed ear behaved much like a normal ear in this respect (see Table 1); i.e., when the *R-r* allele is transmitted through kernels of the tassel seed ear, they express full color, and mottling occurs when *R-r* pollen is crossed to a tassel seed ear with an *r-g* genotype (Fig. 7). From this experiment, we conclude that the position of the flower has no effect on imprinting and that *R*-imprinting is male-

TABLE 1 Effect on *R* Expression in the Kernel following Transmission through the Tassel-seed Ear

Cross	Ear	Phenotype
Ts6/+, R-r/r-g × +/+, r-g/r-g	tassel seed	full color:colorless, 1:1
Ts6/+, R-r/r-g × +/+, r-g/r-g	normal	full color:colorless, 1:1
Ts6/+, R-r/r-g × +/+, R-g/R-g	tassel seed	full color:mottled, 1:1
Ts6/+, R-r/r-g × +/+, R-g/R-g	normal	full color:mottled, 1:1

gametophyte-specific, probably occurring late in the development of the tassel.

The molecular basis for imprinting at this allele is unknown, although methylation of the gene is a possible explanation. Other experiments in progress are designed to determine the point in pollen development in which imprinting occurs.

Transposon Tagging of the Maize *dwarf1* Gene

J. Colasanti, V. Sundaresan

The gibberellins represent a class of phyto-hormones that are important in the growth and development of plants. In maize, the recessive mutation *dwarf1* (*d1*) has been biochemically characterized as being deficient in an enzyme neccesary for the conversion of a precursor gibberellin (GA_{20}) to the active form, GA_1. A new allele of the *d1* gene has been isolated from the F_1 progeny resulting from the cross of a *Mu* line to a normal inbred line. If the mutation is a result of the insertion of a *Mu* element into the wild-type gene (*D1*), it should be possible to detect a band cosegregating with the dwarf phenotype, as detected by Southern blotting with *Mu*-specific probes. This method of gene tagging is complicated in *Mu* lines by the high copy number of elements and their high frequency of transposition. To

FIGURE 7 A mature tassel seed ear exhibiting full-colored and mottled kernels (arrow).

alleviate these difficulties, a dwarf line was selected in which *Mu* transposition had switched "off." In this way, these lines can be out-crossed with lines containing few or no *Mu* elements to dilute the number of elements and facilitate identification of a band that segregates with the dwarf phenotype.

In initial experiments, a *Mu1*-specific probe was used in an attempt to detect dwarf-segregating *Mu1* elements, as this element has been shown to be the most active in terms of transposition (Lillis and Freeling, *Trends Genet. 2:* 183 [1986]). However, although a number of these elements have been reduced to between 5 and 10 copies in most lines, there are no cosegregating bands. The *Mu* elements exist as a family of elements that share terminal inverted repeat sequences but have considerably different internal sequences. At least some and perhaps all of the other elements may be transposing in *Mu* lines and causing insertional mutations. Six other *Mu* elements have been cloned and partially characterized (*Mu3, Mu4, Mu5, Mu6, Mu7,* and *Mu8*). These were also used as probes in an attempt to detect bands cosegregating with the dwarf phenotype. Although none of these have shown evidence of cosegregation with the dwarf phenotype as of yet, other *Mu* elements may be characterized in the future and used as probes.

To approach this problem from another angle, we are also attempting to tag the *d1* gene using the *Spm* transposable element. This element has been used successfully to tag other genes (Cone et al., in *Plant transposable elements.* Plenum Press, New York [1987]) and offers some advantages over *Mu*; i.e., although the mutation rate is lower in an *Spm* stock, the number of active *Spm*-like elements is also small, so that the segregation analysis is simplified. Crosses of *Spm*-active lines with dwarf lines were performed this winter in Florida, and approximately 200,000 F_1 progeny were obtained. These are now being screened for potential new dwarf mutants, and so far, two new *d1* mutants have been identified out of 38,000 progeny screened.

Transposon Tagging of a Quantitative Trait Locus in Maize

V. Sundaresan

Quantitative traits in field crops (height, yield, moisture) have been believed to be controlled by a large number of independent loci. However, recent work using restriction-fragment-length polymorphisms (RFLPs) to study genetic linkage has shown that in many cases, a few genetic loci appear to account for much of the observed variation in the F_2 progeny of heterotic crosses. In particular, a locus linked to an RFLP on chromosome 9 of maize appears to account for about 25% of the variation in height observed (T. Helentjaris, *Trends Genet. 3:* 217 [1987]). This RFLP maps very close to the locus *dwarf3* (*d3*) required for the synthesis of the plant growth regulator gibberellin, and it has been hypothesized that the observed variation in height linked to this locus is due to different alleles of *d3*. We have therefore begun an attempt to isolate this gene using the maize transposable element *Spm* (*Suppressor-mutator*) as a transposon tag. In the summer of 1988, large-scale crosses were performed between *d3* testers and the *Spm* stock *cl-m5 wx-m8*, which carries an *Spm* and a *dSpm* element on chromosome 9. Over 250,000 progeny were obtained, and these will be screened in summer 1989.

Molecular Analysis of the Maize *P* Locus

T. Peterson, C. Lechelt

Although a number of plant genes have been isolated, in few cases do we have a thorough understanding of the mechanisms by which the genes are expressed in specific tissues during development of the plant. The maize *P* gene, which controls pigmentation of certain floral tissues, has several important properties that are useful for studies of plant gene structure and expression. First, *P* is expressed in at least five distinct floral tissues, including the pericarp, glumes of the cob, glumes of the tassel, husks, and silks. Moreover, *P* can be expressed independently in these tissues; e.g., the alleles *P-RR*, *P-RW*, *P-WR*, and *P-WW* specify red pericarp/red cob, red pericarp/white cob, white pericarp/red cob, and white pericarp/white cob, respectively. Second, the *P*-controlled pigments are conspicuous but nonvital, so mutants can be easily isolated and studied. Third, mutations that occur during development of the ear result in sectors of mutant pericarp, and the mutant alleles can be recovered and propagated in the progeny of kernels within the sectors. Finally, the *P* locus is accessible to molecular dissection, since it is "tagged" with the maize transposable element *Ac*.

Using the *Ac* element as a hybridization probe,

we have isolated 27 kb of genomic DNA from the *P* locus. With an overlapping genomic *Bam*HI clone of 10 kb (kindly provided by Jychian Chen and Stephen Dellaporta), the entire cloned region comprises 34 kb. The cloned DNA contains two 5.8-kb homologous regions, in direct orientation, separated by 6.6 kb. The *Ac* element in the original *P-VV* allele is inserted in the 6.6 kb of DNA between the 5.8-kb direct repeats.

We knew from previous experiments that the *Ac* insertion in *P-VV* is correlated with a change in transcriptional pattern around the *Ac* insertion site. For a complete transcriptional analysis of the cloned *P*-locus DNA, restriction fragment probes spanning the 34-kb region were hybridized to Northern blots of RNA from plants carrying the mutant *P-VV* and functional *P-RR* alleles. The results allowed a coarse determination of transcribed regions that are most probably specific for the *P* gene.

We found that probes from a region of 7.3 kb around the *Ac* insertion site detect five transcripts of 7, 6.5, 2, 1.4, and 1 kb in RNA of *P-RR* and *P-RR* revertants derived from *P-VV*. The multiple RNA molecules may be formed by alternative splicing. None of these transcripts are found in RNA from the *P-VV* allele. Instead, a transcript of 9.5 kb in size is detected in *P-VV* RNA by probes located 5′ of *Ac* and by *Ac*-specific probes. The 9.5-kb RNA is a chimeric transcript containing *P*- and *Ac*-specific sequences that most likely terminates within the *Ac* element, since it is not detected by probes 3′ of *Ac*. Hybridization with single-strand-specific M13 probes demonstrated that the direction of transcription of the *P* gene is identical to that of the *Ac* gene in the cloned *P-VV* allele. Thus, the transcriptional start site(s) of the *P* gene is located 5′ of the *Ac* element in the *P-VV* allele used for these studies.

Probes made from DNA fragments outside the 7.3-kb region around the *Ac* insertion site do not detect differences in RNA from the *P-VV* and *P-RR* alleles. Some probes do not hybridize at all to RNA of the two alleles; these sequences might represent introns or nontranscribed DNA. Other probes detect a similar pattern of transcripts in *P-VV* and *P-RR* RNA. These transcripts do not seem to be specific for the *P* gene, since they do not correlate with the phenotype, but we cannot exclude the possibility that they are somehow involved in *P* gene expression.

Although the 7.3-kb region around the *Ac* insertion site is able to code for the largest transcript of 7 kb, we do not yet know whether the promoter of the *P* gene is also located in this region or whether RNA synthesis starts further 5′. At present, it is also unknown which of the five transcripts (or all of them) are important for the expression of the *P* gene.

Short-range Transposition of *Ac* from the *P-OVOV* Allele

T. Peterson, S. Allan

Last year, we reported the isolation and preliminary characterization of an allele termed *P-OVOV* (*o*range *v*ariegated pericarp and cob) derived as a change in state of *P-VV*. *P-VV* specifies colorless pericarp with red sectors, whereas *P-OVOV* specifies orange pericarp with many dark red sectors, and some colorless sectors. Southern analysis showed that the *Ac* transposable element is in the opposite orientation in *P-OVOV* relative to *P-VV*.

Cloning and sequencing show that inversion of *Ac* occurred by short-range transposition and reinsertion in an inverted orientation. The *Ac* element has transposed 160 bp toward the 5′ end of the *P* locus. Although the *Ac* element in *P-VV* is not bordered by host direct repeats, the *Ac* element in the *P-OVOV* allele is flanked by 8-bp direct repeats of a sequence that is present once in the progenitor *P-VV* allele. The orange variegated pericarp phenotype specified by *P-OVOV* may be considered as a mosaic of three phenotypes:

1. The orange background color may be due to a dilution of the red phlobaphene pigments, resulting from a reduced level of expression of *P*. Although we do not yet know why *P-OVOV* allows a moderate level of *P* expression, an orientation-dependent splicing mechanism similar to that proposed by Wessler et al. (*Science 237:* 916 [1987]) seems plausible; i.e., in *P-VV*, transcripts from *P* terminate within the *Ac* element, whereas in *P-OVOV*, the orientation of *Ac* may allow splicing out of element sequences during RNA processing.
2. The numerous red sectors may be due to excision of *Ac* from *P-OVOV*, thereby restoring a *P-RR* allele. We know that this is the case for one germinal *P-RR* revertant from *P-OVOV*.
3. The occasional light sectors may result from a variety of mutations at *P*, including deletions (see below) and short-range transpositions. We have characterized two germinal *P-VV** mutants that have variegated pericarp and cob resembling the progenitor *P-VV* allele. Both cases were derived

from kernels with variegated pericarp on otherwise orange variegated ears. In one case (*P-VV*-4177*), *Ac* has transposed from the site in *P-OVOV* and inserted at a site approximately 700 bp toward the 3′ end of the *P* locus, in the opposite orientation as in *P-OVOV* (i.e., in the same orientation as the *P-VV* "grandparent" allele). In the second case (*P-VV*-4189*), *Ac* has transposed from the site in *P-OVOV* and inserted at a site approximately 4 kb toward the 5′ end of the *P* locus. The orientation of *Ac* in *P-VV*-4189* is not yet known. In these experiments, we have not detected a strict polarity of transposition as might be predicted from Greenblatt's results showing a 4-map-unit region proximal to *P* that contained no *Ac* insertions following transposition from *P-VV* (Greenblatt, *Genetics 108:* 471 [1984]). Rather, our results indicate that *Ac* can transpose in either direction from the site in *P-OVOV* to other sites within the *P* locus.

P-OVOV Mutates to *P-WW* by Deletion

P. Athma, T. Peterson

As mentioned above, the *P-OVOV* allele carries an *Ac* element in the inverted orientation with respect to *P-VV*. The *P-OVOV* allele shows both sporophytic instability (pericarp sectoring; see above) and germinal instability, as evidenced by the progeny of the cross:

P-OVOV/P-OVOV × *P-WW/P-WW*
(both directions)

Among 10,820 progeny ears, 697 (6.4%) had red pericarp and cob (*P-RR*) and 89 (0.8%) had white pericarp and cob (*P-WW*). These frequencies can be compared to those previously reported by Brink (*Genetics 43:* 435 [1958]): Among 4575 offspring of the mating of *P-VV/P-VV* × *P-WW/P-WW*, 125 (2.7%) had red pericarp and cob and 8 (.17%) had white pericarp and cob. Thus, both *P-OVOV* and *P-VV* mutate to *P-WW* at low but detectable frequencies. It is not known whether the different frequencies arise from background effects, direction of the cross, or actual differences in mutation frequency of *P-VV* and *P-OVOV*.

We have investigated the molecular basis of seven *P-WW* alleles derived from *P-OVOV*. Each allele was obtained independently from kernels with mutant pericarp sectors from separate orange variegated ears. Southern analyses indicate that six of the seven *P-WW* mutants have a large deletion at the *P* locus; the seventh mutant has a more complex structure and will not be considered further here. To map the deletion endpoints, restriction fragments to the right and left of the *Ac* insertion site in *P-OVOV* were used as probes. Southern analysis with probes spanning a 3-kb region to the right of *Ac* showed that this region was deleted in the mutants. Similarly, the probes to the left of *Ac* representing a 9.5-kb region were also deleted.

Molecular analysis shows that the *P* locus contains two direct repeats of 5.8 kb, separated by 6.6 kb (see above). The *Ac* element in the *P-OVOV* allele is situated in the 6.6 kb of DNA between the two 5.8-kb repeats. In the *P-WW* mutants, the deletion endpoints lie within the two 5.8-kb homologous direct repeats, on either side of *Ac*. We suspect that the deletions may have occurred by homologous recombination between the two direct repeats such that the 17 kb of intervening DNA, including *Ac* and part or all of the *P* gene, is deleted.

The possible involvement of the *Ac* element in the occurrence of deletions is suggested by the apparent stability of the *P-RR* allele. Although *P-RR* contains the 5.8-kb direct repeats, mutations to *P-WW* are rare. On the other hand, the *P-WW-1112* allele, obtained directly from *P-VV*, has a deletion of the same type as the six *P-WW* mutants derived from *P-OVOV*. We do not know whether the deletions are somehow induced by the presence of an active *Ac* element or the increased length of DNA between the direct repeats.

We thank Rob Fincher and Ruth Meier of Pioneer Hi-Bred for overseeing the maize crosses and isolation fields.

Isolation of the *Hm1* gene by Transposon Tagging

G. Johal, S. Briggs

The *Hm1* locus of maize confers resistance to the fungal pathogen *Helminthosporium carbonum* (race 1). The pathogen causes a severe leaf spot disease, and under ideal conditions, it can cause complete rotting and subsequent blackening of the cob. Nothing is known about the gene product of the *Hm1* locus, which has been characterized phenotyp-

ically and by classic linkage analysis. Since knowledge of a gene's mRNA or protein product is essential for most widely used cloning procedures, there has been little progress to date in cloning these valuable genes.

An alternative gene-cloning strategy has been proposed that is based on gene tagging using mobile genetic elements. Three different transposable element systems have been used to tag the *Hm1* locus. These will be discussed separately, since they differ from each other in technology and strategy for creating mutations and subsequent handling of mutant material to isolate the target gene.

The major advantage of Robertson's Mutator system (*Mu*) is its high rate of mutation. This is perhaps due to the high copy number of *Mu1* elements (about 30) in mutator stocks. However, this high copy number of transposing *Mu* elements makes it difficult to correlate a specific restriction fragment with a mutant allele.

Cloning with *Mu* was initiated by crossing a *Mu Hm1* line with an *hm1* line. Several susceptible mutants were isolated from the cross. These mutants were crossed with a resistant inbred (*Hm1/Hm1*). Two different strategies have been used to identify *Mu* insertions at the *Hm1* locus.

From the outcross progeny of one of the mutants, a plant carrying the mutant allele was identified using restriction-fragment-length polymorphisms (RFLPs). Hybridization to *Mu* (a *Mu* 1.4 internal fragment) revealed 17 insertions. A bacteriophage λ library of 1.3×10^6 phage was constructed from a partial *Sau*3AI digest of genomic DNA. Sixty three *Mu*-homologous clones were recovered, out of which 39 seem to be unique.

The overall objective of this first strategy is to map each *Mu* insertion to its precise chromosome location. This will be done using recombinant inbreds. Each probe from the flanking DNA is hybridized to recombinant inbred parents cut with different enzymes to identify RFLPs. The recombinant inbred DNAs are then cut with the appropriate enzymes and probed with each clone. The pattern of RFLPs in the recombinant inbreds is sent to Brookhaven National Laboratory for entry into the database and placement on the genetic map. Clones that map to the proximal region of chromosome 1L will be tested for linkage with the *Hm1* locus as well as polymorphism between near isogenic inbreds that differ only at the *Hm1* locus. The clones that show tight linkage and/or polymorphism will be considered for further analysis to identify *Hm1* transcripts.

Another objective of this approach is to determine the genomic distribution of *Mu* elements and the pattern of transposition. By hybridizing each probe to siblings of the mutant, we can distinguish which *Mu* insertions were inherited by the mutant and which occurred de novo. This may provide insight into the mechanism of transposition of *Mu*.

During characterization of *Mu*-homologous clones, we have identified other size classes and types of *Mu* elements. One element is 1.2 kb in size and another is about 1.7 kb in size but differs from the previously described 1.7 *Mu*. Another *Mu* element appears to lack the terminal inverted repeats. We are in the process of subcloning these new *Mu* elements for sequencing purposes.

Another interesting observation is the presence of two copies of *Mu* in the same clone. There are at least 3 such clones out of 39. We are presently trying to identify the DNA fragment between the *Mu* elements to determine if it is common to all of the clones containing two copies of *Mu*.

The second strategy is to identify *Mu* restriction fragments that cosegregate with the mutant *Hm1** allele. Progeny from each of several mutants were grown and self-fertilized. The progeny that bear the *Hm1** allele, as opposed to the *hm1* tester allele, are being distinguished using RFLP probes. Among the progeny from the self-fertilization, *Hm1** homozygotes will be identified by inoculation. Hybridization of DNA from several progeny should reveal one or more bands present in all of the susceptible progeny but segregating in the resistant progeny.

The *Ac/Ds* transposable element system has also been used to mutagenize *Hm1*. A series of 2046 families have been generated, each of which is segregating for a different transposed *Ac*. These families were generated as part of a collaboration with Marc Albertson at Pioneer Hi-Bred International, Inc., and Stephen Dellaporta and Jychian Chen at Yale University. One of the families has been found to segregate for susceptibility to *H. carbonum*. Further tests are being done to clarify the relationship between the transposed *Ac* and the mutation.

The transposable element, *Spm*, is the third mutagenic agent we have used. Plants of the genotype *c-m5, Hm1* were crossed with *c, hm* plants. The *c-m5* allele was isolated by Barbara McClintock as an insertion of *Spm* at the *C* locus.

Purple kernels (C germinal revertant) were picked to enrich for transposed *Spm* elements. These kernels were planted in the greenhouse and inoculated as seedlings to score for susceptible mutants. From the

cross where the *Spm* parent was male, we recovered 5 mutants out of 6000 revertant kernels. Another 3 mutants were recovered from the cross where the *Spm* plant was the female parent. Tests are under way to determine whether any of these mutations are associated with transposition of *Spm* to the *Hm1* locus.

Genetic Characterization of Transposed *Ac* Elements in *Rp1* and *Rpp9* Mutant Lines

S. Briggs, B. Bergen

Resistance to infection by the common rust (*Puccinia sorghi*) and southern rust (*Puccinia polysora*) pathogens is conferred by *Rp1* and *Rpp9*, respectively. In collaboration with D. Wilkinson and D. Christiansen at Pioneer Hi-Bred International, Inc., several *Rp1* and *Rpp9* mutants have been identified. During the past year, genetic and molecular studies have been done to determine which, if any, of the mutations have been caused by insertion of an *Ac* element.

Plants of the constitution *r:nj:m1/r, Rpp9* were pollinated by an *r, rpp* tester. The *r-nj:m1* allele was derived by Irwin Greenblatt as an insertion of *Ac* at *R-nj*. Excision of *Ac* from *r-nj:m1* can restore the *R-nj* phenotype. *R-nj* revertant kernels (5300) were selected from 71 ears (families). Approximately 5000 seedlings were inoculated and scored for susceptibility in the greenhouse. Six susceptible progeny were observed from four different families. Seed was obtained from four of the plants, representing three of the families, by crossing to an *r-m3, rpp* tester. Jerry Kermicle derived the *r-m3* allele as a *Ds* insertion at the *R* locus. All four plants transmitted a transposed *Ac* to their offspring according to their ability to destabilize *r-m3*. By counting the members in each progeny class, it was possible to determine the linkage between the transposed *Ac* and *R-nj*. The target locus, *Rpp9*, and the donor locus, *R-nj*, lie on the same chromosome, 61 cM apart. Transposition of *Ac* from *r-nj:m1* to *Rpp9* would result in a transposed *Ac* unlinked to *R-nj*. In two cases, *Ac* was tightly linked to the donor locus *R-nj*, but in the other two cases, the transposed *Ac* was unlinked to *R-nj*. To ascertain whether or not *Ac* was linked to the target locus, *Rpp9*, in the latter two cases, closely linked RFLP probes were hybridized to blots of siblings that were segregating for transposed *Ac*. In one case, the RFLP probe NPI 285 (a gift from NPI, Inc.), which is tightly linked to *Rpp9*, was found to be unlinked to transposed *Ac*. In the other case, the fragment corresponding to the mutant-bearing chromosome was not observed, suggesting that this region of the chromosome may have been deleted.

A similar study has been done with *Rp1*, which is tightly linked to *Rpp9*. Plants of the constitution *r-nj:m1/r, Rp1-d* were pollinated by an *r, rp* tester. *R-nj* revertant kernels (1500) were selected and planted. These kernels came from 47 ears (families). Approximately 1300 plants grew and were screened for susceptibility. A total of 68 susceptible plants from 6 different families were identified. Seed was recovered from 35 of the plants, representing 5 families, either as self or outcross progeny.

Fifteen plants from each of the 35 putative mutant families were crossed with the tester, *r-m3, rp*. Thirteen of the families contained a transposed *Ac*. Seven of the 13 transposed *Ac*s are unlinked to *R-nj*. We are now determining whether any of the 7 unlinked transposed *Ac*s are located at the *Rp1* locus.

Our first tests for cosegregation of each transposed *Ac* with the mutant *Rp1* alleles utilized RFLP probes for the chromosome region of *Rp1*. However, polymorphisms between the disjoining chromosomes have not been found. We are continuing to search for polymorphisms, and, in the meantime, we have begun isolating the transposed *Ac*s as restriction fragments; these can be used for cloning or for generating probes of the flanking DNA with an inverse polymerase chain reaction. In either case, unique probes from the flanking DNAs will be generated for mapping the genetic location of each transposed *Ac* with a highly polymorphic recombinant inbred family.

The transposed *Ac*s are being isolated by taking advantage of the propensity of active *Ac*s to be flanked by hypomethylated DNA. For example, in one family, *Pst*I generated a 9-kb *Ac*-homologous restriction fragment only from siblings that inherited an active *Ac*. Cryptic (homologous but inactive) elements form a high-molecular-weight smear on the blot. Such digests are fractionated on glycerol gradients to isolate the fragment bearing the active *Ac*. We have identified at least one enzyme for isolating the transposed *Ac* from four of the seven cases where *Ac* is unlinked to the donor (*R-nj*) locus.

Quantitative Protein Changes in Response to HC Toxin

B. Elliott, S. Briggs

Resistant (*Hm1*) and susceptible (*hm1*) inbreds were crossed, and the progeny were selfed to generate F_2 immature embryos. The immature embryos were used to initiate embryogenic tissue cultures by Jon Duvick and Joyce Maddox at Pioneer Hi-Bred International, Inc. Each culture line was characterized with regard to HC toxin sensitivity, which segregates with *hm1*.

We have used these culture lines to study changes in protein synthesis or modification following exposure to HC toxin. Proteins were labeled using [^{35}S]methionine. Gels were run and digitized by members of the Quest facility. Sensitive and insensitive lines were compared to control for any nonspecific effects. Samples were taken at different times following exposure to HC toxin. A comparison of three different sensitive and three different resistant lines after 1 hour of treatment revealed a single spot, which decreased approximately 50% in the resistant lines, but increased, remained stable, or decreased slightly in the susceptible lines. After 1 hour of treatment, seven other proteins had increased in only the susceptible line(s). This change peaked between 1 and 5 hours after exposure with a 5- to 20-fold increase, followed by a return toward the control level by 11 hours after exposure. The cultures turn brown 18 hours after exposure, but it is not clear at what time cell death occurs.

Either tunicamycin or cycloheximide will protect sensitive lines from HC-toxin damage. Removal of inhibitor restores toxin sensitivity. [^{3}H]Mannose labeling revealed that one of the proteins that increased dramatically in susceptible cells upon exposure to toxin is a glycoprotein. None of the proteins described appear to be phosphoproteins.

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CELL-CYCLE CONTROL IN *SACCHAROMYCES CEREVISIAE*

B. Futcher R. Nash K. Erickson
G. Tokiwa

This is our first year at Cold Spring Harbor Laboratory, and a very productive year it has been. We were able to clone the *WHI1*$^+$ gene, which had eluded us for several years, and found that it was a cyclin homolog. A year ago, we thought we were alone working on a potentially interesting but overlooked gene; now we find ourselves (and others!) working on a protein that may be the cell-cycle timer in yeast, echinoderms, amphibians, mammals, and probably eukaryotes in general.

Cloning and Partial Characterization of *WHI1*

G. Tokiwa, R. Nash, K. Erickson, B. Futcher

WHI1-1 is a dominant mutation that produces small-size cells ("wee" cells) with very short G_1 phases (Figs. 1 and 2). Mutant cells are small because they commit themselves to cell division prematurely; we interpret this to mean that the cellular measuring

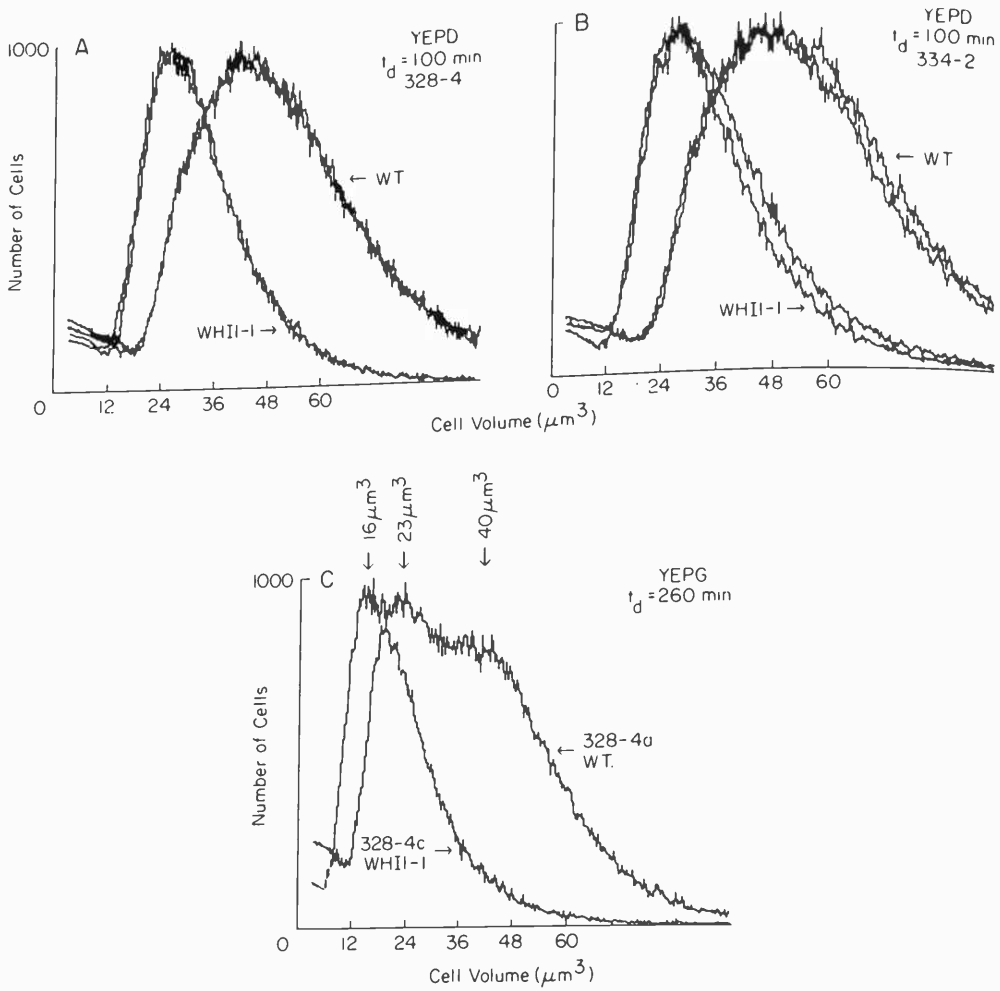

FIGURE 1 Coulter channelyzer plots of cell volume distributions. (*A*) Tetrad BF328-4 segregating 2:2 for WHI1-1; (*B*) tetrad BF334-2. (*C*) Wild-type and a mutant strain growing in a medium containing the relatively poor carbon source glycerol. t_d is the culture doubling time. The modes of the peaks are indicated in *C*.

device that determines the proper time for division is abnormal. We mapped the mutation, and from the map position, we were able to clone the wild-type and mutant genes. Dosage studies were done with the cloned genes. Zero doses (i.e., a deletion of the gene) produced large cells with long G_1 phases—the opposite of the *WHI1-1* phenotype. Increasing the dosage of the wild-type gene (*WHI1*$^+$) decreased cell size and decreased the length of G_1. Increasing the dosage of the mutant gene reduced cell size to about 50% of wild type, and decreased G_1 to nearly 0% of the cycle (Table 1; Fig. 2). Thus, in some sense, the mutant gene is hyperactive, since it behaves like many doses of the wild-type gene. The fact that *WHI1*$^+$ dosage affects the time of commitment generally supports the idea that the WHI1 protein is part of a cellular measuring device.

The wild-type and mutant genes were sequenced, and this gave three important pieces of information. First, the newly installed Fast A program found that *WHI1*$^+$ was a cyclin homolog (Fig. 3), a result confirmed by statistical analysis done by G. Otto. Cyclins (not to be confused with proliferating cell nuclear antigen [PCNA], formerly called cyclin) were first discovered in clams and sea urchins. They are proteins that accumulate with time in newly fertilized oocytes and then are suddenly destroyed at mitosis. This pattern of accumulation and sudden degradation occurs in each cell cycle. Microinjection experiments have shown that quiescent oocytes could be forced through meiosis I by cyclin mRNA (Swenson, Farrell, and Ruderman; Pines and Hunt), and this is consistent with the observation that *WHI1-1* promotes premature division.

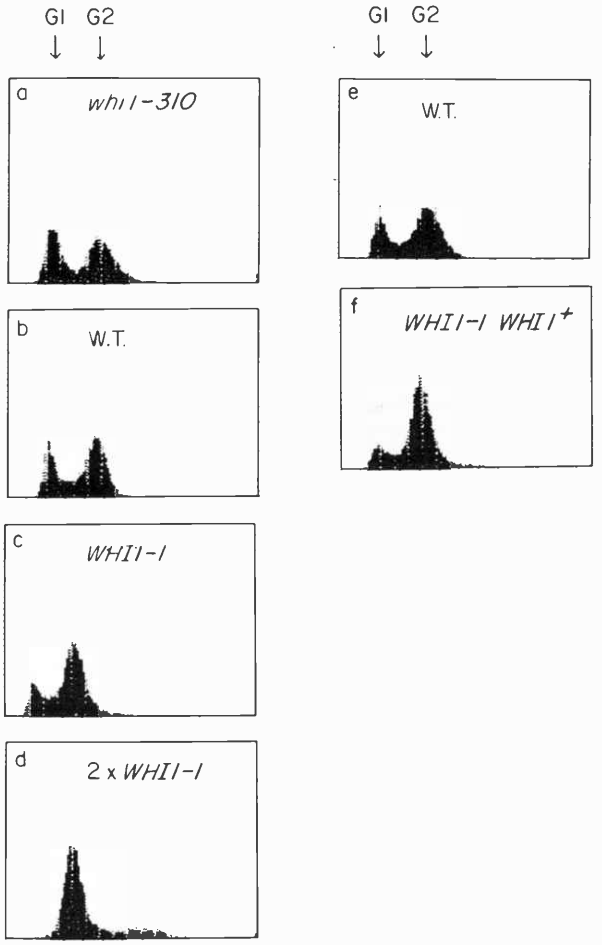

FIGURE 2 Flow cytometry. Cellular DNA was stained with propidium iodide, and the fluorescence per cell was measured. The *y* axis is the number of cells; the *x* axis is the intensity of fluorescence. Peaks due to cells in G_1 and G_2 are indicated. The G_2 peak actually includes cells in G_2, M, and cytokinesis. (*A*) Deletion allele; (*B*) wild-type allele; (*C*) *WHI1-1* mutant allele. (*D*) Haploid containing two copies of the mutant gene; this strain seems to lack G_1 entirely; (*F*) haploid strain containing one wild-type gene and one mutant gene; the short-G_1 phenotype indicates that *WHI1-1* is dominant, as expected for a hyperactive allele.

Second, the *WHI1-1* mutation is a stop codon two thirds of the way through the gene. Thus, the lack of the wild-type carboxyl terminus apparently hyperactivates the protein. Third, this carboxy-terminal third has an unusual amino acid composition. It is very rich in proline, serine, and threonine. Regions of similar composition have been called PEST (*P*roline, *G*lutamate, *S*erine, *T*hreonine) regions by Rogers, Wells, and Rechsteiner; such regions are found in nearly all proteins with short half-lives and may be signals for degradation. PEST regions

TABLE 1 Effect of WHI1 Alleles on Cell Volume and G_1 Phase

Genotype	Cell volume (μm^3)	Normalized volume	% of Cycle spent in G_1
whi1-310 (Δ)	55 ± 5	1.2	37
WHI1$^+$	44 ± 3	1.0	27
2× *WHI1*$^+$	35 ± 3	0.80	n.t.
WHI1-1	27 ± 2	0.61	19
WHI1$^+$ *WHI1-1*	27	0.61	15
2× *WHI1-1*	25	0.57	0–10

n.t. indicates not tested. ± represents the range over at least eight measurements. Where ± is not indicated, less than eight measurements were done. For most constructions, each transformant behaved similarly, but for 2× *WHI1-1* strains, some transformants repeatably had a G_1 of about zero, whereas others had a G_1 of about 10%.

are found in the other sequenced cyclins as well as in *WHI1*$^+$ (Fig. 3).

These observations led to the hypothesis that (1) the PEST regions of *WHI1*$^+$ and other cyclins have something to do with the sudden degradation of these proteins at a certain point in the cell cycle and (2) the hyperactivity of the mutant WHI1-1 protein is due to the loss of this PEST region, so that it can no longer be degraded. We will be testing this hypothesis in the current year.

The *whi3* Mutation

R. Nash, B. Futcher

One of our chief difficulties in working with *WHI1-1* was that it was not deleterious to the cell, and so it could not be cloned by selecting for a complementing plasmid. We wished to find new Whi mutants in a way that would make cloning easy even if no selection could be applied for the wild-type gene. To do this, we used the technique of transposon tagging. A Ty transposon marked with G418 resistance, and carrying a galactose-inducible transposase, was obtained from J. Boeke. Yeast were transformed with this construct, and transposition was induced. An apparently new *whi* mutation was obtained, which we named *whi3*. The mutation was genetically inseparable from G418 resistance, as expected if it were caused by a Ty insertion. The G418-marked Ty element has been cloned out of the mutant strain along with flanking DNA. This flanking DNA has been used as a probe to obtain clones from a library of wild-type DNA. These clones may include the intact *WHI3* gene.

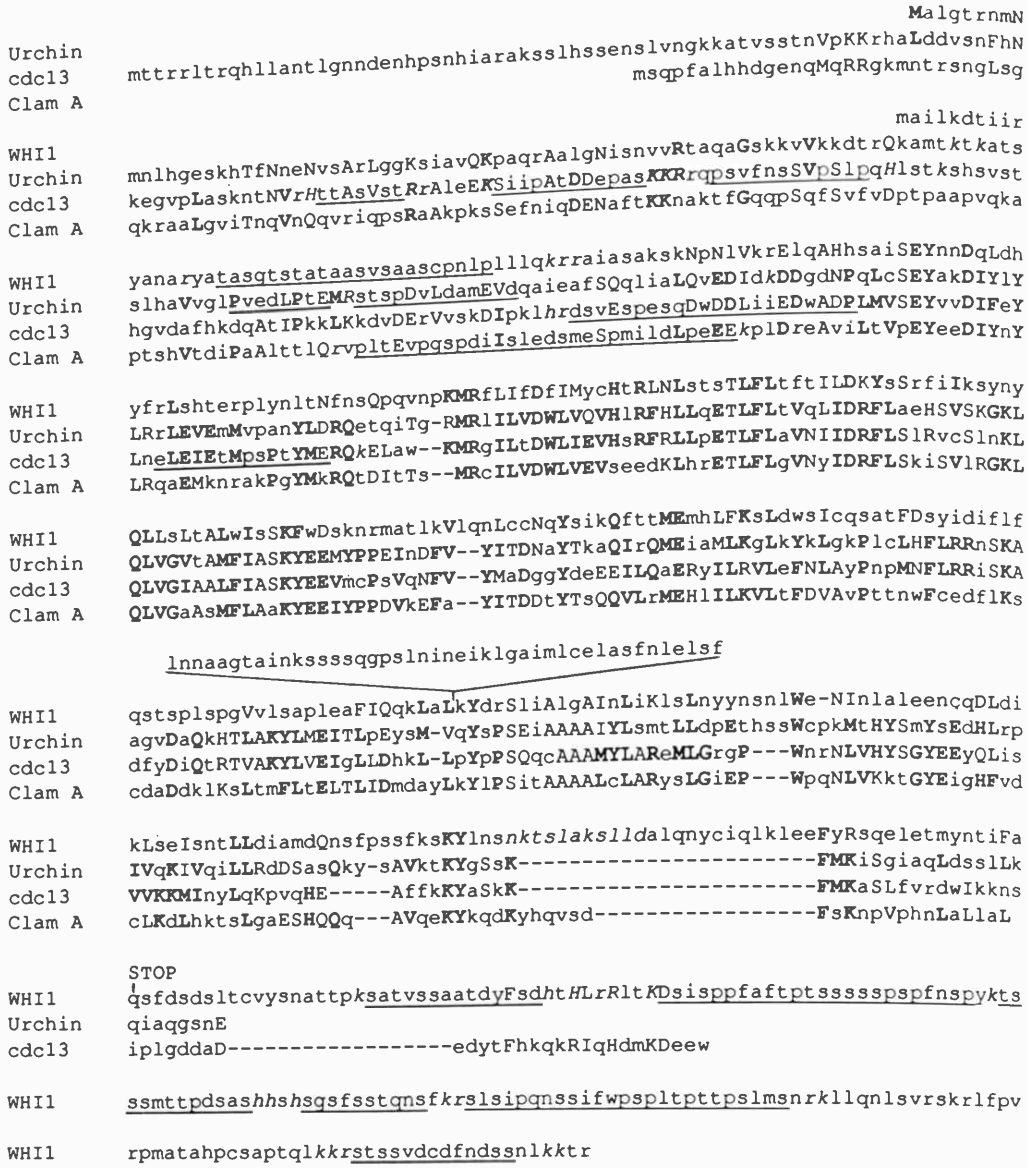

FIGURE 3 Alignment of *WHI1** with three cyclins. The complete sequences of *WHI1**, sea urchin cyclin, clam cyclin A, and *S. pombe cdc13* are shown. Uppercase letters represent conserved residues; bold uppercase letters represent conserved residues with a high Mutation Data Matrix weighting. Possible PEST regions are underlined. Basic residues associated with PEST regions are italicized. Also italicized is the *WHI1** sequence nktslakslld, which is similar to a sequence found 95 residues more amino-terminal in the other cyclins. The position of the stop codon in *WHI1-1* is shown.

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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 lab to study transcriptional 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* regulation 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 (approximately 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. In addition, we have very recently found that BAS1, but not BAS2, regulates pyrimidine biosynthesis. The particular genes under BAS1 and BAS2 regulation in some of these pathways have yet to be determined.

In vitro, BAS2 present in yeast extracts binds to the *HIS4* promoter (at a site different from GCN4) in the presence or absence of BAS1. In vivo, however, both BAS1 and BAS2 are required in order to activate *HIS4* transcription. In contrast, the BAS1 protein is not required for BAS2 to activate *PHO5* transcription. Under conditions where *HIS4* has high basal level transcription, the transcription of the *PHO5* gene is extremely low. *PHO5* transcription derepresses only under conditions of phosphate starvation. This differential activation by BAS2 is very interesting, since the BAS2 protein is homologous to homeobox proteins of *Drosophila* and is most homologous to the engrailed protein. DNase I footprint analysis shows that the engrailed protein binds to the *HIS4* promoter with an affinity equal to or greater than the presently defined engrailed binding sites in *Drosophila*. In addition, the engrailed protein and BAS2 protect identical nucleotides of the *HIS4* promoter from DNase I digestion. We have expressed engrailed in yeast under control of the *GAL1* promoter and are determining whether engrailed can substitute for BAS2 for activation of *HIS4* transcription and if this activation requires BAS1. These experiments are directly relevant for *Drosophila* development, since results in Pat O'Farrell's laboratory show that engrailed by itself binds to the promoter of a reporter gene but does not activate transcription of this reporter gene in *Drosophila* cell culture. Thus, engrailed (like BAS2) may require a protein similar to BAS1 in order to activate transcription. Such a protein is already known to exist in *Drosophila*.

We have sequenced the *BAS1* gene and found that it is homologous to the *myb* oncogene that was originally isolated from a chicken virus and later

shown to have cellular homologs in chickens, mice, humans, and *Drosophila*. The expression of the *Drosophila myb* gene is very high in the embryo but very low in the adult, suggesting that the Myb protein has a role in embryogenesis. We have overproduced the BAS1 protein and various derivatives in bacteria using both λP_L and T7 promoters (BAS1-binding activity is undetectable in extracts prepared from wild-type yeast). We have found that (1) the amino-terminal domain of BAS1, which contains the *myb* repeat motif, binds specifically to the *HIS4* promoter; (2) BAS1 protects a region of 26 bases on the *HIS4* promoter that overlaps with the BAS2-binding site; (3) even though BAS1- and BAS2-binding sites overlap by eight to ten bases, they can bind simultaneously to the promoter; (4) the BAS1 protein and the engrailed protein can also bind simultaneously; and (5) BAS1 and BAS2 do not bind cooperatively to the *HIS4* promoter. Thus, the adjacent binding of BAS1 and BAS2 is required to activate *HIS4* basal transcription. Overproduction in yeast of either protein in the absence of the other only weakly activates *HIS4* transcription. We are currently investigating if the BAS1/BAS2 system is homologous to a Myb/engrailed system. That BAS1 cannot activate *HIS4* transcription without BAS2 suggests why the v-*myb* oncogene transforms myeloid and erythroid hematopoietic cells in culture but cannot transform fibroblasts or other cell lines. Perhaps only a few adult tissues contain the BAS2-like protein necessary for *myb* to cooperatively activate transcription. Presently, we are investigating the details of the BAS1/BAS2 transcriptional activation system.

Another protein present in yeast nuclear extracts, YNF1, binds to a single site in the *HIS4* promoter. When YNF1 binds to this site, it displaces both GCN4 (from its highest-affinity binding site) and BAS2 from their binding sites. In the absence of BAS1, BAS2, and GCN4, YNF1 does not activate *HIS4* transcription. Since YNF1 displaces two activators of transcription (competition determined by in vitro DNase I footprinting), our working model is that YNF1 functions as a repressor of *HIS4* transcription. We have screened 500,000 plaques from a yeast λgt11 expression library using a labeled DNA fragment containing four tandem YNF1-binding sites. However, although positive controls have worked, the *YNF1* gene has not been found among the λgt11 plaques. We are switching to a genetic approach for the isolation of the *YNF1* gene. The type of regulation imposed on *HIS4* transcription by YNF1 will then be determined.

General Transcription Factors in Yeast

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 a 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 increased *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. Another effect of high-copy-number *SIT3* is suppression of the lack of a TATA sequence. In a wild-type cell, both GAL4 protein and GCN4 protein require a TATA sequence to activate transcription. However, high-copy-number *SIT3* is able to increase transcription by GAL4 or GCN4 from promoters containing no 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. 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. We are presently using

two approaches to examine if SIT3 is one of a set of similar general transcription factors that recognize bound transcriptional activation proteins and in turn interact with RNA polymerase II. The first approach is to find other genes that, when mutated, are lethal in combination with a deletion of the *SIT3* gene. A strain with a deletion of the *SIT3* gene is extremely sick but viable. The second approach we are taking is to find other genes in high copy number that can stimulate transcription from defective GCN4 derivatives that bind DNA normally but have very weak acidic activation domains. We will subsequently use direct biochemical analysis to determine if SIT3 and any other factor interact with the acidic domain of the activation proteins and the acidic heptapeptide repeat at the carboxyl terminus of RPB1.

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. We are currently testing this model by Western and immunoprecipitation analyses to determine the phosphorylation state of RPB1 in strains containing wild-type *SIT4*, *sit4* mutations, and overexpression of SIT4.

The SIT4 Protein Phosphatase Is Required for Progression through G_1

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 with spindle pole satellites. These cells continue some growth and become misshapen "shmoos." This G_1 arrest point in *cdc28* mutants has been operationally defined as START. Supposedly, if a normal cell passes this point, it is committed to completion of the cycle. The execution point or the time of action of the *CDC28* kinase is probably at some point before START. Throughout the cell cycle, the amounts of the *CDC28* protein are constant, but its kinase activity occurs almost exclusively during G_1 (and possibly only during early G_1) when *CDC28* is associated with p40 in a higher-molecular-weight complex. Within this higher-molecular-weight complex, p40 itself is phosphorylated, presumably by the *CDC28* kinase. Fission yeast and human cells also contain a kinase (*cdc2*) that is similar to *CDC28*, but in these organisms, this kinase is also required for the G_2/mitosis transition. In this additional role, the Cdc2 protein homolog functions in a complex that was previously identified as MPF (maturation-promoting factor) and histone H1 kinase. The existence of a *cdc2* homolog in human cells implies that certain aspects of cell-cycle control are the same in yeasts and vertebrate organisms. If so, vertebrate cells are likely to have a G_1 decision/commitment point for entry into the cell cycle analogous to the yeast START (and a second control acting at the G_2/mitosis transition).

Most cell-cycle control models include kinases (such as the Cdc2 and CDC28 kinases) that add phosphate groups to their substrate proteins. What is not usually included in these discussions is the involvement of protein phosphatases, which remove phosphate groups from proteins. The only known way to remove phosphates from a protein, other than protein degradation, is via protein phosphatases. The involvement of protein phosphorylation/dephosphorylation in controlling a protein's activity has been recognized for many years.

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. These *sit4*-arrested cells have a diameter about 1.5 times as large as the diameter of *cdc25* or *cdc35* cells at the nonpermissive temperature. In addition, *sit4*-arrested cells contain a single organizing center for microtubules on the surface of their larger than normal single nucleus. Since the spindle pole body is the organizing center for the microtubules, this result probably indicates that spindle pole migration has not occurred. We currently do not know whether or not the spindle pole body has duplicated.

Since *sit4* cells arrest in G_1, we have investigated genetic interactions between *sit4* alleles and *cdc25* or *cdc28* alleles. In crosses of *sit4* with *cdc25*, the expected number (25%) of double mutant progeny were obtained and the haploid double mutants had normal mitotic growth. However, in crosses of *sit4* alleles with *cdc28*, the expected number of double mutant progeny were not obtained. Haploid strains containing a chromosomal *sit4* allele, a chromosomal *cdc28* allele, and the wild-type *SIT4* gene on a *URA3* centromere plasmid have normal mitotic growth. However, this strain cannot grow at all on growth medium that selects against the plasmid *URA3* gene. Thus, the *sit4 cdc28* double mutation is mitotically lethal.

We are currently investigating the role of SIT4 in G_1 progression by both genetic and biochemical approaches. We have taken two genetic approaches. The first approach is to obtain high-copy-number suppressors of the slow growth of *sit4* strains. Using this approach, we have obtained five different genes. One of these genes, which gives weak suppression, is another protein phosphatase (which we call *PPH2*). We have sequenced about half of the *PPH2* gene and found that it is about 90% identical to type-2A protein phosphatases. We are now investigating if *PPH2* is essential and if it is a cell-cycle gene. The second genetic approach, which is in progress, is to obtain second-site suppressors of the temperature-sensitive arrest defect of the *pph1-102* allele. This *PPH1* allele was isolated solely for near-normal growth at permissive temperatures but a temperature-sensitive growth defect at 38°C. At the nonpermissive temperature, this strain arrests in G_1 identically as *sit4* strains. Hopefully, some of the suppressors of the temperature-sensitive arrest phenotype of *pph1-102* strains will be substrates of the phosphatase.

We are also using immunological and biochemical analyses to understand the function of PPH1 (=SIT4). We have prepared antibodies to the amino terminus of PPH1 and have also tagged PPH1 with an epitope that is recognized by a monoclonal antibody. These will serve as tools for immunofluorescence (to determine the localization of PPH1) and immunoprecipitation analyses. In particular, we are looking for interactions of PPH1 with the CDC28 kinase and possibly BCY1 (regulatory subunit of cAMP-dependent protein kinases). Hopefully, our combined genetic and biochemical approach will elucidate the role of the PPH1 phosphatase in G_1 progression.

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A.J.S. Klar J. Wood

The Developmental Fate of Fission Yeast Cells Is Determined by the Patterns of Inheritance of Parental and Grandparental DNA Strands

A.J.S. Klar

Discovering the mechanism by which sister cells gain different developmental fates is central to an understanding of eukaryotic cell differentiation. This issue can be addressed in molecular terms by investigating the pattern of cell fate determination in the fission yeast *Schizosaccharomyces pombe*. Mitotically dividing cells of this ascomycetes fungus exist as one of the two alternate cell types, called *P* (for *P*lus) and *M* (for *M*inus). However, the *P* and *M* mating types are unstable and the cell types interchange spontaneously nearly every other generation. When the phenotype of individual cells is monitored, a remarkable pattern of cell-type switching is observed. Miyata and Miyata (*J. Gen. Appl. Microbiol. 27:* 365 [1981]) found that among a pair of sister cells, one will divide to produce one changed cell and one unchanged cell in about 72–94% of cell divisions, whereas the other sister always produces an unchanged pair of cells. In other words, according to this so-called "one-in-four rule," only one grandchild acquires the switched mating type among the four grandchildren of a cell obtained after two generations.

The *P* and *M* cell types are controlled, respectively, by the alternate *mat1-P* and *mat1-M* alleles of the mating-type locus (*mat1*). Mating-type interchange involves a gene conversion event in which a copy of unexpressed mating-type information residing at the *mat2-P* or the *mat3-M* "donor" locus is transposed to *mat1*, resulting in a switch of cell type. Previous studies have argued that the recombination event required for *mat1* switching is initiated by a site-specific double-stranded DNA break (DSB) found in the *mat1* gene. By assuming that the cut DNA at the receptor site is a necessary precondition for switching by gene conversion, it then follows that the one-in-four switching rule must be a consequence of the observed level of in vivo DSB.

The switching of one in four related cells must be the result of unequal distribution of developmental potential to daughter cells in each of two consecutive apparently asymmetrical cell divisions. A strand segregation model was proposed (Klar, *Nature 326:* 466 [1987]) to explain the developmental asymmetry in which a hypothetical site- and strand-specific "imprinting" of the specific *mat1* DNA strand (say "Watson strand") is assumed to occur in the cell. That cell could generate developmentally specific unequivalent daughter cells, since one daughter inherits the imprinted Watson strand while the other daughter inherits the unimprintable Crick strand.

The strand segregation model makes two specific predictions: one molecular and the other genetic. In an earlier observation (Klar, *Nature 326:* 466 [1987]), it was found by DNA analysis that strains constructed to contain the *mat1* inverted duplication exhibit the DSB in vivo in one cassette or in the other, but *never* simultaneously in both cassettes in a given chromosome. In addition, compared to wild-type strains, twice as many chromosomes contain the DSB, thus satisfying the molecular prediction. Another key genetic prediction of the strand segregation model is that *two*-in-four related cells should switch in strains containing an inverted tandem duplication of *mat1*. This is predicted because, in a given cell, both strands can be imprinted: one strand in one cassette and the other strand in the second cassette. Their segregation will generate two developmentally equivalent sister chromatids, and their inheritance should produce equivalent sister cells. Each of the equivalent sister cells may generate one switched and one unswitched progeny. The essence of the second critical prediction is that two "cousin" cells should switch.

A particularly satisfying result obtained by employing a single-cell assay testing the pattern of switching is that two (cousins)-in-four related cells indeed switch in strains containing an inverted tandem duplication of *mat1*. Thus, both parents of those four cells have become developmentally equivalent. I therefore conclude that the pattern of switching in the fission yeast cell lineage is dictated

by the pattern of inheritance of DNA strands and not because of unequal distribution or expression of factors in sister cells.

In Press, Submitted, and In Preparation

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STRUCTURE

This section includes four laboratories interested in the detailed structure properties of proteins. The Structure Program is relatively new and was organized when Jim Pflugrath and John Anderson joined Cold Spring Harbor Laboratory to set up a new X-ray crystallography laboratory. The other two groups that are associated with this program are those of Mark Zoller, whose laboratory is interested in the structure-function relationships of protein kinases, and Rich Roberts, whose laboratory has had a long-standing interest in restriction enzymes and their associated methylases.

Advances in recombinant DNA technology now allow the ready cloning and overexpression of proteins of interest. This, combined with new methods for purification, offers the possibility that many proteins important for our work can be obtained in quantities sufficient to permit their crystallization. The time is right to capitalize both on the new technology and on the possibilities for analysis and understanding that can be afforded by X-ray crystallography. Over the next few years, we anticipate growing interactions between the members of the Structure Program and all other members of Cold Spring Harbor Laboratory.

NUCLEIC ACID CHEMISTRY

R.J. Roberts L. Hamablet, C. Marcincuk, J. Martling, J. Meyertons, S. Miceli, G. Otto, J. Posfai, D. Roberts, M. Wallace

Cytosine Methylase Domains

J. Meyertons

My research has focused on identifying the protein domains of *Hha*I (recognition sequence: 5′ G^{me}CGC 3′) and *Hpa*II (recognition sequence: 5′ C^{me}CGG 3′) methylases that are responsible for sequence-specific recognition of DNA. The initial step involved constructing a pBR322-based plasmid able to express both methylase genes. The plasmid was constructed with the two methylase genes in tandem and arranged in the same orientation relative to transcription. In one construct, the *Hha*I methylase was upstream of the *Hpa*II methylase; in the second construct, the *Hpa*II methylase was upstream of the *Hha*I methylase. Unique restriction sites between the methylases can be used to linearize the plasmid and allow nuclease BAL-31 digestion. After nuclease BAL-31 digestion, the deleted plasmids can be religated and transformed into *Escherichia coli*. Among the products of these manipulations, there should be hybrid methylases that have the amino-terminal region of the *Hha*I methylase fused to the carboxy-terminal region of the *Hpa*II methylase, and vice versa. In this way, I hope to swap the recognition domains of the two proteins and then to characterize that domain both genetically and biochemically.

Because the hybrid proteins may be less efficient as methylases than their parents, I am setting up an assay system able to detect very low level expression of cytosine methylases. This assay depends on the *Mcr* system of *E. coli*. This is a sequence-specific, modification-dependent restriction system that cleaves DNA at 5-methylcytosine residues. Transformation of a methylase plasmid into an *E. coli* strain with an active *Mcr* system prevents the survival of the methylase because the methylated DNA is cleaved. To detect the hybrid methylases, I am

constructing strains with temperature-sensitive mutations in the *Mcr* genes. Two main *Mcr* systems are known, A and B. The A system is known to restrict DNA methylated by the *Hpa*II methylase, whereas both systems restrict DNA methylated by the *Hha*I methylase. Appropriate *Mcr* A^+ and *Mcr* B^+ strains were mutagenzied to produce temperature-sensitive or cold-sensitive mutations in the *Mcr* genes. The presence of the *Mcr* mutations was confirmed by cross-streaking with T-even phages and with bacteriophage λ methylated by *Hha*I or *Hpa*II and complementation tests.

To aid in the identification of an active hybrid methylase further, an SOS-inducible promoter fused to the *lacZ* gene has been transduced into each of the *Mcr* temperature-sensitive strains. In these strains, β-galactosidase is made in response to any DNA damage, such as cleavage of methylated DNA. The intensity of the blue color can be used as an indicator of the levels of methylase activity. A light-blue colony could represent weak methylation activity that was not strong enough to cause lethality at the nonpermissive temperature. The temperature-sensitive alleles in the *Mcr* genes along with the colony color indicator in the strain will provide two complementary methods to look for methylase activity. The *Mcr* temperature-sensitive genes will be further characterized because these temperature-sensitive strains will have future value in studying other methylases including eukaryotes, such as mammals and plants.

Structure and Function of Integration Host Factor, a Site-specific DNA-binding Protein in *Escherichia coli*

D. Roberts

Integration host factor (IHF) is a site-specific DNA-binding protein that has been shown to play a role both in the regulation of gene expression and in site-specific recombination (for recent review, see Friedman, *Cell 55:* 545 [1988]). IHF was first identified as an *E. coli* factor necessary for bacteriophage λ integration in vitro. IHF binds specifically to DNA, recognizing a 13-nucleotide nonpalindromic consensus sequence. IHF binding causes the DNA to bend at the site of binding.

In vivo, a variety of cellular processes are affected in IHF mutants. In addition to decreased λ integration, there is decreased growth, packaging, or lysogenization of bacteriophages 21, φ80, and Mu. IHF mutants are unable to support replication of the plasmid pSC101, and they exhibit decreased excision or transposition of transposable elements. Finally, expression of several genes is altered, including the *ilv* operon, IHF itself, λcII, Mu early genes, Tn*10* transposase, and the *tra* genes of the F plasmid.

The primary goal of this work is to analyze the structure and function of IHF. To do this, I plan to generate an extensive set of IHF mutants. These mutants will be used to identify the essential structural domains for DNA binding, DNA bending, DNA site recognition, and dimer formation. Of particular interest would be mutants that can bind to DNA but fail to bend it; these mutants should help define the role of DNA bending in the recombination reactions in which IHF is involved. Also of interest would be mutants with altered sequence specificity; these should define the regions of the protein that recognize and interact with DNA. This structural and functional analysis of IHF should provide new information about how a protein can recognize a specific DNA sequence, as IHF is likely to be structurally different from other known sequence-specific DNA-binding proteins.

Measures of Sequence Similarity

G. Otto

In a general sense, taxonomy is the classification of comparable objects into related sets. Although taxonomic analysis is prominent at the organismic level, it is conspicuously underdeveloped in molecular biology. The rising flood of sequence data only emphasizes the need for general methods of analysis that group similar sequences into sets that share and are predictive of given functions. Beyond these predictive goals are questions about the origins of DNA and protein diversity, such as whether existing sequences are descendents of a small set of progenitor domains. Taxonomic analysis of sequences differs from that of organisms in two important respects: (1) The simplicity of sequences allows for measures of similarity that are more rigorous than those available for complex morphological traits and (2) the results of sequence analysis make testable predictions. The goal

of my research is to write a set of programs that systematically analyzes a given database of sequences and returns the taxonomic structure of the database as its output.

The measure of similarity is the critical issue for developing a taxonomy of sequences. Past work has not emphasized probability theory, yet this approach provides a natural and convenient measure of similarity. A probability value can be calculated for every alignment of two sequences; the optimal alignment is the one whose P value is smaller than all the others. Similarity is then defined as this smallest P value and represents the probability that the optimal alignment of two sequences is due to chance. Probabilistic measures can be derived for any criteria of sequence matching. The matching of identical residues is the simplest of these matching criteria. In this case, the calculation of P values reduces to a simple iteration of the binomial formula. Measures for other matching criteria, such as those that allow conservative substitutions, will require more sophisticated techniques. However, this simplest measure of similarity illustrates the main points of the analysis.

Every pairwise comparison is characterized by the P value of its optimal alignment; these values vary between 0 and 1. The closer a P value is to zero, the greater the confidence that two sequences are similar. The distribution of P values expected from the comparison of unrelated sequences will be important to this discussion. If a sequence is randomly generated and compared with a set of unrelated sequences, the resulting P values are expected to be uniformly distributed over the unit interval (0–1). The methods for calculating these probabilistic measures of similarity are tested by comparing large sets of unrelated sequences and asking whether the resulting distributions of P values are uniform. Given a proper measure of similarity, deviations from uniformity provide a basis for doing groupwise comparisons.

The first step is to partition the database into sets of sequences sharing common functions, such as the binding of ATP or NAD or proteolytic activity. The test sequence is then compared with the sequences of each functional set. If the test sequence is unrelated, then we expect to observe a uniform distribution of P values within that set. However, a distribution significantly skewed toward zero suggests that the test sequence is similar to the set even though it may not be especially similar to any member of the set. The subset of similar sequences is then examined to see if they share a common region of alignment that might correspond with the ligand-binding site. Although the majority of sequences in the database may not be obviously similar to any other sequence, there is reason to suspect that relationships will exist between proteins sharing a function. For example, most ATP-binding proteins are not obviously homologous, yet one third of the known sequences share a definite pattern. Moreover, this pattern corresponds to a portion of the ATP-binding site. Conservation of ligand-contacting residues is consistently observed when diverging series of homologous proteins of known structure are compared. In contrast, noncontact residues show very little conservation. Consequently, similarities between isofunctional proteins are expected to involve only the relatively small number of residues that are essential for function. One goal of a systematic analysis is to detect weak similarities that are nevertheless shared by a class of proteins and that may be predictive of function.

Nothing prevents the comparison of a set of sequences with itself or with another set. Given a database partitioned into isofunctional sets, a natural move is to do all pairwise comparisons within these sets. After frankly homologous sequences are identified, the test of similarity is, again, whether the distribution of P values for each comparison is uniform. If a similar subset is identified, a multiple alignment of the sequences is examined for a common region of similarity that may correspond to the ligand-binding site. A consensus pattern of residues that are especially conserved is then sought within this region. Sequences remaining in the original set are searched for matches to this pattern as a test of distant relationships. The final result is a pattern that is predictive of a particular function. Analyzing the database in this way will produce a dictionary of predictive patterns.

The method described above does not require the presence of gross homologies; these would actually hinder the definition of sequence patterns corresponding to ligand-contacting residues. However, the limits of sensitivity have not been exhausted. So far, the focus has been on the optimal alignments from each comparison, yet these are just the best of the ranked lists of alignments. These lesser alignments can also be examined in rank order for consensus patterns. Measures of significance must be developed for this process, since proceeding far enough in these ranked lists would make even random sets appear

to have a common pattern. However, this procedure approximates explicit simultaneous searches of multiple sequences, which is a notoriously intractable problem. Structural data can also be used to increase the sensitivity of these methods. There are additional ways to increase the sensitivity of comparative methods for protein sequences in particular. These include probabilistic measures that allow for conservative substitutions and measures that favor patterns of matching that reflect the natural periodicities of secondary structure.

After isofunctional sets have been compared with themselves, the next step is to compare each set with all the others. Again, if a set is unrelated to another, then the *P* values from all of the pairwise comparisons should be uniformly distributed. The question is whether similarities between isofunctional sets would reflect similarities between the ligands bound. Similarities between the structures of nucleotide-binding proteins at both the primary and tertiary levels argue for such relationships. It is then reasonable to ask whether some portion of the evolutionary history of intermediary metabolism can be deduced from existing sequences. There is a complementary method that should be carried out in parallel to the comparison of functionally defined groups. This approach ignores all additional biological knowledge and compares every sequence with all other sequences in the database. Based only on sequence, this program would attempt to find all clusters of mutually similar sequences. The groups defined by this independent method can then be compared with those defined by the previous approach. There should be substantial agreement between them. The aim of both these approaches is to reduce the apparent diversity of sequences into a taxonomic structure that is both explanatory and predictive.

Algorithms and Computer Programs for the Identification of Functional and Structural Motifs in Protein Sequences

J. Posfai

Common patterns (motifs) in the sequences of functionally related proteins are expected to characterize common functional and/or structural domains of those proteins. If these motifs can be identified, they might be used to localize functional domains in sequences and also to predict possible functions for newly identified proteins. Recently, I have developed different algorithms and computer programs for the semi-automatic generation of such motifs. Beginning with a set of sequences of functionally related proteins, a global alignment of these sequences is performed. Consensus-type patterns are generated from the conserved regions of the alignment. Patterns that discriminate all the proteins with the common function from all other proteins in the PIR database are used as motifs, characteristic of the common function. The main principles behind the algorithms are described below. The use of the programs based on these algorithms is demonstrated by an analysis of the sequences of bacterial methylase enzymes.

GLOBAL SEQUENCE SIMILARITY

A set of related sequences with low-level global similarity is identified using an approach based on graph theory. Sequences are represented as vertices (dots) of a graph, and high (higher than a preset limit) similarity scores from pairwise comparisons of two sequences are represented by edges (lines) connecting the corresponding vertices in the graph. Highly (higher than a second preset limit) connected subgraphs establish subsets of proteins with global sequence similarity. A very low similarity score threshold can be applied if the connectivity threshold is set high. In this way, a set of 13 5C cytosine methylase sequences shows global sequence similarity, since each member of the set has a relatively high score with respect to all other members of the set. In contrast, endonuclease sequences belonging to the same type of restriction-modification system, or having the same DNA recognition specificity, show no sequence similarity.

GLOBAL ALIGNMENT

Unlike other multiple alignment programs, which optimize some abstract similarity score function, our alignment procedure is based on finding common patterns within the sequences being compared. The program scans the sequences to be aligned for identical patterns of three amino acids in a span of 9–11 residues. Segments found to be similar by this criterion are aligned and used as anchor points, or boundaries, for the subsequent alignments. Then, the segments within these boundaries are searched for identical patterns of relaxed specificity (two

amino acids and one or two nucleotides, two amino acids, one amino acid and two nucleotides, etc.). These patterns can then provide further anchor points for the alignment.

This method has identified and aligned ten conserved blocks in the 13 5C cytosine methylase sequences (Fig. 1). Five of these blocks are highly conserved, since each block contains at least three invariant positions. The five other conserved blocks show greater variability. Most likely, the conserved blocks play a role in those enzymatic functions that are common to all methylases such as interaction with S-adenosylmethionine. The highly variable region between conserved blocks VIII and IX is believed to be responsible for specific DNA recognition.

MOTIF DEFINITION AND SEARCH

Similarity in the conserved blocks is described by a consensus-type pattern of specific and nonspecific positions. A position in the conserved block is regarded as specific if the variance at that position is limited either to invariant positions or to positions where no more than four different amino acid residues occur. At nonspecific positions, any amino acid can occur, whereas at specific positions, only residues that are present in at least one of the sequences of the alignment are allowed. A program based on a Depth-First-Search algorithm is used to locate those sequences that contain any of the search patterns. If these patterns do not occur in other sequences, they are regarded as predictive motifs, characteristic of the common function of the related sequences from which they were built.

It has been possible to build characteristic patterns from each of the five highly conserved blocks of the 5C cytosine methylases. The predictive power of the motifs was verified by locating them in the sequences of three newly determined 5C cytosine methylases. Interestingly, with slightly decreased specificity searches, the bacterial motifs can be discovered in the sequence of the murine methylase that is responsible for methylation of the CG dinucleotide. Unidentified open reading frames (ORFs) in two GenBank entries were also found to contain strong matches to the motifs typical of the carboxy-terminal regions of methylases. Unfortunately, the amino-terminal sequences of these ORFs had not been determined. The motifs were also useful in pinpointing sequencing errors in these two GenBank sequences, since reading frame changes were required to accommodate all the detected motifs in the ORFs. These sequencing errors had been noted by the authors in a recent publication.

We are planning to automate these procedures and use them in the construction of a database of predictive functional motifs of protein sequences.

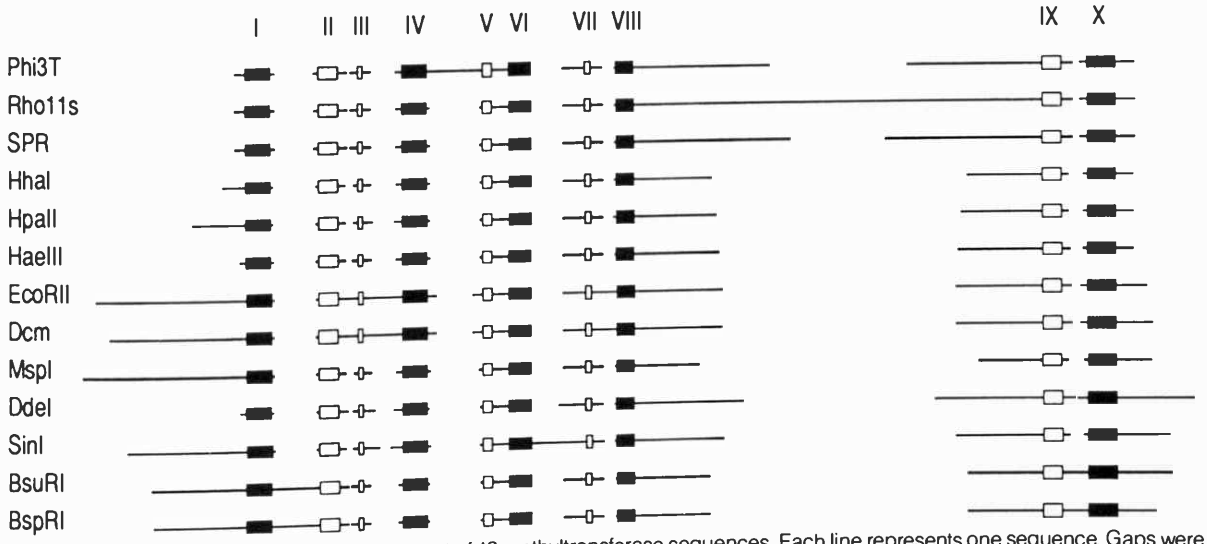

FIGURE 1 Schematic diagram of the alignment of 13 methyltransferase sequences. Each line represents one sequence. Gaps were introduced in the alignment where the lines are interrupted. Boxes indicate where the ten blocks of conserved residues occur. (■) Five highly conserved blocks; (□) five less-conserved blocks. The variable region that is probably the sequence recognition domain lies between blocks VIII and IX.

Restriction Endonucleases

L. Hamablet, J. Martling, J. Meyertons,
S. Miceli, R.J. Roberts

The collection of restriction endonucleases continues to grow, and more than 1100 enzymes have now been characterized; 147 different specificities are known. During the last year, 39 new enzymes have been isolated and characterized as part of a collaborative program with I. Schildkraut and D. Comb (New England Bio-Labs). Among these are six valuable new specificities. Having just received support under the Human Genome Initiative, our screening program is being expanded, and we are focusing our efforts to identify more restriction enzymes like *Not*I and *Sfi*I that recognize octanucleotide sequences. Such enzymes are especially valuable for generating large fragments of DNA that are necessary for the initial physical mapping of large genomes. Recently, we have discovered an enzyme, *Fse*I, from a *Frankia* species that cleaves most of our test DNAs relatively infrequently and has the properties to be expected of an octanucleotide-recognizing enzyme. Further characterization is currently under way.

The restriction enzyme database has undergone a major transition during the last year as we have switched database management systems from INFORMIX to the relational system ORACLE. This has involved a great deal of new codes and extensive checking to ensure that the transfer maintained the integrity of the data, much of which has been collected over a span of 16 years. Now that this transition is complete, the job of data management and distribution will be considerably easier. Our immediate goals are to automate as much as possible the generation of reports from the database and the dissemination of restriction enzyme information to interested individuals. In particular, the data required by computer programs to search DNA sequences for restriction enzyme recognition sites are now available in electronic form. Within a few hours of entry into the database, information about a new enzyme can be sent to any individual with access to a computer network.

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MACROMOLECULAR CRYSTALLOGRAPHY

J.E. Anderson A. Caleca L. Gloss M. Ulman
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J. Kuret C.K. Cheung M. Macias K. Zachmann
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The 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 in our own laboratory and by our collaborators. Together with these data, crystallographic models of protein kinases, proto-oncogene products, and *trans*-acting factors will help us to comprehend the roles of these

macromolecules in cell growth and differentiation, in cell transformation, and, ultimately, in diseases such as cancer and AIDS.

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), we 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 expressing and purifying to crystallographic homogeneity the proteins we are studying.

Overexpression of Nuclear Proto-oncogene Proteins in Bacteria

E. Chang, A. Caleca, L. Gloss, D. Greif, J. Walter
[in collaboration with T. Curran, Roche Institute of Molecular Biology, Nutley, New Jersey, and W.-K. Chan, Cold Spring Harbor Laboratory]

A major goal of our group is to determine the structures of the protein products of the nuclear proto-oncogenes c-*fos*, c-*jun*, and c-*myc*. All of these proteins are present at very low levels in the eukaryotic cells where they naturally occur. To obtain the large amount of material required for crystallization, we overexpressed the proteins in *Escherichia coli* using the bacteriophage T7 expression system. In addition to providing enough of the proteins, bacterial expression eliminates the heterogeneity resulting from posttranslational modifications, such as phosphorylation, that the proteins are subject to in eukaryotic cells.

We obtained rat cDNA encoding c-*fos*, c-*jun*, and the Fos-related antigen *fra1* from T. Curran. SDS-PAGE of lysates of cells containing the c-*jun* or *fra1* expression plasmids and induced with isopropyl-β-D-thiogalactopyranoside (IPTG) shows Coomassie-stained bands that are the size expected for intact Jun or Fra1 protein, respectively. In Western blotting experiments, these proteins react with antibodies made against a Jun peptide (obtained from P. Vogt, University of Southern California) and with antiserum raised against a bacterial *trpE*-v-*fos* fusion protein (obtained from K. Riabowol, Cold Spring Harbor Laboratory), respectively. Fractionation of the sonicated whole-cell lysate by low-speed centrifugation indicates that the Fra1 protein occurs exclusively in the soluble fraction. The Jun protein, however, is produced as insoluble aggregates called inclusion bodies, which often occurs when eukaryotic proteins are overexpressed in bacteria. Purification of inclusion body proteins usually involves solubilization of the insoluble aggregate with denaturants such as urea or guanidine hydrochloride. Since we prefer to avoid these rather harsh treatments, we looked for ways to increase the amount of soluble Jun protein. We found that reducing the growth temperature from 37°C to 30°C increased the proportion of Jun found in the soluble fraction. Furthermore, we found that it is the *post*-induction temperature and not the *pre*-induction temperature that matters; this allows us to avoid reduced yields due to lower cell density at lower pre-induction growth temperature. When cells are grown at 37°C and then shifted to 30°C upon addition of IPTG, about half of the total Jun protein is soluble, with a negligible decrease in total (soluble + insoluble) yield.

We have not yet successfully overexpressed intact Fos protein. In Western blotting experiments using induced whole-cell lysates of cells carrying the c-*fos* expression plasmid, a band of the appropriate molecular weight reacts with Fos antiserum, but no corresponding band is visible on Coomassie-stained gels. A number of additional lower-molecular-weight bands also react with the antiserum, suggesting that intact Fos is degraded by endogenous proteases. We are currently investigating whether expression in protease-deficient strains of *E. coli* will increase the level of intact Fos.

We obtained the human c-*myc*-coding sequence from the plasmid pOTS-*myc* (from W.-K. Chan), which encodes a full-length Myc polypeptide with four additional amino acids on its amino terminus. We used this DNA to construct the T7 expression vector pEC-*myc1.2*. Cells carrying pEC-*myc1.2* make a protein that reacts with anti-Myc monoclonal antibody B3 in Western blots and has the appropriate apparent molecular weight for intact Myc protein. This protein is present in approximately equal amounts in both the soluble and the insoluble fractions of whole-cell lysates. Amino-terminal amino acid sequencing of the protein after purification from cultures carrying pEC-*myc1.2* indicates that its amino-terminal sequence is the same as that of the "authentic" c-Myc protein, without the four additional amino acids encoded by the original construct pOTS-*myc*. We are currently devising

protocols to purify Jun, Fral, and Myc to homogeneity. When we obtain the homogeneous protein, crystallization trials will begin.

Bacterial HIV-1 Tat Protein

C.K. Cheung, J.E. Anderson [in collaboration with C. Rosen, Roche Institute of Molecular Biology, Nutley, New Jersey]

Human immunodeficiency virus type 1 (HIV-1) causes AIDS. HIV-1 usually lies dormant for a variable period of time after infection and then, for reasons that are not entirely clear, begins to multiply and kill T4 lymphocytes, leading to full-blown AIDS. The HIV-1 Tat protein is required for the virus to grow. Therefore, understanding the structure and function of Tat could lead to the development of drugs that inhibit it and thus delay or prevent the onset of AIDS.

HisTat is the bacterially expressed first exon of *tat* (66 of 86 residues) from HIV-1 strain HXB2, with 15 residues (MRGSHHHHHHGSVDE) added onto the amino terminus. This "histidine leader" facilitates purification by chelating Ni^{++} ions when a crude lysate of cells expressing HisTat is passed over a nickel-affinity column. Most of the other proteins pass through the column, and when HisTat is eluted with decreased pH, it is about 90% pure. We obtained HisTat from C. Rosen, after passage over the nickel-affinity column. The histidine leader is removed by treating HisTat with *Staphylococcus aureus* V8 protease at pH 4.0. Under these conditions, V8 protease cleaves only after glutamic acid (E) and does so efficiently only if the following amino acid is *not* proline. Fortuitously, the two glutamic acid residues that occur in the Tat-specific part of HisTat are both followed by proline, and so the major site of cleavage is between the last residue of the leader and the first residue of Tat1-66. Using V8 digestion, we can generate a good yield of Tat1-66 and are currently devising ways to purify Tat1-66 to homogeneity. Tat has a cysteine-rich region in which 7 out of 16 residues are cysteine, and these residues tend to oxidize readily to intermolecular disulfide bonds, producing a collection of Tat oligomers and complicating the purification and handling of the protein. Once we have purified a single oligomeric species, we will attempt to crystallize it and determine its three-dimensional structure.

Restriction-Modification Enzymes

J. Fu, A. Caleca, J.E. Anderson [in collaboration with I. Schildkraut, New England Bio-Labs, and R.M. Blumenthal, Medical College of Ohio]

Restriction endonucleases and modification methylases recognize specific DNA sequences and then cleave or methylate the DNA, respectively. Methylation by a particular methylase protects the site from cleavage by the corresponding endonuclease. Our goal is to solve the structures of some of these enzymes both as free proteins and in complexes with oligonucleotides carrying their recognition sites. These structures will help us to understand how specific DNA sequences are recognized by these proteins, and they will also help us to understand their enzymatic mechanisms.

We have grown 0.2 × 0.2 × 0.8-mm crystals of *Hin*dIII endonuclease (from I. Schildkraut) from a solution of 2.3 M ammonium sulfate and 20 mM glycine (pH 9.60) at 21°C. The crystals are unusually fragile and difficult to mount for diffraction analysis. They do not diffract beyond 5 Å. We are looking for conditions that will improve their order, e.g., the addition of metal ions to help strengthen intermolecular contacts in the crystal.

Small crystals (0.05 × 0.02 × 0.01 mm) of *Pae*R7 endonuclease (from I. Schildkraut) appear in a solution of 1.3 M phosphate (pH 6.40) at 21°C. We are searching for conditions that will produce crystals large enough for diffraction analysis.

We have purified *Pvu*II methylase (from R.M. Blumenthal) to greater than 90% homogeneity. The purification involves cation-exchange/affinity chromatography on phosphocellulose, followed by hydrophobic interaction chromatography on a TSK phenyl-5PW FPLC column and cation-exchange chromatography on a Pharmacia Mono S FPLC column. Once we obtain homogeneous methylase, we will begin crystallization experiments.

Cell Cycle of Fission Yeast

J. W. Pflugrath, M. Macias, D. Sterner [in collaboration with D. Beach, Cold Spring Harbor Laboratory]

The gene product of *cdc2*$^+$ plays an important role in cell-cycle regulation of the fission yeast *Schizosaccharomyces pombe*. Beach and co-workers (Genetics Section) have demonstrated that another gene product, p13^{suc1}, forms a stable complex with the *cdc2*$^+$ protein kinase. Although p13^{suc1} is necessary

for cell-cycle progression, the exact character of its interaction with the kinase is unknown. It is not a substrate of the kinase. To probe and understand this interaction at the molecular level, we have purified with new procedures the yeast $p13^{suc1}$ from an engineered strain of *Escherichia coli* (kindly provided by G. Draetta, L. Brizuela, and D. Beach) and initiated crystallization trials.

Area Detector Software

J. W. Pflugrath, M. Ulman [in collaboration with the EEC Cooperative Workshop on Position-sensitive Detectors]

In the area of computational crystallography, we continue to develop and improve the device-independent area detector software system MADNES. This work is part of an international effort to create software that can be used on any area detector hardware, whether designed privately or commercially. This software is now used by over a dozen laboratories worldwide on several different kinds of area detectors.

Our group coordinates the maintenance and debugging of MADNES. To this end, we edit and distribute a digest about the software to all users via a computer network. Among improvements incorporated into MADNES in the last year are three-dimensional profile analysis, better background estimation, and easier offline processing.

Yeast cAMP-dependent Protein Kinase

J. Kuret

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. I am interested in the structural basis of this "substrate specificity" and wish to identify the amino acid residues responsible for imparting selectivity to the interaction of kinases and their substrates. To achieve this goal, I have initiated a structural study of the cAMP-dependent protein kinase from *Saccharomyces cerevisiae*. This enzyme exists as a heterotetramer of two catalytic and two regulatory subunits (molecular mass = 195,000 daltons). In collaboration with M. Zoller's laboratory, I have designed a yeast-based expression system capable of accumulating the cAMP-dependent protein kinase up to 4% of the total soluble protein. The enzyme produced by this system is fully active, soluble, and responsive to cAMP. To simplify purification and crystallization of the cAMP-dependent protein kinase, I truncated both subunits to produce a fully active heterodimer of 72,000 daltons. The kinase dimer is much smaller, less flexible, and less heterogeneous than the native cAMP-dependent protein kinase tetramer, yet retains nearly identical catalytic properties. Purification of the catalytic subunit has proved to be relatively simple, and my attention is now turned toward obtaining highly purified dimer. Once sufficiently pure kinase is obtained, I will crystallize the protein and determine its three-dimensional structure in collaboration with J. Pflugrath. In addition to elucidating the structural basis of substrate recognition, the three-dimensional structure of the kinase will help us to identify amino acid residues involved in catalysis and cAMP-mediated allosteric regulation.

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STRUCTURE-FUNCTION OF cAMP-DEPENDENT PROTEIN KINASE

M.J. Zoller L.R. Levin K.E. Johnson J. Kuret

Protein kinases are a family of proteins that play an important role in the regulation of cellular processes such as signal transduction, progression through the cell cycle, and metabolism. These enzymes transfer the terminal phosphate from ATP onto serine, threonine, or tyrosine residues of substrate proteins. Phosphorylation/dephosphorylation of proteins is clearly a major cellular mechanism to alter and reversibly control protein function, activity, association, localization, and stability. The important biological questions regarding protein kinases are the identification of the signals that regulate their activities and the identification and localization of the substrate targets. The important structural questions for this class of enzymes concern enzymatic mechanism, regulation of activity, and substrate specificity.

Comparisons between the primary sequences of all known eukaryotic protein kinases have shown that they belong to a superfamily that can be broken down functionally into a group of enzymes that phosphorylate serine and threonine residues and another group of enzymes that phosphorylate tyrosine. Despite the large number of proteins kinases with known primary structures, the functional role for each of these conserved amino acids remains largely unknown. Thus, a large effort is currently aimed at determining the three-dimensional structure for a protein kinase. A detailed structure will begin to address the structural questions outlined above.

The best-understood protein kinase in terms of structure and biochemistry is the cAMP-dependent protein kinase (cAdPK). The protein has been purified from a number of eukaryotic sources, including bovine heart, porcine heart, skeletal muscle, and yeast. Crystallographic studies on the isolated subunits are now in progress and will define more precisely the residues that comprise each functional domain.

In the yeast *Saccharomyces cerevisiae*, three genes, *TPK1*, *TPK2*, and *TPK3*, encode distinct catalytic subunits of cAdPK, C_1, C_2, and C_3, respectively. The three proteins exhibit approximately 75% amino acid identity with each other. Toda et al. (*Cell 50:* 277 [1987]) demonstrated genetically that the three C subunits were functionally similar and that at least one gene was necessary for viability. The cDNAs and genes encoding mammalian C subunits from a number of sources have also been isolated and characterized. This has identified at least two distinct but nearly identical mammalian C subunits termed Cα and Cβ. The level of sequence conservation between the yeast and mammalian C subunits is only 53%. With the isolation of cDNAs and genes for the various subunits, the structure/function of cAdPK can now be approached by isolation of mutants through genetic screens and by use of the techniques of molecular biology and systems for expression of heterologous genes. The first section (Levin and Zoller) involves identification of residues important for R-C interaction. The second section (Johnson and Zoller) demonstrates the use of yeast as a heterologous expression system for studying the mammalian cAdPK.

Association of Catalytic and Regulatory Subunits of cAdPK

L.R. Levin, M.J. Zoller

We have previously reported the isolation and characterization of a mutation in the C subunit of yeast cAdPK that disrupts the tight R-C interaction required to maintain the inactive holoenzyme (Levin et al., *Science 240:* 68 [1988]). In the yeast *S. cerevisiae*, a single gene encoding the R subunit (*BCY1*) and three genes encoding the C subunits (*TPK1*, *TPK2*, and *TPK3*) have been isolated. The C subunit mutation responsible for the unregulated phenotype is a single nucleotide change in *TPK1*, resulting in the substitution of an alanine for the threonine at position 241 in C_1. The mutant, C_1(Ala-241), has a decreased affinity for the R subunit without an appreciable loss in affinity for its substrates. These data suggest that Thr-241 participates in the interaction with the R subunit.

The three yeast C subunits are homologous to the mammalian C subunits, with each exhibiting

approximately 50% amino acid sequence identity with bovine Cα. There are two known sites of phosphorylation in the mammalian C subunit: Ser-338 and Thr-197. The phosphoserine residue is in the carboxyl terminus of the mammalian C subunit and is not conserved in any of the yeast isozymes. The phosphothreonine residue exists in a region that displays 72% amino acid identity and is analogous to Thr-241 of yeast C_1. The yeast and mammalian C subunits are conserved functionally as well as structurally. The mouse Cα can replace the yeast C subunits in vivo and support growth of yeast cells (see below). The mouse C subunit is capable of forming a complex with the yeast R subunit, indicating that the specific contacts responsible for the R-C interaction are conserved between the two species. The phosphorylation of the mammalian analog of Thr-241 and the similarity in the interaction between the subunits in these two species suggest to us that Thr-241 is phosphorylated in yeast C_1. To confirm that this residue is phosphorylated in yeast, we compared the phosphorylation states of wild-type C_1 and the mutant C_1(Ala-241). Two-dimensional gel electrophoresis of ^{32}P-labeled C_1 reveals multiple isoforms, confirming that there are numerous modifications to the protein. C_1(Ala-241) also contains multiple isoforms, but it is missing one of the prominent ^{32}P-labeled forms of C_1, consistent with it having one less-phosphorylated species.

Phosphoamino acid analysis of immunoprecipitated, ^{32}P-labeled C_1 and C_1(Ala-241) revealed a consistent decrease in the amount of phosphothreonine in the mutant protein. Both mutant and wild-type proteins contain phosphothreonine and phosphoserine but no detectable phosphotyrosine. Densitometry revealed that wild-type C_1 contains approximately three times more phosphothreonine than the mutant C_1(Ala-241). Since the only difference between C_1 and C_1(Ala-241) is the substitution of Thr-241 by alanine, we conclude that the loss of phosphothreonine is the direct result of that substitution.

We suspected that the phenotype of C_1(Ala-241) could be due to the loss of the negatively charged phosphothreonine. Using site-directed mutagenesis, we attempted to mimic the acidic moiety by substituting either aspartate or glutamate for Thr-241. Glycine was substituted at this site to investigate the effect of replacement by another small, uncharged amino acid. All three substitutions were made with the use of a single, degenerate oligonucleotide and confirmed by nucleotide sequencing.

To determine the in-vivo-regulated state of each of the C subunits, they were expressed in yeast in the absence of any wild-type C subunit. When nutrient-limited, wild-type yeast cells become resistant to heat shock. Starved cells stop proliferating and enter a cell-cycle state termed G_o, which enables them to survive a period of time at elevated temperatures. Yeast cells containing an activated cAdPK, through mutations that either increase the cellular level of cAMP or decrease the level of functional R subunit, do not enter G_o and are sensitive to heat shock. We have previously shown that the Thr-241 to alanine substitution results in increased catalytic activity in the absence of cAMP regulation. We tested the heat-shock phenotype of cells that express wild-type C_1 or mutant C_1(Ala-241) as their only C subunits. As expected, nutrient-limited yeast cells expressing C_1(Ala-241) are very sensitive to heat shock. They lose viability after just 10 minutes at the elevated temperature, whereas cells expressing wild-type C_1 are able to withstand 40 minutes at 55°C. We then tested the heat-shock sensitivity of the strains expressing the other mutant C subunits. The strain containing C_1(Gly-241) was approximately as sensitive to heat shock as the strain expressing C_1(Ala-241). In contrast, cells expressing either C_1(Asp-241) or C_1(Glu-241) are resistant to heat shock. Both strains retain viability when left at 55°C for up to 40 minutes. These two altered C subunits could be reverting the heat-shock sensitivity by decreasing their catalytic activity or by increasing their affinity for the R subunit. To understand the biochemical effect of each substitution, we purified each of the mutant C subunits and characterized their in vitro affinity for the R subunit and their catalytic activity.

Each of the substituted C subunits was purified from overexpressing strains of yeast. The in vitro kinase activity of each of the purified C subunits was measured using the peptide substrate, Kemptide. Each altered C subunit was preincubated with increasing amounts of the wild-type R subunit to determine the amount required to inhibit catalytic activity. The concentration of R that reduces the activity by 50% is termed the IC_{50}. The IC_{50} of C_1(Ala-241) is 27 times greater than the IC_{50} of wild-type C_1. The IC_{50} of C_1(Asp-241) is increased 7-fold over wild type, and the IC_{50} of C_1(Glu-241) is increased 14-fold over wild type. C_1(Gly-241) was not characterized in vitro. It was not able to be purified by anti-R subunit immunoaffinity chroma-

tography because the holoenzyme was unstable. There is a correlation between decreased affinity for the R subunit and increased heat-shock sensitivity.

The kinetic constants were determined for each C subunit. The K_m for ATP is approximately twofold higher than that for wild type in all of the altered C_1 subunits, whereas the K_m for Kemptide is between three- and fourfold higher than that for wild type in the mutants. The predominant effect of each of the C_1 subunit mutations is the change in affinity for the R subunit and not their catalytic activity.

The substitution of alanine for threonine in C_1(Ala-241) causes a 27-fold reduction in R subunit affinity in vitro and the disruption of cAMP regulation in vivo. We believe that the affected residue, Thr-241, is normally phosphorylated and that it is the acidic threonine-phosphate which is responsible for the tight interaction between the subunits. Figure 1 depicts our model for the inhibition and activation of cAdPK. The negatively charged group in C would be opposed by a positively charged group in R. The positively charged element could exist anywhere within the minimally required region of the R subunit as defined by deletion studies (M.J. Zoller and K.E. Johnson). This positively charged element would be absent from substrates, but could exist in the heat-stable protein kinase inhibitor (PKI). Binding of the effector, cAMP, would stabilize an altered conformation of R, which displaces this positively charged element and would cause R to behave like a substrate. R dissociates from C, leaving C active to phosphorylate other substrates. When Thr-241 is replaced with alanine, there is no negatively charged side chain in C to contact the positively charged element in R, resulting in unregulated C subunit. Replacing Thr-241 with aspartate supplies a negatively charged side chain that would mimic the threonine-phosphate and contact the positively charged element in R. This holoenzyme would be stable and require cAMP for C subunit activation.

Mammalian Catalytic Subunit Functionally Replaces Its Yeast Homolog

K.E. Johnson, M.J. Zoller [in collaboration with W. Yonemoto and S. Taylor, University of California, San Diego]

The expression of eukaryotic protein kinases in *E. coli* has proven to be difficult and unpredictable. Although the *abl* protein kinase was successfully expressed in *E. coli*, our experiments on expression of yeast C subunits in *E. coli* produced large amounts

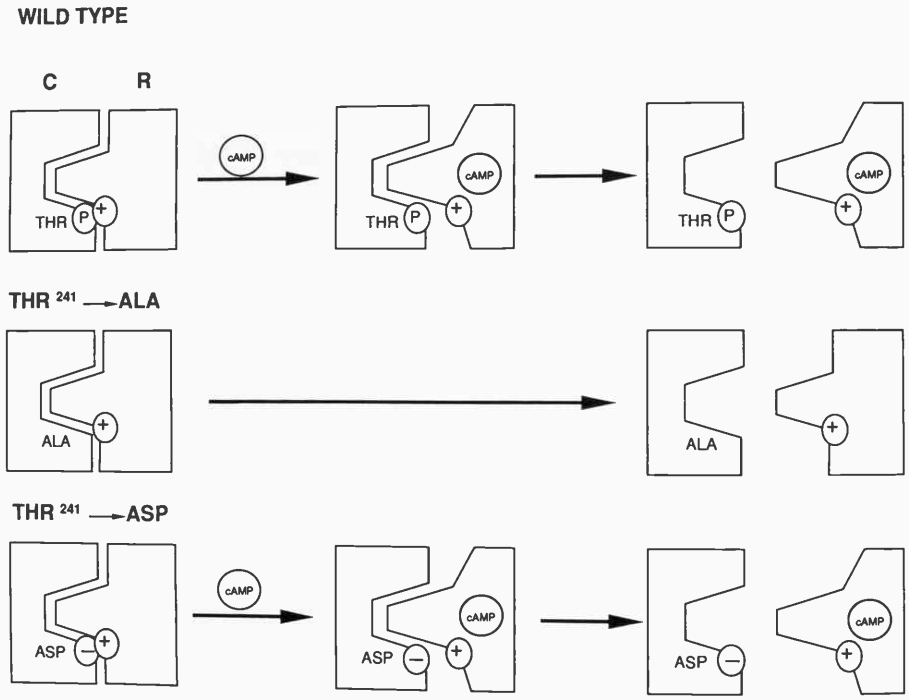

FIGURE 1 Model for the inhibition and activation of cAMP-dependent protein kinase.

of predominantly insoluble and inactive protein. The mouse Cα cDNA expressed in *E. coli* is partially soluble, and the enzyme in the soluble fraction is active (W. Yonemoto and S. Taylor, unpubl.). However, certain mutant forms have proven to be unstable and/or insoluble. In addition, the *E. coli* system cannot be used to study the role of posttranslational modifications specific to eukaryotic systems. Cα and Cβ were also overexpressed in NIH-3T3 cells producing only a tenfold increase in C subunit protein. This approach is not an ideal system for studying mutant proteins, since wild-type C subunits are expressed from the chromosomal copies in the genetic background. Several cell lines with altered subunits of cAdPK have been identified, but a strain completely devoid of the C subunit has not been adequately characterized for protein structure studies.

Yeast provides an ideal experimental system for the study of protein structure and function. By manipulation of chromosomal sequences, mutant proteins can be expressed in yeast and studied in the absence of wild-type proteins. Mutant proteins with desired properties can be obtained using genetic screens. In addition, a number of posttranslational modifications are conserved between yeast and mammalian cells. Recently, we purified one of the three C subunits of yeast cAdPK and demonstrated that in vitro, it was functionally similar to the mammalian catalytic subunit (Zoller et al., *J. Biol. Chem. 263:* 9142 [1988]). We also developed a yeast expression system to produce mutant C subunits in the absence of the wild-type C subunit (Zoller et al. [1989] in prep.). We have extended this yeast expression system to provide a means to study the structure and function of the mammalian C subunit. In the present study, we demonstrate that the mammalian C subunit can functionally replace its yeast homolog and that sufficient amounts of protein can be obtained for enzymatic and structural analyses of mutants we intend to make in the future.

In experiments conducted previously, we demonstrated that the kinetic properties of the two enzymes were similar with respect to k_{cat} and affinity for ATP but were different with respect to affinity for the peptide substrate Kemptide. The yeast enzyme exhibited about a 10-fold higher K_m for this peptide substrate. In addition, the yeast C subunit exhibited a 100-fold lower affinity for a derivative of the heat-stable PKI. These data suggested that the two enzymes exhibit subtle differences in protein substrate specificities.

Since yeast require at least one functional *TPK* gene for viable growth, our strategy was to examine whether yeast cells containing the mammalian C subunit as the sole source of cAdPK were viable. The Cα cDNA was cloned into two different yeast expression vectors: one that expresses Cα using the promoter and terminator from the yeast C subunit gene, *TPK1*, and the other that expresses Cα from the *ADC1* promoter, a strong, constitutive promoter from the gene encoding yeast alcohol dehydrogenase I. These vectors are multicopy episomal plasmids that are maintained by prototrophic selection for leucine. The plasmids were constructed to express the natural Cα protein.

Substitution of the yeast *TPK1* gene with the Cα cDNA was accomplished using a plasmid swap procedure. The starting strain, LL8, contains chromosomal disruptions of all three yeast C subunit (*TPK*) genes. The strain is kept alive by maintenance of a plasmid that contains the wild-type *TPK1* gene. This vector is distinguished by the presence of the *ADE8* gene, which confers adenine prototrophy. LL8 was transformed with the Cα expression vectors, LEU^+,ADE^+ transformants were selected, and the cells were then grown without selection to induce loss of one or the other plasmid. The plasmid present in a particular colony can be identified by its prototrophic markers. Since at least one C subunit gene is required for viability, viable cells must maintain at least one of the two plasmids. Thus, we predicted that following nonselective growth, ade^-,LEU^+ cells would express on the mammalian Cα. Viable cells were obtained that contained the expression vector using the natural *TPK1* promoter. In contrast, no transformants were obtained using the ADH expression vector. We suspected that expression using the *ADC1* promoter resulted in a level of C subunit that was lethal. This was observed with the yeast C subunit using a similar high-level expression vector (unpublished).

Chromatographic analysis of extracts from yeast cells demonstrated that the mammalian C and yeast R subunits associate in vivo to form a tetrameric (R2C2) holoenzyme. We then purified mammalian C from an overexpression strain using the strong constitutive ADH promoter. Overexpression was achieved by coexpression of the ADH-Cα with overexpression of the yeast R subunit, *BCY1*. Purification was accomplished using the two-step immunoaffinity chromatography procedure that we developed for purification of the yeast C subunit. The major protein observed on an SDS-polyacrylamide gel was Cα, which was about 85% pure by Coomassie stain-

ing. The mammalian protein purified from yeast comigrated with the Cα produced in *E. coli*. Recently, we obtained polyclonal antibodies raised against mouse Cα expressed in *E. coli* (W. Yonemoto, unpubl.). The Cα isolated from yeast reacts with the anti-Cα antibodies and not with anti-yeast C antibodies. Biochemical and structural characteristics of yeast-expressed Cα showed that the properties of the yeast-produced C subunit are similar to the published properties of the bovine heart C subunit. The most notable difference between the two enzymes is their affinity for the heat-stable PKI.

Studies on the bovine heart C subunit showed that it contained an amino-terminal myristoyl moiety that was linked to the protein through the glycine that immediately followed the initiator methionine. The enzyme that catalyzes this modification is present in yeast as well. Since we produced the amino acid sequence of the natural mammalian protein, we predicted that the mammalian C subunit expressed in yeast would contain this modification if the initiator methionine was removed posttranslationally. To test this, exponentially growing cells were labeled with [^{3}H]myristic acid, the cells were lysed, and the Cα was then immunoprecipitated from soluble cell extracts and subjected to SDS-PAGE. The labeled proteins were visualized by autoradiography. A ^{3}H-labeled protein with a relative molecular weight of 43,000 was selectively immunoprecipitated using the anti-Cα sera but not using preimmune sera. This radioactive protein comigrated with the purified Cα protein marker.

In summary, we have described an ideal system in which to study the structure and function of the cAdPK. We can now begin to make mutants using yeast genetics or site-directed mutagenesis. Our approach exploits the techniques of protein biochemistry, molecular and cellular biology, and yeast genetics. Information from studying mutant proteins will be integrated into the model derived from the crystal structure that is soon to emerge. Conversely, hypotheses concerning the structure or enzymatic mechanism of the protein that stem from the three-dimensional structure can be tested by expression of mutants in this system. Finally, the expression of the mammalian cAdPK in yeast provides a genetic system to study interactions with mammalian substrates such as the CRE transcription factor.

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In Press, Submitted, and In Preparation

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NEUROSCIENCE

The efforts in neuroscience research have focused on the mechanism of neurotrophic factors of the central nervous system. We have been particularly interested in proteins that cause the initiation of neurite outgrowth from the embryonic cell of the cerebral cortex. This brain region is the seat of higher brain functions, such as learning and memory, and is the target of debilitating diseases, such as Alzheimer's and epilepsy. Our goals are to identify the role of these neural growth factors in the development of the brain and their potential involvement in degenerative processes. In pursuing these objectives, we adopt a molecular approach, employing biochemistry, cell biology, and molecular genetics.

GROWTH AND DIFFERENTIATION OF NEURAL CELLS

D. Marshak J. Figueiredo M. Schmiedeskamp
S. Pesce R. Tonner

Purification and Characterization of Neurotrophic Factors

J. Figueiredo, B. Tonner, S. Pesce, D. Marshak

The differentiation of neurons of the central nervous system includes the extension of neuritic processes, or neurites, from the cell body of a postmitotic neuroblast. These neurites become the axons and dendrites of the mature neuron, receiving and sending signals within a neuronal circuit. We have been purifying proteins from bovine brain extracts that cause neurite outgrowth in model systems of neuronal differentiation. We have used primary cultures of neurons from 7-day-old chicken embryo cerebral cortex, maintained in a serum-free defined medium. At this stage, the cultures are nearly all neurons, with little glial cell contamination. The neurons are postmitotic, and in culture, they attach to poly-L-lysine-coated plates and are flat and phase dark. Upon stimulation with a neurotrophic substance, the cells become rounded and bright, and they send out neurites within 24 hours.

We have fractionated bovine brain extracts to purify substances that cause neurite extension in this assay. One neurotrophic substance is a disulfide form of a calcium and zinc ion-binding protein, known as S100β. This protein is 10.5 kD and forms dimers of 21 kD. Under certain conditions, particularly in acid, the protein can polymerize to higher-molecular-weight forms. The dimer form appears to be the active species in neurite extension. Reduction and alkylation of the cysteine residues result in loss of activity. When protein is purified under nonreducing conditions, there is a significant yield of dimer, as shown on the polyacrylamide gel in Figure 1. This disulfide dimer is predominantly a parallel form, diagramed schematically in Figure 2. Our working model is that the dimer represents a small population of the molecules that are secreted during development and, possibly, at later times in the adult nervous system.

Further fractionation of a heat-stable protein fraction from bovine brain yielded another factor that causes neurite outgrowth at high specific activity. Preliminary characterization of this factor indicates that it has an apparent molecular weight on gel filtration of 97,000 and binds to heparin-Sepharose conjugates. The assay system utilized does not respond to any of the known, soluble neurotrophic proteins, such as nerve growth factor (NGF) and acidic or basic fibroblast growth factors (aFGF, bFGF). Thus, the molecules identified in the chick embryo cortical cell assay are unique among neurotrophic factors.

To confirm the observations in this system, we have used the mouse neuroblastoma cell line, neuro-2a, as a model system for neurite outgrowth. These cells

FIGURE 1 Polyacrylamide gel electrophoresis of S100β isolated from bovine brain in the absence of reducing agents, showing the presence of the disulfide dimer. Molecular-weight standards are shown in the right hand lane.

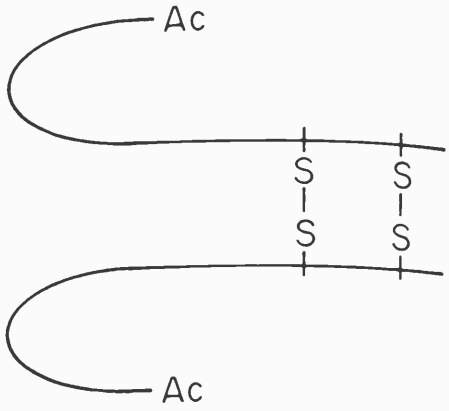

FIGURE 2 A schematic diagram of the disulfide dimer (parallel form) of S100β from brain.

proliferate in culture when grown in media supplemented with serum. However, at low density in serum-free media, they differentiate by sending out neurites in 6–8 hours. Although the mechanism of neurite outgrowth in these cells may be different from that of primary cells, they remain a useful and rapid assay system for neurotrophic factors.

Role of S100β In Vivo

D. Marshak, S. Pesce, M. Schmiedeskamp

During the past year, the role of the S100β protein in vivo has become an important issue, because the gene for this neurotrophic factor has been localized to human chromosome 21 in band 21q22.3 by genetics groups in other laboratories. This is significant because this region of chromosome 21 is involved in Down's syndrome. Our demonstration of a neurotrophic activity for the protein, combined

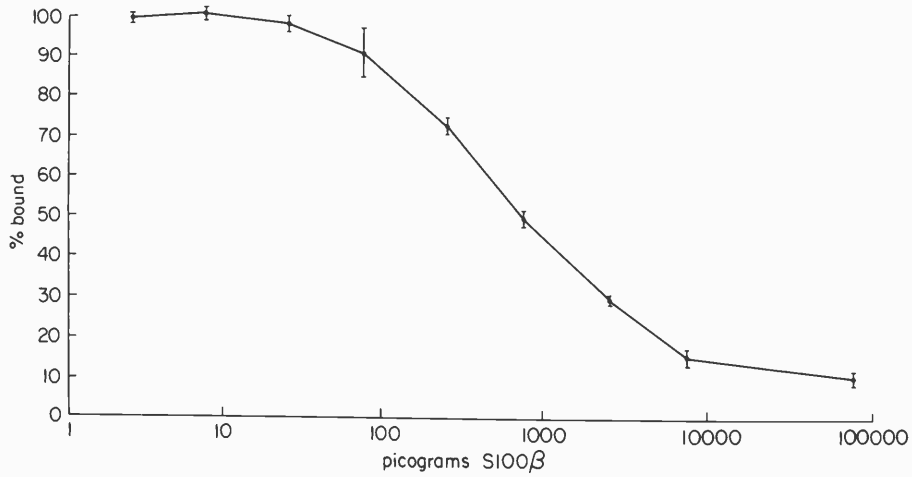

FIGURE 3 Radioimmunoassay of S100β. The vertical axis shows the percentage of radioiodinated S100β bound in the absence of competing antigen. The horizontal axis shows the amount, in picograms, of unlabeled S100β added to the reactions. Each point is an average of duplicate assays with standard errors shown by the bars.

with the abnormal neurological development seen in Down's patients, has led us to investigate the action of S100β in vivo. We raised antibodies in rabbits against native and denatured S100β. These antisera were titered, and the most reactive antiserum can detect the antigen at 1:1000 dilution. Using a competitive inhibition radioimmunoassay, we can detect picogram amounts of S100β, as shown in Figure 3.

In the developing chick embryo, there appears to be a small amount (<20 pg/mg) of S100β at early stages (embryonic day 5–7). Between days 7 and 18, there is an increase in S100β of three- to fivefold, as shown in Figure 4. This increase correlates with the proliferation of astrocytes in the cortex. We are currently extending these observations to examine the developmental pattern in the mouse and in mouse models of Down's syndrome. The availability of a bioassay, a sensitive radioimmunoassay, and a cDNA probe for quantitating mRNA levels allows us to study the changes in S100β at several levels.

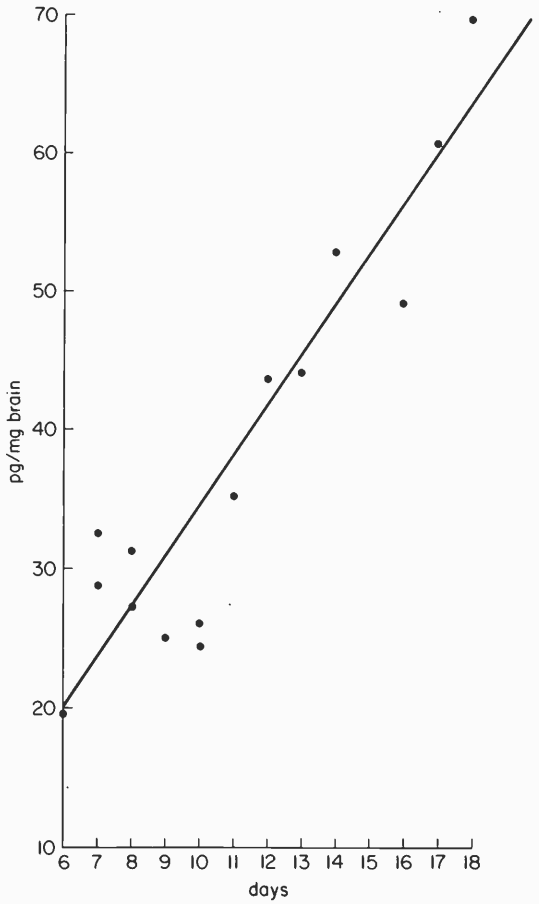

FIGURE 4 Changes in S100β in the developing chicken embryo brain. The vertical axis shows the amount of immunoreactive material in picograms per milligram wet weight of brain tissue. The horizontal axis shows the number of days of embryonic development. Hatching occurs on day 21, and neurotrophic activity is tested on cells from day-7 embryos.

Expression of S100β in Mammalian Cells

D. Marshak, S. Pesce

The cDNA probe for S100β has been characterized in some detail. One clone was selected for further study, and it contains a 1.5-kb insert in an Okayama-Berg expression vector, pcD2. Restriction enzyme mapping of the insert indicates a full-length coding region, with extensive 5′ and 3′ noncoding regions. We have transfected this plasmid into COS I cells, a monkey cell line that contains large amounts of T antigen from SV40. These cells express proteins at high levels from plasmids containing the SV40 early promoter, as in pcD2. The transfected cells express S100β that can be detected in immunoprecipitation experiments. Extracts of transfected cells show neurotrophic activity in the chick embryo assay, whereas mock-transfected and control cell extracts show no activity.

Immunocytochemical localization of S100β in transfected cells resembles that in the astrocytoma cell line, C6, which expresses large amounts of the protein. These experiments demonstrate that neurotrophic activity resides in S100β. Further studies are under way to express the protein in constructs using different tissue-specific promoters. These constructs also provide the basis for construction of transgenic mice that express S100β in overabundance in either neurons or glia. Such animals may provide a new model for the study of developmental and degenerative neurological diseases.

Structural Analysis of a Glial Antigen

D. Marshak [in collaboration with C. Dulac and N. LeDouarin, Nogent-sur-Marne, France]

During an exciting period of 3 months in the spring, Dr. C. Dulac purified and characterized a glycopro-

tein antigen from quail nerves. This protein appears to be a very early developmental marker for glioblasts that are destined to become oligodendrocytes in the central nervous system and Schwann cells of the periphery, both myelin-producing cells. Using a monoclonal antibody produced by Dulac and colleagues at Nogent, we made an immunoaffinity chromatography column and purified sufficient quantities of protein for structural analysis. Amino-terminal sequence analysis yielded information useful for constructing oligonucleotide probes as well as synthetic peptides for production of a site-directed antibody. In addition, sites of N-linked and O-linked oligosaccharides were tentatively identified. This project demonstrated the power of a multidisciplinary approach to protein structural analysis, given the modern tools of protein biochemistry and neurobiology available in our laboratory.

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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. We have recently awarded the 1989 Fellowship to Eric Richards from Fred Ausubel's laboratory at the Massachusetts General Hospital, who will join the laboratory in the summer of 1989 to begin work on the molecular biology of *Arabidopsis*.

A.R. Krainer
C. Greider

Biochemistry of Mammalian Pre-mRNA Splicing

A.R. Krainer, D. Kozak, E. Chan

The 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 factors 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.

ISOLATION OF ACTIVE SMALL NUCLEAR RIBONUCLEOPROTEINS

We have continued our efforts to isolate active small nuclear ribonucleoproteins (snRNPs), which are the only components of known identity that have been clearly shown to be essential splicing factors. For this purpose, we generated a monoclonal antibody that recognizes the distinctive trimethylguanosine (TMG) portion of the snRNA 5′ caps. Because the snRNA trimethylated cap is conserved in all eukaryotes, the anti-TMG monoclonal antibody we generated has also proven useful to other laboratories that are studying snRNPs and snRNAs and other trimethyl capped RNAs from a variety of species, including vertebrates, insects, trypanosomes, nematodes, and yeasts. To carry out immunoaffinity experiments

under optimal conditions, we recently switched the isotype of this monoclonal antibody from IgG1 to IgG2a, by screening for spontaneous heavy-chain switch variants, followed by sequential sublining. The new monoclonal antibody retains its specificity for TMG and can now be efficiently bound to immobilized protein A. The resulting high-efficiency immunoadsorbents are used for preparative purification of all the snRNPs from HeLa cell nuclear extracts in a single step (Fig. 1). The bound snRNPs are eluted under gentle conditions by competition with free nucleoside.

The purified snRNPs retain activity, as demonstrated by their ability to complement a micrococcus-nuclease-treated nuclear extract (Fig. 2). This assay was employed because for unknown reasons, it was not possible to deplete fully the extracts of snRNPs by immunoaffinity chromatography (Fig. 1). Although the nuclease treatment may give rise to snRNP cores or free polypeptides with residual activities, the successful complementation test demonstrates that the purified snRNPs constitute struc-

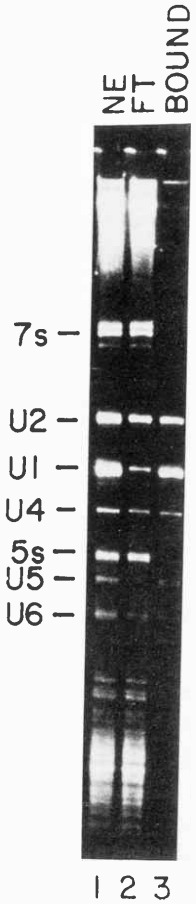

FIGURE 1 RNA composition of anti-TMG column fractions. HeLa snRNPs were purified as described in the text, and the RNAs from 5 × 10^6 cell equivalents of each fraction were extracted and analyzed by PAGE/urea and ethidium bromide staining. (*1*) Crude splicing extract; (*2*) column flowthrough; (*3*) bound fraction eluted by competition with an excess of free nucleoside. The identities of the small RNAs are indicated at the left.

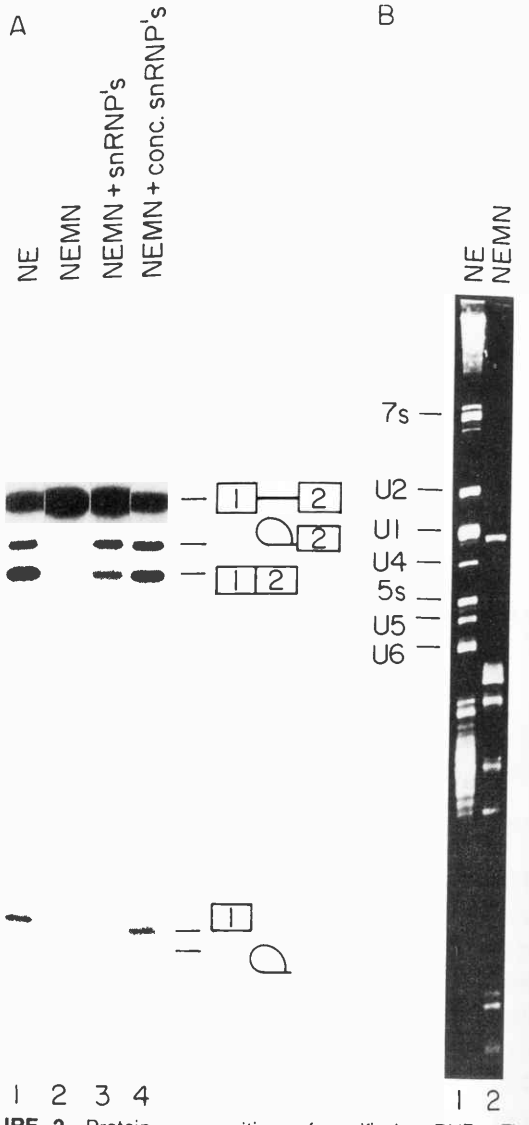

FIGURE 2 Protein composition of purified snRNPs. The indicated fractions were analyzed by SDS-PAGE and Coomassie blue R-250 staining according to the method of Giulian et al. (*A*) or on a 12% gel according to the method of Laemmli (*B*). (*A*) (Lane *1*) Molecular-weight markers, 14.4K–97K; (*2*) 10 μl (2 × 10^6 cell equivalents) of splicing extract; (*3*) 50 μl (2.5 × 10^8 cell equivalents) of concentrated snRNPs; (*4*) markers, 1.7K–17.2K. (*B*) (Lane *1*) Markers, 43K–200K; (*2*) 50 μl of snRNPs; (*3*) 10 μl of splicing extract; (*4*) markers, 14.4K–97K. The identities of the known snRNP polypeptides are indicated at the right of each panel.

tural units with complementable activity. SDS-PAGE analysis of the active snRNPs demonstrates the presence of all known snRNP polypeptides, as well as several minor ones (Fig. 3). Silver staining and two-dimensional gel analysis revealed no additional contaminants. The relative abundances of the characteristic polypeptides suggest heterogeneity in the protein composition of individual snRNPs. Thus, the U1-specific polypeptide C appears to be underrepresented relative to the 70K and A polypeptides, which are also U1-specific (Fig. 3A). Whether this apparent heterogeneity reflects the in vivo stoichiometry, or whether it reflects different stages of assembly, or loss of polypeptide C upon isolation, remains to be determined. Among the previously unidentified polypeptides are four very large polypeptides that are present in small quantities (Fig. 3B). These polypeptides are also obtained by immunoprecipitation of HeLa snRNPs with anti-Sm antibodies. One of these could be the homolog of a U5-associated 260K polypeptide in *Saccharomyces cerevisiae* that is essential for splicing in vivo. Further fractionation of the snRNPs should allow us to determine whether these polypeptides are associated with an individual snRNP and whether they play a role in splicing.

We are fractionating the snRNPs into individual particles, in order to assay the activities of each major particle directly by complementation. This is especially important in the case of U5 snRNP, for

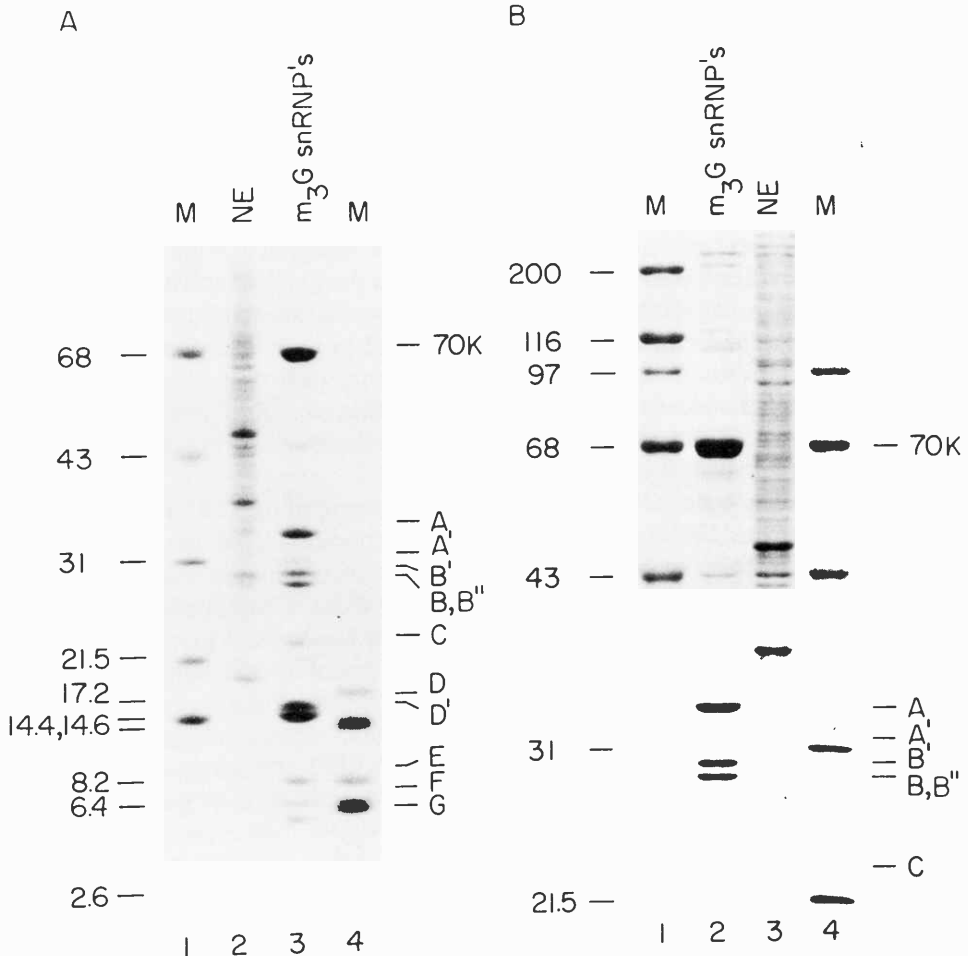

FIGURE 3 (*A*) Assay of snRNP splicing activity. Human β-globin pre-mRNA was incubated under splicing conditions with 15 μl nuclear extract (lane *1*), 15 μl of micrococcus-nuclease-treated nuclear extract (NEMN) (lane *2*), 10 μl NEMN + 1 μl purified snRNPs + 4 μl buffer (lane *3*), and 10 μl NEMN + 5 μl purified snRNPs (lane *4*). The structures and electrophoretic mobilities of the precursor, intermediates, and products of the reaction are shown at the right of the autoradiogram. (*B*) Micrococcal nuclease digestion of snRNAs in the splicing extract. (Lane *1*) 30 μl splicing extract; (lane *2*) 30 μl NEMN.

which a direct involvement in splicing has not been demonstrated. Such fractionation studies may also reveal the involvement in splicing of minor snRNPs. So far, we have obtained highly enriched U1 snRNP by Mono Q chromatography, although this snRNP appears to be very heterogeneous in its polypeptide composition. It may be possible to exploit this heterogeneity to find out which U1 snRNP polypeptides are necessary for its activity.

STUDIES OF snRNP FUNCTION

The functions of U1 and U2 snRNPs are understood at present primarily in terms of specific base pairing between the snRNAs and conserved complementary sequences in the pre-mRNA. The roles of the snRNP polypeptide subunits and of the remaining snRNAs are not understood. One or more of the snRNP polypeptides may have enzymatic activities that are relevant to the overall splicing reaction. In addition, one or more snRNAs may act as a ribozyme to catalyze part of the splicing reaction. We are characterizing the purified particles to uncover such potential activities.

Complementation studies have previously defined four nuclease-resistant activities: Two are necessary for the first cleavage-ligation reaction of splicing and two are necessary for the second cleavage-ligation reaction of splicing. Two of these activities, arbitrarily named SF4A and SF4B, could be chromatographically separated from snRNPs that retained activity and all the known polypeptides, suggesting that these activities are not intrinsic to the snRNPs. In contrast, the activities termed SF2 and SF3 could not be separated from the snRNPs by ion-exchange or gel-filtration chromatography without loss of activity. This raised the possibility that SF2 and SF3 represent known snRNP polypeptides or perhaps factors that are loosely associated with one or more snRNPs. To address this possibility, the purified snRNPs were tested for SF2 and SF3 activities, but neither activity could be detected. Splicing activity could be obtained by providing the snRNPs in one fraction and SF2 and SF3 activities in a separate fraction. We conclude that SF2 and SF3 activities are not encoded by known snRNP polypeptides. If interactions among these factors are necessary for splicing, they can occur during the reaction.

The RNA-binding properties of the purified snRNPs were investigated by incubation with immobilized polynucleotides. All of the snRNPs bind efficiently to poly(G), but not to poly(A) or poly(C), and only U1 snRNP binds to poly(U). The snRNPs can also bind to pre-mRNA splicing substrates, and we are now trying to determine whether purified snRNPs have specific binding sites on pre-mRNA in the absence of other components. There is reason to believe that such binding will be modulated by additional protein factors. Auxiliary activities that enhance specific binding of U1, U2, and U5 snRNPs in crude extracts have been described. One of these is an RNA-binding protein, termed IBP, which has apparent specificity for intron sequences, and has been reported to associate with U5 snRNP under certain conditions. RNA-binding experiments with the purified snRNPs indicate that IBP is not efficiently retained in the immunoaffinity-purified particles. If this factor is necessary for splicing, it may function independently or it may associate with U5 snRNP during the course of the reaction. Additional experiments are required to understand the interactions between IBP and U5 snRNP and their potential significance to splicing.

Since pre-mRNA splicing requires ATP, for reasons that are still unclear, and since at least the U1-specific 70K polypeptide is a phosphoprotein, we assayed the purified snRNPs for ATPase and autophosphorylation activities. Neither activity could be detected in pure, active snRNPs in the absence of additional factors, under splicing conditions. We found that U1 snRNP can bind to immobilized ATP, and we are investigating the specificity of this binding.

Treatment of the snRNPs with *N*-ethylmaleimide, under conditions that inactivate the splicing extract, did not inactivate the snRNPs as assayed by complementation. This observation suggests that snRNP polypeptides present in intact particles do not contribute essential sulfhydryl groups to splicing. Analogous conclusions have been reached in the case of ribosomal protein sulfhydryl groups. In contrast, another RNP particle, the SRP, is inactivated by sulfhydryl modification of its polypeptide constituents.

STUDIES OF PROTEIN SPLICING FACTORS

We have continued our efforts to fractionate several of the protein components that are necessary for the two cleavage-ligation steps of splicing. SF2 is an activity originally defined in a biochemical complementation assay as one of a minimum of two protein components necessary for 5′ splice site

cleavage and lariat formation during pre-mRNA splicing in vitro. The S100 fraction generated during the preparation of splicing extracts is inactive in splicing, even though it contains other components necessary for splicing, such as U snRNPs, SF3, SF4A, SF4B, and creatine phosphokinase. Whereas other splicing factors leak out from the nucleus, SF2 is preferentially retained, suggesting that it may be associated with large RNP complexes or with nuclear structures in vivo. We have extensively purified SF2 from HeLa nuclear extracts, by complementing the S100 fraction for splicing. The most purified, active preparations contain four major polypeptides, which have now been separated to allow determination of their amino-terminal amino acid sequences. We are currently attempting to purify active SF2 to homogeneity. The ongoing physical and biochemical characterization of SF2 includes a comparison with activities characterized in other laboratories, and with major and minor snRNP and hnRNP polypeptides. In addition, purified SF2 is being analyzed to determine its putative enzymatic activities, RNA-binding properties, and specific interactions with pre-mRNA and with purified, active snRNPs, as well as to examine its role in the spliceosome assembly pathway.

Identification and Cloning of the *Tetrahymena* Telomerase RNA Component

C. Greider, L. Sellati

Telomeres have been known to be essential for chromosome stability since the work of Muller, B. McClintock, and others in the 1930s. Chromosomes broken in any of a number of ways undergo rearrangement and fusion events that normal chromosomes do not. In addition to providing stable ends, telomeres must allow the complete replication of linear chromosomes. Since all known DNA polymerases function in the 5′ to 3′ direction and require a primer, one would predict that lagging strand synthesis would result in a region at the end of each chromosome that has not been fully replicated. After a number of rounds of replication, chromosomes would be expected to shorten from their ends. A number of models have been proposed to account for how telomeres overcome this incomplete replication problem. Molecular characterization of the structure and dynamics of telomeres in vivo led to the proposal that de novo addition of telomeric sequences onto the ends of chromosomes balances the loss of sequences from the ends.

Telomeres from all eukaryotes studied, including those as distantly related as *Tetrahymena* yeast, *Arabidopsis*, and humans, consist of tandem repeats of simple C+G-rich sequences, where one strand is very G-rich. In all cases examined, the G-rich strand is oriented 5′→3′ toward the end of the chromosome. The *Tetrahymena* telomere terminal transferase, or telomerase for short, recognizes the 3′ end of telomeric G-strand sequences and adds tandem repeats of the *Tetrahymena* telomeric sequence, TTGGGG, in an apparently template-independent manner. Thus, this enzyme may play a role in establishing a length equilibrium at the ends of chromosomes.

The *Tetrahymena* telomerase exhibits two distinct kinds of primer specificities. Telomere sequence oligonucleotides corresponding to the telomeres from five different organisms are all efficiently elongated by telomerase in vitro. Nontelomeric oligonucleotides and C-rich telomere-strand oligonucleotides are not elongated. In all cases, the sequence added onto the primers is the *Tetrahymena* telomeric TTGGGG sequence. Since the oligonucleotides that are elongated have different primary sequences, some other feature such as the structure, or G-richness, of these oligonucleotides must be specifically recognized by telomerase. The oligonucleotide $d(TTGGGG)_4$ forms a novel intramolecular structure involving G-G Hoogsteen base pairs (Henderson et al., *Cell 51:* 899 [1987]). This unusual structure may be involved both in telomerase recognition and in telomere stability in vivo.

In addition to elongating telomeric primers specifically, telomerase specifically recognizes the sequence at the 3′ end of the primer oligonucleotides. When the 3′ end of the primer has the sequence ...TTGGGG, then the sequence TTGGGG is first added; however, when the primer ends in the sequence ...TTGGG, the sequence that is first added is GTTGGGG. If ...GGGGTT is the primer, GGGGTTGGGG is added. In all cases, after the completion of the first TTGGGG sequence, hundreds of tandem TTGGGG repeats are added.

Biochemical characterization indicated that telomerase is a ribonucleoprotein (RNP) complex and that a specific RNA is required for telomerase activity. To identify the required RNA, telomerase extracts were fractionated using several different chromatographic steps, and the RNAs that copurified were

identified. One small 159-base RNA reproducibly copurified with telomerase over several different five-column series. This RNA was gel-purified and sequenced using enzymatic RNA sequencing techniques.

Since coming to Cold Spring Harbor Laboratory, I have focused on cloning the gene for this RNA and on demonstrating that this RNA is essential for telomerase activity. DNA oligonucleotides complementary to the sequence of the 159-base RNA were synthesized. Southern blot analysis using these oligonucleotides showed that there was a single gene for the 159-base RNA in *Tetrahymena*. A 2-kb *Hin*dIII fragment of macronuclear genomic DNA was cloned, and the sequence of the entire RNA-coding region, as well as some 5′- and 3′-flanking sequences, was obtained. The most notable feature of the RNA sequence was the presence of CAACCCCAA within the coding region for the RNA. This sequence could provide a template for the TTGGGG repeats that are synthesized in vitro.

Oligonucleotides complementary to the 159-base RNA were synthesized that span the entire length of the RNA (Fig. 4). When telomerase was preincubated with these antisense oligonucleotides before the addition of the $d(TTGGGG)_4$ primer and reaction buffer, oligonucleotide 3 and oligonucleotide 8, which hybridized across and near the CAACCCCAA sequence, both dramatically affected telomerase activity. Oligonucleotide 3 inhibited elongation by telomerase, whereas oligonucleotide 8 was itself efficiently elongated. Since oligonucleotide 8 was the only nontelomeric sequence oligonucleotide out of 14 tested that was elongated in vitro, its utilization as a primer may be due to the ability of oligonucleotide 8 to hybridize just 3′ of the CAACCCCAA sequence in the 159-base RNA. This result, along with the inhibition by oligonucleotide 3, suggested that the CAACCCCAA sequence serves as a template for the synthesis of TTGGGG repeats.

As a further test for the involvement of the 159-base RNA in telomerase activity, complementary oligonucleotides were preincubated with telomerase in the presence or absence of RNase H, and the elongation activity was measured. RNase H will cleave the RNA of a DNA/RNA duplex. The competitive inhibition of oligonucleotide 3 was relieved by pelleting telomerase in an ultracentrifuge after preincubation with oligonucleotide 3. Preincubation in the presence of both oligonucleotide 3 and RNase H resulted in cleavage of the telomerase RNA with the CAACCCCAA sequence and concomitant inactivation of the telomerase activity. Preincubation of telomerase with RNase H and most of the other oligonucleotides shown in Figure 1 did not result in cleavage of the 159-base RNA or inactivation of the enzyme activity. The inactivation of telomerase by RNase H and oligonucleotide 3 directly demonstrated that the 159-base RNA is required for telomerase activity.

Preliminary results suggest that TTGGGG repeat addition is processive. A model consistent with this processivity and other telomerase characteristics is presented in Figure 5. The natural ends of chromosomes have a single-strand overhang on the TTGGGG strand. The model suggests that after specific primer recognition (1) the sequence TTGGGG is hybridized to the CAACCCCAA sequence in the RNA; (2) the sequence TTG is then added one nucleotide at a time; (3) translocation then repositions the 3′ end of the TTGGGG strand such that the 3′-most TTG nucleo-

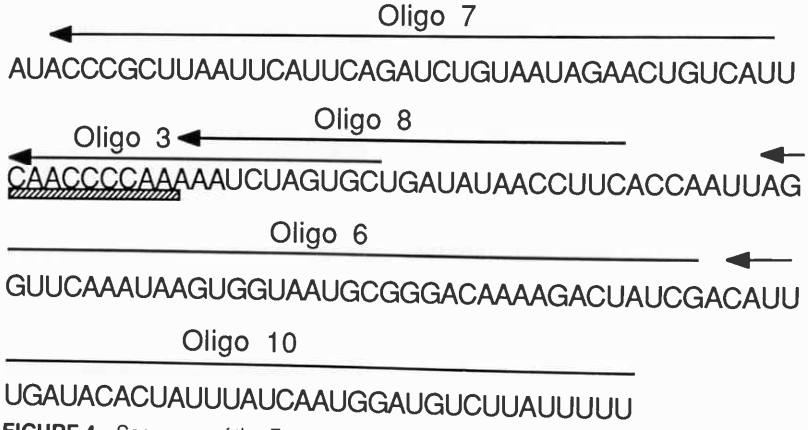

FIGURE 4 Sequence of the *Tetrahymena* telomerase RNA shown along with the DNA oligonucleotides used in cloning and RNase H experiments. The region that may provide a template for synthesis of TTGGGG is underlined.

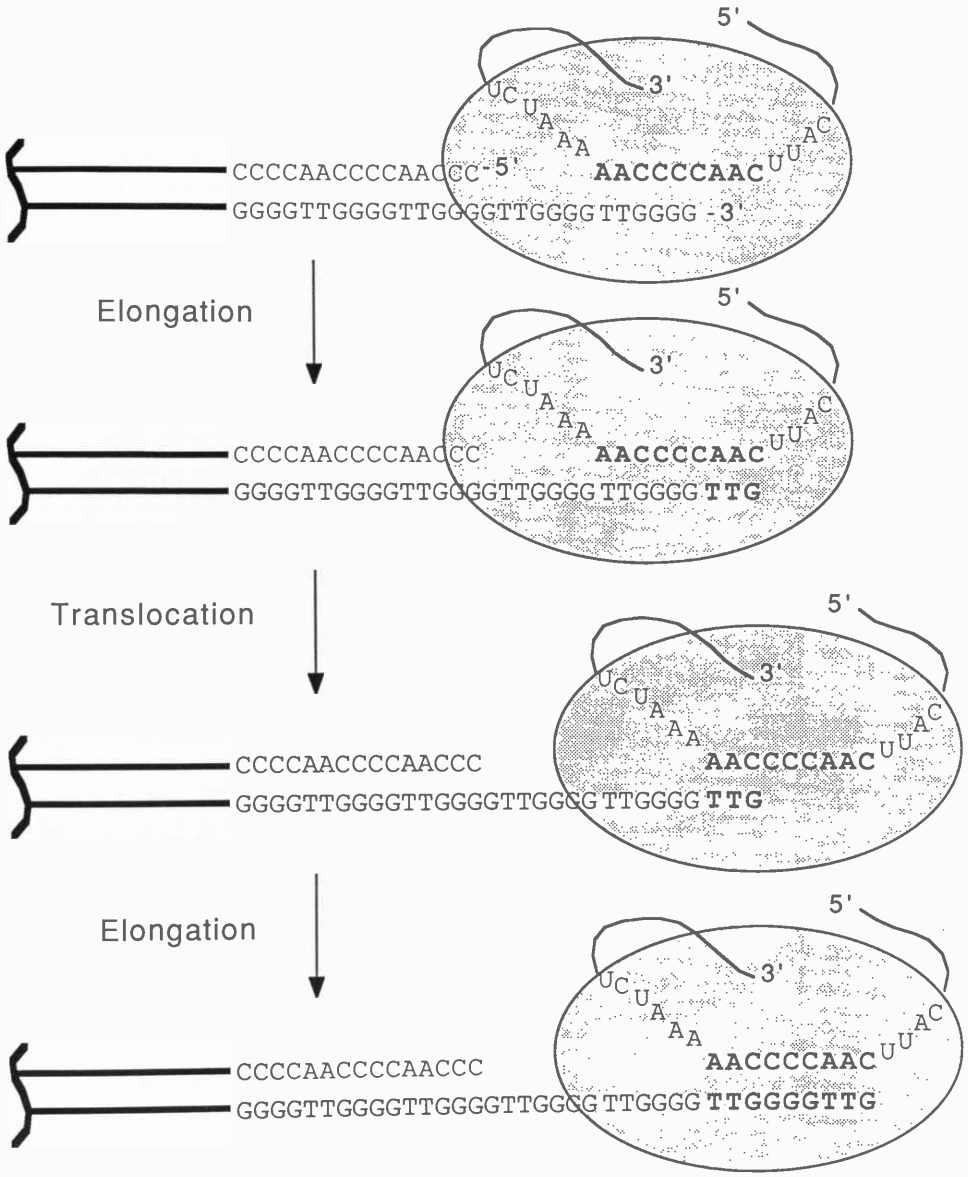

FIGURE 5 Model for processive elongation of telomeres by telomerase. After specific primer recognition, the sequence TTGGGG is hybridized to the CAACCCCAA sequence in the RNA. The sequence TTG is then added one nucleotide at a time. Translocation then repositions the 3′ end of the TTGGGG strand such that the 3′-most TTG nucleotides are hybridized to the RNA component of telomerase. Now elongation can occur again, copying the template sequence to complete the TTGGGGTTG sequence.

tides are hybridized to the RNA component of telomerase; and (4) elongation occurs again, copying the template sequence to complete the TTGGGGTTG sequence.

To test this model, we are currently looking for conditions in which telomerase activity can be reconstituted from isolated protein and synthetic RNA transcripts. In addition, experiments are under way to express the *Tetrahymena* telomerase RNA in yeast cells to determine whether this RNA can compete with a putative yeast telomerase RNA.

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Tschudi, C., A.R. Krainer, and E. Ullu. 1988. The U6 small nuclear RNA from *Trypanosoma brucei*. *Nucleic Acids Res.* **16:** 11375.

In Press, Submitted, and In Preparation

Conway, G., A.R. Krainer, and R.J. Roberts. 1989. Spliceosome formation in splicing extracts depleted of large endogenous RNP complexes. (In preparation.)

Conway, G., A.R. Krainer, D. Spector, and R.J. Roberts. 1989. Multiple splicing factors are released from endogenous RNP complexes at the onset of in vitro pre-mRNA splicing. (Submitted.)

COLD SPRING HARBOR MEETINGS

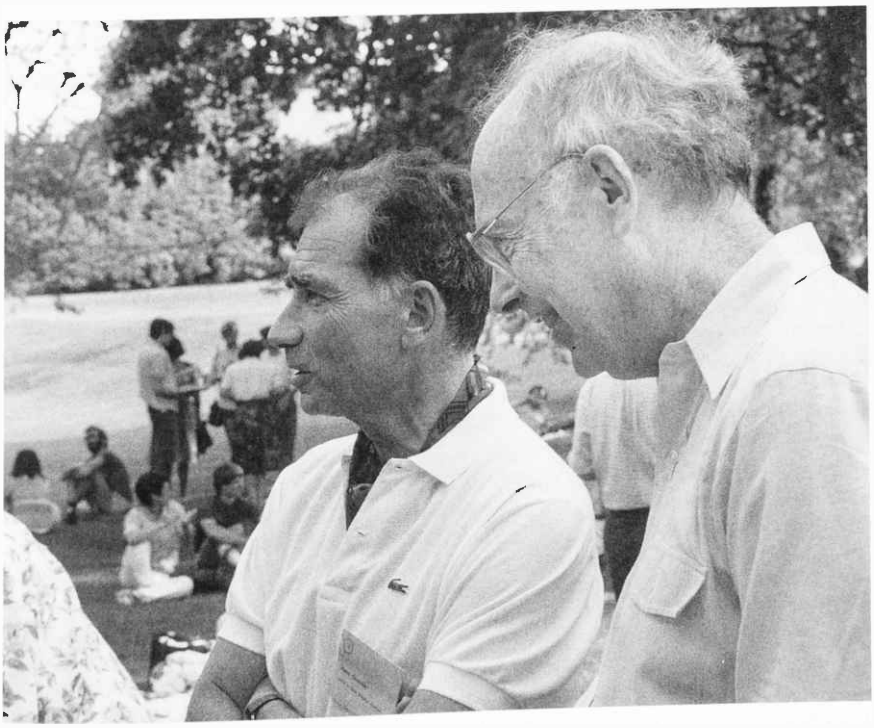
53rd COLD SPRING HARBOR SYMPOSIUM ON QUANTITATIVE BIOLOGY

The Molecular Biology of Signal Transduction

May 25—June 3

430 participants

For many decades, it has been clear that cells have a multitude of ways of sensing their environment and converting a plethora of external signals into measured intracellular responses. Already by 1965 Earl Sutherland had made the distinction between the "first-messenger" role of hormone-like signals and the "second-messenger" role of cyclic AMP, whose concentration was somehow influenced by signals from first messengers. In Sutherland's scheme, the first messenger binds to cellular receptors, with such binding events somehow leading to control of the concentration of the second messenger. Now we realize that many first messengers do not act directly through second messengers, but instead work at the genetic level by binding to cytoplasmically located receptors, which can then bind to DNA and turn on or off the functioning of specific genes. Today, we refer to the way that external signals are passed through various cellular components as signal transduction processes, with receptors and their associated molecules known as biological transducers. Because most transducer molecules are present in very limited amounts, their study at the biochemical level until recently was at best difficult, and hypotheses as to how they functioned were almost impossible to test rigorously.



P. Chambon, J.D. Watson

Today, recombinant DNA techniques have dramatically changed the picture. Even very rare receptors are now open to analysis if their respective genes can be cloned, and virtually every month, the amino acid sequence of a new key biological transducer is established. The time was thus appropriate last June to hold a Cold Spring Harbor Symposium on the Molecular Biology of Signal Tranduction.

The final program consisted of 119 speakers, who spoke before an audience of 439, the largest ever yet to attend a Cold Spring Harbor Symposium. The meeting opened with a series of five virtually electric presentations by Howard Berg. Alfred Gilman, Mark Ptashne, Lubert Stryer, and Keith Yamamoto. A mode of high excitement prevailed throughout the subsequent 14 long sessions, which were concluded by a graciously thoughtful summary by Henry Bourne.

This meeting was supported in part by the National Cancer Institute, a division of the National Institutes of Health; the U.S. Department of Energy; the Lucille P. Markey Charitable Trust; and the National Science Foundation.

Welcoming Remarks: J.D. Watson

SESSION 1 OPENING REMARKS

Berg, H.C., Dept. of Cellular and Developmental Biology, Harvard University and Rowland Institute of Science, Cambridge, Massachusetts: Bacterial chemotaxis.

Gilman, A., Dept. of Pharmacology, University of Texas Southwestern Medical Center, Dallas: Role of G proteins in transmembrane signaling.

Stryer, L., Dept. of Cell Biology, Stanford University School of Medicine, California: Molecular mechanism of visual excitation.

Yamamoto, K.R., Godowski, P.J., Picard, D., Sakai, D.D., Dept. of Biochemistry, University of California, San Francisco: Signal transduction and transcriptional regulation by the glucocorticoid receptor.

Ptashne, M., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: A universal (perhaps) activator of gene expression.

SESSION 2 BACTERIAL CHEMOTAXIS

Chairman: D. Koshland, University of California, Berkeley

Koshland, D.E., Jr., Bollag, G.E., Milligan, D.L., Sanders, D.A., Dept. of Biochemistry, University of California, Berkeley: Sensory transduction in bacterial chemotaxis.

Adler, J., Boileau, A.J., Buechner, M., Delcour, A.H., Li, C., Sager, B.M., Shi, W., University of Wisconsin, Madison: New kinds of behavior of *E. coli*.

Stewart, R., Russell, C.B., Dahlquist, F.W., Institute of Molecular Biology, University of Oregon, Eugene: Feedback events during sensory adaptation by *E. coli*.

Hess, J., Oosawa, K., Simon, M., Division of Biology, California Institute of Technology, Pasadena: Protein phosphorylation and signal transduction in bacteria.

Stock, J., Mottonen, J., Stock, A., Schutt, C., Depts. of Biology and Chemistry, Princeton University, New Jersey: Three-dimensional structure of the chemotaxis response regulator.

Ames, P., Chen, J., Wolff, C., Parkinson, J.S., Dept. of Biology, University of Utah, Salt Lake City: Structure-function studies of bacterial chemosensors.

Macnab, R.M., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: The end of the line in bacterial sensing—The flagellar motor.

SESSION 3 SIGNAL TRANSDUCTION IN YEAST

Chairman: I. Herskowitz, University of California, San Francisco

Marsh, L., Herskowitz, I., Dept. of Biochemistry and Biophysics, University of California, San Francisco: Sites that determine ligand selectivity in the yeast α-factor receptor (*STE2* protein).

Matsumoto, K.,[1] Nakafuku, M.,[2] Nakayama, N.,[1] Miyajima, I.,[1] Kaibuchi, K.,[1] Brenner, C.,[1] Miyajima, A.,[1] Arai, K.,[1] Kaziro, Y.,[2] [1]Dept. of Molecular Biology, DNAX Research Institute, Palo Alto, California; [2]Institute of

D. Koshland, I. Herskowitz, G. Rubin

M. Wigler, H. Hanafusa, S. Numa

Medical Science, University of Tokyo, Japan: Role of G proteins in yeast signal transduction systems.

Dietzel, C., Kurjan, J., Dept. of Biological Sciences, Columbia University, New York, New York: Implication of the *SCG1*-encoded G-protein homolog in pheromone response in yeast.

Reneke, J.E., Blumer, K.J., Courchesne, W.E., Thorner, J., Dept. of Biochemistry, University of California, Berkeley: Receptor desensitization in yeast involves three discrete mechanisms.

Blinder, D., Spatrick, S., Bouvier, S., Sullivan, C., Jenness, D., Dept. of Molecular Genetics Microbiology, University of Massachusetts Medical School, Worcester: Signals controlling α-factor adaptation and receptor internalization.

Clark, K., Davis, N., Wiest, D., Sprague, G., Jr., Institute of Molecular Biology, University of Oregon, Eugene: Activity and structure of yeast α-factor receptor.

Reed, S., de Barros Lopes, M., Ferguson, J., Jahng, K.-Y., Stone, D., Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California: Molecular and genetic analysis of mating pheromone signal transduction in yeast.

Botstein, D.,[1] Segev, N.,[2] Stearns, T.,[3] Hoyt, M.A.,[4] [1]Dept. of Biology, Massachusetts Institute of Technology, Cambridge; [2]Dept. of Biochemistry, Stanford University, California; [3]Genentech, Inc., South San Francisco, California: Diverse biological functions of small G proteins in yeast.

Struhl, K., Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Transcriptional enhancement by the yeast GCN4 activator protein and the related *jun* oncoprotein.

Harshman, K.D., Moye-Rowley, W.S., Parker, C.S., Division of Chemistry, California Institute of Technology, Pasadena: Biochemical and molecular studies on yAP-1, the yeast equivalent to mammalian AP-1.

SESSION 4 NEUROSENSORY TRANSDUCTION

Chairman: M. Bitensky, Los Alamos National Laboratory

Bitensky, M.W., Los Alamos National Laboratory, New Mexico: Shaping of sensory transduction.

Chabre, M., Bigay, J., Bornancin, F., Bruckert, F., Deterre, P., Pfister, C., Vuong, T.M., Biophysique Moleculaire et Cellulaire, CNRS, Grenoble, France: The rhodopsin-transducin-cGMP phosphodiesterase cascade—A model for G-protein-mediated signal transduction.

Baylor, D.A.,[1] Karpen, J.W.,[2] Zimmerman, A.L.,[1] Stryer, L.,[2] Depts. of [1]Neurobiology, [2]Cell Biology, Stanford Medical School, California: Molecular genetics of the cGMP-activated channel of retinal rods.

Selinger, Z.,[1] Minke, B.,[2] Depts. of [1]Biological Chemistry, [2]Physiology, Hebrew University of Jerusalem, Israel: Inositol lipid phototransduction pathway in fly photoreceptor.

Nef, P., Lazard, D., Heldman, J., Khen, M., Lancet, D., Dept. of Membrane Research, Weizmann Institute of Science, Rehovot, Israel: Molecular transduction in smell and taste.

Jones, D.T., Reed, R.R., Dept. of Molecular Biology and Genetics, Howard Hughes Medical Laboratories, Johns Hopkins School of Medicine, Baltimore, Maryland: Olfactory signal transduction utilizes a novel GTP-binding protein.

Khorana, H.G., Flitsch, S., Marti, T., Mogi, T., Stern, L., Subramaniam, S., Depts. of Biology and Chemistry, Massachusetts Institute of Technology, Cambridge: Bacteriorhodopsin, a light-transducing membrane protein that translocates protons.

SESSION 5 SECOND MESSENGER SYSTEMS. I

Chairman: M. Wigler, Cold Spring Harbor Laboratory

Wigler, M.,[1] Cameron, S.,[1] Powers, S.,[1] Field, J.,[1] Toda, T.,[2] Broek, D.,[1] Nikawa, J.,[3] Michaelis, T.,[1] Colicelli,

J.,[1] [1]Cold Spring Harbor Laboratory, New York; [2]Faculty of Science, Kyoto University, Japan; [3]Dept. of Biological Chemistry, Gunma University School of Medicine, Japan: Integration of *RAS* and adenylate cyclase pathways in yeast.

Devreotes, P.,[1] Klein, P.,[1] Lilly, P.,[1] Pitt, G.,[1] Pupillo, M.,[1] Sun, T.,[1] Vaughan, R.,[1] Coukell, B.,[2] Kumagai, A.,[3] Firtel, R.,[3] Saxe, C. III,[4] Kimmel, A.,[4] [1]Johns Hopkins School of Medicine, Baltimore, Maryland; [2]University of York, Toronto, Canada; [3]University of California, San Diego, La Jolla; [4]NIDDK, National Institutes of Health, Bethesda, Maryland: Receptor and G proteins involved in *Dictyostelium* chemotaxis.

Kung, C., Saimi, Y., Martinac, B., Gustin, M.C., Laboratory of Molecular Biology and Dept. of Genetics, University of Wisconsin, Madison: Ion channels of *Pararmecium*, yeast, and *E. coli*.

Kumagai, A.,[1] Mann, S.K.O.,[1] Firtel, R.A.,[1] Pupillo, M.,[2] Pitt, G.,[2] Devreotes, P.,[2] [1]Center for Molecular Genetics, University of California, San Diego, La Jolla; [2]Dept. of Biological Chemistry, Johns Hopkins School of Medicine, Baltimore, Maryland: Molecular analysis of G_α proteins and signal transduction processes controlling early gene expression in *Dictyostelium*.

Murad, F., Stanford University and Veterans Administration Medical Center, Palo Alto, California: The guanylate cyclase—cGMP system mediates the effects of ANF and other vasodilators.

SESSION 6 PROTEIN PHOSPHORYLATION. I

Chairman: E. Krebs, University of Washington

Krebs, E.,[1] Eisenman, R.,[2] Kuenzel, E.,[2] Litchfield, D.,[1] Lozeman, F.,[1] Lüscher, B.,[2] Sommercorn, J.,[1] [1]Howard Hughes Medical Institute and Dept. of Pharmacology, University of Washington, [2]Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle: Casein kinase II as a potentially important enzyme concerned with signal transduction.

Carroll, D., Santoro, N., Marshak, D.R., Cold Spring Harbor Laboratory, New York: Regulating cell growth—Casein-kinase-II-dependent phosphorylation of nuclear oncoproteins.

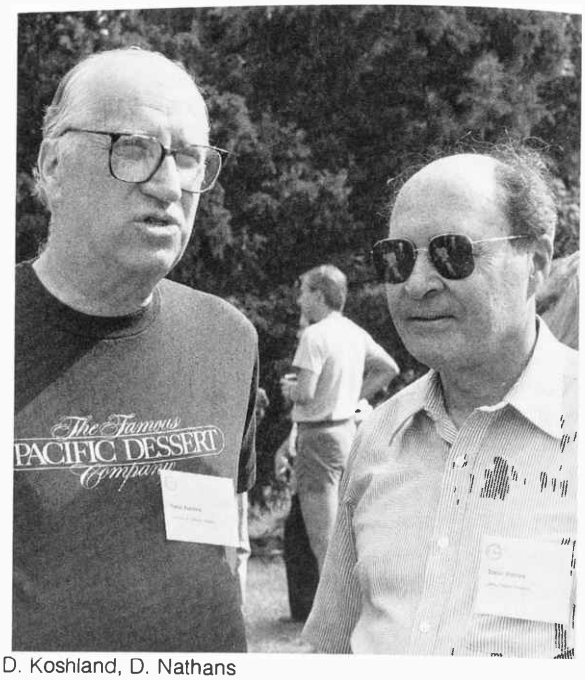
Nishizuka, Y.,[1] Kikkawa, U.,[1] Ono, Y.,[3] Shearman, M.S.,[1] Sekiguchi, K.,[1] Ase, K.,[1] Tanaka, C.,[2] Depts. of [1]Biochemistry, [2]Pharmacology, Kobe University School of Medicine, [3]Biotechnology Laboratories, Central Research Division, Takeda Chemical Industries, Osaka, Japan: The family of protein kinase C in processing and modulating cellular responses.

Bell, R.M., Dept. of Biochemistry, Duke University Medical Center, Durham, North Carolina: Protein kinase C regulation by sphingosine/lysosphingolipids.

McKnight, S., Cadd, G., Otten, A., Chrivia, J., Correll, L., Uhler, M., Clegg, C., Dept. of Pharmacology, University of Washington, Seattle: Analysis of the cAMP-dependent protein kinase system using molecular genetic approaches.

Taylor, S., Buechler, J., Slice, L., Knighton, D., Durgerian, S., Ringheim, G., Neitzel, J., Yonemoto, W., Sowadski, J., Dept. of Chemistry, University of California, San Diego, La Jolla: cAMP-dependent protein kinase—A framework for a diverse family of enzymes.

Hunter, T., Gould, K., Isacke, C., van der Geer, P., Freed, E., Lindberg, R., Boyle, B., Thompson, D., Jähner, D., Salk Institute, San Diego, California: Targets for signal-transducing protein kinases.



D. Koshland, D. Nathans

Riabowol, K.T.,[1] Gilman, M.Z.,[1] Walsh, D.A.,[2] Fink, J.S.,[2] Goodman, R.H.,[3] Feramisco, J.R.,[4] [1]Cold Spring Harbor Laboratory, New York; [2]Dept. of Biological Chemistry, University of California, Davis; [3]Molecular-Medicine, Tufts-New England Medical Center, Boston, Massachusetts; [4]Cancer Center, University of California, San Diego: Catalytic subunit of cAMP-dependent protein kinase induces expression of genes containing cAMP-responsive enhancer elements.

SESSION 7 GROWTH FACTOR AND HORMONE RECEPTORS. I

Chairman: M. Brown, University of Texas Southwestern Medical Center

Brown, M.S., Goldstein, J.L., University of Texas Southwestern Medical Center, Dallas: The LDL receptor—Prototype for receptor-mediated endocytosis.

Escobedo, J.A., Williams, L.T., University of California and Howard Hughes Medical Institute, San Francisco: Intracellular structural domains of the PDGF receptor have distinct functions.

Gill, G.N.,[1] Glenney, J.R., Jr.,[2] Chen, W.S.,[1] Lazar, C.S.,[1]

Wiley, H.S.,[3] Rosenfeld, M.G.,[1] [1]University of California School of Medicine, [2]Salk Institute, San Diego; [3]University of Utah School of Medicine, Salt Lake City: Regulation of the EGF receptor.

Chao, M.V., Dept. of Cell Biology and Anatomy and Hematology/Oncology Division, Cornell University Medical College, New York, New York: The NGF receptor.

Dixon, R.A.F.,[1] Sigal, I.S.,[1] Strader, C.D.,[2] Depts. of [1]Molecular Biology, [2]Biochemistry, Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania, and Rahway, New Jersey: Molecular genetic analysis of the β-adrenergic receptor.

Ross, E.M., Higashijima, T., Rubenstein, R.C., Wong, S.K.-F., Dept. of Pharmacology, University of Texas Southwestern Medical Center, Dallas: Functional domains of the β-adrenergic receptor.

Lefkowitz, R.J., Kobilka, B.K., Benovic, J.L., Bouvier, M., Cotecchia, S., Hausdorff, W.P., Dohlman, H.G., Caron, M.G., Depts. of Medicine, Biochemistry, and Physiology, and HHMI, Duke University Medical Center, Durham, North Carolina: Molecular and regulatory properties of adrenergic receptors.

SESSION 8 GROWTH FACTOR AND HORMONE RECEPTORS. II

Chairman: M. Waterfield, Ludwig Institute for Cancer Research

Pallen, C.J., Sahlin, L., Waterfield, M.D., Ludwig Institute for Cancer Research, London, England: Purification and characterization of membrane tyrosine phosphatases.

Schlessinger, J.,[1,2] Honegger, A.M.,[1] Ullrich, A.,[3] [1]Rorer Biotechnology, Inc., Research Laboratories, King of Prussia, Pennsylvania; [2]Weizmann Institute of Science, Rehovot, Israel; [3]Genentech, Inc., South San Francisco, California: The protein tyrosine kinase activity of EGF receptor is essential for signal transduction.

Sherr, C.J.,[1] Downing, J.R.,[1,2] Rettenmier, C.W.,[1] Roussel, M.F.,[1] Depts. of [1]Tumor Cell Biology, [2]Pathology, St. Jude Children's Research Hospital, Memphis, Tennessee: Signal transduction by the c-*fms* (CSF-1 receptor) proto-oncogene and the v-*fms* oncogene.

Ullrich, A.,[1] Hudziak, R.,[1] Massoglia, S.,[1] Gray, A.,[1] Lee, J.,[1] Riedel, H.,[1] Shepard, H.M.,[1] Slamon, D.,[2] Schlessinger, J.,[3] Graf, T.,[4] Beug, H.,[4] Khazaie, K.,[4] Vennstrom, B.,[4] [1]Genentech, Inc., South San Francisco, California; [2]University of California, Los Angeles; [3]Rorer Biotechnology, Inc., King of Prussia, Pennsylvania; [4]EMBL, Heidelberg, Federal Republic of Germany: Transforming potential of growth-factor receptors.

Hall, D.J., Rollins, B.J., Jones, S.D., Irminger, J.C., Stiles, C.D., Dept. of Microbiology and Molecular Genetics, Harvard Medical School and Dana-Farber Cancer Institute, Boston, Massachusetts: PDGF-inducible genes respond differentially to at least two distinct intracellular second messengers.

Steele-Perkins, G.,[1] Turner, J.,[2] Edman, J.C.,[2] Hari, J.,[1] Kovacina, K.,[1] Pierce, S.B.,[1] Stover, C.,[1] Rutter, W.J.,[2] Roth, R.A.,[1] [1]Stanford University School of Medicine, [2]University of California, San Francisco: Insulin and insulin-like growth-factor receptors and responses.

Katan, M.,[1] Meldrum E.,[1] Totty, N.,[1] Philp, R.,[1] Kriz, R.,[2] Knopf, J.,[2] Parker, P.J.,[1] [1]Ludwig Institute for Cancer Research, London, England; [2]Genetics Institute, Boston, Massachusetts: PI-specific phospholipase C.

Rosenfeld, M.G., Albert, V., Adler, S., Chen, W., Crenshaw, B., Elsholtz, H., Glass, C., He, X., Ingraham, H., Lu, H., Mangalam, H., Gill, G.N., Howard Hughes Medical Institute, Eukaryotic Regulatory Biology Program, University of California School of Medicine, San Diego, La Jolla: Developmental and homeostatic regulation of neuroendocrine gene expression.

Levy, D., Kessler, D., Pine, R., Reich, N., Darnell, J., Jr., Rockefeller University, New York, New York: Interferon signals regulation of gene expression through modulation of promoter-recognition factors.

SESSION 9 MECHANISM OF ACTION OF G PROTEINS

Chairman: Y. Kaziro, University of Tokyo

Itoh, H., Kozasa, T., Toyama, R., Tsukamoto, T., Matsuoka, M., Hernandez, R., Nakafuku, M., Obara, T., Takagi, T., Kaziro, Y., Institute of Medical Science, University of Tokyo, Japan: Structure of the genes coding for G-protein α-subunits from mammalian and yeast cells.

Bourne, H.R.,[1,2] Masters, S.B.,[2] Miller, R.T.,[2] Sullivan, K.A.,[2] [1]Depts. of Pharmacology and Medicine, University of California; [2]Cardiovascular Research Institute, San Francisco, California: Mutations test a structural and functional model of the α chain of G_s (α_s).

Birnbaumer, L.,[1,2] Codina, J.,[1] Yatani, A.,[2] Mattera, R.,[1] Olate, J.,[1] Sanford, J.,[1] Brown, A.M.,[2] Depts. of [1]Cell Biology, [2]Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, Texas: Structural aspects of G proteins regulating ion channels.

Neer, E.J.,[1] Kim, S.Y.,[1] Bloch, D.,[2] Logothetis, D.,[1] Clapham, D.,[3] Seidman, J.G.,[2] Depts. of [1]Medicine, [2]Genetics, Harvard Medical School, Boston, Massachusetts; [3]Dept. of Pharmacology, Mayo Foundation, Rochester, Minnesota: Function of G-protein subunits.

Schultz, G.,[1] Rosenthal, W.,[1] Hescheler, J.,[2] Hinsch, K.-D.,[1] Trautwein, W.,[2] [1]Institut für Pharmakologie, Freie Universität Berlin, [2]Physiologisches Institut, Universität des Saarlandes, Homberg, Federal Republic of Germany: Hormonal modulations of calcium channel activity via G proteins.

Katada, T.,[1] Ui, M.,[2] [1]Dept. of Life Science, Faculty of Science, Tokyo Institute of Technology, Yokohama, [2]Dept. of Physiological Chemistry, Faculty of

Pharmaceutical Sciences, University of Tokyo, Japan: Unique properties of a new GTP-binding protein purified from porcine brain membranes.

Kim, S.-H.,[1] de Vos, A.,[1] Tong, L.,[1] Milburn, M.,[1] Matias, P.,[1] Noguchi, S.,[2] Nishimura, S.,[2] [1]Dept. of Chemistry and Lawrence Berkeley Laboratory, University of California, Berkeley; [2]Biology Division, National Cancer Center Research Institute, Tokyo, Japan: Crystal structures of human c-H-*ras* p21 proteins.

SESSION 10 STEROID HORMONE RECEPTORS

Chairman: R.M. Evans, Salk Institute

Evans, R.M., Salk Institute, San Diego, California: Molecular genetics of the steroid receptor superfamily.

Chambon, P., CNRS, INSERM, Strasbourg, France: Constitutive and inducible transcriptional enhancers.

O'Malley, B., Tsai, S., Bagchi, M., Wang, L.-H., Bradshaw, S., Tsai, M.-J., Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas: Combinatorial interactions of proteins and DNA at transcriptional regulatory elements of the ovalbumin gene.

Schütz, G., Strähle, U., Münsterberg, A., Becker, P.B., Schmid, W., German Cancer Research Center, Institute of Cell and Tumor Biology, Heidelberg, Federal Republic of Germany: Synergistic action of the glucocorticoid receptor with a CCAAT-box transcription factor.

Goldstein, J.L., Brown, M.S., University of Texas Southwestern Medical Center, Dallas: Sterol-dependent repression of genes mediating cholesterol homeostasis.

DORCAS CUMMINGS LECTURE

E. Kandel, Columbia University: The Long and Short of Long-term Memory.

SESSION 11 NEUROTRANSMITTERS AND RECEPTORS

Chairman: S. Numa, Kyoto University

Numa, S.[1] Fukuda, K.,[1] Jubo, T.,[1] Maeda, A.,[1] Akiba, I.,[1] Bujo, H.,[1] Nakai, J.,[1] Mishina, M.,[1] Higashida, H.,[1] [1]Depts. of Medical Chemistry and Molecular Genetics, Kyoto University Faculty of Medicine, [2]Dept. of Biophysics, Neuroinformation Research Institute, Kanazawa University School of Medicine, Japan: Molecular Basis of the functional heterogeneity of the muscarinic acetylcholine receptor.

Brown, A.M.,[1] Yatani, A.,[1] Kirsch, G.,[1] Van Dongen, T.,[1] Codina, J.,[2] Mattera, R.,[2] Birnbaumer, L.,[2] Depts. of [1]Physiology and Molecular Biophysics, [2]Cell Biology, Baylor College of Medicine, Houston, Texas: Direct G-protein gating of ionic channels.

Brown, D.A.,[1] Higashida, H.,[2] Adams, P.R.,[3] Marrion, N.V.,[4] Smart, T.G.,[4] [1]Dept. of Pharmacology, University College, London, England; [2]Neuroinformation Research Institute, University of Kanazawa School of Medicine, Ishikawa, Japan; [3]Howard Hughes Medical Institute Research Laboratories, Dept. of Neurobiology and Behavior, State University of New York, Stony Brook; [4]Dept. of Pharmacology, School of Pharmacy, London, England: Role of G-protein-coupled PI system in signal transduction in vertebrate neurones—Experiments on neuroblastoma hybrid and ganglion cells.

Julius, D.,[1] MacDermott, A.,[1,2] Jessell, T.,[1,3] Axel, R.,[1,3] [1]Howard Hughes Medical Institute, Depts. of [2]Physiology and Cellular Biophysics, [3]Biochemistry, Columbia University College of Physicians & Surgeons, New York, New York: Molecular characterization of a functional cDNA encoding the serotonin 1c receptor.

Sweatt, D., Volterra, A., Siegelbaum, S., Kandel, E., Howard Hughes Medical Institute and Center for Neurobiology and Behavior, Columbia University, New York, New York: Molecular convergence of presynaptic facilitation and presynaptic inhibition on common substrate proteins of individual sensory neurons in *Aplysia*.

Kaupp, U.B., Universität Osnabruck, Federal Republic of Germany: Molecular and electrical properties of the cGMP-gated channel from photoreceptors.

Bowtell, D., Simon, M., Rubin, G.M., Howard Hughes Medical Institute and Dept. of Biochemistry, University of California, Berkeley: Sevenless, a gene encoding a putative receptor for positional information.

Schwartz, J.H.,[1] Shapiro, E.,[1] Piomelli, D.,[1] Feinmark, S.,[2] Vogel, S.S.,[1] [1]Howard Hughes Medical Institute, [2]Dept. of Pharmacology, Columbia University College of Physicians & Surgeons, New York, New York: Lipoxygenase metabolites of arachidonic acid as second messengers mediating presynaptic inhibition in *Aplysia*.

SESSION 12 PROTEIN PHOSPHORYLATION. II

Chairman: J. Feramisco, Cold Spring Harbor Laboratory

Erikson, R.,[1] Alcorta, D.,[1] Bedard, P.-A.,[1] Blenis, J.,[3] Erikson, E.,[2] Jones, S.,[1] Maller, J.,[2] Martins, T.,[1] Simmons, D.,[1] [1]Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts; [2]Dept. of Pharmacology, University of Colorado Medical School, Denver; [3]Dept. of Molecular Biology, Northwestern University Medical School, Chicago, Illinois: Molecular analyses of gene products involved in the response of cells to mitogenic stimulation.

Kypta, R.M., Ulug, E.T., Goldberg, Y., Courtneidge, S.A., EMBL, Heidelberg, Federal Republic of Germany: Interactions between the middle T antigen of polyomavirus

and host-cell proteins.

Morrison, D.,[1] Kaplan, D.,[1] Piwnica-Worms, H.,[1] Keller, T.,[1] Mamon, H.,[1] Cohen, B.,[2] Rapp, U.,[3] Schaffhausen, B.,[2] Cantley, L.,[2] Roberts, T.,[1] [1]Dana-Farber Cancer Institute, Harvard Medical School, [2]Tufts University Medical School, Boston, Massachusetts; [3]NCI-Frederick Cancer Research Facility, Frederick, Maryland: Tyrosine phosphorylation in signal transduction.

Watterson, D.M.,[1] Haiech, J.,[2] Zimmer, W.,[1] Shattuck, R.,[1] Shoemaker, M.,[1] Lau, W.,[1] Craig, T.,[1] Lukas, T.,[1] [1]Dept. of Pharmacology, Vanderbilt University, Nashville, Tennessee; [2]CNRS, Montpellier, France: Molecular biology of a calmodulin-regulated protein kinase system.

SESSION 13 GROWTH CONTROL

Chairman: **R. Weinberg,** Whitehead Institute, Massachusetts Institute of Technology

Weinberg, R.A.,[1] Friend, S.,[1] Horowitz, J.,[1] Bernards, R.,[1] Dryja, T.,[2] Kimchi, A.,[3] Cheifetz, S.,[4] Massague, J.,[4] [1]Whitehead Institute and Dept. of Biology, Massachusetts Institute of Technology, Cambridge; [2]Massachusetts Eye and Ear Infirmary, Boston; [3]Weizmann Institute, Rehovot, Israel; [4]University of Massachusetts Medical School, Worcester: The retinoblastoma gene as a model anti-oncogene.

McCormick, F.,[1] Adari, H.,[1] Trahey, M.,[1] Wong, G.,[1] Rubinfeld, B.,[1] Willumsen, B.,[2] Lowy, D.R.,[3] [1]Cetus Corporation, Emeryville, California; [2]University of Copenhagen, Denmark; [3]NCI, National Institutes of Health, Bethesda, Maryland: GTPase-activating protein may be the *ras* effector.

Hall, A., Calés, C., Hancock, J.F., Lloyd, A., Marshall, C.J., Morris, J., Price, B., Self, A., Institute of Cancer Research, Chester Beatty Laboratories, London, England: Analysis of mammalian *ras* effector function.

Sigal, I.S., Marshall, M.S., Schaber, M., Allard, W.J., Vogel, U., Scolnick, E.M., Gibbs, J.B., Dept. of Molecular Biology, Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania: Interaction of mammalian *ras* with target and regulatory proteins.

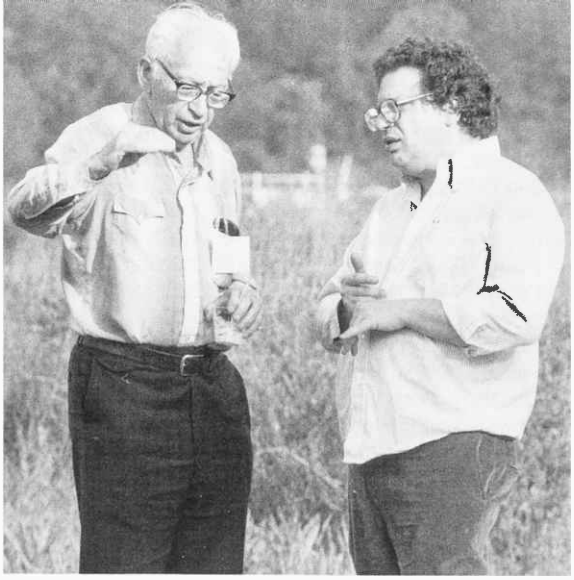
Downward, J., de Gunzberg, J., Weinberg, R.A., Whitehead Institute for Biomedical Research, Cambridge,

SESSION 14 NUCLEAR SIGNALING

Chairman: **R. Tjian,** University of California, Berkeley

Bohmann, D.,[1] Turner, R.,[1] Admon, A.,[1] Bos, T.,[2] Vogt, P.,[2] Tjian, R.,[1] [1]Dept. of Biochemistry, Howard Hughes Medical Institute, University of California, Berkeley; [2]Dept. of Microbiology, University of Southern California School of Medicine, Los Angeles: Transcriptional regulation by an oncoprotein complex involving the AP1/*jun* family of enhancer-binding factors and Fos protein—A potentially important nuclear target for signal transduction.

Vogt, P.K.,[1] Bos, T.J.,[1] Tsuchie, H.,[1] Nishimura, T.,[1] Bohmann, D.,[2] Tjian, R.,[2] [1]Dept. of Microbiology, University of Southern California School of Medicine, Los Angeles; [2]Dept. of Biochemistry, University of California,



E. Racker, M. Wigler

Massachusetts: Investigation of the biochemical function of *ras* proteins.

Stacey, D.W.,[1] Yu, C.-L.,[1] Smith, J.K.,[2] Tsai, M.-H.,[1] [1]Dept. of Molecular Biology, Cleveland Clinic Foundation, Ohio; [2]Roche Institute of Molecular Biology, Nutley, New Jersey: Cellular *ras* activity performs a critical function in proliferative signal transduction.

Nathans, D., Christy, B., Hartzell, S., Nakabeppu, Y., Ryder, K., Dept. of Molecular Biology, Johns Hopkins University School of Medicine, Baltimore, Maryland: Genomic response to growth factors.

Bravo, R., Ryseck, R.P., Chavrier, P., Charnay, J., Almendral, J., Zerial, M., European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: Complexity of the genomic response to growth factors in mouse fibroblasts.

Mayer, B.J., Hamaguchi, M., Hanafusa, H., Rockefeller University, New York, New York: Modulation of tyrosine phosphorylation by an oncogene product related to phospholipase C.

Bristol, A., Hall, S., Sultzman, L., Knopf, J., Genetics Institute, Cambridge, Massachusetts: Expression of mutant PLC phospholipase C.

Berkeley: The oncogene *jun*—A transcriptional regulator becomes oncogenic.

Treisman, R., Norman, C., Runswick, M., Wilson, T., MRC Laboratory of Molecular Biology, Cambridge, England: Proteins involved in serum-regulated c-*fos* gene transcription.

Ziff, E., Gizang-Ginsberg, E., Gorham, J., Greenberg, M.E., Kouzarides, T., Metz, R., Siegfried, Z., Thompson, M.A., Dept. of Biochemistry and Kaplan Cancer Center, New York University Medical Center, New York: Gene regulation by growth factors.

Prywes, R., Fisch, T.M., Roeder, R.G., Laboratory of Biochemistry and Molecular Biology, Rockefeller University,

New York, New York: Transcriptional regulation of c-*fos*.

Verma, I.M., Lamph, W.W., Visvader, J., Raymond, V., Atwater, J., Sisson, J., Sassone-Corsi, P., Salk Institute, San Diego, California: Regulation of *fos* gene—Role as a *trans*-regulator of transcription.

Gilman, M., Berkowitz, L., Graham, G., Riabowol, K., Ryan, W., Jr., Franza, B.R., Jr., Cold Spring Harbor Laboratory, New York: Intracellular mediators of c-*fos* induction.

Curran, T.,[1] Rauscher, F.J. III,[1] Cohen, D.R.,[1] Ferreira, P.C.P.,[1] Franza, B.R., Jr.,[2] [1]Dept. of Molecular Oncology, Roche Institute of Molecular Biology, Nutley, New Jersey; [2]Cold Spring Harbor Laboratory, New York: *fos*—A nuclear messenger in signal transduction.

Franza, B.R., Jr., Cold Spring Harbor Laboratory, New York: Identification and analysis of inducible cellular proteins that interact with genetically defined transcription control elements.

Baeuerle, P., Baltimore, D., Whitehead Institute, Massachusetts Institute of Technology, Cambridge: Activity of the NF-*K*B transcription factor is controled by an inhibitory protein.

K Yamamoto

SESSION 15 SECOND MESSENGER SYSTEMS. II

Chairman: R. Tsien, University of California, Berkeley

Harootunian, A.T., Tsien, R.Y., Dept. of Molecular and Cell Biology, University of California, Berkeley: Sustained calcium oscillations in fibroblasts are produced by a combination of mitogen application and depolarization.

Rozengurt, E., Nanberg, E., Erusalimsky, J., Morris, C., Mehmet, H., Woll, P., Millar, J., Sinnett-Smith, J., Imperial Cancer Research Fund, London, England: Early signals in the mitogenic response.

Hanley, M.,[1] Goedert, M.,[2] Carpenter, D.,[3] Cheung, P.,[1] Dreher, M.,[1] Gatti, A.,[1] Hawkins, P.,[1] Jackson, T.,[1] Patterson, S.,[1] Vallejo, M.,[1] [1]MRC Molecular Neurobiology Unit, [2]Laboratory of Molecular Biology, Medical School, Cambridge, [3]AFRC Institute for Animal Disease Research, Huntingdon, England: Molecular mechanisms and genetic manipulation of phospholipid signaling pathways in mammalian nerve cells.

Saltiel, A.R., Rockefeller University, New York, New York: Function of glycosyl-phosphoinositides in hormone action.

Larner, J.,[1] Huang, L.,[1] Tang, G.,[1] Suzuki, S.,[1] Shen, T.Y.,[2] Oswald, A.S.,[2] Schwartz, C.F.W.,[1] Romero, G.,[1] Roulidis, Z.,[2] Zeller, K.,[1] Leef, J.W.,[1] Depts. of [1]Pharmacology, [2]Chemistry, University of Virginia School of Medicine, Charlottesville: Insulin mediators—Structure and formation.

Low, M.G., Dept. of Physiology and Cellular Biophysics, Columbia University College of Physicians & Surgeons, New York, New York: Functions of the glycosyl-phosphatidylinositol anchor of cell-surface proteins.

Raetz, C.R.H., Dept. of Biochemistry, University of Wisconsin, Madison: Gram-negative endotoxin—A biologically active lipid.

SESSION 16 SECOND MESSENGER SYSTEMS. III

Chairman: M. Berridge, Agricultural and Food Research Council

Berridge, M.J., Taylor, C.W., Dept. of Zoology, Agricultural and Food Research Council, Cambridge, England: Inositol trisphosphate and calcium signaling.

Gomperts, B.D., University College, London, England: Phosphorylation/de-phosphorylation, Ca^{++} and G protein (G_E), in the control of exocytosis.

Garbers, D.L.,[1] Lowe, D.G.,[2] Dangott, L.J.,[1] Chinkers, M.,[1] Thorpe, D.S.,[1] Bentley, K.,[1] Ramarao, C.S.,[1] Goeddel, D.V.,[2] Singh, S.,[1] [1]Vanderbilt University Medical Center, Nashville, Tennessee; [2]Genentech, Inc., South San Francisco, California: Regulation of the membrane form of guanylate cyclase.

Sardet, C., Franchi, A., Pouysségur, J., Centre de Biochimie, CNRS, Nice, France: Molecular cloning of the growth-factor-activatable Na^+/H^+ antiporter.

Summary: H. Bourne, University of California, San Francisco

MEETINGS

Genome Mapping and Sequencing

April 27—May 1

ARRANGED BY

Charles Cantor, Columbia University
Maynard Olson, Washington University
Richard Roberts, Cold Spring Harbor Laboratory

221 participants

The last few years have seen the gradual development of a variety of tools that make the possibility of mapping and sequencing whole genomes a realistic possibility. Although many small workshops and meetings have been held to discuss the possibility of sequencing the human genome, most have been politically oriented. In contrast, this year's meeting at Cold Spring Harbor Laboratory on Genome Mapping and Sequencing was the first to focus entirely on science. A full gamut of techniques in this area were described and their use for preparing maps of small and large genomes was presented. A significant highlight was the announcement of the cloning of a stretch of DNA that hybridizes to all human telomeres. By providing a probe for the ends of human chromosomes, this development should greatly aid physical mapping of the human genome. An informal session within the meeting saw the birth of HUGO, the HUman Genome Organization.

M. Olson, J. Watson, F. Ruddle, V. McKusick

SESSION 1 MAPPING. I: Cutting and Separation of Large DNA Fragments

Chairman: R. Roberts, Cold Spring Harbor Laboratory

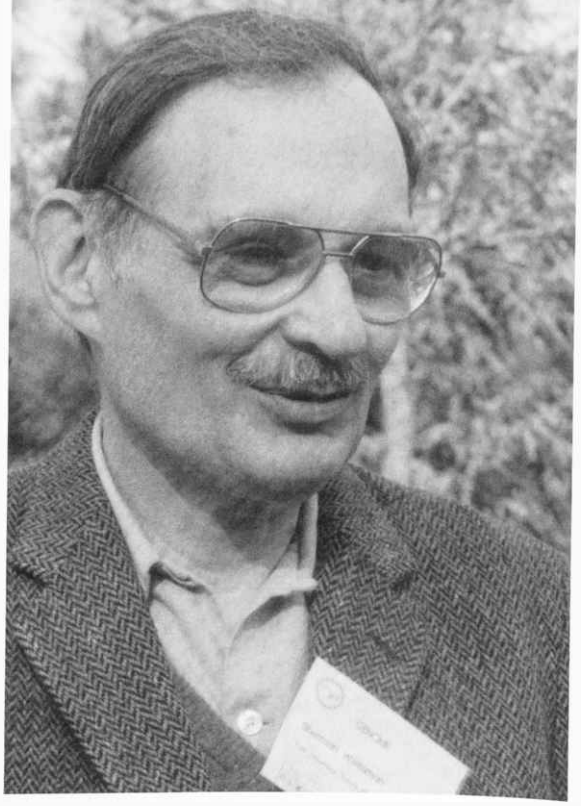
Cantor, C.R., Doggett, N., Abad, P., Mathew, M.K., Fan, J.B., Smith, C.L., Depts. of Microbiology, Psychiatry, and Genetics and Development, Columbia University College of Physicians & Surgeons, New York, New York: Progress in large DNA methods and their application to megabase restriction mapping.

Lai, E.,[1] Woolf, T.,[1] Kronenberg, M.,[2] Hood, L.,[1] [1]Division of Biology, California Institute of Technology, Pasadena; [2]Dept. of Microbiology and Immunology, University of California, Los Angeles: Mapping genomic organization by field inversion and two-dimensional gel electrophoresis—Application to the murine T-cell receptor Y gene family.

Marchuk, D.,[1] Cole, J.,[1] Cantor, C.,[2] Weissman, S.,[3] Collins, F.,[1] [1]University of Michigan, Ann Arbor; [2]Columbia University, New York, New York; [3]Yale University, New Haven, Connecticut: Coincidence cloning—A new method for selective cloning of sequences shared between DNA samples.

Jones, R., Dept. of Mathematics, University of Southern California, Los Angeles: Macrorestriction mapping of prokaryote genomes.

Helene, C., Laboratoire de Biophysique, INSERM, Museum National d'Histoire Naturelle, Paris, France: Sequence-specific recognition and cleavage of double-stranded DNA by oligonucleotides covalently linked to photosensitizers or phenanthroline-copper complexes.



S. Weissman

Sigman, D.S. Dept. of Biological Chemistry, University of California School of Medicine, Los Angeles: Targeting the nuclease activity of 1,10-phenanthroline-copper.

SESSION 2 MAPPING. II: Linked Libraries and Large Clones

Chairman: Maynard Olson, Washington University School of Medicine

Burke, D.T., Carle, G.F., Olson, M.V., Washington University School of Medicine, St. Louis, Missouri: Construction of libraries from eukaryotic genomes as yeast artificial chromosomes.

Little, R.D.,[1] Carle, G.,[2] Olson, M.V.,[2] Schlessinger, D.,[1] Depts. of [1]Microbiology, [2]Genetics, Washington University School of Medicine, St. Louis, Missouri: Screening of YAC libraries containing human DNA inserts.

Hieter, P., Shero, J. McCormick, M., Connelly, C., Vollrath, D., Dept. of Molecular Biology and Genetics, Johns Hopkins School of Medicine, Baltimore, Maryland: Physical mapping of large DNA by chromosome fragmentation in *S. cerevisiae*.

Sternberg, N., Sauer, B., Central Research and Development Dept., E.I. du Pont de Nemours and Company, Inc., Wilmington, Delaware: A new cloning system that permits the efficient cloning, isolation, amplification, and recovery of big DNA inserts.

Lehrach, H.,[1] Zehetner, G.,[1] Nicetic, D.,[1] Craig, A.,[1] Michiels, F.,[2] [1]Imperial Cancer Research Fund, London, England; [2]Plant Genetic Systems, Gent, Belgium: Oligonucleotide fingerprinting, a parallel approach to establish ordered clone libraries.

Livak, K.J.,[1] Korolkoff, P.N.,[1] Brenner, S.,[2] [1]Central Research and Development Dept., E.I. du Pont de Nemours and Co., Wilmington, Delaware; [2]Molecular Genetics Unit, Medical Research Council, Cambridge, England: Use of fluorescent DNA terminators to map overlapping DNA fragments.

SESSION 3 POSTER SESSION

Avdalovic, N., Burns, J., Beckman Instruments, Palo Alto, California: Automation of Sanger's protocols for high thruput DNA sequencing.

Birren, B.W., Lai, E., Clark, S.M., Hood, L., Simon, M.I., Division of Biology, California Institute of Technology, Pasadena: A new gel electrophoresis apparatus with versatile applications for DNA separation.

Bowcock, A.,[1] Farrer, L.,[2] Hebert, J.,[1] Sternlieb, I.,[3] Scheinberg, I.,[3] Frydman, M.,[4] Bonne-Tamir, B.,[4] Cavalli-Sforza, L.,[1] [1]Stanford University School of Medicine, California; [2]Boston University Medical Center, Massachusetts; [3]Albert Einstein College of Medicine,

Bronx, New York; [4]Tel-Aviv University, Ramat-Aviv, Israel: DNA markers closely linked to Wilson's disease.

Braaten, D.C., Little, R.D., Schlessinger, D., Dept. of Microbiology, Washington University School of Medicine, St. Louis, Missouri: Human 5S rDNA repeat—Sequence and genomic clusters.

Branscomb, E.W., Slezak, T.R., Pae, R., Carrano, A.V., Biomedical Sciences Division, Lawrence Livermore National Laboratory, California: Optimal overlap detection in genomic ordering.

Canard, B., Cole, S.T., Institut Pasteur, Paris, France: Long-range restriction mapping of the *C. perfringens* genome.

Carle, G.F., Little, R., Olson, M.V., Washington University School of Medicine, St. Louis, Missouri: Analysis of a YAC clone possessing homology with a member of the class I HLA gene family.

Carle, G.F., Olson, M.V., Washington University School of Medicine, St. Louis, Missouri: Physical characterization of a 3-Mb yeast chromosome (XII) containing the rDNA cluster from *S. cerevisiae*.

Chai, J., Institute of Genetics, Fudan University, Shanghai, People's Republic of China: Creating a linear physical map of human chromosome X.

Chandrasekharappa, S.C.[1] Westbrook, C.,[1] McClelland, M.,[2] Depts. of [1]Medicine, [2]Biochemistry and Molecular Biology, University of Chicago, Illinois: Production of partial *Not I* enzyme digests by methylation competition.

Chimini, G.,[1] Marguet, D.,[2] Djabali, M.,[1] Jordon, B.,[1] Lauquin, G.,[2] [1]Centre d'Immunologie INSERM-CNRS, [2]Physiologie Cellulaire, Faculte des Sciences, Marseille, France: Human genomic library in yeast artificial chromosomes.

de Jong, P.J., Pederson, L., Summers, L., Biomedical Sciences Division, Lawrence Livermore National Laboratory, California: Long-range restriction mapping of human chromosome 19 using *Not I*-linking probes.

Dulbecco, R., Salk Institute, La Jolla, California: A mapping and sequencing project of a consortium of Italian universities.

Evans, G.A., Lewis, K., Rothenberg, B.E., Evans, K.C., Salk Institute, La Jolla, California: Genomic mapping by cosmid multiplex analysis.

Fields, C.A., Coombs, M.J., Hartley, R.T., Knowledge Systems Group, CRL, NMSU, Las Cruces, New Mexico: Automated construction of functional models of multi-kilobase regions of genomic DNA sequence.

Ganguly, S., Chaganti, R.S.K., Memorial Sloan-Kettering Cancer Center, New York, New York: Nucleotide sequence of an unusual t(14;18) translocation junction in follicular lymphoma.

Grant, S.G., Mullins, L.J. Stephenson, D.A., Chapman, V.M. Dept. of Molecular and Cellular Biology, Roswell Memorial Institute, Buffalo, New York: Cumulative molecular mapping of the mouse X chromosome—Implications for the human genome.

Hochgeschwender, U., Sutcliffe, J.G., Brennan, M.B., Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California: An essentially complete library of mouse chromosome 16—A systematic method for production and linking of chromosome-specific libraries.

Hozier, J.,[1·2] Hall, B., [2] Marzluff, W.,[1] [1]Florida State University, Tallahassee; [2]Applied Genetics Labs, Inc., Melbourne, Florida: Mapping of histone genes in man and mouse—A model for comparative mapping of complex gene families.

Lander, E.S.,[1·2] Botstein, D., [3·4] [1]Whitehead Institute for Biomedical Research, [2]Harvard University, Cambridge, Massachusetts, [3]Massachusetts Institute of Technology, Cambridge, Massachusetts; [4]Genentech, South San Francisco, California: Methods for mapping the genes involved in polygenic traits in mammalian genomes by using a complete RFLP linkage map.

Lench, N.J.,[1] Estivill, X.,[2] Scambler, P.J.,[1] Williamson, R.,[1] [1]Cystic Fibrosis Genetics Research Group, St. Mary's Hospital Medical School, London, England; [2]Hospital de la Santa Creu I Sant Pau, Barcelona, Spain: Identification of rare cutter restriction sites using short synthetic oligonucleotide probes.

Litt, M.[1] Kondoleon, S.,[1] Luty, J.,[1] Carrero-Valenzuela, R.,[1] Buder, A.,[1] Ramsay, A.,[1] Vissing, H.,[2] vanTuinen, P.,[3] Ledbetter, D.H.,[3] [1]Oregon Health Sciences University, Portland; [2]State University of New York Downstate Medical Center, Brooklyn; [3]Baylor College of Medicine, Houston, Texas: Three cosmids from a chromosome-17 library identify compound polymorphic loci on the long arm of this chromosome.

Martin, R.K., Barker, P.E., Laboratory of Medical Genetics and UAB Cystic Fibrosis Research Center, University of Alabama, Birmingham: Deletion mapping with a series of human chromosome-7 DNA markers.

McKusick, V.A., Dept. of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland: Morbid anatomy of the human genome—Current status of the human gene map.

Mead, D., Promega Corporation, Madison, Wisconsin: A unique λ vector engineered for high-resolution restriction mapping of genomic inserts.

Nadeau, J.,[1] Carlson, G.,[1] Figueroa, F.,[2] Henson, V.,[1] Kasahara, M.,[3] Klein, J.,[2·3] [1]Jackson Laboratory, Bar Harbor, Maine; [2]Max Planck Institut fur Biologie Abteilung Immungenetik, Tubingen, Federal Republic of Germany; [3]Dept. of Microbiology and Immunology, University of Miami School of Medicine, Florida: Meiotic and mitotic linkage maps of mouse chromosome 17.

Ott, J., Sandkuyl, L.A., Depts. of Genetics and Development and Psychiatry, Columbia University, New York, New York: Computer simulation methods for mapping human disease loci by genetic linkage analysis.

Pearson, P.L.,[1] Kidd, K.K.,[2] Willard, H.F.,[3] [1]Dept. of Human Genetics, University of Leiden, The Netherlands; [2]Dept. of Human Genetics, Yale University, New Haven, Connecticut; [3]Dept. of Medical Genetics, University of Toronto, Ontario, Canada: ARPmap—A proposed hierarchical information system for physical mapping of the human genome.

Poustka, A.,[1] Lehrach, H.,[2] [1]Max Planck Institut fur medizinische Forschung, Heidelberg, Federal Republic of Germany; [2]Imperial Cancer Research Fund Laboratories, London, England: Rare cutter chromosome jumping—A long-range cloning technique.

Ragsdale, C., Chu, D., Freeby, S., Zoller, P., Garfin, D., Bio-Rad Laboratories, Richmond, California: Genome

mapping and sequencing conference.

Richterich, P. Pohl, F.M., Faculty of Biology, University of Constance, Federal Republic of Germany: Direct blotting electrophoresis—Recent improvements for colorimetric DNA sequencing.

Sikela, J.M.,[1] Law, M.L.,[2] Kao, F.T.,[2] Hartz, J.H.,[2] Wei, Q.,[2] Hahn, W.E.,[1] [1]University of Colorado School of Medicine, Boulder; [1]Eleanor Roosevelt Institute for Cancer Research, Denver, Colorado: The gene encoding CaM kinase IV, a new brain Ca++/calmodulin-dependent protein kinase, maps to human chromosome 5q21-q23.

Siniscalco, M., Memorial Sloan-Kettering Cancer Center, New York, New York: A population approach to human genome sequencing.

Sirotkin, K., Goad, W., Los Alamos National Laboratory, New Mexico: Computer simulation of genomic mapping.

Smith, G.P., Division of Biological Sciences, Tucker Hall, University of Missouri, Columbia: The Lehrach probe hybridization map—Algebra of consistency and statistics of inconsistencies.

Stewart, G.D., Kurachi, S., Kang, H., Baldori, N., Kahl, T., Kurnit, D.M., Howard Hughes Medical Institute, University of Michigan, Ann Arbor: Rapid identification and isolation of transcribed sequences.

Stormo, G.D., Dept. of Biology, University of Colorado, Boulder: Determining the sequence specificity of DNA-binding proteins.

Testa, J.R.,[1] Park, M.,[1] Blair, D.,[2] Vande Woude, G.,[1] [1]BRI-Basic Research Program, [2]Laboratory of Molecular Oncology, Frederick, Maryland: FIGE analysis of two rearranged *met* proto-oncogene alleles in a chemically treated human osteosarcoma cell line, MNNG-HOS, and use of a derivative chromosome 7 to map DNA markers linked to the cystic fibrosis locus.

Toneguzzo, F.,[1] Danby, P.,[1] McKenny, K.,[2] [1]EG&G Biomeolecular, Natick, Massachusetts; [2]National Bureau of Standards, Gaithersburg, Maryland: An automated system for detection of radiolabeled nucleic acids—DNA sequencing and other applications.

Waye, J.S., Greig, G.M., Willard, H.F., Dept. of Medical Genetics, University of Toronto, Canada: Molecular organization of the human β satellite DNA family.

Weber, J.L., May, P.S., Marshfield Medical Research Foundation, Wisconsin: A new type of polymorphic human DNA marker.

Wenzel, R., Herrmann, R., Dept. of Microbiology, University of Heidelberg, Federal Republic of Germany: Construction of a physical map of the *Mycoplasma pneumoniae* genome.

Westbrook, C., Le Beau, M.M., Chandrasekharappa, S.C., Firak, T., Dept. of Medicine, University of Chicago, Illinois: Physical mapping of the long arm of human chromosome 5.

Zhao, Y.,[2] Chai, J.,[1] Wang, X.,[1] Weng, X., Xou, Q.,[2] Shen, G.,[2] Tang, S.,[2]; [1]Institute of Genetics, Fudan University, Shanghai, [2]Dept. of Biology, Hangzhou University, People's Republic of China: Construction of cosmid library and detailed physical map of rice chloroplast genome.

SESSION 4 COMPLETE MAPS AND MODEL SYSTEMS

Chairman: **J. Sulston,** MRC Laboratory, Cambridge

Kohara, Y.,[1] Akiyama, K.,[1] Isono, K.,[2] [1]Dept. of Molecular Biology, Nagoya University, Japan; [2]Dept. of Biology, Kobe University, Japan: Physical map of the whole *E. coli* chromosome.

Coulson, A.,[1] Sulston, J.,[1] Waterston, R.,[2] [1]MRC Laboratory of Molecular Biology, Cambridge, England; [2]Washington University Medical School, St. Louis, Missouri: The genome of *Caenorhabditis*.

Link, A., Dutchik, J.E., Riles, L., Olson, M.V., Dept. of Geneticks, Washington University School of Medicine, St. Louis, Missouri: Physical mapping of the yeast genome.

Hauge, B.M., Yett, D., Fritze, C., Nam, H.-G., den Boer, B., Goodman, H.M., Dept. of Genetics, Harvard Medical School, and Dept. of Molecular Biology, Massachusetts General Hospital, Boston: Progress toward a physical map of the *Arabidopsis thaliana* genome.

Smith, C.L., Condemine, G., Ringquist, S., Yu, M.T., Abad, P., Fan, J.B., Cantor, C.R., Depts. of Microbiology, Psychiatry, and Genetics and Development, Columbia University College of Physicians & Surgeons, New York, New York: Molecular biological studies of whole chromosomes.

Chikashige, Y., Matsumoto, T., Niwa, O., Yanagida, M., Dept. of Biophysics, Faculty of Science, Kyoto University, Japan: Creation of new *Not I restriction sites on S. pombe* chromosome for genome mapping and artificial chromosome.

Weil, M.D., Patel, Y., Nelson, M., McClelland, M., Dept. of Biochemistry and Molecular Biology, University of Chicago, Illinois: Cleavage methods for megabase mapping.

SESSION 5 MAMMALIAN MAPS OF WHOLE CHROMOSOMES

Chairman: E. Lander, Massachusetts Institute of Technology

Shimizu, N., Minoshima, S., Kudoh, J., Kawasaki, K., Fukuyama, R., Maekawa, M., Dept. of Molecular Biology, Keio University School of Medicine, Tokyo, Japan: Sorting of single homologs of human chromosomes 21 and 22 to use for physical mapping.

Gardiner, K.,[1] Watkins, P.,[2] Patterson, D.,[1] [1]Eleanor Roosevelt Institute for Cancer Research, Denver, Colorado; [2]Integrated Genetics, Framingham, Massachusetts: Mapping human chromosome 21.

Carrano, A.V., Branscomb, E.W., de Jong, P.J., Watkins, B.E., Mohrenweiser, H.W., Slezak, T., Biomedical Sciences Division, Lawrence Livermore National Laboratory, California: Creating an ordered cosmid set for chromosome 19.

Hildebrand, C.E.,[1] Stallings, R.L.,[1] Deaven, L.L.,[1] Longmire, J.L.,[1] Cram, L.S.,[1] Meyne, J.,[1] Moyzis, R.K.,[1] Callen, D.,[2] [1]Los Alamos National Laboratory, New Mexico; [2]Adelaide Childrens Hospital, Australia: Physical maps of human chromosome 16—Status and perspectives.

Gusella, J.F.,[1] Haines, J.,[1] Tanzi, R.E.,[1] Rouleau, G.,[1] Watkins, P.,[2] Sacchi, N.,[3] Wertelecki, W.,[4] Wexler, N.,[5] Conneally, P.M.,[6] [1]Massachusetts General Hospital and Harvard Medical School, Boston; [2]Integrated Genetics, Inc. Framingham, Massachusetts; [3]NCI, Frederick, Maryland; [4]University of South Alabama, Mobile; [5]Columbia University, New York, New York; [6]Indiana University School of Medicine, Indianapolis: Genetic linkage maps of chromosomes 21 and 22.

Green, P., Helms, C., Cartinhour, S., Weiffenbach, B., Stephens, K., Keith, T., Bowden, D., Smith, D., Donis-Keller, H., Dept. of Human Genetics, Collaborative Research, Inc., Bedford, Massachusetts: A genetic linkage map of the human genome.

Casey, G.,[1] Thompson, T.G.,[1] Gross, M.,[2] Dowdy, S.F.,[2] Stanbridge, E.J.,[2] [1]Pharmaceutical Division, Imperial Chemical Industries plc, England; [2]Dept. of Microbiology and Molecular Genetics, University of California, Irvine: Development and application of a monochromosome hybrid panel in which every human chromosome is represented.

SESSION 6 MAMMALIAN MAPS—LARGE REGIONS

Chairman: C. Cantor, Columbia University

Barlow, D.P.,[1] Lehrach, H.,[2] [1]Institute of Molecular Pathology, Vienna, Austria; [2]ICRF, London, England: Pulsed-field gel mapping of the mouse t-complex.

Cohen, D.,[1] Albertsen, H.,[1] Abderrahim, H.,[1] Bougueleret, L.,[1] Carroll, M.,[2] Claverie, J.M.,[1] Dembic, Z.,[3] Legall, I.,[1] Le Paslier, D.,[1] Marcadet, A.,[1] Millasseau, P.,[1] Prieur, S.,[1] Rodriguez-Tome, P.,[1] Steinmetz, M.,[3] Strominger, J.L.,[2] Uematsu, Y.,[3] Dausset, J.,[1] [1]CEPH, Annexe du College de France, Paris; [2]Harvard University, Boston; [3]Basel Institute for Immunology, Switzerland: Primary structure of the human MHC.

Cox, D.R.,[1] Price, E.R.,[1] Burmeister, M.,[2] Murray, C.,[1] Myers, R.M.,[2] Depts. of [1]Pediatrics, [2]Physiology, University of California, San Francisco: A genetic map of human chromosome 21 constructed by analysis of marker segregation in hamster-human somatic-cell hybrids.

Gemmill, R.M.,[1] Smith, D.I.,[2] Drabkin, H.A.,[3] [1]Southwest Biomedical Research Institute, Scottsdale, Arizona; [2]Wayne State University, Detroit, Michigan; [3]University of Colorado Health Sciences Center, Denver, Colorado: Physical mapping within human chromosomal region 3p14 to p21.

Dracopoli, N.C., Rose, E., Stanger, B.Z., Ito, C.Y., Glaser, T., Whitfield, G.K., Guidon, P.T., Kourides, I.A., Housman, D.E., Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: A genetic linkage map of 27 loci on chromosome 1p, including a detailed analysis of a thyroid-hormone-regulated gene cluster in 1p22.

Sawada, I.,[1] Chorney, M.,[1] Gillespie, G.,[1] Kandpal, R.,[1] Swaroop, A.,[1] Shukla, H.,[1] Weissman, S.M.,[1] Tam, A.,[2] [1]Dept. of Human Genetics, Yale University School of Medicine, New Haven, Connecticut; [2]Gene Labs, Redwood City, California: Approaches to the large-scale gene mapping and applications to human MHC.

SESSION 7 MAMMALIAN MAPS—SMALL REGIONS

Chairman: R. Dulbecco, Salk Institute

Brown, W.R.A., Dept. of Biochemistry, Oxford University, England: Molecular map of the human pseudoautosomal region.

Petit, C., Levilliers, J., Weissenbach, J., INSERM, CNRS, Institut Pasteur, Paris, France: Mapping the human pseudoautosomal region by pulsed-field gel electrophoresis.

Rappold, G.A., Lehrach, H., ICRF, London, England: Long-range restriction mapping by partial digestion from a chromosome telomere.

Caskey, C.,[1] Nelson, D.,[1] Corbo, L.,[1] Webster, T.,[1] Edwards, A.,[1] Ansorge, W.,[2] [1]Institute for Molecular Genetics and Howard Hughes Medical Institute, Houston, Texas; [2]European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: Mapping, cloning, and sequencing in human Xq.

Nguyen, C.,[1] Chimini, G.,[2] Pontarotti, P.,[2] Djabali, M.,[2] Jordan, B.R.,[2] [1]INSERM, La Timone, [2]CIML INSERM-CNRS, Luminy, Marseille, France: Large-scale mapping in the HLA and fragile-X regions.

Arveiler, B., Chaboute, M.E., Oberle, I., Vincent, A., Mandel, J.L., LGME/CNRS, INSERM, Institut de Chimie Biologique, Strasbourg, France: Genetic and physical mapping of the Xq27-q28 region.

Warren, S.T., Zhang, F., Peters, J.F., Consalez, G., Depts. of Biochemistry and Pediatrics, Emory University School of Medicine, Atlanta, Georgia: Isolation of Xq28 within a somatic-cell hybrid—Use as a prototype for human genomic mapping and sequencing.

Davis, L.M., Nowak, N.J., Shows, T.B., Dept. of Human Genetics, Roswell Park Memorial Institute, New York State Dept. of Health, Buffalo: Isolation and regional assignment of chromosome 11 anonymous DNA segments—Two map near the aniridia gene in the WAGR locus.

Porteous, D.J., Bickmore, W., Maule, J., van Heyningen, V., Hastie, N.D., MRC Clinical and Population Cytogenetics Unit, Edinburgh, Scotland: Mapping band 11p13 deletions and translocations associated with the WAGR syndrome—Use of HRAS1-selection in cell fusion and chromosome-mediated gene transfer for enrichment cloning.

Bucan, M.,[1] Zimmer, M.,[1] Youngman, S.,[2] MacDonald, M.,[3] Smith, B.,[4] Pohl, T.,[5] Poustka, A.,[6] Bates, G.,[1] Volinia, S.,[1] Searle, S.,[1] Ormonroyd, E.,[1] Shaw, D.,[2] Harper, P.,[2] Wasmuth, J.,[4] Gusella, J.,[3] Lehrach, H.,[1] Frischauf, A.-M.,[1] [1]ICRF, London, England; [2]University of Wales, Cardiff; [3]Massachusetts General Hospital, Boston; [4]University of California, Irvine; [5]EMBL, Heidelberg, Federal Republic of Germany; [6]Max-Planck Institute fur Medizinishe Forschung, Heidelberg, Federal Republic of Germany: Linking and jumping clones in the construction of a long-range restriction map of 4p.

Myers, R.M.,[1] Pritchard, C.,[1] Casher, D.,[1] Vulpe, C.,[1] Uglum, E.,[2] Shampay, J.,[2] Kobori, J.,[2] Sheffield, V.,[2] Cox, D.R.,[2] Depts. of [1]Physiology, [2]Pediatrics, University of California, San Francisco: Isolation and physical mapping of DNA markers in the vicinity of the Huntington disease locus.

SESSION 8 GENOME PATTERNS AND FUNCTIONAL UNITS (Chromosome Structure)

Chairman: V. McKusick, Johns Hopkins University Hospital

Nadeau, J., Jackson Laboratory, Bar Harbor, Maine: Progress toward saturated maps of linkage and synteny homologies for mouse and man.

Willard, H.F., Dept. of Medical Genetics, University of Toronto, Ontario, Canada: Mapping and sequencing of long tandem arrays of satellite DNA in the human genome.

Allshire, R.C.,[1] Fantes, P.A.,[2] Hastie, N.D.,[1] [1]MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Edinburgh, [2]Dept. of Zoology, University of Edinburgh, Scotland: From yeast chromosomes to human telomeres.

Bernardi, G., Institut Jacques Monod, Paris, France: The organization of the human genome.

Hamkalo, B.A., Narayanswami, S., Lundgren, K., Dvorkin, N., Dept. of Molecular Biology and Biochemistry, University of California, Irvine: High-resolution sequence mapping by electron microscopy in situ hybridization.

van Ommen, G.J.B., den Dunnen, J.T., Wapenaar, M.C., Blonden, L., Ginjaar, H.B., van Paassen, M., Grootscholten, P., Bakker, E., Pearson, P.L., Dept. of Human Genetics, University of Leiden, The Netherlands: FIGE and CHEF study of a deletion hot spot in the *DMD* gene.

SESSION 9 SEQUENCING

Chairman: **L. Hood,** California Institute of Technology

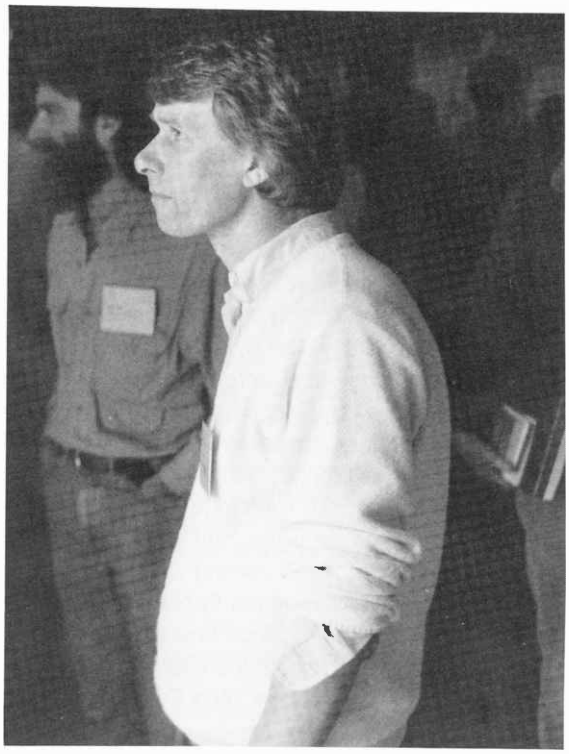
Church, G.M., Gryan, G., Juang, G.J., Kieffer-Higgins, S., Mintz, L., Rubenfield, M.J., Temple, M., Wang, M.X., Dept. of Genetics, Harvard Medical School, Boston, Massachusetts: Multiplex DNA sequencing.

Schwager, C.,[1] Sproat, B.,[1] Stegemann, J.,[1] Voss, H.,[1] Kristensen, T.,[2] Ansorge, W.,[1] [1]European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany; [2]Research Institute for Internal Medicine, University of Oslo, Norway: Development of DNA sequencing techniques at the European Molecular Biology Laboratory.

Weiss, R., Wills, N., Velick, S., Dunn, D., Gesteland, R., Howard Hughes Medical Institute, Dept. of Human Genetics, University of Utah Medical Center, Salt Lake City: Multiplexed dideoxy DNA sequencing.

Trainor, G.L.,[1] Prober, J.M.,[2] Dam, R.J.,[2] Jensen, M.A.,[3] Depts. of [1]Central Research and Development, [2]Engineering, [3]Medical Products, E.I. du Pont de Nemours and Co., Inc., Experimental Station, Wilmington, Delaware: An automated system for fluorescence-based nucleic acid analysis.

Soeda, E., Imai, T., Furuno, N., Institute of Physical and Chemical Research, Tsukuba Science City, Japan: Feasibility study of the automated sequencing machines using a cosmid clone containing human genomic DNA.



R. Roberts

Cell and Molecular Biology of *Chlamydomonas*

May 4—May 8

ARRANGED BY

Robert Bloodgood, University of Virginia
Ursula Goodenough, Washington University
Joel L. Rosenbaum, Yale University

126 participants

Although yeast is in many respects a superb model organism for the analysis of eukaryotic cells, it is not useful for the study of such important eukaryotic activities as photosynthesis, phototaxis, ciliary motility and assembly, centriole and basal-body function, and membrane-mediated cell-cell interactions. All of these functions are under intensive study using the unicellular green alga *Chlamydomonas*, which, like yeast, is readily manipulated in the laboratory and has well-characterized genetics. The Third International *Chlamydomonas* meeting took place at Cold Spring Harbor Laboratory in May, 1988, and was attended by 125 researchers from the United States and many foreign countries.

Students of *Chlamydomonas* have been frustrated by the lack of a reliable

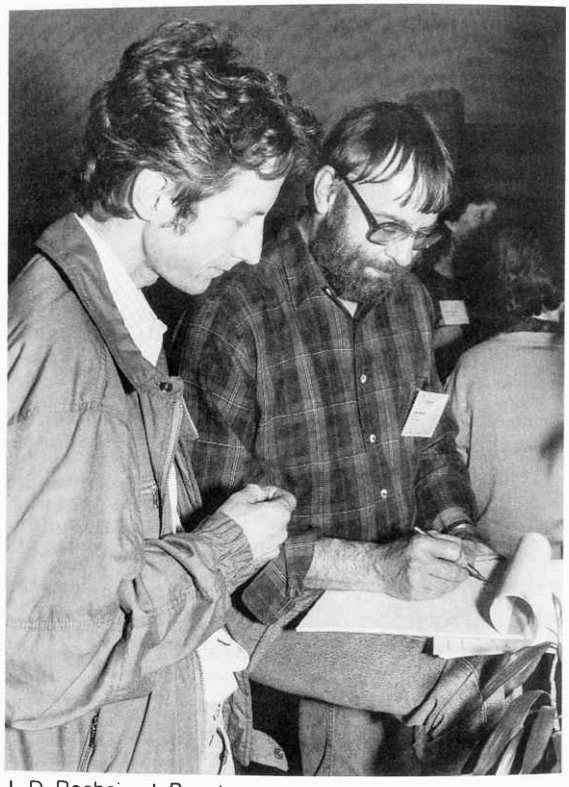
transformation system to analyze its many identified genes. Since standard transformation vectors fail to be expressed in *Chlamydomonas*, many laboratories have been working to develop systems using homologous selectable genes. Reports of successful chloroplast DNA transformation (Boynton, Gillham, Harris, Sanford, and colleagues, Duke and Cornell) and nuclear gene transformation (Mayfield, Scripps Institute) using this approach were most encouraging, and several other laboratories either have cloned selectable genes or are close to that goal. There were also two reports of endogenous transposable elements in *Chlamydomonas*, one a retrotransposon (Rochaix, Geneva) and one an *Ac*-like element (Ferris, Washington University); such elements may prove useful in developing a general vector system.

The remaining sessions were devoted to numerous research reports on the topics listed above, and significant progress was repeatedly documented: For example, several flagellar genes have been cloned, RFLP maps have been constructed, the chloroplast genome has been exhaustively mapped and physically characterized, roles for cyclic AMP, rhodopsin, calcium, and other interesting effectors are being found. There was considerable optimism the *Chlamydomonas* is indeed coming into its own.

This meeting was supported in part by funds by Amoco Technology Company, the National Science Foundation, the Department of Energy, the Foundation for Microbiology, the International Society for Plant Molecular Biology, Monsanto Company, Nippon Zeon Company, Ltd., and Zoecon Research Institute.

SESSION 1 KEYNOTE ADDRESS -
J.-D. Rochaix, University of Geneva

Rochaix, J.-D., Kuchka, M., Choquet, Y., Goldschmidt-Clermont, M., Day, A., Mayfield, S., Erickson, J., Girard-Bascou, J., Bennoun, P., Depts. of Molecular and Plant Biology, University of Geneva, Switzerland and Institut de Biologiei physico-chimique, Paris, France: Photosynthetic mutants of *C. reinhardtii*—Tools for studying the interactions between the chloroplast and nucleocytoplasmic genetic systems.



J.-D. Rochaix, J. Boynton

SESSION 2 FLAGELLAR APPARATUS AND CYTOSKELETON

Chairman: **G. Witman,** Worcester Foundation for Experimental Biology

Kamiya, R., Kurimoto, E., Sakakibara, H., Dept. of Molecular Biology, Nagoya University, Japan: Mutants deficient in inner and outer dynein arms.

Piperno, G., Rockefeller University, New York, New York: Molecular complexity and regulatory function of axonemal dyneins located in the inner row of arms.

King, S.M., Witman, G.B., Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Molecular structure of outer-arm dynein from *Chlamydomonas* flagella.

Mitchell, D., Dept. of Anatomy and Cell Biology, State University of New York Health Science Center, Syracuse: Characterization of the outer-arm dynein α and β heavy-chain genes.

Wilkerson, C., Piperno, G., Luck, D., Rockefeller University, New York, New York: Isolation and characterization of an outer-arm dynein heavy-chain subunit.

Williams, B.D., Velleca, M.A., Curry, A.M., Rosenbaum, J.L., Yale University, New Haven, Connecticut: Cloning and sequence analysis of the *Chlamydomonas pf-14* gene reveals a nonsense mutation.

Salisbury, J.L., Laboratory for Cell Biology, Dept. of Developmental Genetics and Anatomy, Case Western Reserve University, Cleveland, Ohio: *Chlamydomonas* centrin—A calcium-sensitive cytoskeletal system that links the flagellar apparatus to the nucleus.

Huang, B., Mengersen, S., Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California: Further characterization of a *Chlamydomonas* basal-body-associated 20/kD CA++-binding protein.

SESSION 3 FLAGELLAR DEVELOPMENT

Chairman: P. Lefebvre, University of Minnesota

James, S.W., Lefebvre, P.A., Silflow, C.D., Dept. of Genetics and Cell Biology, University of Minnesota, St. Paul: *C. reinhardtii* mutants resistant to antimicrotubule drugs.

Peet, R.C.,[1] Beck, C.,[2] West, J.,[1] Opp, L.,[1] Kuker, K.,[1] Weeks, D.P.,[1] [1]Zoecon Research Institute, Sandoz Crop Protection Corp., Palo Alto, California; [2]Albert-Ludwigs Universitat, Freiberg, Federal Republic of Germany: Characterization of oryzalin-resistant mutants of *C. reinhardtii*.

Jarvik, J., Wright, R., Allen, J., Bruckner, M., Dept. of Biological Sciences, Carnegie Mellon University, Pittsburgh, Pennsylvania: Analysis of the basal body cycle in *C. reinhardtii*.

Lee, V.D., Schibler, M.J., Huang, B., Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California: Colchicine-resistant β-tubulin mutants in *C. reinhardtii*.

Dutcher, S.K., Dehmer, K., Gibbons, W., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Isolation of dominant selectable mutations in *Chlamydomonas*.

Diener, D.R.,[1] Baker, E.J.,[2] Rosenbaum, J.L.,[1] [1]Dept. of Biology, Yale University, New Haven, Connecticut: Differential translation of α1- and α2-tubulin mRNAs in *Chlamydomonas*.

Baker, E.,[1] Rosenbaum, J.,[2] [1]Dept. of Biology, University of Nevada, Reno; [2]Dept. of Biology, Yale University, New Haven, Connecticut: Role of poly(A) in tubulin mRNA stability.

Lefebvre, P., Barsel, S., Larkin, J., Silflow, C., Wexler, D., Dept. of Genetics and Cell Biology, University of Minnesota, St. Paul: Flagellar regeneration mutants—Defects in flagellar protein synthesis and length control.

SESSION 4 TRANSFORMATION

Chairman: D. Weeks, Zoecon Research Institute

Transformation and Transformation Techniques

Boynton, J.E.,[1] Gillham, N.W.,[1] Harris, E.H.,[1] Hosler, J.P.,[1] Johnson, A.M.,[1] Jones, A.R.,[1] Randolph-Anderson, B.L.,[1] Robertson, D.,[1] Klein, T.M.,[2] Shark, K.B.,[2] Sanford, J.C.,[2] [1]Depts. of Botany and Zoology, Duke University, Durham, North Carolina; [2]New York Agricultural Experiment Station, Cornell University, Geneva: Chloroplast transformation in *Chlamydomonas*.

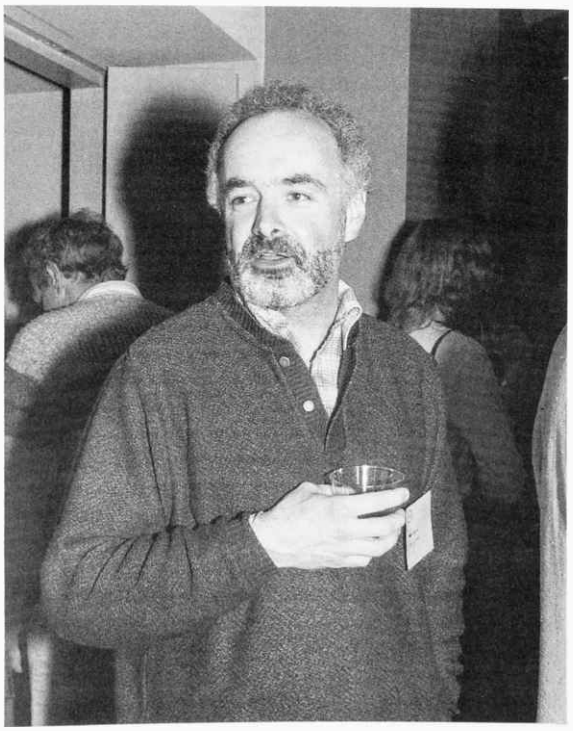
Mayfield, S., Research Institute of Scripps Clinic, La Jolla, California: Approaches to nuclear genome transformation.

Ferris, P., Dept. of Biology, Washington University, St. Louis, Missouri: Characterization of a *Chlamydomonas* transposon.

Leung, W.-C., Depts. of Medicine, Medical Microbiology, and Infectious Diseases, University of Alberta, Edmonton, Canada: Expression of HSV thymidine kinase gene in recombinant *C. reinhardtii*.

Homologous Selectable Marker Genes

Fernandez, E., Ranum, L.P.W., Silflow, C.D., Lefebvre, P.A., Dept. of Genetics and Cell Biology, Bioscience Center, University of Minnesota, St. Paul: Cloning of the



P. Lefebvre

structural gene for nitrate reductase in *C. reinhardtii*.

Beaumont, M.,[1] Hodson, R.,[2] [1]Dept. of Food Service, [2]School of Life and Health Sciences, University of Delaware, Newark: Isolation of acetamidase from *C. reinhardtii*.

de Hostos, E.L.,[1,2] Grossman, A.,[1] [1]Dept. of Biological Sciences, Stanford University, California; [2]Dept. of Plant Biology, Carnegie Institution, Stanford, California: Structure and expression of the *C. reinhardtii* arylsulfatase gene.

Dutcher, S.K., Dehmer, K., Gibbons, W., Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Isolation of dominant selectable mutations in *Chlamydomonas*.

Thiry-Blaise, L.M., Dept. of Biology, University of Liege, Belgium: Selection of *Chlamydomonas* DNA sequence with promoter activity.

SESSION 5 CELL AND MOLECULAR BIOLOGY OF MITOCHONDRIA AND CHLOROPLASTS

Chairman: J. Boynton, Duke University

Lee, R.W.,[1] Lemieux, C.,[2] Turmel, M.,[2] [1]Dept. of Biology, Dalhousie University, Halifax, [2]Dépt. de biochinie, Faculté des sciences et génie, Université Laval, Québec, Canada: Physical characterization of the mitochondrial genome in *C. moewusii* and *C. eugametos* and its transmission in high-viability backcrosses.

Matagne, R.F., Bovie, C., Rongvaux, D., Michel-Wolwertz, M.-R., Loppes, R., Genetics of Microorganisms, Dept. of Botany, University of Liege, Belgium: Mitochondrial DNA inheritance in *Chlamydomonas*.

Wu, M., Chang, C.H., Wang, Z.F., Dept. of Biological Sciences, University of Maryland-Baltimore County: Regulation of the initiation of chloroplast DNA replication in *C. reinhardtii*.

Thompson, R.J., Davies, J.P., Mosig, G., Dept. of Molecular Biology Vanderbilt University, Nashville, Tennessee: Torsional stress in the chloroplast DNA of *C. reinhardtii* differentially affects promoter activity in vivo.

Erickson, J.M., Dept. of Biology, University of California, Los Angeles: Molecular and genetic analysis of photosystem II polypeptides and genes.

Lemieux, C., Gauthier, A., Mercier, J.-P., Turmel, M., Dept. of Biochemistry, Universite Laval, Quebec, Canada: Evidence for the spreading of group I introns in the chloroplast rRNA operon of *Chlamydomonas*.

Boynton, J.E., Gillham, N.W., Harris, E.H., Liu, X.-Q., Dept. of Botany and Zoology, Duke University, Durham, North Carolina: Involvement of chloroplast genes in biogenesis of chloroplast ribosomes in *C. reinhardtii*.

Roitgrund, C., Mets, L.J., Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois: Deletion and linkage analysis define a 5-kb region of *C. reinhardtii* chloroplast DNA necessary both for the *trans*-splicing of *ps1A1* transcripts and for the light-independent synthesis of chlorophyll.

SESSION 6 TECHNIQUES IN *CHLAMYDOMONAS* RESEARCH

Chairman: E. Harris, Duke University

Togasaki, R., Indiana University, Bloomington: Isolation of cloroplasts.

Kindle, K., Cornell University, Ithaca, New York: Particle gun technology for transformation.

Harris, E., Duke University, Durham, North Carolina: Traditional genetic analysis.

Ranum, L.P.W., Lefebvre, P.A., Silflow, C.D., Dept. of Genetics and Cell Biology, University of Minnesota, St. Paul: Mapping genes in *Chlamydomonas* using RFLPs.

Johnson, D.W., Mueh, K.E., Dutcher, S.K., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Use of repetitive DNA probes to construct a molecular map of *Chlamydomonas* rapidly.

Bloodgood, R.A., University of Virginia, Charlottesville: Fluorescent antibody techniques and other fluorescent staining methods.

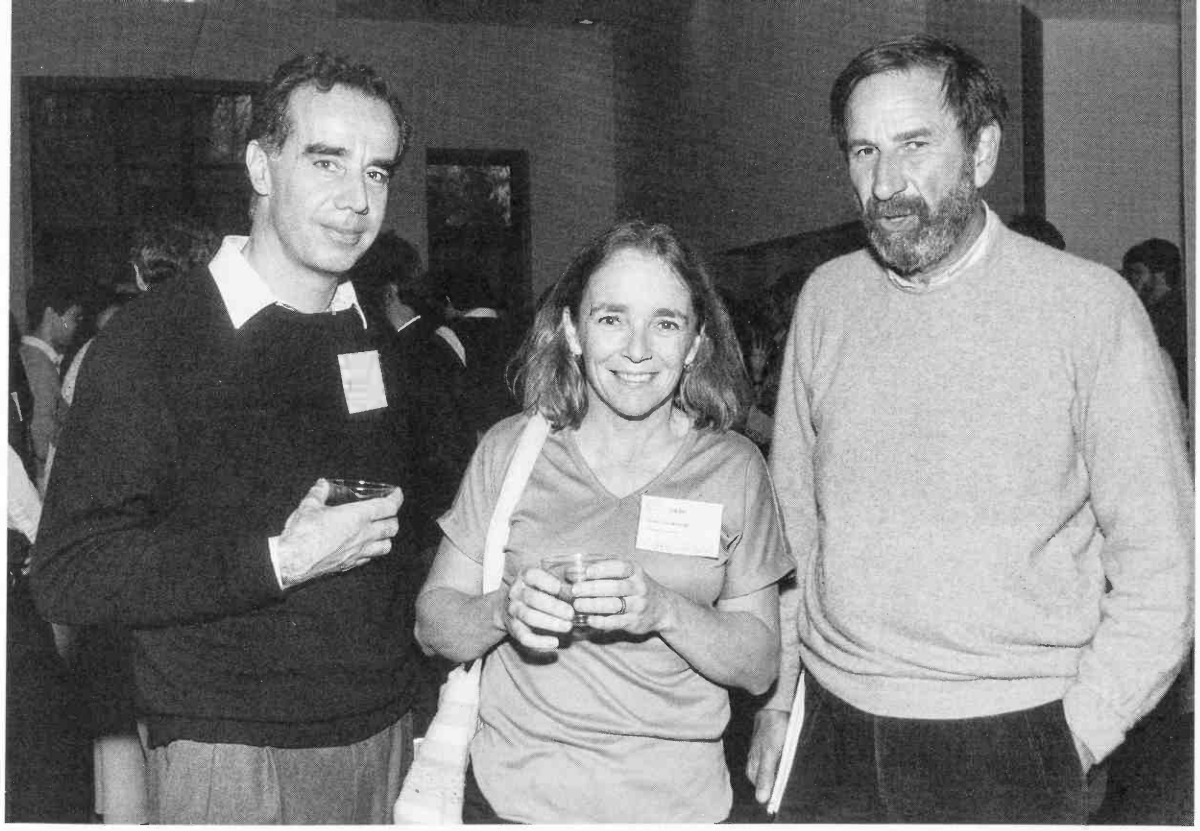
SESSION 7 CHLOROPLASTS AND PHOTOSYNTHESIS

Chairman: R. Togasaki, Indiana University

Spreitzer, R.J., Chen, C., Chastain, C.J., Chollet, R., Al-Abed, S.R., Zhang, D., Huether, M.J., Dept. of Biochemistry, University of Nebraska, Lincoln: Chloroplast mutations alter the substrate specificity of RuBisCO.

Beasley, E.M., Mets, L.J., Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois: Nuclear mutations affecting RuBisCO assembly in *C. reinhardtii*.

Kuchka, M.R.,[1] Mayfield, S.P.,[2] Rochaix, J.-D.,[1] [1]Dept. of Molecular Biology, University of Geneva, Switzerland; [2]Dept. of Molecular Biology, Scripps Clinic and Research Institute, La Jolla, California: Nuclear mutations specifically affect the synthesis of D2, a chloroplast-encoded polypeptide of the



G. Piperno, U. Goodenough, D. Luck

photosystem II complex.

Wang, W.-y, Chang, T.-E., Wegmann, B., Dept. of Botany, University of Iowa, Iowa City: First two enzymes of the chlorophyll biosynthetic pathway.

Merchant, S., Dept. of Chemistry and Biochemistry, University of California, Los Angeles: Transcriptional regulation of gene expression by Cu.

Herrin, D.L., Battey, J.F., Greer, K., Schmidt, G.W., Dept. of Botany, University of Georgia, Athens: Pigment control of photosynthetic gene expression in *C. reinhardtii.*

SESSION 8 CELL SURFACE AND MATING

Chairman: U. Goodenough, Washington University

Pasquale, S.M., Goodenough, U.W., Dept. of Biology, Washington University, St. Louis, Missouri: Sexual signaling in *C. reinhardtii* gametes.

Snell, W.J.,[1] Imam, S.H.,[2] Buchanan, M.J.,[1] Eskue, W.A.,[1] [1]University of Texas Southwestern, Dallas; [2]Plant Polymer Research, Agricultural Research Service, Peoria, Illinois: Lysin release and wall degradation during the mating reaction in *Chlamydomonas.*

Matsuda, Y., Dept. of Biology, Faculty of Science, Kobe University, Japan: Agglutinin and cell-wall lytic enzyme; key molecules to investigate the sexual differentiation in *C. reinhardtii.*

Musgrave, A., Kooijman, Dept. of Molecular Cell Biology, University of Amsterdam, The Netherlands: Agglutinin complexes in *C. eugametos* flagellar membranes.

Goodenough, U., Dept. of Biology, Washington University, St. Louis, Missouri: Effects of elevated cAMP levels on flagellar agglutinability of *Chlamydomonas* gametes.

Woessner, J.P., Goodenough, U.W., Dept. of Biology, Washington University, St. Louis, Missouri: Characterization of zygote wall proteins in *C. reinhardtii.*

Bloodgood, R.A., Dept. of Anatomy and Cell Biology, University of Virginia School of Medicine, Charlottesville: Flagellar glycoprotein dynamics as the basis for gliding motility in *C. reinhardtii.*

SESSION 9 POSTER SESSION

Bingham, S.E., Strem, M.D., Martek Corporation, Columbia, Maryland: Studies on oritidine-5′-phosphate decarboxylase as a selectable marker in *C. reinhardtii.*

Coleman, A.W., Moore, L.J., Division of Biology and Medicine, Brown University, Providence, Rhode Island: Mitochondrial variation within a species.

S. Waffenschmidt, A. Coleman

S. Bingham, T. Osafune

Dutcher, S.K., Lux, F.G., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Inhibition of nuclear fusion to analyze diploid *Chlamydomonas*.

Forest, C., Dept. of Biology, Brooklyn College of the City University of New York, Brooklyn: Freeze-fracture of wild-type and fusion-defective *C. reinhardtii*.

Graves, D.A., Greenbaum, E., Chemical Technology Division, Oak Ridge National Laboratory, Tennessee: Method for measuring in situ absolute photosynthetic rates of individual colonies of *Chlamydomonas*.

Hasnain, S.E., vanWinkle Swift, K., Dept. of Biology, Texas A&M University, College Station: Derivation of a *C. reinhardtii* prototrophic strain from an arginine auxotroph by second-site chromosomal integration of wild-type transforming DNA.

Herrin, D.L., Schmidt, G.W., Dept. of Botany, University of Georgia, Athens: RNA splicing of chloroplasts—Evidence of involvement of nuclear and chloroplast gene products.

Kamiya, R., Hasegawa, E., Dept. of Molecular Biology, Nagoya University, Japan: Intrinsic difference in beat frequency between the two flagella.

Kindle, K.L., Section of Biochemistry, Molecular, and Cell Biology, Cornell University, Ithaca, New York: Expression of a nuclear gene for a thylakoid membrane chlorophyll-*a*/*b*-binding protein—Different mechanisms in the dark and light.

Klein, U., Fischer, P., Botanical Institute, University of Bonn, Federal Republic of Germany: Compartmentation of nitrogen-assimilating enzymes in *C. reinhardtii*.

Leu, S., Michaels, A., Dept. of Biology, Ben-Gurion University of the Negev, Beer Sheva, Israel: Influence of membranes on translation of chloroplast mRNAs.

Loppes, R.,[1] Dumont, F.,[1] Peers, B.,[1] Piette, J.,[2] [1]Laboratory of Molecular Genetics, Dept. of Botany, [2]Laboratory of Microbiology, Institute of Pathology, University of Liege, Belgium: Characterization of new *Chlamydomonas* DNA sequences conferring autonomous replication in *S. cerevisiae*.

Manuel, L.J., Mason, C.B., Moroney, J.V., Dept. of Botany, Louisiana State University, Baton Rouge: Inorganic carbon uptake by *C. reinhardtii*—New proteins are made during adaptation to low CO_2.

Marcus, Y., Dept. of Plant Biology, Carnegie Institute of Washington, Stanford, California: Adaptation of low CO_2 concentration during the cell cycle in *C. reinhardtii*.

Matters, G.L., Woessnew, J.P., Dept. of Biology, Washington University, St. Louis, Missouri: Characterization of zygote-specific genes in *C. reinhardtii*.

Melkonian, M.,[1] Schulze, D.,[1] McFadden, G.I.,[2] Robenek, H.,[3] [1]Botanisches Institut, Munster, Federal Republic of Germany; [2]Plant Cell Biology Centre, Melbourne, Australia; [3]Arbeitsgruppe Zellbiologie, Munster, Federal Republic of Germany: Localization and possible function of centrin, a calcium-modulated contractile protein, in the flagellar apparatus of green algae.

Mergenhagen, D., Mergenhagen, E., Institut für Allgemeine Botanik, Hamburg, Federal Republic of Germany: Physiological clock of *C. reinhardtii* in a microgravity environment.

Orr, E., Dept. of Biology, Washington University, St. Louis, Missouri: Evaluation of a *Chlamydomonas* transposon as a transformation vector.

Park, P., Ford, C., Dept. of Genetics, Iowa State University, Ames: Isolation and characterization of histone genes from *C. reinhardtii*.

Schmitt, R.,[1] Kirk, D.,[2] [1]Universitat Regensberg, Federal Republic of Germany; [2]Dept. of Biology, Washington University, St. Louis, Missouri: Molecular analysis of the relationship of *C. reinhardtii* and *Volvox carteri* to each other and to other organisms.

Spreitzer, R.J., Zhang, D., Chen, C., Dept. of Biochemistry, University of Nebraska, Lincoln: Heteroplasmic suppression of a missense mutation in the chloroplast gene that encodes the RuBisCO large subunit.

Su, X., Kaska, D., Gibor, A., Dept. of Biological Sciences, University of California, Santa Barbara: Characterization of mRNA isolated during wall regeneration in *Chlamydomonas*.

Surzycki, S.J., Fong, S., Hong, T-H., Opperman, T., Dept. of Biology, Indiana University, Bloomington: Identification and analysis of expression of chloroplast genes involved in transcription, DNA replication, and DNA repair.

VanWinkle-Swift, K.,[1] Burrascano, C.,[2] Maddock, J.,[2] Slack, S.,[1] Yoas, K.,[1] Salinger, A.,[1] [1]Dept. of Biology, Texas A&M University, College Station; [2]Dept. of Biology, San Diego State University, California: Genetic dissection of zygosporulation in *Chlamydomonas*.

Voigt, J., Vogeler, H.-P., Institut fur allgemeine Botanik, Universitat Hamburg, Federal Republic of Germany: Structure, biosynthesis, and turnover of the cell wall of the unicellular green alga *C. reinhardtii*.

Waffenschmidt, S., Kuhne, W., Spessert, R., Jaenicke, L., Institut für Biochemie, Universität Köln, Federal Republic of Germany: Autolysins in *C. reinhardtii*.

Wakarchuk, W.W., Gromoff, E.D.v., Beck, C.F., Institut für Biologie III, University of Freiburg, Federal Republic of Germany: Identification of v-*myc* homologous genes in *C. reinhardtii*.

Yu, L., Selman, B., Dept. of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin, Madison: Nucleotide sequence of the cDNA of the γ subunit from the ATP synthase of *C. reinhardtii*.

SESSION 10 CELL CYCLE AND CIRCADIAN CLOCKS

Chairman: **P.C.L. John,** Australian National University

John, P.C.L., Research School of Biological Sciences, Australian National University, Canberra City: *Chlamydomonas* and controls in plant cell division.

Michaels, A., Leu, S., White, D., Dept. of Biology, Ben-Gurion University of the Negev, Beer Sheva, Israel: Chloroplast mRNA abundance and transcription in the cell cycle of *Chlamydomonas*.

Ehara, T.,[1] Osafune, T.,[1] Hase, E.,[2] [1]Dept. of Microbiology, Tokyo Medical College, [2]Chemistry Laboratory, Faculty of Medicine, Teikyo University, Japan: Formation of giant mitochondrion in an early phase of the cell cycle of *C. reinhardtii* in synchronized culture.

Mergenhagen, D., Institut für Allgemeine Botanik, Hamburg, Federal Republic of Germany: Physiological and genetic characterization of the circadian clock in a metabolic mutant of *C. reinhardtii*.

Voigt, J., Munzner, P., Institut für Allgemeine Botanik, Universität Hamburg, Federal Republic of Germany: An extranuclear gene regulates cell division in *C. reinhardtii*.

Mihara, S.[1] , Hase, E.,[2] [1]Institute of Applied Microbiology, University of Tokyo, [2]Chemistry Laboratory, Faculty of Medicine, Teikyo University, Japan: Regulation of cell-cycle revolution in *C. reinhardtii*—A circadian timing mechanism and a sequence of adenine-involving reactions related to initiation of nuclear division.

Kondo, R.,[1] Johnson, C.,[2] Hastings, J.W.,[3] [1]National Institute for Basic Biology, Okazaki, Japan; [2]Vanderbilt University, Nashville, Tennessee; [3]Harvard University, Cambridge, Massachusetts: Photoreceptor systems for the circadian clock of *Chlamydomonas*.

Hegemann, P.,[1] Foster, K.W.,[2] [1]Max-Planck-Institut für Biochemie, Martinsried, Federal Republic of Germany; [2]Dept. of Physics, Syracuse University, New York: Preparation of eyespots and identification of a rhodopsin-like protein.

Foster, K.W.,[1] Saranak, J.,[1] Derguini, F.,[2] Nakanishi, K.,[2] [1]Dept. of Physics, Syracuse University, [2]Dept. of Chemistry, Columbia University, New York, New York: *cis-trans*-Isomerization is not required for activation of *Chlamydomonas* rhodopsin.

Wegener, D., Gromoff, E.D.v., Müller, F., Fuchs, S., Treier, U., Beck, C.F., Institut für Biologie III , University of Freiburg, Federal Republic of Germany: The sexual cycle, light, and heat stress control the expression of defined *C. reinhardtii* genes.

Wegener, D., Müller, F., Treier, U., Beck, C.F., Institut für Biologie III , University of Freiburg, Federal Republic of Germany: Light-induced germination of *C. reinhardtii* zygotes—Physiology and changes in gene expression.

Summary: **D. Kirk,** Washington University

RNA Processing

May 11—May 15

ARRANGED BY

Michael Green, Harvard University
Christine Guthrie, University of California, San Francisco
Alan Lambowitz, Ohio State University

375 participants

The 1988 RNA Processing meeting processed a record number of abstracts in its seventh year at Cold Spring Harbor Laboratory. The spotlight was again focused on the catalytic possibilities of RNA. This year, new examples of self-

cleaving RNAs were introduced, including the provocative hepatitis delta agent, and the possibility was raised that catalysis can be mediated by structural motifs more diverse than the simple "hammerhead." Although the RNA from "telomerase" has not yet been shown to be the catalytically active partner of the RNP enzyme, its sequence analysis revealed the potential for templating the telomeric DNA sequence. Further inroads in mRNA splicing derived substantially from mounting evidence that the yeast and mammalian splicing pathways are fundamentally the same, thus allowing data from each system to inform the other. In particular, despite earlier emphasis on the differences in branchpoint recognition, it is now clear that mammalian introns obey the same sequence preferences as yeast and that yeast U2 (which is known to base pair with the TACTAAC box), although six times larger than human, can be deleted to the same size with no ill effects. On the other hand, there is as yet no yeast analog for a specific factor (U2AF) that mediates assembly at the 3′ splice site in mammalian cells. Especially encouraging was the observation that antibodies to a yeast U5-specific snRNP protein cross-react with a similarly sized (>200 kD) protein in HeLa extracts. A most exciting breakthrough in alternative splicing was provided by the case of sex determination in flies, where decades of genetics can now be brought to bear on a biochemical solution. *trans*-splicing in worms and trypanosomes appears to utilize an snRNA linked in *cis* to the 5′ exon; this surprising finding suggests an interesting evolutionary link to self-splicing introns. Finally, the audience was stunned by the announcement that the central dogma is once again under attack—this time by the observation that trypanosome transcripts undergo extensive posttranscriptional "editing" at the RNA level.

This meeting was supported in part by funds from the National Science Foundation and the National Institute of General Medical Sciences, a division of the National Institutes of Health.

J. Beggs, M. Rosbash

SESSION 1 CATALYTIC RNA AND tRNA PROCESSING

Chairman: N. Pace, Indiana University

Guerrier-Takeda, C.,[1] van Belkum, A.,[2] Pleij, C.W.A.,[2] Altman, S.,[1] [1]Dept. of Biology, Yale University, New Haven, Connecticut; [2]Dept. of Biochemistry, University of Leiden, The Netherlands: Novel reactions of RNase P.

Waugh, D.S.,[1] Green, C.J.,[2] James, B.D.,[1] Olsen, G.J.,[1] Vold, B.S.,[2] Pace, N.R.,[1] [1]Dept. of Biology, Indiana University, Bloomington; [2]SRI International, Menlo Park, California: Design and catalytic properties of an abbreviated ribonuclease P RNA.

Wang, M.J., Li, X.-Q., Gegenheimer, P., Depts. of Biochemistry and Botany and Molecular Genetics Program, University of Kansas, Lawrence: Chloroplast RNase P does not have a catalytic RNA subunit.

Winey, M., Culbertson, M.R., Laboratories of Genetics and Molecular Biology, University of Wisconsin, Madison: Mutations affecting the tRNA-splicing endonuclease activity of *S. cerevisiae*.

Thompson, L.D., Daniels, C.J., Dept. of Microbiology, Ohio State University, Columbus: Unique substrate recognition properties of an archaebacterial tRNA intron endonuclease.

Forster, A.C., Davies, C., Sheldon, C.C., Jeffries, A.C., Symons, R.H., Dept. of Biochemistry, University of Adelaide, Australia: A structural model for the active sites of self-cleaving viroid and newt RNAs.

Ruffner, D.E., Uhlenbeck, O.C., Dept. of Chemistry, and Biochemistry, University of Boulder, Colorado: Nucleotide sequence requirements for a self-cleaving RNA.

Feldstein, P.A., Buzayan, J.M., Bruening, G., Dept. of Plant Pathology, University of California, Davis: A ribonuclease activity derived from autolytic processing sequences of satellite tobacco ringspot virus RNA.

Shareem, L.,[1] Kuo, M.,[1] Dinter-Gottlieb, G.,[2] Taylor, J.,[1] [1]Fox Chase Cancer Center, [2]Drexel University, Philadelphia, Pennsylvania: Self-processing of human HDV RNAs.

Dreyfus, D.H., Emmons, S.W., Dept. of Molecular Biology and Genetics, Albert Einstein College of Medicine, Bronx, New York: A repetitive element in *C. elegans* with similarity to both transposable elements and plant viroids encodes a self-cleaving RNA.

Greider, C.,[1] Blackburn, E.,[2] [1]Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; [2]Dept. of Molecular Biology, University of California, Berkeley: Characterization of telomerase RNP.

Bennett, J.L., Chang, D.D., Fisher, R.P., Stohl, L.L., Topper, J.N., Clayton D.A., Dept. of Pathology, Stanford University School of Medicine, California: Mitochondrial enzymes with nuclear RNA components

SESSION 2 SPLICING OF GROUP I AND GROUP II INTRONS

Chairman: C. Peebles, University of Pittsburgh

Burgin, A.B.,[1] Parardos, K.,[2] Lane, D.J.,[2] Pace, N.R.,[1] [1]Dept. of Biology, Indiana University, Bloomington; [2]Gene-Trak System, Framingham, Massachusetts: Excision of intron-like elements from *Salmonella* 23S rRNA precursors.

Shub, D.A.,[1] Goodrich, H.E.,[1] Gott, J.M.,[1] Xu, M.-Q.,[1] Scarlato, V.,[2] [1]Dept. of Biological Sciences, State University of New York, Albany; [2]Dept. of Biology, University of California, San Francisco, and Institute of Genetics and Biophysics, Naples, Italy: A self-splicing group I intron in the DNA polymerase gene of the *B. subtilis* bacteriophage SPO1.

Flanegan, J.B.,[1] Cech, T.R.,[2] [1]Dept. of Immunology and Medical Microbiology, University of Florida, Gainesville; [2]Dept. of Chemistry and Biochemistry, University of Colorado, Boulder: *trans*-Splicing of model oligoribonucleotide substrates by *Tetrahymena* ribozyme.

Kay, P.S., Menzel, P., Inoue, T., Salk Institute, La Jolla, California: Two guanosine-binding sites in the group I IVS RNA and their roles in the mechanism of self-splicing.

Burke, J., Williamson, C., Desai, N., Chemistry Dept., Williams College, Williamstown, Massachusetts: Compensatory mutations in P6 and P8 of self-splicing tetrahymena pre-rRNA.

Coetzee, T.,[1,2] Salvo, J.G.,[1] DiMarla, P.,[1,3] Belfort, M.,[1] [1]Wadsworth Center of Laboratories and Research, New York State Dept. of Health, [2]Albany Medical College, [3]Dept. of Biology, State University of New York, College at Fredonia: Deletion analysis and *trans*-splicing of the group I *td* intron.

Akins, R., Majumder, A.L., Cherniack, A., Ericson, J., Kelley, R., Snook, A., Lambowitz, A., Depts. of Molecular Genetics and Biochemistry, Ohio State University, Columbus: Involvement of an aminoacyl-tRNA synthetase in splicing of group I mitochondrial introns.

Herbert, C.J., Labouesse, M., Dujardin, G., Slonimski, P.P., Centre de Génétique Moléculaire du CNRS, France: Evidence for the involvement of the mitochondrial leucyl tRNA synthetase in yeast mitochondrial RNA splicing.

Peebles, C.L.[1] Stoops, M.J.,[1] Perlman, P.S.,[2] [1]Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania; [2]Dept. of Molecular Genetics, Ohio State University, Columbus: The 3′-terminal ribose of exon 1 is a crucial determinant for conformational switching by self-splicing group II introns.

Altura, R., Rymond, B., Seraphin, B., Rosbash, M., Dept. of Biology, Brandeis University, Waltham, Massachusetts: The 5′ end of the intron suppresses hydrolysis of group II 5′ splice sites.

Perlman, P.S., Hebbar, S.K., Dietrich, R.C., Jarrell, K.A., Dib-Hajj, S.D., Dept. of Molecular Genetics, Ohio State University, Columbus: Studies of the structure and function of domain 5 of a group II intron of yeast mitochondrial DNA.

SESSION 3 POSTER SESSION

Abmayr, S., Reed, R., Maniatis, T., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Functional analysis of mammalian splicing complexes.

Acheson, N.H., Lanoix, J., Dept. of Microbiology and Immunology, McGill University, Montreal, Quebec, Canada: A rabbit β-globin polyadenylation signal directs efficient termination of transcription on polyomavirus DNA.

Adami, G.,[1] Nevins, J.,[2] [1]Rockefeller University, New York, New York; [2]HHMI, Duke University, Durham, North Carolina: Adenovirus mRNA processing—In a regulated manner, a splice-site choice dominates over selection of a poly(A) site located in an intron.

Apostol, B., Belford, H., Greer, C., Dept. of Biological Chemistry, University of California, Irvine: Characterization of pre-tRNA binding by yeast ligase.

Arrigo, S., Beemon, K., Dept. of Biology, Johns Hopkins University, Baltimore, Maryland: Negative regulation of RSV RNA splicing.

Augustin, S.,[1] Morl, M.,[2] Muller, M.W.,[2] Schmelzer, C.,[2] [1]Universitat Wien, Austria; [2]Universitat Munchen, Federal Republic of Germany: Effect of mutations at the 3′ end of group II intron bI1 on splicing efficiency and 3′ splice-site selection.

Banroques, J., Abelson, J., California Institute of Technology, Pasadena, California: RNA4 protein and yeast spliceosome.

Bartkiewicz, M., Gold H., Altman, S., Yale University, New Haven, Connecticut: RNA subunit of RNase P from HeLa cells.

Baas, P.D., Adema, G.J., Bovenberg, R.A.L., Jansz, H.S., University of Utrecht, The Netherlands: Processing of CALC-I RNA in vitro.

Bewley, G.C., Cook, J.L., Dept. of Genetics, North Carolina State University, Raleigh: *Drosophila* sn-glycerol-3-phosphate dehydrogenase isozymes are generated by alternate pathways of RNA processing, resulting in different carboxy-terminal amino acid sequences.

Bond, U., Steitz, J.A., Yale University Medical School, New Haven, Connecticut: Differential effects of stress on pre-mRNA processing in HeLa cells.

Branch, A.D.,[1] Benenfeld, B.J.,[1] Robertson, H.D.,[1] Baroudy, B.M.,[2] Buckler-White, A.,[2] Gerin, J.L.,[2] [1]Rockefeller University, New York, New York; [2]Georgetown University, Rockville, Maryland: A hammerhead structure from RNA of the delta agent.

Castaño, J.G., Universidad Autonoma de Madrid, Spain: Purification and characterization of the 3′-pre-tRNAse from Ehrilich ascites cells.

Chauhan, A.K., Subbarao, M.N., Miczak, A., Apirion, D., Dept. of Microbiology, Washington University, St. Louis, Missouri: Cloning and sequencing the gene for a small abundant RNA of *E. coli*.

Connelly, S., Manley, J.L., Dept. of Biological Sciences, Columbia University, New York, New York: Poly(A) site-dependent transcription termination by RNA polymerase II—A role for promoter-proximal DNA sequences.

Conway, G., Roberts, R.J., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: A splicing factor(s) is associated with large RNP complexes and is released in a soluble active form in the presence of ATP.

Conway, L., Wickens, M., Dept. of Biochemistry, University of Wisconsin, Madison: Identification of bases and phosphates of SV40 late pre-mRNAs that are required for 3′-end formation in vitro.

Cooke, N.E.,[1,2] Ray, J.,[2,3] Estes, P.A.,[2] Emery, J.G.,[1,2,3] Liebhaber, S.A.,[1,2,3] Depts. of [1]Medicine, [2]Human Genetics, [3]Howard Hughes Medical Institute, University of Pennsylvania School of Medicine, Philadelphia: Alternative mRNA processing within the human growth-hormone gene family.

Corell, R.A., Friedlander, L.H., Rice, P.W., Gross, R.H., Dept. of Biological Sciences and Molecular Genetics Center, Dartmouth College, Hanover, New Hampshire: Diffusible factors involved in RNA processing.

Craig, N.,[1] Kass, S.,[2] Sollner-Webb, B.,[2] [1]Dept. of Biological Sciences, University of Maryland, Baltimore County; [2]Dept. of Biological Chemistry, Johns Hopkins School of Medicine, Baltimore, Maryland: Sequences required for processing of mouse rRNA.

Cusick, M., Abelson, J., Division of Biology, California Institute of Technology, Pasadena: Search for yeast hnRNPs.

Daar, I.O., Lim, S., Maquat, L.E., Dept. of Human Genetics, Roswell Park Memorial Institute, Buffalo, New York: Premature translation termination mediates mammalian mRNA degradation.

Das, G., Henning, D., Reddy, R., Baylor College of Medicine, Houston, Texas: Involvement of components of both RNA polymerase II and III transcription machineries in U6 snRNA gene transcription.

Dauwalder, B., Kubli, E., Institute of Zoology, University of Zurich, Switzerland: *D. melanogaster* U6 snRNA genes—Control of *Drosophila* U6 gene transcription is different from that in vertebrates.

deLannoy, P., Caruthers, M.H., Dept. of Biochemistry, University of Colorado, Boulder: Partial purification of a factor required for 5′ splice-site cleavage and IVS-exon formation.

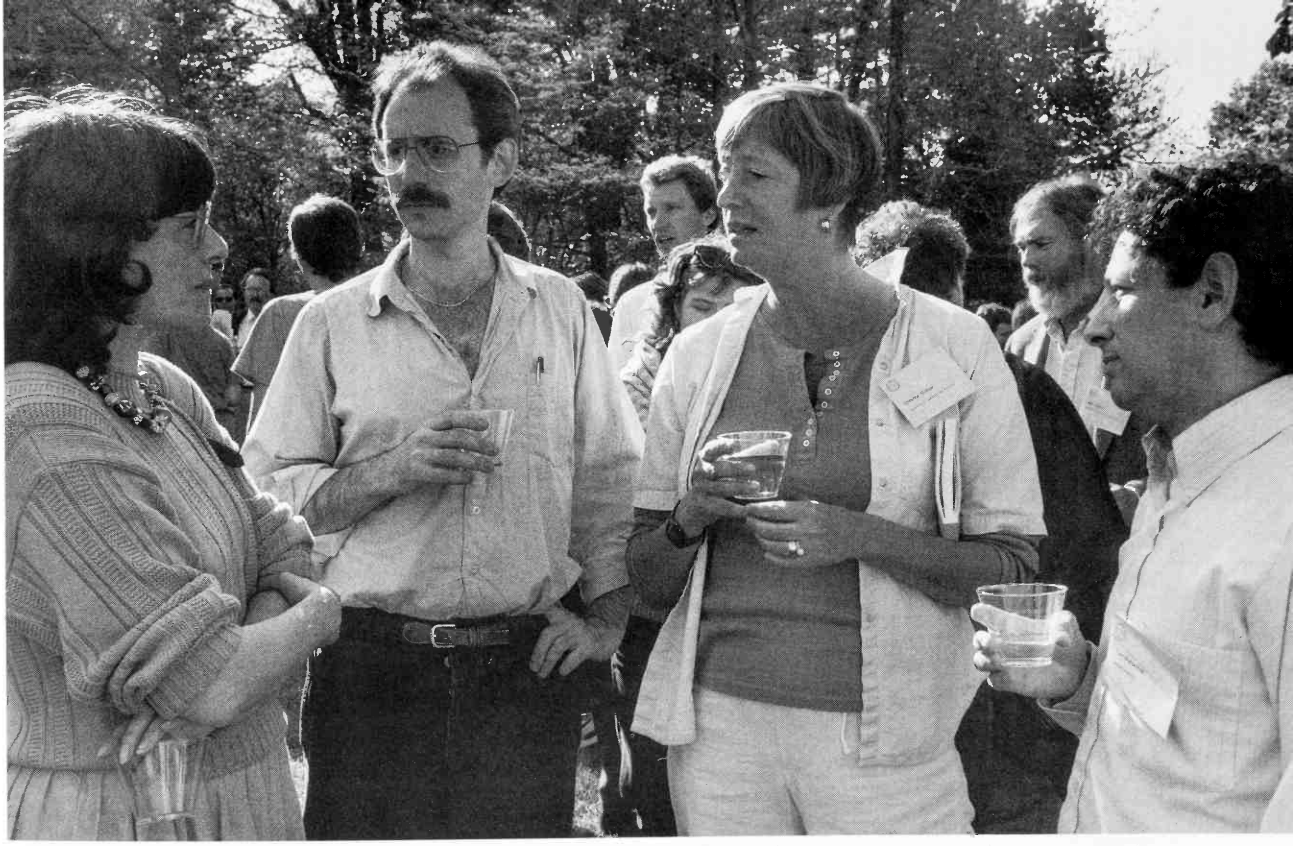
de Mars, M., Sterner, D.A., Murphy, E.C., Jr., University of Texas System Cancer Center, M.D. Anderson Hospital, Houston: Changes in nonconserved intron sequences alter MSV RNA splicing.

Deshler, J.O., Rossi, J.J., Dept. of Microbiology, University of California, Los Angeles, and Beckman Research Institute, City of Hope, Duarte: Characterizing the heterologous splicing capabilities of two budding yeasts—*S. cerevisiae* and *K. lactis*.

Deutscher, M.P., Marshall, G.T., Cudny, H., Dept. of Biochemistry, University of Connecticut Health Center, Farmington: RNase PH—A new phosphate-dependent nuclease implicated in tRNA processing.

DeZazzo, J.D., Wilson-Gunn, S.I., Hales, K.H., Imperiale, M.J., Dept. of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor: Regulation of polyadenylation within the adenovirus major late transcription unit.

Drabkin, H.J., RajBhandary, U.L., Dept. of Biology,



T. Grodzicker, M. Green, C. Guthrie, T. Blumenthal

Massachusetts Institute of Technology, Cambridge: Introduction of an intervening sequence into a mammalian serine amber suppressor tRNA gene.

Erster, S.H., Finn, L.A., Helfman, D.M., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: Alternative 3′-end processing of tropomyosin pre-mRNA in vivo.

Fabrizio, P., McPheeters, D., Abelson, J., Division of Biology, California Institute of Technology, Pasadena: In vitro assembly of yeast snRNPs.

Flaspohler, J.A., Milcarek, C., Dept. of Microbiology, Biochemistry, and Molecular Biology, University of Pittsburgh, Pennsylvania: Transcription termination in the murine immunoglobulin γ2b and γ2a genes.

Francis, M.A., RajBhandary, U.L., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Functional expression of a mammalian RNA polymerase III gene in yeast.

Frank D., Guthrie, C., Dept. of Biochemistry, University of California, San Francisco: Structural analysis of yeast snRNAs and snRNPs.

Fresco, L.D., Keene, J.D., Dept. of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina: Molecular analysis of the U2 snRNP unique protein A′.

Freyer, G.A., O'Brien, J.P., Balogh, L.A., Kurwitz, J., Cold Spring Harbor Laboratory, New York: Effects of alterations in the polypyrimidine sequence of the splicing of single- and double-intron-containing pre-mRNAs.

Gallego, M.E., Nadal-Ginard, B., Howard Hughes Medical Institute, Dept. of Cardiology, Children's Hospital, Harvard Medical School, Cambridge, Massachusetts: Mutually exclusive splicing of MLC1/3 transcripts is *cis*-regulated.

Genovese, C., Milcarek, C., Dept. of Microbiology, Biochemistry, and Molecular Biology, University of Pittsburgh, Pennsylvania: Alterations in immunoglobulin mRNA stability during B-cell development.

Goodall, G., Wiebauer, K., Filipowicz, W., Friedrich Miescher-Institut, Basel, Switzerland: Specificity of nuclear pre-RNA splicing in plants.

Goux-Pelletan, M.S., Brody, E., Marie, J., Institut de Biologie Physico-Chimique, Paris, France: In vitro splicing of the chicken β-tropomyosin pre-mRNA.

Green, C.,[1] Vold, B.S.,[1] Morch, M.D.,[2] Joshi, R.L.,[2] Haenni, A.-L.,[2] [1]SRI International, Menlo Park, California: [2]Institut Jacques Monod, CNRS and Université Paris, France: Processing of the tRNA-like structure of TYMV RNA by the catalytic RNA component of RNase P.

Habets, W.J., Sillekens, P.T., Hoet, M.H., Beyer, R.P., van Venrooij, W.J., Dept. of Biochemistry, University of Nijmegen, The Netherlands: Molecular cloning of U1 and U2 snRNP-associated proteins.

Hall, B., Milcarek, C., Dept. of Microbiology, Biochemistry, and Molecular Biology, University of Pittsburgh, Pennsylvania: Sequence and secondary structure of the membrane 3′ UT region of the murine immunoglobulin γ2a gene.

Hampson, R.K., Rottman, F.M., Dept. of Molecular Biology and Microbiology, Case Western Reserve University School of Medicine, Cleveland, Ohio: Alternative processing of bovine growth-hormone precursor mRNA

is strongly influenced by sequences within the downstream exon.

Hanley, B.A., Egeland, D.B., Schuler, M.A., Depts. of Plant Biology and Biochemistry, University of Illinois, Urbana: Plant introns and snRNAs.

Hanna, M., Cherry, M., Doudna, J., Green, R., Szostak, J.W., Dept. of Molecular Biology, Massachusetts General Hospital, Boston: Mutational analysis of tetrahymena rRNA intron core.

Hartmuth, K., Barta, A., Institut fur Biochemie, Vienna, Austria: Possible base-pairing interactions between mammalian branch point sequences and U2 RNA.

Haynes, S.,[1] Johnson, D.,[2] Raychaudhuri, G.,[3] Beyer, A.,[3] [1]NICHD, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Biological Sciences, George Washington University, Washington, D.C.; [3]Dept. of Microbiology, University of Virginia School of Medicine, Charlottesville: Molecular characterization of *Drosophila* genes for proteins related to the mammalian hnRNP A1 protein.

Hebbar, S.K., Perlman, P.S., Dept. of Molecular Genetics, Ohio State University, Columbus: Auto-catalytic reactions of a maturase encoding group II intron.

Henchcliffe, C., Paolella, G., Barone, M.V., Baralle, F.E., Sir William Dunn School of Pathology, University of Oxford, England: An investigation of *cis*- and *trans*-acting elements involved in alternative splicing of the primary transcript of the human fibronectin gene.

Hernandez, N., Lucito, R., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: Promoter sequences required for initiation and 3′-end formation of the human U2 snRNA.

Herrick, D., Jacobson, A., Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester: Use of chimeric mRNAs to study the structural determinants of mRNA stability in yeast.

Hirsh, D.I., Bektesh, S.L., Van Doren, K., Synergen, Inc., Dept. of Developmental Biology, Boulder, Colorado: Presence of the 22-nucleotide *C. elegans* spliced leader in mRNAs of other nematodes.

Ho, C.K., Vijayraghavan, U., Abelson, J., Division of Biology, California Institute of Technology, Pasadena: A yeast mutant that accumulates pre-tRNA splicing "2/3" intermediates.

Hwang, S.-P.L., Shelness, G.S., Binder, R., Eisenberg, M., Williams, D.L., Dept. of Pharmacological Sciences, State University of New York, Stony Brook: Secondary structure of apolipoprotein II mRNA. Evidence for base pairing between the 3′-noncoding region and the coding region near the termination codon.

Inoue, K., Ohno, M., Sakamoto, H., Shimura, Y., Dept. of Biophysics, Faculty of Science, Kyoto University, Japan: Effect of the cap structure on pre-mRNA in *Xenopus* oocytes.

Jarrell, K.A., Dietrich, R.C., Hebbar, S.K., Perlman, P.S., Dept. of Molecular Genetics, Ohio State University, Columbus: *trans*-Splicing experiments with a group II intron reveal an essential function for intron domain 5.

Jones, M.H., Guthrie, C., University of California, San Francisco: Biochemical and genetic analysis of yeast Sm snRNPs.

Joyce, G.F., Inoue, T., Nucleotide Chemistry Laboratory, Salk Institute of Biological Studies, La Jolla, California: Deletion of nonconserved portions of a self-splicing group I intron using a novel in vitro mutagenesis technique.

Käufer, N.F., Gatermann, K., Rosenberg, G., Hoffmann, A., Dept. of Bioscience and Biotechnology, Drexel University, Philadelphia, Pennsylvania: Features and "make up" of introns in the fission yeast *S. pombe*.

Kedes, D.H., Steitz, J.A., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: In vivo versus in vitro 5′ splice-site selection in the mouse immunoglobulin κ light-chain transcript—Why the difference?

Kessler, M., Resnekov, O., Ben-Asher, E., Aloni, Y., Weizmann Institute of Science, Rehovot, Israel: A novel transcription elongation block is active within the late leader sequences of SV40.

Kessler, M., Bengal, E., Aloni, J., Weizmann Institute of Science, Rehovot, Israel: In vitro structural analysis of a eukaryotic RNA polymerase II termination signal.

Kirsebom, L.A., Baer, M., Altman, S., Yale University, New Haven, Connecticut: Kinetic studies of the RNase P reaction using mutants of both the substrate and the enzyme.

Kiss, T., Jakab, G., Antal, M., Pálfi, Z., Hegyi, H., Kiss, M., Solymosy, F., Institute of Plant Physiology, Biological Research Center, Hungarian Academy of Science, Szeged: Plant snRNAs. U4 RNA is present in plants—Primary and possible secondary structure as well as base pairing with plant U6 RNA.

Kleinschmidt, A.M., Patton, J.R., Pederson, T., Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: In vitro assembly of the U2 snRNP particle.

Kloetzel, P.M., Haass, C., ZMBH, Molekulare Genetik, Heidelberg, Federal Republic of Germany: The small heat-shock proteins of *D. melanogaster* form globular cytoplasmic 16S RNP particles—Molecular analysis of the protein components and cDNA cloning of the RNA component.

Knaack, D., Breitbart, R., Nadal-Ginard, B., Howard Hughes Medical Institute, Harvard Medical School, Boston, Massachusetts: Identification of an intron sequence with developmentally regulated effects on the splicing of muscle-specific exons.

Köhrer, K., Löw, A., Domdey, H., Genzentrum der Ludwig-Maximilians-Universität Mu̇nchen, Federal Republic of Germany: Dependence of yeast pre-mRNA splicing and polyadenylation from transcription.

Konarska, M.M., Sharp, P.A., Center for Cancer Research and Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Associations of snRNPs in the formation of pseudospliceosomes.

Koster, J.G.,[1] Hanover, J.A.,[2] Zasloff, M.,[1] [1]NICHHD, [2]NIDDKD, National Institutes of Health, Bethesda, Maryland: Inhibition of tRNA nuclear transport by the lectin wheat-germ agglutinin.

Kreivi, J.-P., Svensson, C., Larsson, S., Akusjarvi, G., Dept. of Microbial Genetics, Karolinska Instituet, Stockholm, Sweden: Regulation of adenovirus-2 L1 pre-mRNA splicing.

Krol, A.,[1] Carbon, P.,[1] Murgo, S.,[1] Lescure, A.,[1] Dathan, N.,[2] Kazmaier, M.,[2] Parry, H., [2] Tebb, G.,[2] Mattaj, I.W.,[2] [1]IBMC, Strasbourg, France; [2]EMBL, Heidelberg, Federal Republic of Germany: Expression of *Xenopus* U6 snRNA genes.

Kwakman, J.H.J.M., Pel, H.J., Grivell, L.A., Dept. of Molecular Cell Biology, University of Amsterdam, The Netherlands: Determination of the secondary structure of a group II intron by chemical modification.

Lang, K.M., Keller, W., Dept. of Cell Biology, Biocenter of the University, Basel, Switzerland: Interaction of splicing components with pre-mRNA substrates—Investigations with modification/interference techniques.

Lassman, C., Milcarek, C., Dept. of Microbiology, Biochemistry, and Molecular Biology, University of Pittsburgh, Pennsylvania: Regulation of immunoglobulin γ2b mRNA processing.

Lavery, D., Chen-Kiang, S., Immunobiology Center and Dept. of Microbiology, Mt. Sinai Medical School, New York, New York: Lymphoid-cell-specific regulation of nuclear-cytoplasmic mRNA transport.

SESSION 4 PRE-mRNA SPLICING MECHANISMS

Chairman: A. Weiner, Yale University School of Medicine

Siliciano, P.G., Guthrie, C., Dept. of Biochemistry and Biophysics, University of California, San Francisco: Base pairing between yeast U1 and the 5′ splice site dictates cleavage efficiency but not fidelity.

Seraphin, B., Kretzner, L., Rosbash, M., Dept. of Biology, Brandeis University, Waltham, Massachusetts: Role of U1 snRNA during splicing in yeast.

Nelson, K.K., Zamore, P.D., Ruskin, B., Green, M.R., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Mechanistic aspects of U2 snRNP binding.

Zhuang, Y., Weiner, A.M., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Sequence specificity of branch-site selection in mammalian cells.

Reed, R., Maniatis, T., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: The branchpoint sequence plays a role in 3′ splice-site selection in vitro.

Lotan, A.,[1] Sperling, J.,[2] Sperling, R.,[1] [1]Dept. of Genetics, Hebrew University of Jerusalem, [2]Dept. of Organic Chemistry, Weizmann Institute of Science, Rehovot, Israel: Localization of a base-paired region of U2 snRNA with pre-mRNA in an in-vitro-splicing complex by UV-induced psoralen-mediated cross-linking.

Garcia-Blanco, M.A., Konarska, M.M., Sharp, P.A., Center for Cancer Research, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Two proteins in HeLa cell nuclear extracts interact specifically with pre-mRNAs.

Kramer, A., Frick, M., Utans, U., Keller, W., Dept. of Cell Biology, Biocenter of the University, Basel, Switzerland: Analysis of protein factors from HeLa cells involved in the assembly of splicing complexes with nuclear pre-mRNA.

Sawa, H., Ohno, M., Sakamoto, H., Shimura, Y., Dept. of Biophysics, Faculty of Science, Kyoto University, Japan: Requirement of ATP in the second step of the pre-mRNA splicing reaction.

Gutman, D., Goswami, P., Goldenberg, C.J., Dept. of Microbiology and Immunology, University of Miami School of Medicine, Florida: Purification and characterization of an RNA helicase involved in pre-mRNA splicing.

Zapp, M.L., Berget, S.M., Dept. of Biochemistry, Baylor College of Medicine, Houston, Texas: Factors required for recognition of 5′ splice junctions.

Zeitlin, S., Wilson, R.C., Parent, A., Efstratiadis, A., Dept. of Genetics and Development, Columbia University, New York, New York: In vivo or in vitro assembled self-splicing and complementable complexes.

SESSION 5 SPLICEOSOME ASSEMBLY AND *TRANS*-SPLICING

Chairman: P. Sharp, Massachusetts Institute of Technology

Michaeli, S., Watkins, K.P., Agabian, N., Intercampus Program, Molecular Parasitology, University of California, San Francisco: SL RNP—A unique small ribonucleoprotein particle in *Trypanosomes*.

Bruzik, J.P.,[1] Van Doren, K.,[2] Hirsh, D.,[2] Steitz, J.A.,[1] [1]Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut; [2]Synergen, Inc., Boulder, Colorado: *trans*-Spliced leader RNAs exist as covalently linked exon-snRNPs incorporated into Sm particles.

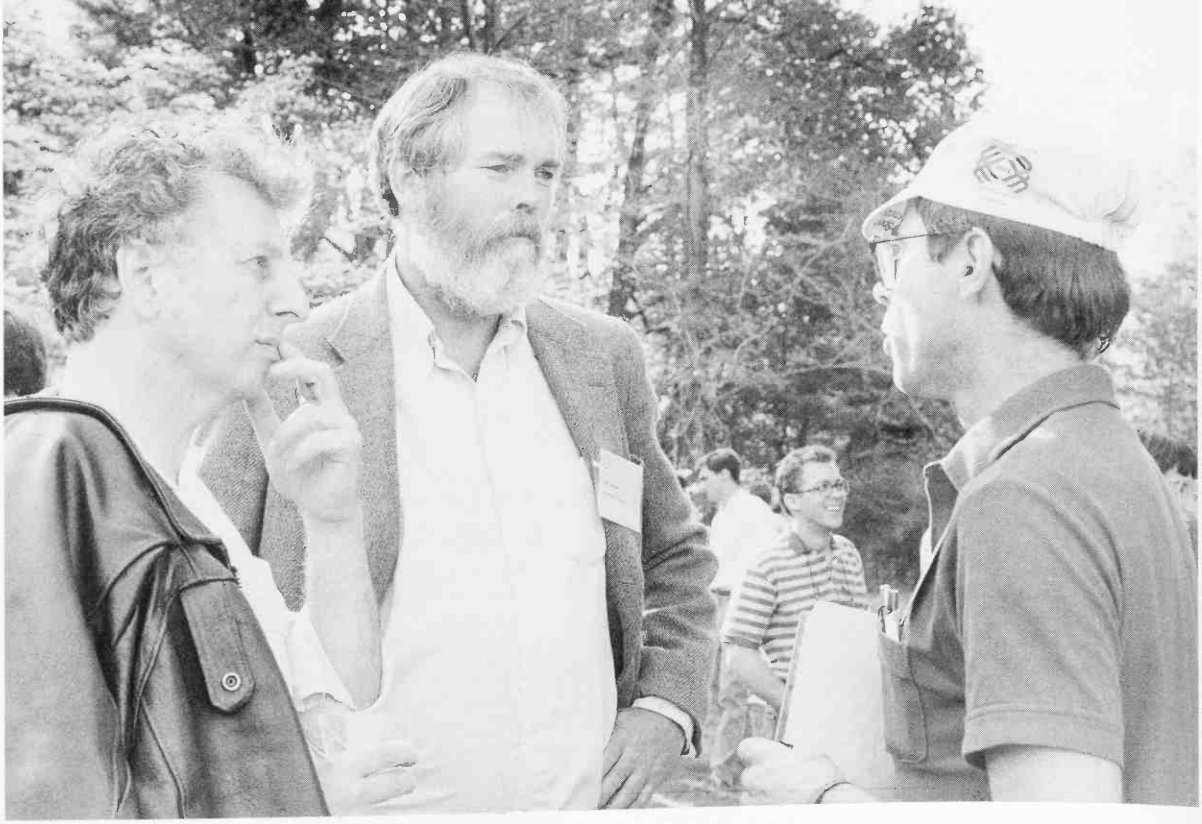
Thomas, J., Conrad, R., Blumenthal, T., Dept. of Biology, Indiana University, Bloomington: *C. elegans* snRNAs—The *trans*-spliced leader precursor is bound to Sm antigen and may have a TMG cap.

Osheim, Y.N., Amero, S.A., Beyer, A.L., Dept. of Microbiology, University of Virginia, Charlottesville: Visualizing the splicing process.

Jamieson, D., Beggs, J., Dept. of Molecular Biology, University of Edinburgh, Scotland: Identification and cloning of the *ssp1* gene whose product suppresses the defect of the *rna8-1* mutant of *S. cerevisiae*.

Brow, D.A., Guthrie, C., Dept. of Biochemistry and Biophysics, University of California, San Francisco: Conserved features of the synthesis and structure of U6 snRNA.

Bindereif, A., Pikielny, C.W., Green, M.R., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: In vitro reconstitution of functional snRNPs and multi-snRNP complexes.



E. Brody, J. Abelson, N. Pace

Lassota, P., Pruzan, R., Belgado, N., Hurwitz, J., Memorial Sloan-Kettering Cancer Center, New York, New York: Pre-spliceosome formation in vitro using purified U2 RNA.

Legrain, P., Seraphin, B., Fromherz, S., Rosbash, M., Dept. of Biology, Brandeis University, Waltham, Massachusetts: Yeast pre-mRNA is committed to the spliceosome pathway before formation of U2 snRNP-containing complexes.

Ruby, S.W., Goelz, S., Abelson, J.N., Division of Biology, California Institute of Technology, Pasadena: Yeast U1 snRNP binding is required for other snRNPs to bind during in vitro splicing.

Padgett, R.A., Wang, X., Dept. of Biochemistry, University of Texas Southwestern Medical Center, Dallas: Chemical footprinting of splicing factors on pre-mRNA.

SESSION 6 POSTER SESSION

Layden, R., Eisen, H., Fred Hutchinson Cancer Research Center, Seattle, Washington: Does *trans*-splicing in trypanosomes require base pairing between the two spliced RNAs?

Lee, S.,[1] Murthy, S.,[2] Trimble, J.,[2] Desrosiers, R.C.,[2] Steitz, J.A.,[1] [1]Howard Hughes Medical Instituet, Dept. of Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Connecticut; [2]New England Regional Primate Research Center, Harvard Medical School, Cambridge, Massachusetts: A virus encodes four small U RNAs.

Legrain, P., Rosbash, M., Dept. of Biology, Brandeis University, Waltham, Massachusetts: Splicing and nuclear-cytoplasmic transport.

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Ware, V.C., Dept. of Biology and Center for Molecular Bioscience and Biotechnology, Lehigh University, Bethlehem, Pennsylvania: rRNA processing—Structure of the gap region in domain IV of *T. thermophila* 26S rRNA.

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Zassenhaus, P., Dept. of Microbiology, St. Louis University Medical Center, St. Louis, Missouri: An ATP-dependent 5′ exoribonuclease from yeast mitochondria.

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Zwieb, C., European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: Toward the three-dimensional structure of 7SL RNA—Functional implications.

SESSION 7 snRNP AND hnRNP STRUCTURE AND SYNTHESIS

Chairman: D. Clayton, Stanford University Medical School

Lührmann, R., Bach, M., Heyer, A., Kastner, B., Winkelmann, G., Max-Planck-Institut für Molekulare Genetik, Berlin, Federal Republic of Germany: The major UsnRNPs from HeLa Cells—Heterogeneity of protein composition, immunoelectron microscopic investigation, and contribution of individual snRNPs to splicing as investigated by a complementation assay.

Krainer, A.R., Kozak, D., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: Purification of active mammalian m^3G snRNPs.

Pan, Z., Prives, C., Columbia University, New York, New York: Assembly of functional U1 and U2 human-amphibian hybrid snRNPs in *X. laevis* oocytes.

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Carbon, P.,[1] Westhof, E.,[1] Ebel, J.P.,[1] Bach, M.,[2] Luhrmann, R.,[2] Krol, A.,[1] [1]IBMC, Strasbourg, France; [2]MPI, Berlin, Federal Republic of Germany: Three-dimensional model of U1 snRNA.

Petersen-Bjorn, S., Friesen, J.D., Dept. of Medical Genetics, University of Toronto, Canada: Analysis of the roles of *RNA4* and *ORF2* in yeast mRNA splicing.

Lund, E., Dahlberg, J.E., Dept. of Physiological Chemistry, University of Wisconsin, Madison: Efficient and accurate transcription of exogenous U1 RNA genes in isolated nuclei of *X. laevis* oocytes.

Shuster, E.O., Guthrie, C., University of California, San Francisco: Yeast U2 has two functionally important domains separated by a large nonessential region.

Frendewey, D., Li, R., Potashkin, J., Cold Spring Harbor Laboratory, New York: Isolation of a mutant defective in U2 RNA sysnthesis from *S. pombe*.

Swanson, M.S., Dreyfuss, G., Dept. of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, Illinois: Specific binding and ATP-dependent complex formation of hnRNP proteins at the 3′ end of introns.

Barnett, S.F., Friedman, D.L., LeStourgeon, W.M., Dept. of Molecular Biology, Vanderbilt University, Nashville, Tennessee: The C proteins of core 40S hnRNP particles exist as four tetramers of 3C1-1C2 with a Stokes' radius of 6.2 nm and an RNA-binding potential of 115 nucleotides per tetramer.

SESSION 8 ALTERNATIVE PROCESSING PATHWAYS

Chairman: A. Beyer, University of Virginia

Ge, H., Fu, X.-Y., Manley, J.L., Dept. of Biological Sciences, Columbia University, New York, New York: A varient U5 found in human 293 cells but not HeLa cells may influence alternative splicing of SV40 early pre-mRNA.

Smith, C.W.J., Gooding, C., Nadal-Ginard, B., Dept. of Cardiology, Children's Hospital, Howard Hughes Medical Institute, Boston, Massachusetts: Mechanism of alternative splicing in a α-tropomyosin—Mutually exclusive splicing is enforced by an abnormally positioned lariat branch point distance from the acceptor.

Helfman, D.M., Ricci, W.M., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: Studies of alternative RNA splicing of tropomyosin pre-mRNAs in vitro.

Cooper, T.A., Cardone, M.H., Ordahl, C.P., Dept. of Anatomy, University of California, San Francisco: *cis* requirements for alternative splicing.

Eng, F.J., Johnson, S.P., Warner, J.R., Depts. of Cell Biology and Biochemistry, Albert Einstein College of Medicine, Bronx, New York: Mechanism of the autogenous regulation of mRNA splicing by a yeast ribosomal protein.

Bingham, P., Chou, T.-B., Mims, I., Zachar, Z., Dept. of Biochemistry, State University of New York, Stony Brook: Analysis of autoregulation of a regulatory gene at the level of splicing.

Bell, L., Maine, E., Cline, T., Schedl, P., Dept. of Biology, Princeton University, New Jersey: Sex-specific splicing of a *Drosophila* sex-determination gene, sex-lethal, that shows sequence similarity of RNA-binding proteins.

Brady, H., Wold, W., Institute of Molecular Virology, St. Louis University School of Medicine, Missouri: Competition between splicing and polyadenylation determines which adenovirus region E3 mRNAs are synthesized.

Rivkin, E.,[1] Galli, G.,[1] Tucker, P.W.,[2] Nevins, J.R.,[3] [1]Howard Hughes Medical Institute, Rockefeller University, New York, New York; [2]Dept. of Microbiology, University of Texas, Southwestern Medical Center, Dallas; [3]Howard Hughes Medical Institute, Duke University Medical Center, Durham, North Carolina: Efficiency of poly(A)-site utilization, dictated by downstream sequence elements, determines immunoglobulin μ-gene control.

Peterson, M.L., Perry, R.P., Institute for Cancer Research, Philadelphia, Pennsylvania: On the developmentally regulated processing of immunoglobulin mRNA.

SESSION 9 RNA TRANSPORT AND TURNOVER

Chairman: D. Cleveland, Johns Hopkins University School of Medicine

Aebi, M.[1] Vijayraghavan, U.,[1] Jacobson, A.,[2] Abelson, J.,[1] [1]Division of Biology, California Institute of Technology, Pasadena; [2]University of Massachusetts Medical School, Worcester: A search for mutants of *S. cerevisiae* defective in transport of mRNA from the nucleus to the cytoplasm.

Hopper, A.K., Traglia, H.M., Dept. of Biological Chemistry, M.S. Hershey Medical Center, Pennsylvania State University, Hershey: Intracellular location of the yeast RNA1 protein.

von Gabain, A., Dept. of Bacteriology, Karolinska Institute, Stockholm, Sweden: The growth-dependent stability of *E. coli ompA* mRNA is regulated by a site-specific endonuclease.

Yen, T.J., Cleveland, D.W., Dept. of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland: Autoregulated degradion of β tubulin mRNA requires its presence on polysomes and is achieved through the recognition of nascent n-terminus of β tubulin.

Casey, J.L., Hentze, M.W., Koeller, D.M., Caughman, S.W., Rouault, T.A., Klausner, R.D., Harford, J.B., NICHD, National Institutes of Health, Bethesda, Maryland: Similar iron-responsive RNA elements are implicated in the control of transferrin receptor mRNA levels and ferritin mRNA translation.

Tatro, T.A., Zhang, Y., Schneider, R.J., Dept. of Biochemistry and Kaplan Cancer Center, New York University Medical Center, New York: Targeted degradation of RNAs by an AU-rich sequence is independent of translation, splicing, and polyadenylation.

Brewer, G., Ross, J., McArdle Laboratory for Cancer Research and Dept. of Pathology, University of Wisconsin, Madison: The c-*myc* mRNA decay rate is accelerated by labile cytosolic factor(s) in a cell-free mRNA decay system.

Binder, R., Hwang, S.-P.L., MacDonald, C.C., Williams, D.L., Dept. of Pharmacological Sciences, State University of New York, Stony Brook: Degradation of the estrogen-induced mRNA for chick apolipoprotein II occurs via cleavage at AAUs with loop structures of the 3′-noncoding region.

Munroe, D., Jacobson, A., Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester: Direct evidence for translational role for the poly(A) tract of mRNA.

SESSION 10 3′-END FORMATION

Chairman: J. Manley, Columbia University

Takagaki, Y., Ryner, L.C., Manley, J.L., Dept. of Biological Sciences, Columbia University, New York, New York: Separation and further purification of a poly(A) polymerase and a cleavage-specificity factor required for

pre-mRNA polyadenylation.

McDevitt, M.A., Gilmartin, G.M., Lakota, J., Nevins, J.R., Howard Hughes Medical Institute, Duke University Medical Center, Durham, North Carolina: Identification of multiple factors involved in poly(A)-site formation.

Virtanen, A.,[1] Gil, A.,[2] Sharp, P.A.,[2] [1]Dept. of Medical Genetics, Uppsala University, Sweden; [2]Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Characterization of factors responsible for cleavage and polyadenylation of pre-mRNA in HeLa nuclear extract.

Clements, J.B., Simpson, S., McLauchlan, J., MRC Virology Unity, University of Glasgow, Scotland: Temporal regulation of HSV poly (A)- site selection.

Moore, C., Chen, J., Whoriskey, J., Dept. of Molecular Biology and Microbiology, Tufts Medical School, Boston, Massachusetts: Two proteins cross-linked to RNA containing the adenovirus L3 polyadenylation site require the AAUAAA sequence for binding.

Wilusz, J., Shenk, T., Dept. of Molecular Biology, Princeton University, New Jersey: The downstream element of the polyadenylation signal is required for efficient cross-linking of hnRNP C proteins to polyadenylation substrate RNAs.

Wickens, M., Bardwell, V., Conway, L., Fox, C., Ogg, S., Sheets, M., Wigley, P., Zarkower, D., Dept. of Biochemistry, University of Wisconsin, Madison: Formation of SV40 late pre-mRNA 3′ termini in vitro—Complexes and critical features of the substrate.

Rose, S.D., Berget, S.M., Baylor College of Medicine, Houston, Texas: Evidence for classes of polyadenylation sites.

Marzluff, W.F.,[1] Pandey, N.,[2] [1]Dept. of Chemistry, [2]Institute of Molecular Biophysics, Florida State University, Tallahassee: Intervening sequences interfere with formation of 3′ ends of histone mRNAs.

Proudfoot, N.J., Levett, N.C., Briggs, D., Sir William Dunn School of Pathology, University of Oxford, England: A-rich sequences in RNA polymerase II termination.

Mowry, K.L., Oh, R., Steitz, J.A., Howard Hughes Medical Institute, Dept. of Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Connecticut: The mammalian U7 snRNP and at least one additional factor are required for 3′-end processing of mammalian histone pre-RNAs in vitro.

RNA Tumor Viruses

May 17—May 22

ARRANGED BY

Stephen Goff, Columbia University
Naomi Rosenberg, Tufts University Medical School

363 participants

The 1988 RNA Tumor Virus meeting brought together several hundred virologists, biochemists, geneticists, and clinicians to discuss progress in the field

of retroviruses. This year's meeting continued the trends of recent years in emphasis of two major areas: biochemical analysis of retroviral replication, on the one hand, and analysis of viral pathogenesis, on the other. Important progress was reported on the identification of the murine viral receptor; on the mechanism of integration of the proviral DNA, facilitated by the development of an in vitro recombination system; on the identification of "hot spots" in the cellular genome for retroviral integration; on the formation and translation of viral mRNAs; and on the assembly of virion particles, especially the selective encapsidation of genomic viral RNA. The interactions of the viruses with cellular oncogenes—both insertional activation and transduction—remained significant fields of study.

A large portion of the meeting was devoted to the biology of the human and simian retroviruses. Major debates centered around the mechanisms of regulation of expression of the viral gene products, under the control of (at a minimum) the *tat* and *rev* proteins. The identification of new gene products, perhaps with regulatory functions, encoded by selected isolates of the HIV family was reported. The rapid mutability of the viral genome continues to be documented, foreboding difficulties in preparation of vaccines. Specific alterations (truncations) of the SIV *env* gene that arise spontaneously were found to be essential for replication of the virus in culture; the complete gene was essential for full pathogenicity in animals.

M. Linial, G.S. Martin

SESSION 1 EARLY EVENTS: REVERSE TRANSCRIPTION AND INTEGRATION

Chairmen: **P. Brown,** Stanford University
H. Temin, University of Wisconsin

Leis, J.,[1] Baltimore, D.,[2] Bishop, J.M.,[3] Coffin, J.,[4] Fleissner, E.,[5] Goff, S.P.,[6] Oroszlan, G.,[7] Robinson, H.,[8] Skalka, A.M.,[9] Temin, H.M.,[10] Vogt, V.,[11] [1]Dept. of Biochemistry, Case Western Reserve University, School of Medicine, Cleveland, Ohio; [2]Massachusetts Institute of Technology, Cambridge; [3]University of California, San Francisco; [4]Tufts University School of Medicine, Boston, Massachusetts; [5]Memorial Sloan-Kettering Cancer Center, New York, New York; [6]Dept. of Biochemistry, Columbia University College of Physicians & Surgeons, New York, New York; [7]NCI-Frederick Cancer Research Facility, Frederick, Maryland; [8]University of Massachusetts Medical Center, Worcester; [9]Fox Chase Cancer Center, Institute for Cancer Research, Philadelphia, Pennsylvania; [10]McArdle Laboratory for Cancer Research, University of Wisconsin, Madison; [11]Section of Biochemistry, Cornell University, Ithaca, New York: A standardized and simplified nomenclature for proteins common to all retroviruses.

Albritton, L.A.,[1] Tseng, L.,[1] Kozak, C.A.,[2] Cunningham, J.,[1] [1]Howard Hughes Medical Institute and Dept. of Medicine, Brigham and Women's Hospital, Boston,

Massachusetts; [2]NIAID, National Institutes of Health, Bethesda, Maryland: Identification of the gene encoding the murine ecotropic retroviral receptor.

Tanese, N., Roth, M., Yang, W., Goff, S.P., Dept. of Biochemistry, Columbia University College of Physicians & Surgeons, New York, New York: Mutational analysis of the reverse transcriptase domain of Mo-MLV—Enzymes with DNA polymerase but no RNase H activity.

Fiore, D., Talbot, K., Panganiban, A., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: I. Ordered inter- and intrastrand DNA transfer during reverse transcription. II. A cell-specific determinant of retroviral DNA integration.

Bowerman, B., Brown, P.O., Bishop, J.M., Varmus, H.E., University of California, San Francisco: Biochemical and structural characterization of the nucleoprotein complex active in the integration of retroviral DNA.

Brown, P.,[1,2] Bowerman, B.,[2] Varmus, H.E.,[2] Bishop, J.M.,[2] [1]Stanford University, California; [2]University of California, San Francisco: Structure of the precursor and initial product of retroviral integration.

Fujiwara, T., Mizuuchi, K., NIDDK, National Institutes of Health, Bethesda, Maryland: Retroviral DNA integration—Structure of an integration intermediate.

Lee, Y.M.H., Coffin, J.M., Dept. of Molecular Biology and Microbiology, Tufts University of School of Medicine, Boston, Massachusetts: High-efficiency autointegration in vitro of avian retroviral DNA.

Shih, C.-C., Coffin, J.M., Dept. of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts: Characterization of highly preferred cellular target sites of retroviral integration in vivo.

Silver, J., Keerikatte, V., NIAID, National Institutes of Health, Bethesda, Maryland: Powerful method for analyzing proviral integration sites using the polymerase chain reaction.

Barklis, E.,[1] Comer, J.,[1] Sobel, S.,[2] Jaenisch, R.,[2] [1]Oregon Health Sciences University, Portland; [2]Whitehead Institute, Cambridge, Massachusetts: Integration sites permitting retroviral expression in embryonal carcinoma cells.

SESSION 2 LATE EVENTS: TRANSCRIPTION, SPLICING, RNA PROCESSING

Chairmen: **H. Fan,** University of California, Irvine
A. Skalka, Fox Chase Cancer Center

Gama Sosa, M.A., Rosas, H.D., Ruprecht, R.M., Division of Cancer Pharmacology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Slipped DNA structures in the LTR enhancer region of Mo-MLV.

Flanagan, J.R.,[1] Krieg, A.M.,[2] Max, E.E.,[1] Khan, A.S.,[1] [1]NIAID, [2]NIAMSD, National Institutes of Health, Bethesda, Maryland: Murine and human nuclear factors bind a highly conserved sequence at the 5′ end of the MLV LTR—Evidence for a negative control region.

Manley, N., O'Connel, M., Sharp, P., Hopkins, N., Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Identification of novel cellular proteins that bind specifically to MLV enhancer regions.

Lenz, J., Okenquist, S., Boral, A., Cupelli, L., Morrison, H., LoSardo, J., Dept. of Genetics, Albert Einstein College of Medicine, Bronx, New York: Identification of cell-type-specificity-determining elements in MLV LTRs.

Dutta, A., Dorai, T., Hanafusa, H., Rockefeller University, New York, New York: The putative *trans*-activator in the *gag* region of RSV is not required for transformation of primary CEFs.

Katz, R.A., Kotler, M., Skalka, A.M., Fox Chase Cancer Center, Institute for Cancer Research, Philadelphia, Pennsylvania: Genetic selection strategy for identification of sequences required for regulated ASV splicing.

Stoltzfus, C.M., Berberich, S.L., Fogarty, S.J., Dept. of Microbiology, University of Iowa, Iowa City: Deletions in the RSV v-*src* intron affect spliced to unspliced RNA levels—Correlation with infectivity.

Arrigo, S., Beemon, K., Dept. of Biology, Johns Hopkins University, Baltimore, Maryland: Negative regulation of RSV RNA splicing.

Feng, Y.X.,[1] Hatfield, D.,[2] Rein, A.,[3] Levin, J.G.,[1] [1]NICHD, [2]LEC, NCI, National Institutes of Health, [3]NCI-Frederick Cancer Research Facility, Frederick, Maryland: Analysis of tRNA involved in suppression of the MLV amber codon at the *gag-pol* junction.

Derse, D., NCI-Frederick Cancer Research Facility, Frederick, Maryland: *cis*- and *trans*-Acting regulation of BLV mRNA 3′-end formation.

Swain, A., Coffin, J.M., Dept. of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts: Correct polyadenylation is not required for retroviral replication.

SESSION 3 POSTER SESSION: Receptors, Viral Genes, and Expression

Basu, S., Varmus, H.E., Dept. of Microbiology and Immunology, University of California, San Francisco: Studies on the Mo-MLV integrase produced in yeast.

Blair, D.G., Dunn, K.J., O'Hara, B.M., NCI-Frederick Cancer Research Facility, Frederick, Maryland; Lederle Laboratories, Pearl River, New York: Glycosylation inhibition alters retroviral receptor specificity on mouse cells.

Sommerfelt, M.A.,[1] Williams, B.P.,[2] Goodfellow, P.N.,[2] Weiss, R.A.,[1] [1]Institute of Cancer Research, [2]Imperial Cancer Research Fund, London, England: Receptors for retroviruses on human cells.

Goodrich, D., Duesberg, P., Dept. of Molecular Biology, University of California, Berkeley: Retroviruses use RNA template switching for homologous, but not for nonhomologous, recombination.

Posters

Pryciak, P.M., Varmus, H.E., Depts. of Biochemistry and Biophysics, and Microbiology and Immunology, University of California, San Francisco: In vitro dissection of the mechanism of MLV replicative restriction by the mouse *Fv-1* locus.

Chalker, D.L., Haywood, L.J., Sandmeyer, S.B., Dept. of Microbiology and Molecular Genetics, University of California, Irvine: Position-specific transposition of a yeast retrotransposon, Ty3.

Haywood, L., Chalker, D., Sandmeyer, S., University of California, Irvine: The yeast retrotransposon, Ty3, is homologous to animal retroviruses.

Youngren, S.D., Garfinkel, D.J., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Organization of the *TyB* gene from the *S. cerevisiae* retrotransposon Ty1.

McClure, M.A., Johnson, M.S., Feng, D.-F., Doolittle, R.F., Dept. of Chemistry and Center for Molecular Genetics, University of California, San Diego: Sequence comparisons of retroviral proteins—Relative rates of change, recombination, and general phylogeny.

Moustakas, A., Hackett, P.B., Dept. of Genetics and Cell Biology, University of Minnesota, St. Paul: Modification of the first and second open reading frames on RSV RNA affects viral propagation.

Shoji, A., Park, H.T., Kaji, A., Dept. of Microbiology, University of Pennsylvania School of Medicine, Philadelphia: Unusual properties of RSV particles produced at early period postinfection—Presence of viral DNA and reduced amount of *env* protein.

Pryciak, P.M., Jacks, T.E., Varmus, H.E., Depts. of Biochemistry and Biophysics, and Microbiology and Immunology, University of California, San Francisco: Mutations in the site of ribosomal frameshifting in RSV and their effects on viral replication.

Felsenstein, K.M., Goff, S.P., Dept. of Biochemistry, Columbia University, College of Physicians & Surgeons, New York, New York: RNA sequence requirements for *pol* gene expression via translational readthrough of the *gag* terminator codon in Mo-MLV.

Donehower, L.A., Dept. of Virology and Epidemiology, Houston, Texas: Analysis of linker insertion mutants in the integrase-encoding region of Mo-MLV.

Mumm, S., Grandgenett, D., St. Louis University, Institute for Molecular Virology, Missouri: Synthesis of avian retrovirus pp32 protein by in vitro translation-purification and mapping of DNA-binding domains.

Karnitz, L., Ip, T., Chalkley, R., Dept. of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, Tennessee: Chromatin structure of the 5′ end of the RSV provirus in BHK cells.

Speck, N., Renjifo, B., Golemis, E., Hartley, J., Hopkins, N., Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, and NCI, National Institutes of Health, Bethesda, Maryland: Functional contribution of enhancer-binding nuclear factors to the transcriptional activity of the Mo-MLV enhancer.

Shimkus, M., Boulden, A., Sealy, L., Dept. of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, Tennessee: *trans*-Acting factors that interact with the RSV LTR enhancer.

Kitado, H., Fan, H., Dept. of Molecular Biology and

Biochemistry, University of California, Irvine: Chromatin structure of chimeric Mo-MLV proviruses containing pX-responsive sequences from HTLV-II.

Mondal, D., Prakash, O., Laboratory of Molecular Oncology, Alton Ochsner Medical Foundation, New Orleans, Louisiana: Regulation of MMTV LTR-directed gene expression by phorbol esters.

Gowland, P., Diggelmann, H., Buetti, E., Swiss Institute for Experimental Cancer Research, Epalinges, Switzerland: Mutations in the hormone regulatory element of MMTV differentially affect the response to progestins and androgens as compared to glucocorticoids.

Golemis, E.,[1] Li, Y.,[2] Hartley, J.W.,[3] Hopkins, N.,[1] [1]Center for Cancer Research, Massachusetts Institute of Technology, Cambridge; [2]New England Regional Primate Center, Harvard Medical School, Southboro, Massachusetts; [3]NCI, National Institutes of Health, Bethesda, Maryland: Disease specificity of MLVs—Interactions between discrete segments within the enhancer are involved in targeting.

Theunissen, H.J.M., Paardekooper, M., Michalides, R.J.A.M., Nusse, R., Dept. of Molecular Biology, Antoni van Leeuwenhoekhuis, Netherlands Cancer Institute, Amsterdam: TPA-inducible cell-type-specific expression of MMTV variants.

van Klaveren, P., Kneppers, A.L.J., Bentvelzun, P., Dept. of Retrovirology, Radiobiological Institute TNO, Rijswijk, The Netherlands: *trans*-Activating potential of the LTR of the MMTV.

Dorn, P.L.,[1] Derse, D.,[2] [1]NCI and Dept. of Zoology, University of Maryland, College Park; [2]NCI-Frederick Cancer Research Facility, Frederick, Maryland: *cis*- and *trans*-Acting regulation of gene expression of EIAV.

Crowell, R.C.,[1] Wolfes, H.,[2] Cooper, G.M.,[2] Kiessling, A.A.,[1] [1]Dept. of Obstetrics, Gynecology, and Reproductive Biology, [2]Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: The epididymis is a preferred site of synthesis of a unique retroviral transcript.

Tupper, J., Diem, K., Yoshimura, F., Dept. of Bio Structure, University of Washington, Seattle: Differences in protein-DNA complexes generated by LTR sequences of MLVs with different pathogenicities.

Falzon, M., Kuff, E.L., NCI, National Institutes of Health, Bethesda, Maryland: Characterization of nuclear protein-binding domains within the mouse intracisternal A-particle LTR.

Villar, C.J., Kozak, C.A., NIAID, National Institutes of Health, Bethesda, Maryland: Transcriptional activity of the MCF-related proviruses of *Mus spretus* and their potential for recombination.

Brack-Werner, R.,[1] Barton, D.E.,[4] Werner, T.,[2] Foellmer, B.E.,[4] Leib-Mösch, C.,[3] Francke, U.,[4] Erfle, V.,[1] Hehlmann, R.,[3] [1]Abt. f. Molekulare Zellpatholgie, [2]Institut f. Saeugetiergenetik, Neuherberg, [3]Medizinische Poliklinik der Universitaet Muenchen, Munich, Federal Republic of Germany; [4]Dept. of Human Genetics, Yale University School of Medicine, New Haven, Connecticut: A human endogenous LTR-like sequence is located on chromosome 18Q21.

Fredholm, M.,[1,2] Policastro, P.F.,[1] Wilson, M.C.,[1] [1]Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California; [2]Dept. of Animal Genetics, Royal Veterinary and Agricultural University, Copenhagen, Denmark: Recombination and transposition of murine endogenous retroviral sequences.

Boone, L.R.,[1] Glover, P.L.,[1] Innes, C.L.,[1] Niver, L.A.,[1] Bondurant, M.C.,[2] Yang, W.K.,[3] [1]National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina; [2]Veterans Administration Medical Center, Nashville, [3]Oak Ridge National Laboratory, Tennessee: Analysis of *Fv-1* N- and B-tropic-specific sequences in MLV and related endogenous proviral genomes.

Olsen, H.S., Lovmand, S., Baekgard, A., Dai, H.Y., Jorgensen, P., Kjeldgaard, N.O., Pedersen, F.S., Dept. of Molecular Biology, University of Aarhus, Denmark: Transcriptional control of Akv and related MLVs—Role of nuclear-factor-I-like DNA-binding activity.

SESSION 4 REGULATION: *TAT* AND *ART*

Chairmen: **W. Haseltine,** Dana Farber Cancer Institute, Harvard Medical School
P. Jolicoeur, Clinical Research Institute of Montreal

Dokhelar, M.C., Sodroski, J., Haseltine, W., Dana-Farber Cancer Institute, Boston, Massachusetts: HTLV-I p27 protein regulates *gag-env* and *tat* expression.

Tan, T.-H., Roeder, R.G., Rockeller University, New York, New York: Identification and purification of nuclear factors interacting with HTLV-I *tat*-I-responsive element within the HTLV-I LTR.

Ruben, S.,[1] Poteat, H.,[2] Tan, T.H.,[3] Hurst, H.,[4] Hazeltine, W.,[2] Roeder, R.,[3] Jones, N.,[4] Rosen, C.,[1] [1]Roche Institute of Molecular Biology, Nutley, New Jersey; [2]Dana-Farber Cancer Institute, Boston, Massachusetts; [3]Rockefeller University, New York, New York; [4]Imperial Cancer Research Fund, London, England: Identification and characterization of transcription factors required for regulation of viral and cellular gene expression by the HTLV-I *tax* protein.

Chen, I.S.Y., Rosenblatt, J., Lugo, J., Williams, J., Depts. of Medicine and Microbiology and Immunology, University of California School of Medicine, Los Angeles: Regulation of HTLV gene expression.

Ikawa, Y.,[1] Katoh, I.,[1] Shoji, A.,[1] Yoshinaka, Y.,[2] [1]Tsukuba Life Science Center, Riken, [2]Japan Immunoresearch Laboratories, Takasaki: BLV *tat* protein *trans*-activates heterologous promoters with cAMP-responsive elements.

Seiki, M., Inoue, J., Hidaka, M., Yoshida, M., Dept. of Viral Oncology, Cancer Institute, Tokyo, Japan: A unique posttranscriptional mechanism controls viral mRNA levels and replication of HTLV-I.

Williams, J.L., Chen, I.S.Y., Depts. of Medicine and Microbiology and Immunology, University of California School of Medicine, Los Angeles: Studies of heterologous promoter *trans*-activation by the HTLV-II *x* protein.

Ohta, M., Akagi, T., Nyunoya, H., Tanaka, H., Okamoto, T., Shimotohno, K., Division of Virology, National Cancer Center Research Institute, Tokyo, Japan: The *rex* gene products of HTLV-II may be involved in stabilization of the unspliced viral RNAs.

Ruben, S.,[1] Perkins, A.,[1] Purcell, R.,[2] Rosen, C.,[1] [1]Dept. of Molecular Oncology, Roche Institute of Molecular Biology, [2]Dept. of Protein Chemistry, Roche Research Center, Nutley, New Jersey: Mutational analysis of the HIV *trans*-acting regulatory proteins *tat* and *art*.

Kao, S.-Y., Selby, M.J., Peterlin, B.M., Howard Hughes Medical Institute, Dept. of Medicine, University of California, San Francisco: Transcriptional elongation effect of HIV-1 by *tat* gene product.

Dayton, A.I., Terwilliger, E.,[1] Potz, J., Rosen, C.,[2] Kowalski, M., Haseltine, W.A.,[1] [1]Dana-Farber Cancer Institute, Boston, Massachusetts; [2]Dept. of Molecular Oncology, Roche Institute of Molecular Biology, Nutley, New Jersey: On the nature of *ART/CAR*—The second *trans*-activation axis of HIV-1.

Ahmad, N., Mervis, R.J., Venkatesan, S., NIAID, National Institutes of Health, Bethesda, Maryland: The B(3′)-ORF product of HIV suppresses transcription from HIV LTR containing the negative regulatory element.

SESSION 5 RNA PACKAGING, VIRAL VECTORS, AND GENE TRANSFER

Chairmen: **M. Linial,** Fred Hutchinson Cancer Research Center
H. Hanafusa, Rockefeller University

Cobrinik, D., Leis, J., Dept. of Biochemistry, Case Western Reserve University School of Medicine, Cleveland, Ohio: A retroviral RNA secondary structure near the primer-binding site is required for efficient priming of reverse transcriptase.

Malone, R.,[1] Felgner, P.,[2] Verma, I.M.,[1] [1]Salk Institute and Dept. of Biology, University of California, San Diego, La Jolla, [2]Syntex Corporation, Palo Alto: A novel approach to study packaging of retroviral RNA by RNA transfection.

Danos, O., Mulligan, R.C., Whitehead Institute for Biomedical Research and Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Safe and efficient generation of recombinant retroviruses with amphotropic and ecotropic host range.

Markowitz, D., Goff, S., Bank, A., Dept. of Genetics and Development, Biochemistry and Molecular Biophysics, and Medicine, Columbia University, College of Physicians & Surgeons, New York, New York: Construction of a safe and efficient amphotropic retrovirus-packaging cell line.

Shackleford, G., Varmus, H., Dept. of Microbiology and Immunology, University of California, San Francisco: Construction of a fully clonable provirus that produces infectious, tumorigenic MMTV and derivation of an MMTV vector.

Dornburg, R., Temin, H.M., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: A retroviral vector system to study formation of cDNA genes.

Aronoff, R.,[1] Adam, M.,[1] Katz, R.,[2] Miller, D.,[1] Linial, M.,[1] [1]Fred Hutchinson Cancer Research Center, Seattle, Washington; [2]Institute for Cancer Research, Philadelphia, Pennsylvania: Development and analysis of retrofection in avian and mammalian cell lines.

von Ruden, T.,[1] Gilboa, E.,[2] [1]EMBL, Heidelberg, Federal Republic of Germany; [2]Memorial Sloan-Kettering Cancer Center, New York, New York: Inhibition of HTLV-I replication in primary human T cells expressing antisense RNA.

Lobel, L.I., Schwartzberg, P., Parker, R., Dept. of Biochemistry, Columbia University, College of Physicians & Surgeons, New York, New York: Construction of retroviral vectors for the isolation of constitutive or inducible promoters of a Mud-*lac*-like vector for murine cells.

SESSION 6 POSTER SESSION: Regulation, Expression, and Gene Transfer

Ratner, L., Niederman, T., Dept. of Medicine, Washington University, St. Louis, Missouri: Analysis of the functions of the HIV-1 R and F gene products.

Viglianti, G.A., Mullins, J.I., Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts: SIV and HIV-1 *tat* have different requirements for activity.

Bacheler, L., Strehl, L., Ferguson, B., Neubauer, R., Eustice, D., Dept. of Medical Products, E.I. du Pont de Nemours & Co., Inc., Wilmington, Delaware: Heterologous gene expression under HIV LTR/*tat* control.

Ahmad, N., Mervis, R.J., Lillehof, E.P., and Venkatesan, S., NIAID, National Institutes of Health, Bethesda, Maryland: *art/trs* protein of HIV is essential for splicing and/or transport of HIV transcripts containing *env* sequences.

Jeang, K.-T.,[1] Shank, P.,[2] Rabson, A.,[3] Kumar, A.,[4] [1]Laboratory of Molecular Virology, [2]Division of Biology and Medicine, Brown University, [3]Laboratory of Molecular Microbiology, [4]NIAID, Dept. of Biochemistry, George Washington University, Washington, D.C.: Expression of HIV *trans*-activator protein in baculovirus vector system.

Pavlakis, G.N., Felber, B.K., Cladaras, M., Cladaras, C., Wright, C.M., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Feedback regulation of HIV-1 gene expression by *trs*.

Cann, A., Koyanagi, Y., Zack, J., Chen, I.S.Y., Depts. of Medicine and Microbiology and Immunology, University of California School of Medicine, Los Angeles: Regulation of HIV gene expression in primary isolates.

Cochrane, A.,[1] Terwilliger, E.,[2] Hazeltine, W.,[2] Rosen, C.,[1] [1]Dept. of Molecular Oncology, Roche Institute of

Molecular Biology, Nutley, New Jersey; [2]Dana-Farber Cancer Institute, Boston, Massachusetts: Localization of intragenic *cis*-acting repressive and *art*-responsive sequences within the HIV genome.

Garcia, J.A., Harrich, D., Wu, F., Jackson, D., Gaynor, R.B., Dept. of Medicine, University of California School of Medicine, Los Angeles: Characterization of DNA-binding proteins and regulatory sequences involved in HIV transcriptional regulation.

Weighous, T.F.,[1] Tomich, C.-S.C.,[2] Tarpley, W.G.,[1] [1]Cancer and Infectious Diseases Research, [2]Molecular Biology Research, Upjohn Company, Kalamazoo, Michigan: Synthesis at high levels in *E. coli* of a soluble, truncated HIV *trans*-activator.

Radonovich, M., Jeang, K.-T., Rasheed, S., Zhou, J.-T., NCI, National Institutes of Health, Bethesda, Maryland: Activation of the HTLV-I LTR by phorbol ester.

Inoue, J., Seiki, M., Fujisawa, J., Yoshida, M., Dept. of Viral Oncology, Cancer Institute, Tokyo, Japan: Further studies on *trans*-activation of IL-2 receptor (Tac antigen) gene expression by $p40^{tax}$ of HTLV-1.

Gore, I.,[1] Hui, H.,[1] Wong-Staal, F.,[2] Gallo, R.C.,[2] Shaw, G.M.,[1] Hahn, B.H.,[1] [1]Dept. of Medicine, University of Alabama, Birmingham; [2]NCI, National Institutes of Health, Bethesda, Maryland: Molecular characterization of a variant of HTLV-I with sequence homology with normal human DNA.

Lagarias, D., Radke, K., Dept. of Avian Sciences, University of California, Davis: Transcriptional activation of BLV in blood cells from infected animals.

Krump-Konvalinkova, V.,[1] Gilboa, E.,[2] [1]Radiobiological Institute TNO, Rijswijk, The Netherlands; [2]Memorial Sloan-Kettering Cancer Center, New York, New York: Killer vectors—Retroviral vectors inhibiting the growth of HTLV-I *trans*-activation protein producing cells.

Bovenhoff, M.E., Visser, T.P., de Groene, E.M., Dorssers, L.C.J., Valerio, D., Wagemaker, G., Dept. of Radiobiology, Erasmus University and Radiobiological Institute TNO, Rijswijk, The Netherlands: Friend-virus-derived retroviral vectors for gene transfer into hematopoietic stem cells.

Noël, G.,[1] Zillinger, L.,[1] Laliberté, F.,[1] Crine, P.,[1] Boileau, G.,[1] Rassart, E.,[2] [1]Dép. de biochimie, Université de Montréal, [2]Dép. Sciences biologiques, Universite du Québec, Montréal, Canada: Expression and processing of pro-opiomelanocortin in neural cell lines using a retroviral vector.

Bradac, J.A., Hughes, S.H., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Replication enhancement of avian retroviral vectors containing the polymerase gene derived from the Bryan high-titer strain of RSV.

Hunt, L.A.,[1] Brown, D.W.,[2] Robinson, H.L.,[2] Naeve, C.W.,[3] Webster, R.G.,[3] [1]University of Louisville School of Medicine, Kentucky; [2]University of Massachusetts Medical Center, Worcester; [3]St. Jude Children's Research Hosptial, Memphis, Tennessee: An avian leukosis virus vector expressing H7 hemagglutinin protects chickens against lethal influenza infections.

Hantzopolous, P., Bordignon, C., Smith, C.A., Yu, S.F., Ungers, G.E., O'Reilly, R., Gilboa, E., Memorial Sloan-Kettering Cancer Center, New York, New York: Retroviral vector-mediated expression of adenosine deaminase in long-term bone marrow cultures from patients affected by the ADA-deficient variant of severe combined immunodeficiency.

Evrard, C., Galiana, E., Rouget, P., Laboratoire de Biochimie Cellulaire, Collége de France, Paris: Establishment of permanent and genetically marked neural cell lines after oncogene transfection or retroviral vector transduction.

Morgenstern, J.P., Land, H., Imperial Cancer Research Fund, London, England: An advanced retrovirus-based gene-transfer system for mammalian cells.

Young, J.A.T., Bates, P., Varmus, H.E., Dept. of Microbiology and Immunology, University of California, San Francisco: An attempt to alter retroviral tropism specifically using *egf*-envelope chimeras.

Alford, R.L.,[1] MacGregor, G.R.,[2] Moore, K.A.,[2] Fletcher, F.A.,[2] Hawkins, D.,[3] Caskey, C.T.,[1,2,3] Belmont, J.W.,[2,3] [1]Dept. of Biochemistry, [2]Institute for Molecular Genetics, Baylor College of Medicine, [3]Howard Hughes Medical Institute, Houston, Texas: Long-term expression of human adenosine deaminase in murine hematopoietic cells by retroviral vector-mediated gene transfer.

Kerner, N., Decaux, J.F., Kahn, A., Weber-Benarous, A., INSERM, Paris, France: Expression and regulation of a hepatic gene transferred into primary culture of adult rat hepatocytes using a retroviral vector.

Scadden, D., Cunningham, J., Howard Hughes Medical Institute and Dept. of Medicine, Brigham and Women's Hospital, Boston, Massachusetts: Identification of a defective MCF RNA efficiently packaged into recombinant retroviruses.

Murphy, J.E., Kalnik, S.T., Goff, S.P., Dept. of Biochemistry, Columbia University, College of Physicians & Surgeons, New York, New York: Analysis of *cis*-acting sequences in the 5′-noncoding regions of Mo-MLV.

SESSION 7 VIRAL PROTEINS

Chairmen: **V. Vogt,** Cornell University
J. Coffin, Tufts University

Wills, J.W., Weldon, R.A., Jr., Craven, R.C., Achacoso, J.A., Dept. of Biochemistry and Molecular Biology, Louisiana State University Medical School, Shreveport: Creation of novel myristic addition sites on the RSV *gag* gene product and their expression in mammalian cells.

Rhee, S.S., Hunter, E., Dept. of Microbiology, University of Alabama, Birmingham: Mutagenic analysis of the membrane-binding protein, p10, of MPMV.

Jentoft, J., Fu, X.-D., Smith, L., Johnson, M., Leis, J., Dept. of Biochemistry, Case Western Reserve University, School of Medicine, Cleveland, Ohio: Conserved Cys and His residues of avian retrovirus nucleocapsid protein

J. Coffin, J. Stoye

are essential for viral replication but do not form Zn-binding fingers.

Meric, C., Fisher, J., Goff, S.P., Dept. of Biochemistry, Columbia University, College of Physicians & Surgeons, New York, New York: Site-directed mutagenesis of the nucleocapsid p10 of Mo-MLV.

Gorelick, R.J., Henderson, L.E., Rein, A., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Point mutations preventing encapsidation of Mo-MLV RNA—Evidence for role of "finger-like" sequence in recognition of genomic RNA.

Darlix, J.L., Prats, A.C., Constantin, S., Bieth, E., Gabus, C., Centre de Recherche de Biochimie et Génétique Cellulaires, CNRS, Toulouse, France: Small finger protein of avian and murine retroviruses causes two RNA conformational changes required for the production of infectious viral particles.

Vogt, V.M., Schatz, G., Putterman, D., Section of Biochemistry, Cornell University, Ithaca, New York: Viral protease and *gag* protein cleavage in ASV and ALV.

Loeb, D.,[1] Hutchison, C.,[1] Stamper, S.,[1] Edgell, M.,[1] O'Bryan, J.,[2] Everitt, L.,[2] Swanstrom, R.,[2] Depts. of [1]Microbiology, [2]Biochemistry, University of North Carolina, Chapel Hill: Genetic analysis of the HIV-1 protease.

Vicenzi, E., Obata, M., Garon, C., Khan, A.S., NIAID, National Institutes of Health, Bethesda, Maryland: Isolation and characterization of MLV-related "solo" *pol* genes.

Dong, Y.-Y., Dubay, J.W., Perez, L.G., Hunter, E., Dept. of Microbiology, University of Alabama, Birmingham: Mutations in the proteolytic cleavage site of the RSV envelope glycoprotein.

Einfeld, D., Hunter, E., Dept. of Microbiology, University of Alabama, Birmingham: The RSV glycoprotein complex is an oligomeric structure.

Tsai, W.-P., Oroszlan, S., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Identification of novel glycosylation pathways and characterization of an Endo-H-resistant *env* precursor polyprotein in avian REV.

SESSION 8 ONCOGENES

Chairmen: **G.S. Martin,** University of California, Berkeley
N. Teich, Imperial Cancer Research Fund

Engelman, A., Rosenberg, N., Dept. of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts: Ab-MLV mutants temperature-sensitive for transformation are defective in kinase activity.

Franz, W.M., Wang, J.Y.J., Dept. of Biology, University of California, San Diego, La Jolla: Deletion of the tyrosine kinase regulatory domain of the *c-abl* protein activates its oncogenic potential.

Poirier, Y., Jolicoeur, P., Clinical Research Institute of Montreal, Canada: Difference in helper-virus requirements between Abelson-induced pre-B- and T-cell lymphomas.

Green, P.L., Kaehler, D.A., Bennett, L.M., Risser, R., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Clonal selection and progression in Ab-MLV tumorigenesis.

Hoffmann, F.M., Henkemeyer, M.J., Bennett, R.L., Risser, R., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: The Abelson tyrosine kinase of

Drosophila—Fly/mammalian chimeric genes function in transformation of mouse cells and in the terminal differentiation of neural cells of *Drosophila.*

DeClue, J.E., Martin, G.S., University of California, Berkeley: Linker insertion mutagenesis of the v-*src* gene—Isolation of host- and temperature-dependent mutants.

Fuhrmann, U., Ness, S., Beug, H., Differentiation Programme, European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: Oncogenic potential of the v-*fms* oncogene in the chicken hematopoietic system.

Lipsick, J., Garcia, A., Grasser, F., Ibanez, C., Lane, T., Stober-Grasser, U., Veteran's Administration Medical Center and Dept. of Pathology, University of California, San Diego, La Jolla: Molecular genetics of the v-*myb* of AMV.

Neuberg, M.,[1] Lucibello, F.C.,[1] Hunter, J.B.,[1] Jenuwein, T.,[1] Wallich, R.,[1] Schuermann, M.,[1] Stein, B.,[2] Schönthal, A.,[2] Herrlich, P.,[2] Müller, R.,[1] [1]European Molecular Biology Laboratory, Heidelberg, [2]Institut für Genetik und Toxikologie Kernforschungszentrum Karlsruhe, Federal Republic of Germany: *trans*-Activation of gene expression by *fos* proteins—Involvement of a binding site for the transcription factor AP-1.

Judelson, C., Boucher, P., Privalsky, M., Depts. of Bacteriology and Biochemistry and Biophysics, University of California, Davis: Determinants of the AEV genome involved in erythroid transformation—Dissection of the v-*erb* A and v-*erb* B oncogenes.

Bar-Sagi, D.,[1] Cockcroft, S.,[2] [1]Cold Spring Harbor Laboratory, New York; [2]Dept. of Experimental Pathology, University College London, England: H-*ras* proteins can stimulate polyphosphoinositide phosphodiesterase activity in HL-60 membranes.

SESSION 9 POSTER SESSION: Viral Proteins, Pathogenesis, and Oncogenesis

Prats, A.C., DeBilly, G., Darlix, J.L., Labo-Tetro, Centre de Recherche de Biochimie et de Genetique Cellulaires, CNRS, France: Translation initiation of the novel CUG codon in MLV and RNA sequences involved in translation and encapsidation regulations.

Hecht, S., Coffin, J.M., Tufts University School of Medicine, Boston, Massachusetts: Expression and processing of the retroviral *gag* gene in *E. coli.*

Christensen, J.R., Balduzi, P.C., Ooi, P., Dept. of Microbiology and Immunology, University of Rochester, New York: Removal of nonexpressed *env* sequences from ASV U R2 produces a defect in viral replication (encapsidation?).

Mitchell, T.C., Risser, R., McArdle Laboratory, University of Wisconsin, Madison: Inducible cell fusion and viral interference with controlled expression of MLV envelope glycoproteins.

Morisson, M.,[1] Mamoun, R.Z.,[1] Rebeyrotte, N., Busetta, B.,[2] Hospital, M.,[2] Guillemain, B.,[1] [1]INSERM, Bordeaux, [2]CNRS, Bordeaux, France: Localization of antigenic epitopes of the BLV glycoproteins using a tridimensional model.

Kotler, M., Katz, R.A., Burstein, H., Skalka, A.M., Forx Chase Cancer Center, Philadelphia, Pennsylvania: Activity of ASV protease expressed in *E. coli.*

Culp, P., Talbott, R., Trauger, R., Wilson, M., Elder, J., Research Foundation of Scripps Clinic, La Jolla, California: Characterization of the retroviral repertoire of a highly tumorigenic cell line derived from a spontaneous AKR lymphoma.

Miura, T., Tsujimoto, H., Shibuya, M., Fukasawa, M., Hayami, M., Institute of Medical Science, University of Tokyo, Japan: Molecular cloning and partial sequencing of a FeLV provirus integrated adjacent to the c-*myc* gene in feline T-cell leukemia cell line.

Brightman, B.K., Davis, B.R., Fan, H., Dept. of Molecular Biology and Biochemistry, University of California, Irvine: Study of preleukemima induced by Mo-MLV.

Martinelli, S.C., Koehne, C.F., Alves, K., O'Donnell, P.V., Memorial Sloan-Kettering Cancer Center, New York, New York: Genetic characterization of multistage leukemogenesis in AKR mice.

Racevskis, J., Beyer, H., Depts. of Oncology and Medicine, Montefiore and Albert Einstein Medical Centers, Bronx, New York: Presence of amplified MMTV proviruses with altered LTRs in a pituitary tumor cell line.

Ru, M.,[1] Zheng, B.-F.,[2] Pattengale, P.K.,[3] Fan, H.,[1] [1]Dept. of Molecular Biology and Biochemistry, University of California, Irvine: [2]Dept. of Biophysics, Shanghai Medical University, People's Republic of China; [3]Dept. of Pathology, University of Southern California School of Medicine, Los Angeles: MLVs that induce erythroid and myeloid leukemia—The SRS complex.

Runnels, J., Rosenberg, N., Dept. of Pathology, Tufts University School of Medicine, Boston, Massachusetts: Mo-MLV immortalizes B lymphocytes in vitro.

Ruscetti, S.,[1] Ruscetti, F.,[2] [1]NCI, National Institutes of Health, Bethesda, [2]NCI-Frederick Cancer Research Facility, Frederick, Maryland: Apparent Epo independence of SFFV-infected erythroid cells is not due to Epo production or change in Epo receptors.

Smith, E.J., Crittenden, L.B., Fadly, A.M., USDA-Agricultural Research Service, Regional Poultry Research Laboratory, East Lansing, Michigan: Influence of host-cellular resistance genes on congenital transmission of endogenous virus (EV21) and induction of tolerance to exogenous ALVs.

Paquette, Y., Hanna, Z., Savard, P., Jolicoeur, P., Clinical Research Institute of Montreal, Canada: Determinant of paralysis of the neurotropic Cas-Br-E MLV maps within *env.*

Zhang, J., Bose, H.R., Jr., Dept. of Microbiology, University of Texas, Austin: Acquisition of additional proviral copies in transformed lymphoid cell lines persistently infected by REV.

Sitbon, M.,[1] Nishio, J.,[2] Hayes, S.F.,[3] Wehrly, K.,[2] Pozo, F.,[1] Evans, L.H.,[2] Tambourin, P.,[1] Chesebro, B.,[2] [1]INSERM, Hôpital Cochin, Paris, France; [2]LPVD, [3]LBP, National Institutes of Health, Rocky Mountain Laboratories, Hamilton, Montana: Early and late pathogenic effects of Fr-MLV and Mo-MLV are influenced by different viral sequences.

Posters

Voytek, P., Kozak, C., NIAID, National Institutes of Health, Bethesda, Maryland: Pathological evaluation and sequence analysis of a wild mouse MLV from *Mus hortulanus*.

Contag, C.H., Plagemann, P.G.W., Dept. of Microbiology, University of Minnesota, Minneapolis: Increased replication of endogenous MLV as a predisposing factor to lactate-dehydrogenase-elevating virus-induced polioencephalomyelitis.

Rassart, E., Bergeron, R., Lambert, J., Poliquin, L., Dép. Sciences biologiques, Université du Québec, Canada: Leukemogenic potential of the envelope gene of the passaged RadLV BL/VL3.

Boyce-Jacino, M., Faras, A.J., Institute of Human Genetics and Dept. of Microbiology, University of Minnesota, Minneapolis: Structure and conservation of the *env*-gene-like region of the novel avian endogenous retrovirus EV-0.

Frankel, W.N., Stoye, J.P., Coffin, J.M., Tufts University School of Medicine, Boston, Massachusetts: Genetics of endogenous nonecotropic murine proviruses—Linkage analysis in recombinant inbred mice.

Amanuma, H., Watanabe, N., Ikawa, Y., Tsukuba Life Science Center, Institute of Physical and Chemical Research, Japan: Essential role of a deletion in the sequence of gp55 of Fr-SFFV for its biological activity.

Ruddell, A., Linial, M., Groudine, M., Fred Hutchinson Cancer Research Center, Seattle, Washington: Liability of LTR enhancer-binding proteins in ALV lymphomagenesis.

Siegel, M.,[1] Brown, C.,[1] Siden, E.,[2] [1]University of Florida College of Medicine, Gainesville; [2]Mt. Sinai School of Medicine, New York, New York: Midgestation hematopoietic development—Comparison of normal precursors and Ab-MLV-transformed cells.

Glass, D.J., Rees-Jones, R., Depts. of Biochemistry and Medicine, Columbia University, College of Physicians & Surgeons, New York, New York: Isolation and characterization of flat revertant cell lines from Ab-MLV-transformed fibroblasts.

Geryk, J.,[1] Dezelee, P.,[1] Barnier, J.-V.,[1] Nehyba, J.,[2] Karakoz, I.,[2] Svoboda, J.,[2] Calothy, G.,[1] [1]Institut Curie-Biologie, Orsay, France; [2]Institute of Molecular Genetics, Czechoslovak Academy of Sciences, Praha: Transduction of the c-*src* gene and 3′ adjacent sequences in ASV Pr2257.

McClure, M.A.,[1] Perrault, J.,[2] [1]Dept. of Chemistry and Center for Molecular Genetics, University of California, [2]Dept. of Biology and Molecular Biology Institute, San Diego State University: Two different tyrosine protein-kinase-like domains in the VSV polymerase.

Ben-David, Y.,[1] Prideaux, V.R.,[1] Chow, V.,[1] Benchimol, S.,[2,3] Bernstein, A.,[1,3] [1]Division of Molecular and Developmental Biology, Mount Sinai Hospital Research Institute, [2]Ontario Cancer Institute, [3]Dept. of Medical Genetics and Medical Biophysics, University of Toronto, Canada: Inactivation of the p53 oncogene by internal deletion or retroviral integration in erythroleukemic cell lines induced by FrLV.

Heidecker, G., Huleihel, M., Cleveland, J.L., Beck, T.W., Rapp, U.R., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Structure/function analysis of *raf* family proteins.

Rovigatti, U.G., Dept. of Human Neoplasia, Alton Ochsner Medical Foundation, New Orleans, Louisiana: Variation of the N-*myc* gene structure in neuroblastoma cells.

Yuan, C.C.,[1] Kan, N.,[2] Papas, T.,[1] Blair, D.G.,[1] [1]NCI-Frederick Cancer Research Facility, Frederick, Maryland; [2]Milton Hershey Medical Center, Pennsylvania State University, Hershey: A biologically active murine retrovirus derived from ALV E26.

Ratner, L., Dept. of Medicine, Washington University, St. Louis, Missouri: Characterization of the c-*sis* enhancer—Activation by the HTLV-I *tat* product.

Smidt, M., Ratner, L., Dept. of Medicine, Washington University, St. Louis, Missouri: Consistent deletion within the 5th intron of the c-*sis* gene in a familial form of meningioma.

Van den Ouweland, A.M.W., Schalken, J.A., de Jong, M.E.M., Van Groninger, J.J.M., Van Bokhoven, A., Van de Ven, W.J.M., Dept. of Biology, University of Nijmegen, The Netherlands: Conserved close linkage in avian and mammalian species of the *fes/fps*

proto-oncogene and the *fur* gene.

Trauger, R.J.,[1] Wilson, S.,[2] Elder, J.,[1] [1]Dept. of Molecular Biology, Scripps Clinic and Research Foundation, La Jolla, California; [2]NCI, National Institutes of Health, Bethesda, Maryland: Immunological relatedness between the recombinant retrovirus gp70s and the group-A heterogeneous nuclear ribonucleoproteins.

Morris, D.W., Bradshaw, H.D., Jr., Billy, H.T., Hendrix, K.M., Young, L.J.T., Munn, R.J., Cardiff, R.D., Dept. of Pathology, University of California School of Medicine, Davis: Infection and transformation of mouse mammary epithelial cells with MMTV from cloned proviral DNA.

SESSION 10 PATHOGENESIS AND INSERTIONAL ACTIVATION

Chairmen: **N. Hopkins,** Massachusetts Institute of Technology
W. Hayward, Memorial Sloan-Kettering Cancer Center

Sharpe, A.H., Jaenisch, R., Whitehead Institute, Cambridge, Massachusetts: Studies on the pathogenesis of the murine neurotropic retrovirus Cas-Br-E.

Koehne, C.F., Alves, K., O'Donnell, P.V., Memorial Sloan-Kettering Cancer Center, New York, New York: DNA rearrangements of 12 common sites of proviral integration observed at different stages of leukemogenesis in AKR mice.

Clurman, B.E., Hayward, W.S., Sloan-Kettering Memorial Cancer Center, New York, New York: Multiple common integration sites in ALV-induced bursal lymphomas—Identification of stage-specific proto-oncogene activations.

Newstein, M.C., Montigny, W.J., Shank, P.R., Division of Biology and Medicine, Brown University, Providence, Rhode Island: Molecular analysis of osteopetrosis-determining regions of ALV.

Wagemaker, G., Valerio, D., Bovenhoff, M.E., Dept. of Radiobiology, Eramus University, and Radiobiological Institute TNO, Rijswijk, The Netherlands: Effect of a deletion mutant of replication-defective Friend virus on hematopoietic cell differentiation in genetically defective W/Wv mice.

Hsu, C.-L.L., Fabritius, C., Dudley, J.P., Dept. of Microbiology, University of Texas, Austin: MMTV proviruses from T-cell lymphomas lack a negative regulatory element in the LTR.

Marchetti, A.,[1] Robbins, J.,[2] Smith, G.,[2] Squartini, F.,[1] Callahan, R.,[2] [1]University of Pisa, Italy; [2]National Cancer Institute, Bethesda, Maryland: Effect of the host genetic background on the frequency of MMTV insertion at the *int*-loci in mammary tumor DNA.

Coppola, M.A.,[1] Holland, C.A.,[2] Thomas, C.Y.,[1] [1]Depts. of Medicine and Microbiology, University of Virginia, Charlottesville; [2]Dept. of Radiation Oncology, University of Massachusetts, Worcester: A host gene linked to MHC controls the envelope gene structure of tumor-associated recombinant MLV.

Smith, M.R.,[1] Smith, R.E.,[2] Hayward, W.S.,[1] [1]Dept. of Molecular Biology, Memorial Sloan-Kettering Cancer Center, New York, New York; [2]Dept. of Microbiology, Colorado State University, Fort Collins: Genetic determinants of rapid lymphomagenesis in an ALV.

Pizer, E., Humphries, E.H., University of Texas Southwest Medical Center, Dallas: Infection of chick embryos with RAV-1 results in a novel B-cell lymphoma that expresses elevated levels of c-*myb*.

Stoye, J.P., Fenner, S., Frankel, W.N., Coffin, J.M., Dept. of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts: Use of naturally occurring retroviral integrations to access the mouse genome.

SESSION 11 HUMAN IMMUNODEFICIENCY VIRUSES: MOLECULAR BIOLOGY

Chairmen: **E. Hunter,** University of Alabama
H. Robinson, University of Massachusetts

Cohen, E.A.,[1] Parkin, N.T.,[2] Sonenberg, N.,[2] Haseltine, W.,[1] [1]Dana-Farber Cancer Institute, Boston, Massachusetts; [2]Dept. of Biochemistry, McGill University, Montreal, Canada: Translational effects of the 5′ leader region of HIV-1.

Strebel, K., Martin, M., NIAID, National Institutes of Health, Bethesda, Maryland: Functional analysis of the HIV-1 "A" (SOR) gene product.

Hansen, J., Schulze, T., Billich, S., Moelling, K., Max-Planck-Institut für Molekulare Genetik, Berlin, Federal Republic of Germany: Characterization of bacterially expressed reverse transcriptase, RNase H and protease of HIV.

Prasad, V., Goff, S.P., Dept. of Biochemistry, Columbia University, College of Physicians & Surgeons, New York, New York: Analysis of mutants of HIV reverse transcriptase expressed in bacteria.

Freed, E.O., Risser, R., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Effects of point mutations in two functional domains of the HIV envelope glycoprotein.

Dubay, J.W.,[1] Kong, L.,[2] Kappes, J.,[2] Shaw, G.,[2] Hahn, B.,[2] Hunter, E.,[1] Depts. of [1]Microbiology, [2]Medicine, University of Alabama, Birmingham: A functional role for the carboxy-terminal domain of the HIV-1 gp41 glycoprotein.

Windheuser, M.G., Wood, C., University of Kansas, Lawrence: Cloning, expression, and characterization of HIV-1 gp41 immunoreactive epitopes in *E. coli*.

Bosch, M.L., Earl, P., Giombini, F., Wong-Staal, F., Fargnoli, K., Gallo, R.C., Franchini, G., National Institutes of Health, Bethesda, Maryland: In vitro mutagenesis and functional characterization of the fusion peptide of SIV.

Franchini, G.,[1] Fargnoli, K.,[1] Giombini, F.,[1] O'Keeffe, T.,[2]

Rusche, J.,[2] Wong-Staal, F.,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Repligen Corporation, Cambridge, Massachusetts: Identification of a novel protein encoded by HIV-2 X and study of its function in a biologically active genotype of HIV-2.

Kappes, J.C.,[1] Morrow, C.D.,[2] Lee, S.-W.,[1] Jameson, B.A.,[3] Kent, S.,[3] Hood, L.E.,[3] Shaw, G.M.,[1] Hahn, B.H.,[1] Depts. of [1]Medicine, [2]Microbiology, University of Alabama, Birmingham; [3]California Institute of Technology, Pasadena: Characterization of a novel retroviral gene product unique for HIV-2 and SIV.

SESSION 12 POSTER SESSION: Human Immuno-deficiency Virus Biology and Evolution

Kumar, R., Hughes, S., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Rapid detection of a single HIV-1-infected cell in the presence of 10^5 normal cells.

Darke, P.L., Nutt, R., Leu, C.-T., Heimbach, J., Brady, S.F., Garsky, V.M., Davis, L., Dixon, R.A.F., Verber, D.F., Sigal, I., Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania: HIV-1 protease—Characterization of *E. coli* expressed and chemically synthesized enzyme.

Lillehoj, E.,[1] Salazar, R.,[2] Mervis, R.J.,[3] Ahmad, N.,[3] Chan, H.,[2] Venkatesan, S.,[3] [1]NCI-Frederick Cancer Research Facility, Frederick, Maryland; [2]Syntex Corp., Palo Alto, California; [3]NIAID, National Institutes of Health, Bethesda, Maryland: Characterization of HIV *gag* proteins and the *gag-pol* protease.

Giam, C.-Z.,[1] Boros, I.,[2] [1]Dept. of Biochemistry, University of Nebraska Medical Center, Omaha; [2]NCI, National Institutes of Health, Bethesda, Maryland: In vivo and in vitro autoprocessing of HIV protease expressed in *E. coli*.

West, A.B., Roberts, T.M., Dana-Farber Cancer Institute, Boston, Massachusetts: Characterization of HIV *pol* ORF proteins produced in a eukaryotic expression vector system.

Terwilliger, E., Sodroski, J., Haseltine, W., Dana-Farber Cancer Institute, Boston. Massachusetts: Construction and characterization of a replication competent HIV-1 provirus expressing the CAT enzyme.

Haffar, O., Dowbenko, D., Berman, P., Genentech, Inc., South San Francisco, California: Topogenic analysis of the HIV-1 envelope glycoprotein, gp160, in microsomal membranes.

Chang, K.S.,[1] Gao, C.,[1] Wang, L.,[1] Li, Y.,[2] [1]Laboratory of Cellular Oncology, NCI, Bethesda, Maryland; [2]New England Regional Primate Research Center, Harvard Medical School, Southborough, Massachusetts: Replication and cytopathic effects of HIV and SIV in human B and monocytic cell lines.

Buckheit, R.,[1] Cloyd, M.,[2] Swanstrom, R.,[1] [1]Dept. of Biochemistry, University of North Carolina, Chapel Hill; [2]Dept. of Microbiology, University of Texas Medical Branch, Galveston: Characterization of an HIV-1 variant with an apparent absence of reverse transcriptase activity.

Perez, L.G.,[1] Mehta, S.,[1] Resnick, L.,[1] Wain-Hobson, S.,[2] [1]Mount Sinai Medical Center and University of Miami, Florida; [2]Institut Pasteur, Paris, France: Selective down-regulation of the HIV *env* gene expressed from a vaccinia virus vector in lymphoid cells.

Goudsmit, J.,[1] Meloen, R.,[2] Boucher, C.,[1] [1]Human Retrovirus Laboratory, AMC, Amsterdam, [2]Central Veterinary Laboratory, Lelystad, The Netherlands: Definition of a HIV-1 neutralizing-antibody-binding site on the external envelope.

Murphey-Corb, M.,[1] Kornfeld, H.,[2] Martin, L.N.,[1] Bachmann, M.,[2] Donahue, P.R.,[2] Gallo, M.V.,[2] Mullins, J.I.,[2] [1]Delta Regional Primate Research Center, Covington, Louisiana; [2]Dept. of Cancer Biology, Harvard University, Boston, Massachusetts: Structure and biological activity in vivo of molecularly cloned SIV_{mac} isolate BK28.

Naidu, U.M., Kestler, H.W. III, Li, Y., Daniel, M.D., Desrosiers, R.C., New England Regional Primate Research Center, Harvard Medical School, Southborough, Massachusetts: Characterization of infectious molecular clones of SIV_{mac} and HIV-2.

Kraus, G., Werner, A., Baier, M., Hartung, S., Norley, S., Mikschy, U., König, H., Kurth, R., Paul Ehrlich Institute, Frankfurt, Federal Republic of Germany: Isolation of HIV-1- and HIV-2-related retroviruses from African green monkeys.

Anand, R.,[1] VedBrat, S.S.,[2] Whatt, R.J.,[1] [1]Neuropsychiatry Branch, National Institute of Mental Health, Washington, D.C.; [2]Braton Biotech Inc., Rockville, Maryland: Cellular interaction of noncytocidal and cytocidal isolates of HIV from patients with neurological disorders.

Li, Y.,[1] Naidu, Y.M.,[1] Durda, P.J.,[2] Kestler, H.W. III,[1] Desrosiers, R.C.,[1] Daniel, M.D.,[1] [1]New England Regional Primate Research Center, Harvard Medical School, Southborough, [2]Dupont, North Billerica, Massachusetts; SIV from African green monkeys.

Rasheed, S., Zhou, J.-T., Laboratory of Viral Onocology anc AIDS Research, University of Southern California, Los Angeles: Full-length HIV-related DNA sequence in a naturally occurring monoclonal B-cell lymphoma of a patient with AIDS.

Tersmette, M.,[1] Winkel, I.,[1] Meloen, R.,[2] Deleys, R.J.,[3] van der Groen, G.,[4] de Goede, R.,[1] Miedema, F.,[1] Huisman, J.G.,[1] [1]Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory of Experimental and Clinical Immunology of the University of Amsterdam, [2]Central Veterinary Institute, Lelystad, [3]Innogenetics, Antwerp, [4]Institute of Tropical Medicine and University of Antwerp, Belgium: Differential recognition of Dutch and Central African HIV isolates by a characterized panel of monoclonal antibodies to HIV p24.

Terwilliger, E., Lu, Y., Haseltine, W., Dana-Farber Cancer Institute, Boston, Massachusetts: Mapping of genetic determinants responsible for phenotypic differences between two divergent clones of HIV-1.

Tsujimoto, H.,[1] Fukasawa, M.,[1] Miura, T.,[1] Hasegawa, A.,[2] Morikawa, S.,[3] Hayami, M.,[1,3] [1]Institute of Medical Science, Tokyo University, [2]Tao Nenryo Kogyo K.K.,

[3]National Institute of Health, Tokyo, Japan: Molecular characterization of SIV from naturally infected African green monkeys and mandrills.

Hasegawa, A.,[1] Tsujimoto, H.,[2] Maki, N.,[1] Ishikawa, K.,[2] Fukasawa, M.,[2] Miki, A.,[1] Hayami, M.,[2] [1]Foundamental Research Laboratory, Kogyo; [2]Institute of Medical Science, Tokyo University, Japan: Sequence of HIV-2 from an AIDS patient in Ghana and its relationship to HIV/SIV group viruses.

Heidecker, G.,[1] Dorn, P.L.,[1] Derse, D.D.,[1] Morton, W.R.,[2] Benveniste, R.E.,[1] [1]NCI-Frederick Cancer Research Facility, Frederick, Maryland; [2]University of Washington Regional Primate Research Center, Seattle: Molecular characterization of SIV/*Mne*.

Kestler, H.W. III, Li, Y., Naidu, Y.M., Butler, C.V., Ochs, M.F., Jaenel, G., King, N.W., Daniel, M.D., Desrosiers, R.C., New England Regional Primate Research Center, Harvard Medical School, Southborough, Massachusetts: Comparison of SIV isolates.

Kong, L.I.,[1] Lee, S.-W.,[1] Decker, D.,[1] Parkin, J.,[1] Kumar, P.,[1] Hui, H.,[1] Kappes, J.C.,[1] Hoxie, J.,[2] Hahn, B.H.,[1] Shaw, G.M.,[1] [1]Dept. of Medicine, University of Alabama, Birmingham; [2]Dept. of Medicine, University of Pennsylvania, Philadelphia: Naturally occurring West African HIV-2-related human retrovirus with attenuated cytopathicity and genetic and structural polymorphism.

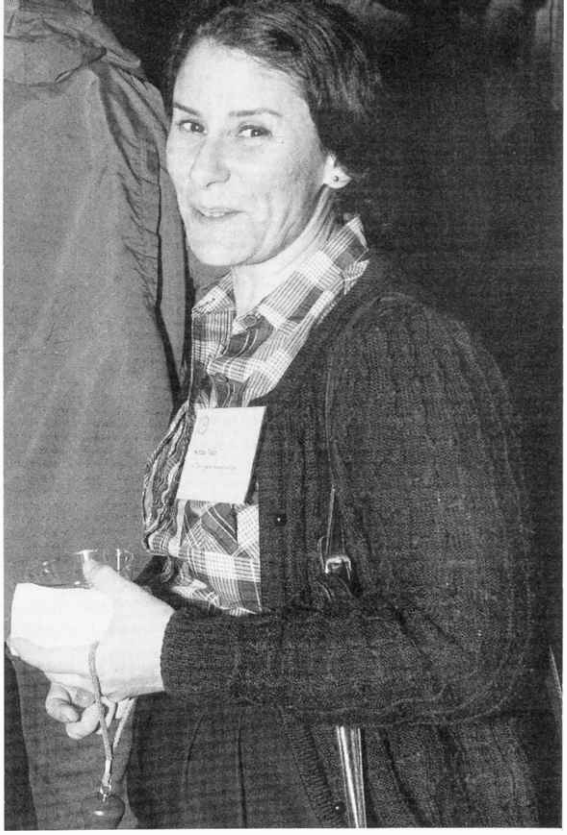
O'Brien, W.A., Koyanagi, Y., Chen, I.S.Y., Depts. of Medicine and Microbiology and Immunology, University of California School of Medicine, Los Angeles: Cellular DNA fragments that have homology with HIV LTR.

Srinivasan, A., Jannoun-Nasr, R., Kalyanaraman, S., York, D., Bohan, C., Dorsett, D., Butler, D., Centers for Disease Control, Retrovirus Diseases Branch, Atlanta, Georgia: Homologous recombination between HIV DNAs—An approach to generate hybrid HIV.

Chiodi, F.,[1] Albert, J.,[1] Norkrans, G.,[2] Hagberg, L.,[2] Sonnerborg, A.,[3] Asjo, B.,[1] Fenyo, E.,[1] [1]Dept. of Virology, Karolinska Institute, Stockholm, [2]Dept. of Infectious Diseases, Ostra Hospital, Gothenberg, [3]Central Microbiological Laboratory, Stockholm, Sweden: Isolation frequency and characterization of HIV from cerebrospinal fluid and blood of patients with varying severity of HIV infection.

Barry, P., Marthas, M., Banapour, B., Luciw, P., Dept. of Medical Pathology, University of California, Davis: Interactions of SIV and DNA viruses.

O'Connor, T.,[1] Smith, R.,[1] Tanguay, S.,[1] Tonelli, Q.,[1] Lawrence, K.,[1] Steinman, R.,[1] Seymour, C.,[1] Goodness, J.,[1] Pedersen, N.,[2] Andersen, P.,[1] [1]AgriTech Systems, Inc., Portland, Maine; [2]University of California, Davis: Characterization of the major structured proteins of FTLV.



N. Teich

Hoover, E.A.,[1] Quackenbush, S.L.,[1] Overbaugh, J.M.,[2] Donahue, P.R.,[2] Mullins, J.I.,[2] [1]Colorado State University, Fort Collins; [2]Harvard School of Public Health, Boston, Massachusetts: Molecularly cloned immunodeficiency-inducing feline retroviruses are cytopathic for intestinal epithelium as well as T lymphocytes.

Bates, P.,[1] Safran, N.,[2] Perk, K.,[2] Varmus, H.E.,[1] [1]Dept. of Microbiology and Immunology, University of California, San Francisco; [2]Koret School of Veterinary Medicine, Hebrew University of Jerusalem, Rehovot, Israel: Isolation of cDNA clones of a visna-related retrovirus associated with sheep pulmonary carcinoma.

Gourdou, I., Mazarin, V., Querat, G., Sauze, N., Vigne, R., Laboratoire de Virologie, Marseille, France: Early genes of visna virus—Structure and expression.

SESSION 13 HUMAN IMMUNODEFICIENCY VIRUS PATHOLOGY

Chairmen: **I.S.Y. Chen,** University of California, Los Angeles
J. Mullins, Harvard School of Public Health

Hirsch, V.[1] Arbeille, B.,[21] Mullins, J.I.,[1] [1]Dept. of Cancer Biology, Harvard University, Boston, Massachusetts; [2]Unité de Microscopie Electronique, UER Médecinen, Tours, France: Truncation of the SIV_{BK28} transmembrane protein is required for full infectivity.

Zack, J., Cann, A., Lugo, J., Chen, I.S.Y., Depts. of Medicine and Microbiology and Immunology, University of California School of Medicine, Los Angeles: Investigation of HIV persistence/latency in vitro.

Kowalski, M.M., Dorfman, T., Basiripour, L., Potz, J.,

Bergeron, L., Haseltine, W., Sodroski, J., Division of Biochemical Phymacology, Dana-Farber Cancer Institute, Boston, Massachusetts: Molecular events important for HIV-1 entry into host cells and cytopathicity.

Clavel, F., Hoggan, M.D., Willey, R.L., Strebel, K., Martin, M.A., Repaske, R., NIAID, National Institutes of Health, Bethesda, Maryland: Genetic recombination of HIV.

Fenyö, E.M.,[1] Schwartz, S.,[1] Felber, B.K.,[2] Pavlakis, G.N.,[2] [1]Dept. of Virology, Karolinska Institute, Stockholm, Sweden; [2]NCI-Frederick Cancer Research Facility, Maryland: HIV-1 strains with differences in replicative capacity show distinct tropism on indicator cell lines.

Tersmette, M.,[1] Gruters, R.A.,[1] de Goede, R.E.Y.,[1] de Wolf, F.,[2] Goudsmit, J.,[3] Cuypers, H.T.,[1] Huisman, J.G.,[1] Miedema, F.,[1] [1]Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory of Experimental and Clinical Immunology of the University of Amsterdam, [2]Municipal Health Service, [3]Academic Medical Centre, Amsterdam, The Netherlands: Evidence for a role of virulent HIV strains in the development of AIDS.

Koyanagi, Y., O'Brien, W.A., Golde, D.W., Gasson, J.C., Chen, I.S.Y., Depts. of Medicine and Microbiology and Immunology, University of California School of Medicine, Los Angeles: Mechanism of monocyte activation of HIV replication.

Matsuyama, T.,[1,2] Okamoto, T.,[1] Hamamoto, Y.,[2] Kobayashi, N.,[2] Josephs, S.F.,[3] Wong-Staal, F.,[3] Yamamoto, N.,[2] Miwa, M.,[1] Shimotohno, K.,[1] [1]Division of Virology, National Cancer Center Research Institute, Tokyo, [2]Dept. of Virology and Parasitology, Yamaguchi, Japan; [3]National Institutes of Health, Bethesda, Maryland: Enhancement of HIV gene expression by tumor necrosis factors α and β.

Donahue, P.R.,[1] Quackenbush, S.L.,[2] Overbaugh, J.,[1] de Noronha, C.M.C.,[1] Hoover, E.A.,[2] Mullins, J.I.,[1] [1]Harvard University, Boston, Massachusetts; [2]Colorado State University, Fort Collins: T-cell cytopathic determinants of an immunodeficiency-inducing FeLV variant.

Aziz, D.C., Jolicoeur, P., Clinical Research Institute of Montreal, Canada: Murine AIDS (MAIDS) induced by the Duplan strain of RadLV—Molecular cloning of a defective provirus.

SV40, Polyoma, and Adenoviruses

August 10—August 14

ARRANGED BY

Terri Grodzicker, Cold Spring Harbor Laboratory
Michael Botchan, University of California, Berkeley

393 participants

The Tumor Virus meeting on SV40, polyoma and adenoviruses, which has been held for 15 years, continues to bring together a large group of scientists to discuss their latest findings. This year's meeting was attended by more than 400 scientists who use the small DNA tumor viruses as model systems to analyze mechanisms of eukaryotic transcription, replication, RNA processing, and transformation.

Much excitement at this year's meeting focused on mechanisms of transformation and the finding that the adenovirus E1A proteins, SV40 large T antigen, and the human papillomavirus type-16 E7 protein all bind to the product of the retinoblastoma gene, a protein whose inactivation is involved in tumor growth. Much research continues to focus on the purification and analysis of cellular transcription factors that bind to viral promoters and enhancers and the cloning of the genes that encode these factors; on viral proteins such as the adenovirus E1A proteins that interact with and/or affect the activity of transcription factors; and on the use of in vitro systems to analyze the role of purified viral and cellular proteins on viral DNA replication. Talks were also given on splicing, transport, and stability of viral mRNAs; translational control; the role of protein

modifications and different domains on the function of transcription factors and transforming proteins; the interaction of viral transforming proteins with additional cellular proteins such as p53 and tyrosine kinase; and the role of different viral proteins in the host's immune response.

SESSION 1 MECHANISMS OF TRANSFORMATION

Chairman: H. Ginsberg, Columbia College of Physicians & Surgeons

Buchkovich, K.J.,[1] Whyte, P.,[1] Dyson, N.,[1] Horowitz, J.M.,[2] Friend, S.H.,[2] Raybuck, M.,[1] Weinberg, R.A.,[2] Harlow, E.,[1] [1]Cold Spring Harbor Laboratory, New York; [2]Whitehead Institute and Massachusetts Institute of Biology, Cambridge: Transforming proteins of three DNA tumor viruses interact with the retinoblastoma gene product.

DeCaprio, J.,[1] Ludlow, J.,[1] Figge, J.,[1] Marsilio, E.,[1] Shew, J.-Y,[2] Lee, W.-H,[2] Paucha, E.,[1] Livingston, D.,[1] [1]Neoplastic Disease Mechanisms, Dana-Farber Cancer Institute, Boston, Massachusetts; [2]Experimental Pathology Program and Center for Molecular Genetics, University of California, San Diego: Specific association of SV40 large T antigen with the retinoblastoma locus product.

Kuo, A.,[1] Worrell, M.,[1] Kohl, N.,[2] O'Brien, M.,[1] Ruley, H.E.,[1] [1]MIT Center for Cancer Research, Cambridge, Massachusetts; [2]Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania: Mechanisms of oncogene collaboration—Activation and inactivation of a dominant repressor.

Moran, E.,[1] Corrigan, M.,[1] Zerler, B.,[2] [1]Cold Spring Harbor Laboratory, New York; [2]Molecular Therapeutics, Inc., West Haven, Connecticut: A region of SV40 large T antigen can substitute for a transforming domain of the adenovirus E1A products.

Srinivasan, A.,[1] Peden, K.W.C.,[2] Pipas, J.M.,[1] [1]Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania; [2]Dept. of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, Maryland: Transforming functions of the SV40 large T antigen.

Cheng, S.H., Harvey, R., Espino, P.C., Smith, A.E., Laboratory of Cellular Regulation, Integrated Genetics Inc., Framingham, Massachusetts: pp59$^{c\text{-}fyn}$ is capable of complex formation with the middle T antigen of polyomavirus.

Young, A.T.,[1] Talmage, D.,[2] Freund, R.,[2] Benjamin, T.L.,[1-2] [1]Harvard University, Cambridge, [2]Harvard Medical School, Boston, Massachusetts: Phosphorylation of polyomavirus middle T on tyrosine 315 is required for efficient tumor induction and for association with type I phosphatidylinositol kinase activity.

Piwnica-Worms, H.,[1] Williams, N.G.,[1] Cheng, S.H.,[2] Roberts, T.M.,[1] [1]Dana-Farber Cancer Institute and Dept. of Pathology, Harvard Medical School, Boston; [2]Laboratory of Cellular Regulation, Integrated Genetics, Inc., Framingham, Massachusetts: Regulation of pp60$^{c\text{-}src}$ and its association with polyomavirus middle T antigen in insect cells.

Lamberti, C., Williams, J., Dept. of Biological Sciences, Carnegie Mellon University, Pittsburgh, Pennsylvania: Differential transforming capacity of Ad12 EIA proteins.

Ikeda, M.,[1-2] Yamada, S.,[1-2] Koyama, T.,[1-3] Sawada, Y.,[4] Fujinaga, K.,[4] Tsuchida, N.,[1] [1]Dept. of Oral Microbiology, [2]Institute of Stomatognathic Science, [3]1st Dept. of Oral and Maxillofacial Surgery, Tokyo Medical and Dental University, Tokyo; [4]Dept. of Molecular Biology, Cancer Institute, Sapporo Medical College, Japan: Ad12 E1A collaborated with viral-promoter-linked and nonlinked human c-*myc* to transform established rat cells.

Silverstein, G., Kohrman, D., Christensen, J., Elenich, L., Imperiale, M.J., Dept. of Microbiology and Immunology, University of Michigan, Ann Arbor: Analysis of SV40 transformation-resistant cell lines.

SESSION 2 TRANSCRIPTION I: ADENOVIRUSES

Chairman: J. Nevins, Duke University Medical School

Hai, T., Liu, F., Green, M.R., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: A family of immunologically related transcription factors that includes multiple forms of ATF and AP-1.

Jones, R.H.,[1] Moreno, S.,[2] Nurse, P.,[2] Jones, N.C.,[1] Imperial Cancer Research Fund, [1]Gene Regulation Laboratory, London, [2]Cell Cycle Control Laboratory, Dept. of Biochemistry, University of Oxford, England: Identification and characterization of yeast ATF and AP-1 transcription factors.

Manohar, C.F.,[1] SivaRaman, L.,[1] Kratochvil, J.,[1] Kwast-Welfeld, J.,[2] Jungmann, R.A.,[2] Thimmappaya, B.,[1] Depts. of [1]Microbiology and Immunology, [2]Molecular Biology, Northwestern University Medical School, Evanston, Illinois: Transcriptional activation of the adenovirus EII early promoter—*cis*-acting control elements and the cognate host transcription factors.

Marton, M., Hardy, S., Shenk, T., Dept. of Molecular Biology, Princeton University, New Jersey: Characterization of the cellular putative transcription factor E2F.

Neill, S.D.,[1] Simon, M.C.,[2] Reichel, R.,[2] Kovesdi, I.,[2] Raychaudhuri, P.,[1] Nevins, J.R.,[1] [1]Dept. of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina; [2]Rockefeller University, New York, New York: The adenovirus E4 gene, in

addition to the E1A gene, is required for *trans*-activation of E2 transcription and E2F activation.

Boeuf, H., Kédinger, C., LGME, CNRS, INSERM, Strasbourg, France: Adenovirus early gene expression in mouse teratocarcinoma cells.

Murray, E.J., Rigby, P.W.J., National Institute for Medical Research, London, England: Mutational analysis of the sequence requirements for adenovirus E2A transcription in F9 embryonal carcinoma cells.

La Thangue, N.B., National Institute for Medical Research, London, England: Cell-specific and ubiquitous factors regulate the transcription of the adenovirus E2A promoter in embryonal carcinoma stem cells.

Bruder, J., Hearing, P., Dept. of Microbiology, Health Science Center, State University of New York, Stony Brook: Nuclear factor EF-1A binds to the Ad5 E1A core enhancer element I and to the Ad5 E4 and polyomavirus enhancer regions and the β-interferon interferon response element.

Vales, L.D.,[1] Harter, M.L.,[2] Babiss, L.E.,[1] Darnell, J.E.,[1] [1]Rockefeller University, New York, New York; [2]University of Medicine and Dentistry of New Jersey, Piscataway: Regulation of the adenovirus polypeptide IX gene.

Venkatesh, L.K., Chinnadurai, G., Institute for Molecular Virology, St. Louis University Medical Center, Missouri: Transcriptional regulation of Ad2 protein IX promoter by a silencer element.

Weisshaar, B., Hoeveler, A., Müller, U., Dobrzanski, P., Langner, K.-D., Doerfler, W., Institute of Genetics, University of Cologne, Federal Republic of Germany: Inactivation by sequence-specific methylations of adenovirus promoters—A reversible signal.

SESSION 3 POSTER SESSION

Amemiya, K., Traub, R., Durham, L., Major, E.O., NINCD, National Institutes of Health, Bethesda, Maryland: Identification of binding sites of nuclear proteins by DNase I protection in the regulatory region of the human papovavirus JC.

Batson, S.,[1] Heath, C.,[1] Samuels, M.,[2] Hansen, U.,[1] [1]Dana Farber Cancer Institute, Harvard Medical School, [2]Dept. of Biology, Massachusetts Institute of Technology, Boston: Initiation of transcription from SV40 minichromosomes in vitro.

Bennett, E.R., Hassell, J.A., Dept. of Microbiology and Immunology, McGill University, Montreal, Canada: Activation of the polyomavirus origin core for DNA replication by heterologous enhancer elements.

Berko-Flint, Y., Karby, S., Hassin, D., Lavi, S., Dept. of Microbiology, Tel Aviv University, Israel: An in vitro system to study carcinogen-induced SV40 amplification in Chinese hamster cells.

Bourgaux, P., Gendron, D., Bourgaux-Ramoisy, D., Dept. of Microbiology, Université de Sherbrooke, Québec, Canada: A sequence that promotes recombination in polyomavirus DNA.

Bullock, P., Hurwitz, J., Graduate Program in Molecular Biology, Memorial Sloan-Kettering Cancer Center, New York, New York: Identification of an unwound topological form that is an initial substrate for SV40 DNA replication in vitro.

Caruso, M., Felsani, A., Maione, R., Amati, P., Dipt. di Biopatologia Umana, Sezione Biologia Cellulare, Università La Sapienza, Roma, Italy: Interactions of nuclear proteins with wild-type and mutant polyomavirus enhancers.

Chakraborty, T., Das, G.C., Dept. of Molecular Biology, University of Texas Health Center, Tyler: *cis*- and *trans*-acting elements of the BK virus early promoter.

Cheng, S., Blume, M., Vogel, R., Lee, S., Hung, P., Division of Biotechnology and Microbiology, Wyeth-Ayerst Research, Radnor, Pennsylvania: DNA transfection studies of simian adenovirus.

Chu, Y., Huang, T.S., Hsu, M.T., Dept. of Microbiology, Mt. Sinai School of Medicine, New York, New York: P1 nuclease makes a single cleavage of a subfraction of SV40 chromatin with high superhelical density at the origin of replication or at the enhancers.

Couture, L.A., Lehman, J.M., Albany Medical College, New York: Regulatory region modifications necessary for T-antigen expression and replication of polyomavirus in PCC4-aza1 cells.

Das, G.D., Dept. of Molecular Biology, University of Texas Health Center, Tyler: DNA-protein interaction in the polyomavirus early promoter.

DasGupta, S., Das, G.C., Dept. of Molecular Biology, University of Texas Health Center, Tyler: Functional analysis of the enhancer region of polyomavirus-spanning F9 mutation.

Delmas, V.,[1] Gardes, M.,[1] Goutebroze, L.,[1] Scherneck, S.,[2] Feunteun, J.,[1] [1]Laboratoire d'Oncologie Moléculaire, Institut Gustave Roussy, Villejuif, France; [2]Central Institute of Molecular Biology, Academy of Sciences, Berlin-Buch, German Democratic Republic: Regulation of the hamster polyomavirus early gene expression.

Del Vecchio, A.M., Steinman, R.A., Ricciardi, R.P., Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania: BK virus enhancer sequences required for DNA replication.

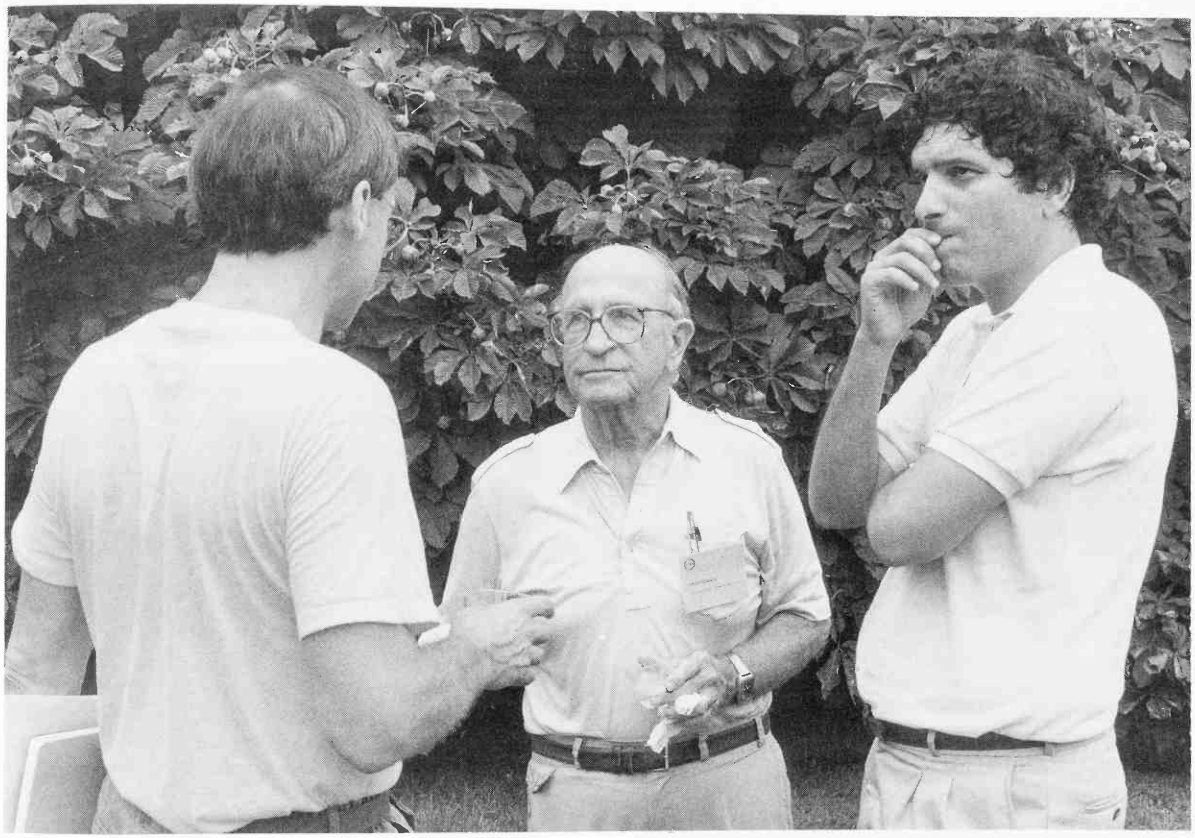
Dörries, K., Möllers, U., Schäffner, M., Institut für Virologie und Immunbiologie der Universität, Würzburg, Federal Republic of Germany: Expression of human polyomavirus JC (GS) genes in eukaryotic cells.

Fairman, M., Din, S., Tsurimoto, T., Prelich, G., Smith, S., Stillman, B., Cold Spring Harbor Laboratory, New York: Early events in the initiation of DNA replication at the SV40 origin.

Frappier, D., Bourgaux, P., Dept. of Microbiology, Université of Sherbrooke, Quebéc, Canada: Deletion analysis of the sites involved in the resolution of a polyoma-mouse hybrid replicon.

Friedrich, T.D., Laffin, J.A., Lehman, J.M., Dept. of Microbiology and Immunology, Albany Medical College, New York: Phorbol-ester-induced changes in SV40 T antigen expression and cellular DNA synthesis during lytic infection.

Guo, Z.-S., Heine, U., Gutierrez, C., DePamphilis, M.L., Dept. of Cell and Developmental Biology, Roche Institute



W. Wold, H. Ginsberg, R. Gaynor

of Molecular Biology, Nutley, New Jersey: Auxiliary sequences strongly facilitate SV40 *ori*-core activity in vitro as well as in vivo.

Hadlock, K.G., Lutter, L.C., Division of Molecular Biology Research, Henry Ford Hospital, Detroit, Michigan: Association of SV40 large T antigen with minichromosomes actively transcribing in vivo.

Hassin, D.,[1,2] Karby, S.,[1] Berko-Flint, Y.,[1] Lavi, S.,[1] [1]Dept. of Microbiology, Tel Aviv University, [2]Sheba Medical Center, Israel: In vitro replication of SV40 in extracts from carcinogen-treated Chinese hamster C060 cells—Onion skin mode of DNA replication.

Hendrickson, F.M., Cole, R.D., Dept. of Biochemistry, University of California, Berkeley: Histone H1 has an aversion to the origin of replication of SV40.

Hirai, S.,[1] Mechta, F.,[1] Piette, J.,[2] Ryseck, R.-P.,[3] Bravo, R.,[3] Yaniv, M.,[1] [1]Dept. of Molecular Biology, [2]Biotechnology, Institut Pasteur, Paris; [3]EMBL, Heidelberg, Federal Republic of Germany: Mouse PEA 1 (AP-1) factor is coded by several genes of the jun family.

Hoess, A., vanZee, K., Arthur, A., Schneider, J., Liebstein, A., Fanning, E., Institute for Biochemistry, Munich, Federal Republic of Germany: Activation of SV40 late gene expression by mutant large T antigens.

Iggo, R., Ford, M., Anton, I., Gough, G., Lane, D.P., ICRF Clare Hall Laboratories, South Mimms, England: A growth-regulated nuclear antigen, p68, cross-reacts with SV40 large T and is a member of a helicase superfamily.

Jessberger, R., Spies, A., Heuss, D., Rauskolb, C., Doerfler, W., Institute of Genetics, University of Cologne, Federal Republic of Germany: Recombination in hamster cell nuclear extracts between Ad12 DNA and a hamster preinsertion sequence.

Kelly, J., Wildeman, A., Dept. of Molecular Biology and Genetics, University of Guelph, Ontario, Canada: Analysis of the effect of replication on T-antigen *trans*-activation of the SV40 late and late-early promoters in different cell lines.

Kenny, M., Lee, S.-H., Dean, F., Hurwitz, J., Graduate Program in Molecular Biology, Memorial Sloan-Kettering Cancer Center, New York, New York: Characterization of the single-stranded DNA-binding protein required for SV40 DNA replication.

Kumar, R.,[1] Subramanian, K.N.,[2] [1]NCI-Frederick Cancer Research Facility, Frederick, Maryland; [2]Dept. of Microbiology and Immunology, University of Illinois, Chicago: Identification of loci exhibiting sequence-directed curvature in SV40 DNA.

Lapidot, A., Baran, N., Baru, M., Tenzer, M., Snir, A., Manor, H., Dept. of Biology, Technion, Haifa, Israel: Induction of polyomavirus DNA replication in the LPT line of polyomavirus-transformed rat cells.

Lashgari, M.S., Tada, H., Khalili, K., Dept. of Biochemistry and Molecular Biology, Jefferson Institute of Molecular Medicine, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania: *trans*-actions of

the JC virus late promoter by T antigen is not cell-type-specific.

Lawlor, K.G.,[1] Dermody, J.J.,[1] Du, H.,[1] Jha, K.K.,[1] Malkas, L.,[2] Hickey, R.,[2] Baril, E.F.,[2] Ozer, H.L.,[1] [1]Dept. of Biological Sciences, Hunter College, City University of New York, New York; [2]Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Polyomavirus DNA synthesis in *ts*DNA mutants in vitro.

Leu, M.-H., Chen-Kiang, S., Immunobiology Center and Dept. of Microbiology, Mt. Sinai School of Medicine, New York, New York: T-cell-specific requirement of E1B 21-kD polypeptide for adenovirus DNA replication.

Lutter, L.C.,[1] Franken, N.,[1] Keshavarzi, S.,[1] Petryniak, B.,[2] [1]Molecular Biology Research Division, Henry Ford Hospital, Detroit; [2]Howard Hughes Medical Institute, University of Michigan, Ann Arbor: Thermally induced changes in the topology of SV40 transcription complex DNA.

Manor, H., Lapidot, A., Baran, N., Dept. of Biology, Technion, Haifa, Israel: $(dG\text{-}dA)_n$ and $(dT\text{-}dC)_n$ tracts arrest DNA replication in vitro.

Markowitz, R.-B., Dynan, W.S., Dept. of Chemistry and Biochemistry, University of Colorado, Boulder: Comparison of the regulatory regions in different strains of BK virus—A possible example of viral evolution.

Martinez-Salas, E.,[1] Cupo, D.Y.,[2] DePamphilis, M.L.,[1] [1]Dept. of Cell and Developmental Biology, Roche Institute of Molecular Biology, Nutley, New Jersey; [2]Dept. of Chemistry, Bates College, Lewiston, Maine: The need of enhancers to activate the polyomavirus origin of replication is acquired in mammalian development with the formation of a diploid nucleus.

Mastrangelo, I.,[1] Hough, P.,[1] Wall, J.,[1] Dodson, M.,[2] Dean, F.,[3] Hurwitz, J.,[3] [1]Dept. of Biology, Brookhaven National Laboratory, Upton, New York; [2]Dept. of Biochemistry, Stanford University School of Medicine, California; [3]Graduate Program in Molecular Biology, Memorial Sloan-Kettering Cancer Center, New York, New York: SV40 T antigen—ATP-dependent assembly of hexamers in solution and double hexamers at the viral core origin of replication.

Maulbecker, C., Bartholomew, J., Botchan, M., Dept. of Molecular Biology, University of California, Berkeley: Study of a new SV40 T-antigen mutant that replicates well in human cells but is replication-defective in CV-1 cells.

Meisterernst, M., Rogge, L., Donath, C., Gander, I., Stelzer, G., Foeckler, R., Winnacker, E.-L., Institute of Biochemistry, Munich, Federal Republic of Germany: Nuclear factor I—Isolation and characterization of the gene.

Morin, N., Cleghon, V., Klessig, D.F., Waksman Institute, Rutgers University, Piscataway, New Jersey: Study on the importance of the phosphorylation of the DNA-binding protein of adenovirus.

Neale, G.A.M., Kitchingman, G.R., Dept. of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, Tennessee: Site-directed mutants of the adenovirus single-stranded DNA-binding protein that are functionally deficient and structurally altered.

Nilsson, M., Magnusson, G., Dept. of Medical Virology, Uppsala University, Sweden: Activity of the polyomavirus enhancer in early transcription and DNA replication.

Omilli, F., Scieller, P., May, E., Unité d'Oncologie Moléculaire, IRSC, Villejuif, France: Characterization of the SV40 late promoter elements involved in the T-antigen-mediated stimulation of late genes.

Ondek, B., Herr, W., Cold Spring Harbor Laboratory, New York: SV40 enhancer structure and function—Enhanson spacing and factor interactions.

O'Neill, F., Stevens, R., Miller, T., Xu, X.-L., Veterans Administration Medical Center and Dept. of Cellular, Viral and Molecular Biology, University of Utah, Salt Lake City: SV40 Vp_2 mutants produce persistent infections in green monkey cells.

Pettit, S.C.,[1] Abraham, C.,[1] Horwitz, M.S.,[2] Engler, J.A.,[1] [1]Dept. of Biochemistry, University of Alabama, Birmingham; [2]Dept. of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York: Effects of mutations in the precursor to the terminal protein (pTP) gene on adenovirus DNA replication activity in vitro.

Rainwater, R.M., Mann, K., Biology Dept., University of Alaska, Anchorage: Localization of topoisomerase I and topoisomerase II in high-salt extracts from SV40-infected TC7 cells.

Rajadhyasksha, A., Ambrose, C., Bina, M., Dept. of Chemistry, Purdue University, West Lafayette, Indiana: Position of nucleosomes assembled in vivo near the terminus of SV40 DNA replication and transcription.

Ramanujam, P.,[1] Chandrasekharappa, S.,[2] Subramanian, K.,[1] Depts. of [1]Microbiology and Immunology, [2]Medicine, University of Illinois, Chicago: Functional anatomy of the SV40 21- and 72-bp repeats vis-à-vis their replication enhancement function.

Satake, M., Ibaraki, T., Yamaguchi, Y., Ito, Y., Institute for Virus Research, Kyoto University, Japan: Modulation of nuclear factors interacting with the polyomavirus enhancer A element upon transformation by the Ha-*ras* oncogene.

Shigesada, K., Kamachi, Y., Imai, M., Satake, M., Ito, Y., Institute for Virus Research, Kyoto University, Japan: Purification and functional characterization of new protein factors binding to the polyomavirus enhancer A element.

Snapka, R.M.,[1] Mehta, V.B.,[2] Muller, M.T.,[2] [1]Dept. of Radiology, [2]Dept. of Molecular Genetics, Ohio State University, Columbus: Type I and type II topoisomerases are trapped on different SV40 DNA replication intermediates by specific inhibitors.

Stetter, G., Montenarh, M., Dept. of Biochemistry, University of Ulm, Federal Republic of Germany: Binding of subclasses of SV40 large T antigen to isolated binding site I, II, or III.

Stern, S., Herr, W., Cold Spring Harbor Laboratory, New York: Activation of transcription in yeast by SV40 enhancer elements.

Sturm, R., Herr, W., Cold Spring Harbor Laboratory, New York: Octamer-binding protein—Isolation of a human cDNA clone.

Tada, H., Lashgari, M., Khalili, K., Dept. of Biochemistry and Molecular Biology, Jefferson Institute of Molecular Medicine, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania: Cell-type-specific

expression of JC virus early protein is under positive and negative regulation.

Tanaka, M., Hernandez, N., Herr, W., Cold Spring Harbor Laboratory, New York: SV40 enhancer sequences display both cell-specific and promoter-specific transcriptional activation.

Tang, W.-J., Folk, W.R., Dept. of Microbiology, University of Texas, Austin: Polyomavirus origin of DNA replication—Functional organization and interaction with *trans*-acting factors.

Traut, W., Alliger, P., Carstens, E., Dornreiter, I., Fanning, E., Institute for Biochemistry, Munich, Federal Republic of Germany: Purification and characterization of a cellular protein that binds specifically to the SV40 core origin of DNA replication.

Xu, F.-Y., Hu, C.-H., Bennett, K., Pearson, G.D., Dept. of Biochemistry and Biophysics, Oregon State University, Corvallis: Adenovirus-displaced strands participate in two separate DNA replication pathways.

Zhang, L., Gralla, J.D., Dept. of Chemistry and Biochemistry, University of California, Los Angeles: Nucleoprotein structures in the SV40 control region during the late phase of the lytic cycle.

Zhao, L., Irie, K., Padmanabhan, R., Dept. of Biochemistry, University of Kansas Medical Center, Kansas City: Nuclear localization and biological activity of adenovirus preterminal protein and DNA polymerase transiently expressed in CV-1 cells.

Zhu, J., Cole, C.N., Dept. of Biochemistry and Molecular Genetics Center, Dartmouth Medical School, Hanover, New Hampshire: Mapping the transcriptional *trans*-activation function of SV40 large T antigen.

SESSION 4 REPLICATION

Chairman: M. Botchan, University of California, Berkeley

Tsurimoto, T., Fairman, M., Prelich, G., Smith, S., Din, S., Stillman, B., Cold Spring Harbor Laboratory, New York: Identification of multiple cellular replication factors required for SV40 DNA in vitro.

Ishimi, Y., Lee, S.-H., Bullock, P., Kenny, M., Hurwitz, J., Graduate Program in Molecular Biology, Memorial Sloan-Kettering Cancer Center, New York, New York: Complete enzymatic synthesis of DNA containing the SV40 origin of replication.

Scheffner, M., Wiekowski, M., Wessel, R., Stahl, H., Faculty of Biology, University of Konstanz, Federal Republic of Germany: Sequence-independent duplex DNA opening activity of SV40 large T antigen.

Borowiec, J., Dean, F., Hurwitz, J., Dept. of Molecular Biology, Memorial Sloan-Kettering Cancer Center, New York, New York: SV40 large T antigen locally melts and untwists the SV40 origin of replication in the presence of ATP.

Gutierrez, C., Guo, Z.-S., DePamphilis, M.L., Dept. of Cell and Developmental Biology, Roche Institute of Molecular Biology, Nutley, New Jersey: Sequences flanking SV40 *ori*-core facilitate initiation of replication after the T-antigen initiation complex binds to the origin.

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Mermod, N.,[1] Santoro, C.,[1] Tanese, N.,[1] Andrews, P.,[2] Tjian, R.,[1] [1]Dept. of Biochemistry, University of California, Berkeley; [2]Dept. of Biochemistry, Purdue University, West Lafayette, Indiana: Multiple cDNAs encode a family of human CCAAT-box-binding factors active in both transcription and adenovirus DNA Creplication.

Chen, M., Meyers, M., Horwitz, M.S., Depts. of Cell Biology and Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York: Identification of functional domains in cloned adenovirus DNA polymerase by in-phase insertion mutagenesis.

SESSION 5 TRANSCRIPTION II: SV40, POLYOMA

Chairman: D. Livingston, Dana Farber Cancer Institute

Williams, T., Kadonaga, J., Courey, A., Jackson, S., Admon, A., Lüscher, B., Mitchell, P., Bohmann, D., Turner, R., Tjian, R., Dept. of Biochemistry, University of California, Berkeley: Structure and functional analysis of transcription factors that activate SV40 early gene expression.

Davidson, I., Fromental, C., Xiao, J.H., Kanno, M., Macchi, M., Rosales, R., Nomiyama, H., Vigneron, M., Bornert, J.M., Chambon, P., LGME/CNRS, INSERM, Faculté de Medecine, Strasbourg, France: Functional organization of the SV40 enhancer and purification of cell-specific SV40 enhancer-binding proteins.

Baumruker, T., Sturm, R., Herr, W., Cold Spring Harbor Laboratory, New York: Octamer-binding protein displays remarkably degenerate sequence binding specificity through interaction with flanking sequences.

Clark, L., Hay, R.T., Dept. of Biochemistry and Microbiology, University of St. Andrews, Fife, Scotland: Interactions of EBP1 with the SV40 enhancer—Contact point and mutational analysis of the binding site.

Loeken, M.R.,[1-2] Bikel, I.,[2-3] Livingston, D.M.,[2-3] Brady, J.,[4] [1]Joslin Diabetes Center, [2]Harvard Medical School,

[3]Dana Farber Cancer Institute, Boston, Massachusetts; [4]NCI, National Institutes of Health, Bethesda, Maryland: SV40 small t antigen *trans*-activates pol II and III promoters.

Gallo, G.J., Gilinger, G., Manuppello, J., Alwine, J.C., Dept. of Microbiology, University of Pennsylvania School of Medicine, Philadelphia: Gene expression activation and the alteration of DNA-binding characteristics of host-cell transcription factors mediated by SV40 large T antigen.

Dynan, W.S., Ayer, D., Dept. of Chemistry and Biochemistry, University of Colorado, Boulder: Analysis of two regions controlling SV40 late transcription.

Musial, S.,[1] St. Louis, J.,[1] Ro, H.-S.,[2,3] Speigelman, B.,[2,3] Cherington, V.,[1] [1]Dept. of Pathology, Tufts University School of Medicine, [2]Dana Farber Cancer Institute, [3]Dept. of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts: An early event in adipocyte differentiation blocked by SV40 large T antigen.

Ostapchuk, P., Scheirle, T., Hearing, P., Dept. of Microbiology, State University of New York, Health Science Center, Stony Brook: Analysis of the EF-C binding sites in the polyomavirus and HBV enhancer regions.

Martin, M., Yoo, W., Folk, W., Dept. of Microbiology, University of Texas, Austin: Developmental regulation of three cellular factors important in activation of the polyomavirus enhancer.

Furukawa, F., Satake, M., Ito, Y., Institute for Virus Research, Kyoto University, Japan: Positive and negative factors interacting with the polyomavirus enhancer involved in developmental regulation.

Villarreal, L.P., Campbell, B.A., Sun, R.R., Davis, K., DePolo, N., Dept. of Molecular Biology and Biochemistry, University of California, Irvine: In vivo tissue specificity of polyomavirus DNA replication; modular enhancers and the natural strategy of papovaviruses.

SESSION 6 POSTER SESSION

Ackrill, A.M., Blair, G.E., Dept. of Biochemistry, University of Leeds, Scotland: Regulation of transcription of MHC class I genes in adenovirus-transformed rat cells.

Allard, A., Wadell, G., Dept. of Virology, University of Umeå, Sweden: Physical organization of the enteric Ad41 early region E1.

Ballmer-Hofer, K., Moroni, C., Muser, J., Friedrich Miescher Institute, Basel, Switzerland: Effect of polyomavirus middle T antigen on the growth factor requirement of FDCP-1 cells.

Burns, J.S., Williams, E.D., Wynford-Thomas, D., Dept. of Pathology, University of Wales College of Medicine, Cardiff: SV40 large T transformation of rat thyroid epithelial cells.

Carbone, M., Chattopadhyay, S.K., Lewis, A.M., Jr., NIAID, National Institutes of Health, Bethesda, Maryland: Peculiar persistence and expression of viral genomes in Ad5-transformed BALB/c mouse cells.

Carlin, C.R., Tollefson, A.E., Brady, H.A., Hoffman, B.L., Ranheim, T.S., Wold, W.S.M., Institute for Molecular

D. Livingston, H. Ozer, Y. Gluzman, Y. Aloni

Virology, St. Louis University Medical Center, Missouri: Epidermal growth factor receptor is down-regulated by a 10,400-molecular-weight protein encoded in region E3 of adenovirus.

Clever, J., Kasamatsu, H., Dept. of Biology, University of California, Los Angeles: The carboxy-terminal 40 amino acids of SV40 VP3 confer upon β-galactosidase the ability to bind DNA and change its conformation.

Deckhut, A.,[1] Tevethia, M.J.,[1] Haggerty, S.,[2] Frisque, R.,[2] Tevethia, S.S.,[1] [1]Dept. of Microbiology, Penn State College of Medicine, Hershey; [2]Dept. of Molecular and Cellular Biology, Penn State University, University Park, Pennsylvania: Analysis of JC virus T-antigen epitopes recognized by MHC-restricted cytotoxic T lymphocytes.

Deminie, C.A., Norkin, L.C., Dept. of Microbiology, University of Massachusetts, Amherst: SV40 infection of semi-permissive human cells studied by in situ hybridization and simultaneous immunocytochemistry.

Egan, C.,[1-3] Jelsma, T.N.,[1] Howe, J.A.,[1] Bayley, S.T.,[1] Branton, P.E.,[2-3] Depts. of [1]Biology, [2]Pathology, [3]Molecular Virology and Immunology Program, McMaster University, Hamilton, Canada: Mapping of the binding sites for cellular proteins on the E1A products of human Ad5.

Evrard, C., Galiana, E., Rouget, P., Laboratoire de Biochimie Cellulaire, Université Paris et Collège de France, France: Immortalization of murine brain cells after transfer of the adenovirus E1A or the polyomavirus large T genes.

Friedman, P., Wang, E., Prives, C., Dept. of Biological Sciences, Columbia University, New York, New York: The SV40 T antigen–p53 complex—Its formation and biochemical properties in vitro.

Glenn, G., Eckhart, W., Molecular Biology and Virology Lab, Salk Institute, San Diego, California: Regulation of *fos* expression by polyomavirus and SV40.

Herbst, R.S.,[1] Hermo, H.,[2] Fisher, P.B.,[2] Babiss, L.E.,[1] [1]Rockefeller University, [2]Dept. of Pathology and Urology, Columbia University College of Physicians & Surgeons, New York, New York: Regulation of adenovirus and cellular gene expression and cellular transformation by the E1B-encoded 175R protein.

Herrmann, C.H., Mathews, M.B., Cold Spring Harbor Laboratory, New York: Effects of the E1B 19K protein on transcription and DNA stability.

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Huang, M.M., Hearing, P., Dept. of Microbiology, Health Science Center, State University of New York, Stony Brook: Genetic analysis of Ad5 early region 4.

Li, Q.-G., Wadell, G., Dept. of Virology, University of Umeå, Sweden: Molecular epidemiology and genetic variability of adenoviruses of subgenera B and E.

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Raptis, L.,[1] Garcea, R.,[2] Bolen, J.,[3] [1]Dept. of Microbiology and Immunology, Queen's University, Kingston, Canada; [2]Dana-Farber Cancer Institute, Boston, Massachusetts; [3]NCI, National Istitutes of Health, Bethesda, Maryland: Transformation of mouse NIH-3T3 cells by polyomavirus middle T antigen.

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Segawa, K.,[1] Yoshitake, Y.,[1] Takano, T.,[1] Asada, M.,[2] Furukawa, K.,[2] Kobata, A.,[2] Sato, C.,[1] [1]Dept. of Microbiology, Keio University, Tokyo, [2]Dept. of Biochemistry, Institute of Medical Science, Tokyo, [3]Laboratory of Experimental Radiology, Aich Cancer Research Institute, Nagoya, Japan: Biological functions of SV40 large T antigen having the membrane

attachment sequence of polyomavirus middle T antigen.

Slavicek, J.M.,[1] Jones, N.,[2] Richter, J.D.,[1] [1]Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts; [2]Imperial Cancer Research Fund, London, England: A karyophilic signal sequence in Ad5 E1A located in the 13S unique region is functional in *Xenopus oocytes* but not somatic cells.

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St-Onge, L., Bouchard, L., Bastin, M., Dept. of Microbiology, University of Sherbrooke, Quebec, Canada: Mechanism of transcriptional activation by polyomavirus large T antigen.

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Tack, L.,[1] Frankel, C.,[1] Gurney, E.,[2] [1]Laboratory of Molecular Biology and Virology, Salk Institute, La Jolla, California; [2]Dept. of Biology, University of Utah, Salt Lake City: Properties of SV40 T and T + p53 complexes during lytic infection.

Westphal, K.-H., Espen, J., Laboratorium für moleculare Biologie-Genzentrum, Martinsried, Federal Republic of Germany: Interaction of SV40 T antigen with cellular RNA—Inactivation of tumor suppressors?

Westphal, K.-H., Muller, K., Mitreiter, R., Espen, J., Laboratorium für molekulare Biologie, Genzentrum, Martinsried, Federal Republic of Germany: SV40 T antigen inhibits the differentiation of thymic mouse muscle cells. This inhibition might be suppressed by transfected DNA.

Nakshatri, H., Pater, M.M., Pater, A., Memorial University of Newfoundland, St. John's, Canada: Characterization of the function of tumor antigens of BK virus in transformation by antisense RNA and in vitro mutagenesis.

SESSION 7 POSTER SESSION

Albin, R.,[1] Harter, M.L.,[2] Flint, S.J.,[1] [1]Dept. of Biology, Princeton University, [2]Dept. of Microbiology and Molecular Genetics, New Jersey Medical School, Newark: Bacterially synthesized 289R E1A protein directly *trans*-activates Ad2 major late transcription.

Bandea, C., Wu, M.-W.H., Wu, G.-J., Dept. of Microbiology and Immunology, Emory University School of Medicine, Atlanta, Georgia: Sequence requirement for a functional B block promoter element in the VA $_{I}$RNA1 gene.

Berkner, K.L., Beech, K.D., Harkonen, S.K., Zymogenetics, Inc., Seattle, Washington: Analysis of translation initiation facilitated by the tripartite leader, at ATGs downstream from the start codon.

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Bohan, C.,[1] Srinivasan, A.,[2] Robinson, R.,[3] [1]Dept. of Pathology, Emory University School of Medicine, [2]Centers for Disease Control, Atlanta, Georgia; [3]Dept. of Microbiology, University of Texas Southwestern Medical Center, Dallas: Differential effects of adenovirus 13S and 12S E1A gene products on HIV gene expression.

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Carcamo, J., Lobos, S., Merino, A., Reinberg, D., Dept. of Biochemistry, University of Medicine and Dentistry of New Jersey, Piscataway: Role of MLTF in transcription from the IVa2 promoter.

Carswell, S., Alwine, J.C., Dept. of Microbiology, University of Pennsylvania School of Medicine, Philadelphia: Analysis of the efficiency of utilization of the SV40 late polyadenylation site—Involvement of upstream sequences.

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Dolph, P.J., Haung, J., Schneider, R.J., Dept. of Biochemistry and Kaplan Cancer Center, New York University Medical Center, New York: Characterization of cap-binding protein-independent translation conferred by the adenovirus tripartite leader.

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Gharakhanian, E., Kasamatsu, H., Dept. of Biology, University of California, Los Angeles: Two independent signals—a nuclear localization signal and a VP1-interactive signal—reside within the carboxy-terminal 35 amino acids of SV40 VP3.

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Gorman, C., Huang, M., Dept. of Cell Genetics, Genentech, Inc., South San Francisco, California: The SV40 small t intron, present in most CAT vectors, leads to aberrant splicing.

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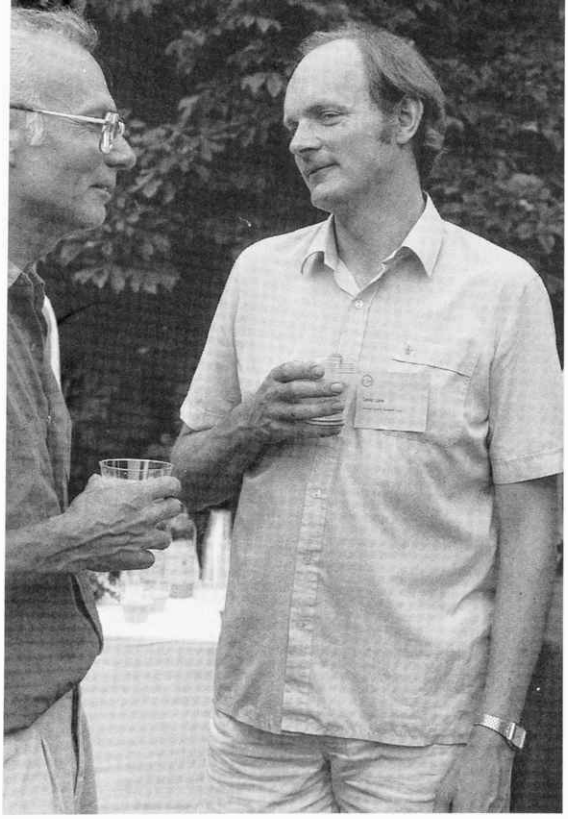

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Wu, G.-J., Dept. of Microbiology and Immunology, Emory University School of Medicine, Atlanta, Georgia: Effects of mutations on assembly of transcription machinery containing the VA RNA1 gene.

SESSION 8 EARLY PROTEINS: STRUCTURE AND FUNCTION

Chairman: M. Fried, Imperial Cancer Research Fund

McVey, D.,[1] Gluzman, Y.,[1] Mohr, I.,[1] Pizzolato, M.,[1] Strauss, M.[2] [1]Cold Spring Harbor Laboratory, New York; [2]Zentralinstitut fur Molekularbiologie, East Berlin, German Democratic Republic: Interaction of SV40 large Tag with origin-specific and single stranded DNA.

Loeber, G., Parsons, R., Tegtmeyer, P., Dept. of Microbiology, State University of New York, Stony Brook: Genetic analysis of the "zinc finger" of SV40 large T antigen.

Bradley, M.K.,[1] Wyatt, J.,[1] Weiner, B.,[2] Kurihara, T.,[1] Depts. of [1]Pathology, [2]Biological Chemistry and Molecular Pharmacology, Dana-Farber Cancer Institute and Harvard Medical School, Boston, Massachusetts: Effective choices for site-directed mutagenesis of the SV40 T-antigen gene were made according to a model for the tertiary structure of the ATP-binding site.

Stacy, T., Chamberlain, M., Cole, C.N., Dept. of Biochemistry and Molecular Genetics Center, Dartmouth Medical School, Hanover, New Hampshire: Analysis of SV40

M. Imperiale, J. DeCaprio

K. Rundel, M. Pater

mutants that do not express the carboxyl terminus of large T antigen and are deficient in the host range/adenovirus helper function.

van Zee, K., Schindewolf, C., Schneider, J., Fanning, E., Institute for Biochemistry, Munich, Federal Republic of Germany: A mutant SV40 T antigen interferes with nuclear localization of heterologous proteins and activation of SV40 late gene expression by wild-type T antigen.

Maxwell, S.A., Butel, J.S., Dept. of Virology, Baylor College of Medicine, Houston, Texas: Dynamic and growth-dependent expression of SV40 large T antigen in protein complexes on the surface of SV40-transformed cells.

Linder, S., Nilsson, M., Martens, I., Magnusson, G., Dept. of Medical Virology, Uppsala University Biomedical Centre, Sweden: Genetic analysis of the involvement of polyomavirus large T antigen and small T antigen in transformation and viral growth.

Bockus, B., Rose, P., Holman, P., Day, R., Schaffhausen, B., Dept. of Biochemistry, Tufts Medical School, Boston, Massachusetts: Mutational analysis of polyomavirus large T antigen.

Dumont, D.J., Tremblay, M.L., Branton, P.E., Molecular Virology and Immunology Program, Dept. of Immunology, McMaster University, Hamilton, Canada: Analysis of the role of Ad5 E1A protein phosphorylation by site-directed mutagenesis.

Cleghon, V., Morin, N., Delsert, C., Voelkerding, K., Klessig, D.F., Waksman Institute, Rutgers University, Piscataway, New Jersey: Active nuclear localization of adenovirus DNA-binding protein is complex and is non-essential for viral viability.

White, E., Cipriani, R., Cold Spring Harbor Laboratory, New York: Regulation of gene expression by the adenovirus E1B 19K tumor antigen.

SESSION 9 TRANSCRIPTION III: *TRANS*-ACTIVATION

Chairman: **P. Hearing,** State University of New York, Stony Brook

Kovelman, R., Hoeffler, W.K., Workman, J.L., Cromlish, W.A., Abmayr, S.M., Roeder, R.G., Laboratory of Biochemistry and Molecular Biology, Rockefeller University, New York, New York: In vitro transcriptional regulation by viral immediate early proteins.

Datta, S.,[1] Chatterjee, P.,[2] Losada, M.C.,[1] Flint, S.J.,[2] Harter, M.L.,[1] [1]Dept. of Microbiology and Molecular Genetics, New Jersey Medical School, Newark; [2]Dept. of Molecular Biology, Princeton University, New Jersey: An *E. coli*-produced E1A 289R protein and a synthetic E1A 49R peptide variably regulates pol II and pol III transcription in vitro.

Maguire, K., Rivlin, M., Levy, D., Weinmann, R., Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania: Analysis of transcription factors interacting with adenovirus E1A.

Loewenstein, P.M., Pusztai, R., Green, M., Institute for Molecular Virology, St. Louis University Medical Center, Missouri: In vivo and in vitro *trans*-activation by synthetic peptides encoded in Ad5 E1A region 3—Mechanism and mutational analysis.

Webster, L.C., Ricciardi, R.P., Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania: Distinct sub-domains within the *trans*-activating domain of E1A.

Martin, K.J., Lillie, J.W., Lee, K.A.W., Green, M.R., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Mechanisms of transcriptional activation by E1A.

Engel, D.A., Shenk, T., Dept. of Molecular Biology, Princeton University, New Jersey: E1A-dependent induction of E1A and E1A-regulated adenovirus early genes by cAMP.

Raychaudhuri, P., Bagchi, S., Yee, A.S., Nevins, J.R., Duke University Medical Center, Durham, North Carolina: Phosphorylation of E2F and E4F transcription factors as a mechanism for E1A-mediated *trans*-activation.

Buckbinder, L., Cortes, P., Reinberg, D., University of Medicine and Dentistry of New Jersey, Piscataway: Specific factors involved in transcription of the adenovirus EIII promoter.

Leong, K., Berk, A.J., Dept. of Microbiology and Molecular Biology Institute, University of California, Los Angeles: Interaction of infection-induced factors with sequences within the first intron on the adenovirus MLP results in elevated accumulation of RNA synthesized from the MLP in late adenovirus-infected cells.

SESSION 10 POST-TRANSCRIPTIONAL REGULATION

Chairman: C. Prives, Columbia University

Huang, M., Gorman, C., Dept. of Cell Genetics, Genentech, South San Francisco, California: Splicing contributes to the stability and/or transport of mRNA in 293 cells.

Ryu, W.-S., Mertz, J.E., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Transcripts synthesized from cDNA mutants of SV40 late mRNA are defective in both stability in the nucleus and transport to the cytoplasm.

Hyde-DeRuyscher, R., Carmichael, G., Dept. of Microbiology, University of Connecticut Health Center, Farmington: An unusual mechanism for the temporal regulation of polyomavirus gene expression.

Kessler, M., Resnekov, O., Bengal, E., Aloni, Y., Weizmann Institute of Science, Rehovot, Israel: In vitro structural analyses of the SV40 attenuation signal.

Larsson, S., Akusjarvi, G., Dept. of Microbial Genetics, Karolinska Institute, Stockholm, Sweden: Activation of the adenovirus major late transcription unit—Accumulation of late mRNA is controlled at multiple levels.

Mellits, K.H., Kostura, M., Mathews, M.B., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: Structure and function of adenovirus VA RNA_I.

Furtado, M.R.,[1] Subramanian, S.,[1] Bhat, R.A.,[1] Safer, B.,[2] Thimmappaya, B.,[1] [1]Dept. of Microbiology and Immunology, Northwestern University Medical School, Chicago, Illinois; [2]NHLB Institute, National Institutes of Health, Bethesda, Maryland: Adenovirus VA_I RNA and translation control—Structure rather than the sequence per se may be critical for its function.

Slavicek, J.M.,[1] Jones, N.,[2] Richter, J.D.,[1] [1]Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts; [2]Imperial Cancer Research Fund, London, England: Rapid turnover of adenovirus E1A is determined through a cotranslational mechanism that requires an amino-terminal domain.

Lavery, D., Leu, M.-H., Chen-Kiang, S., Immunobiology Center and Dept. of Microbiology, Mt. Sinai School of Medicine, New York, New York: Enhancement of T-cell-specific block of adenoviral mRNA transport in the absence of E1B.

SESSION 11 TRANSFORMATION AND VIRUS–HOST CELL INTERACTIONS

Chairman: Y. Gluzman, Lederle Laboratories

Kaplan, P.,[1] Small, M.B.,[2] Li, G.,[1] Orlian, M.,[1] Pardinas, J.,[1] Resnick-Silverman, L.,[1] Zainul, B.,[1] Jha, K.K.,[1] Ozer, H.L.,[1] [1]Dept. of Biological Sciences, Hunter College, City University of New York, New York; [2]G.S. Hooper Foundation, Dept. of Microbiology and Immunology, University of California Medical Center, San Francisco: Role of SV40 T antigen in immortalization of human fibroblasts.

Westphal, K.-H., Kolbeck, C., Mitreiter, R., Laboratorium für molekulare Biologie, Genzentrum, Martinsried, Federal Republic of Germany: Expression of SV40 T antigen in human embryonic cells in human malignant melanoma and normalized derivatives.

Butel, J.S.,[1] Slagle, B.L.,[1] Sepulveda, A.,[2] Clift, S.M.,[3] Shen, R.-F.,[3] DeMayo, J.L.,[3] Finegold, M.J.,[2] Woo, S.L.C.,[3] Depts. of [1]Virology, [2]Pathology, [3]Cell Biology, Baylor College of Medicine, Houston, Texas: Multiple tumors induced in transgenic mice by the expression of SV40 large T antigen controlled by the regulatory elements of the human alpha-antitrypsin gene.

Dubensky, T.W., Freund, R., Dawe, C.J., Benjamin, T.L., Dept. of Pathology, Harvard Medical School, Boston, Massachusetts: Polyomavirus tumor induction in mice—Influences of viral replication on tumor profiles.

Yamashita, T., Kato, H., Fujinaga, K., Dept. of Molecular Biology, Cancer Research Institute, Sapporo Medical College, Japan: Collaborative transformation of rat cells by Ad12 E1A and v-*abl* oncogenes.

Subramanian, T., Chinnadurai, G., Institute for Molecular Virology, St. Louis University Medical Center, Missouri: Suppression of T24 *ras* oncogene-mediated tumorigenesis by an Ad2 E1A protein region.

Ginsberg, H.S.,[1] Beauchamp, U.,[1] Wold, W.S.,[2] Chanock, R.M.,[3] Pernis, B.,[1] Prince, G.,[3] [1]Columbia University, New York, New York; [2]Institute for Molecular Virology, St. Louis University, Missouri; [3]NIAID, National Institutes of Health, Bethesda, Maryland: Function of adenovirus E3 in pathogenesis.

Gooding, L.R.,[1] Tollefson, A.E.,[2] Elmore, L.,[1] Horton, T.,[1] Duerksen-Hughes, P.,[1] Wold, W.S.M.,[2] [1]Dept. of Microbiology, Emory University School of Medicine, Atlanta, Georgia; [2]Institute for Molecular Virology, St. Louis University Medical Center, Missouri: The 14.7K protein encoded in region E3 of adenovirus is a conditional inhibitor of TNF-mediated cytolysis.

Cook, J.,[1] May, D.,[1] Wilson, B.,[1] Chen, M.-J.,[2] Shalloway, D.,[3] Walker, T.,[1] [1]National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado; [2]Smith Kline and French Laboratories, King of Prussia, Pennsylvania; [3]Pennsylvania State University, University Park: E1A gene expression in transformed cells induces cytolytic susceptibility by TNF-dependent and -independent mechanisms.

Atwood, W.J., Norkin, L.C., Dept. of Microbiology, University of Massachusetts, Amherst: Interaction of SV40 with class I MHC antigens on rhesus monkey kidney cells.

Tevethia, S.,[1] Tanaka, Y.,[1] Anderson, R.,[2] Maloy, L.,[2] [1]Dept. of Microbiology, Penn State College of Medicine, Hershey, Pennsylvania; [2]NIAID, National Institutes of Health, Bethesda, Maryland: Synthetic peptides as probes for the localization of SV40 T antigen epitopes recognized by cytotoxic lymphocyte clones.

Molecular Genetics of Bacteria and Phages

August 16—August 21

ARRANGED BY

Gary Gussin, University of Iowa
Gisela Mosig, Vanderbilt University
Andrew Wright, Tufts Medical School

287 participants

The 1988 meeting on Molecular Genetics of Bacteria and Phages attracted over 300 scientists, including many young investigators in the field. Topics discussed at the meeting included DNA replication, recombination, various aspects of transcriptional control, RNA processing, and membrane proteins. The common thread throughout the meeting was again the mechanism by which protein-DNA interactions influence processes such as DNA replication, DNA partitioning, site-specific recombination, and regulation of gene expression. Of particular interest was the role that proteins such as IHF and FIS play as accessory factors in many of these processes. Exciting advances were reported in the understanding of the molecular mechanisms of signal transduction in *E. coli* by the regulatory pairs of proteins EnvZ/OmpR, NtrB/NtrC, and CheA/CheY, which function through protein phosphorylation to regulate important cellular processes. At least two of these systems were shown to exhibit crosstalk, sharing a common transphosphorylation step. It was evident that molecular techniques are now being applied to a wider range of microorganisms than ever before. Impressive advances were reported in the understanding of gene regulation relating to pathogenesis in organisms such as *Vibrio cholerae* and *Bordetella pertussis*. The quality of presentations at the meeting was of uniformly high quality.

SESSION 1 DNA REPLICATION

Wickner, S., Hoskins, J., Chattoraj, D., McKenney, K., NCI, National Institutes of Health, Bethesda, Maryland: Replication of mini-P1 plasmid DNA in vitro.

Pal, S.K., Dasgupta, S., Chattoraj, D.K., NCI, National Institutes of Health, Bethesda, Maryland: P1 plasmid replication—Control by steric hindrance.

Davis, M., Martin, K., Austin, S., NCI-Frederick Cancer Research Facility, Frederick, Maryland: A host factor that binds to the partition site of the P1 plasmid.

Martin, K., Davis, M., Fernandez, L., Austin, S., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Mutagenesis of the P1 plasmid-partition site.

Novick, R.P., Iordanescu, S., Projan, S.J., Edelman, I., Public Health Research Institute, New York, New York: pT181 replication is regulated by countertranscript-induced transcriptional attenuation.

Pelletier, A.J., Hill, T.M., Kuempel, P.L., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Studies in a plasmid system of termination sites that inhibit DNA replication in *E. coli*.

Lin, G., Gruidl, M., Luder, A., Mosig, G., Dept. of Molecular Biology, Vanderbilt University, Nashville, Tennessee: Novel intermediates in phage T4 DNA replication.

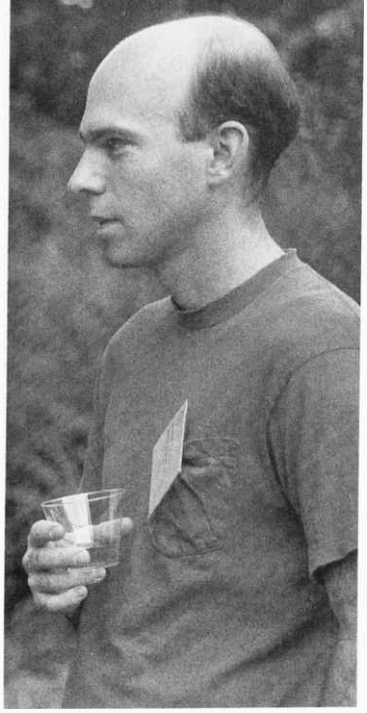
Andrake, M.,[1] Hsu, T.,[1] Guild, N.,[2] Dawson, M.,[1] Gold, L.,[2] Karam, J.,[1] [1]Dept. of Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston; [2]Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Control of T4 DNA polymerase biosynthesis.

Polayes, D.A., Martin-Moe, S.A., Ruscitti, T.M., Linn, S.M., Dept. of Biochemistry, University of California, Berkeley: Transcriptional control of the *polA* gene in *E. coli*.

Imamoto, F., Kano, Y., Wada, M., Kohno, K., Goshima, N., Tsukuba Life Science Center, Riken, Japan: Genetic characterization and transcriptional regulation of the *hup* genes encoding histone-like protein HU of *E. coli*.

Di Laurenzio, L., Frost, L., Finlay, B., Paranchych, W., Dept. of Biochemistry, University of Alberta, Edmonton, Canada: Interaction of the mating signal protein, TraMp, with the origin of transfer of the conjugative plasmid pED208.

T. Atkins



T. Elliot

SESSION 2 RECOMBINATION

Hughes, R., Hatfull, G., Sanderson, M., Freemont, P., Rice, P., Goldman, A., Steitz, T., Grindley, N., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Recombinationally deficient mutants of the γδ resolvase that are deficient in protein/protein interactions in the γδ resolvasome.

Lee, E., Gardner, J., Dept. of Microbiology, University of Illinois, Urbana: Genetic analysis of λ integrase arm-type binding-site interactions.

Ball, C.A.,[1] Saccone, G.D.,[2] Johnson, R.C.,[1,2] [1]Molecular Biology Institute, [2]Dept. of Biological Chemistry, University of California, Los Angeles: Genetic analysis of the operon encoding Fis—In vivo function of Fis in Hin-mediated DNA inversion and λ excision.

Ramaiah, N.,[1] Yagil, E.,[2] Kislev, N.,[1] Dolev, S.,[2] Weisberg, R.,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Tel Aviv University, Israel: Localization of the determinants of specificity of site-specific recombination.

Shapiro, J.A.,[1] Higgins, N.P.,[2] [1]Dept. of Biochemistry and Molecular Biology, University of Chicago, Illinois; [2]Dept. of Biochemistry, University of Alabama Medical School, Birmingham: Mu*dlac* replication/transposition and *lacZ* expression in *E. coli* colonies.

Schumann, W., Apfel, C., Mummenthey, K., Kirschner, P., Lehrstuhl für Genetik, Universiät Bayreuther, Federal Republic of Germany: Identification of two functions involved in the regulation of the transposition functions of phage Mu.

Sonti, R.V., Keating, D.H., Roth, J.R., Dept. of Biology, University of Utah, Salt Lake City: Rec-dependent transposition of Mu*d* phages of transducing fragments is due to increased transposition in *recA*^{1} and in *recBC*^{1} strains.

Sandler, S.J., Clark, A.J., Dept. of Molecular Biology, University of California, Berkeley: Factors influencing inhibition of overexpression of the *recF* gene of *E. coli* K-12.

Poteete, A.R., Volkert, M.R., Dept. of Molecular Genetics and Microbiology, University of Massachusetts, Worcester: Activation of RecF-dependent recombination in *E. coli* by bacteriophage λ- and p22-encoded functions.

Kobayashi, I., Takahashi, N., Dept. of Infectious Diseases Research, National Children's Research Center and Hospital, Tokyo, Japan: Double-strand gap repair by *E. coli* and λ.

Brusca, J.S., Chastain, C.J., Hale, M.A., Carrasco, C., Golden, J.W., Dept. of Biology, Texas A&M University, College Station: Expression of the *xisA* gene of *Anabaena* 7120 causes excision of the *nifD* element in vegetative cells.

SESSION 3 POSTER SESSION

Alifano, P., Nappo, A.G., Bruni, C.B., Carlomagno, M.S., Dept. of Biology, University of Naples, Italy: Polar effects of nonsense mutations within a cistron.

Anderson, R., Young, K.D., Dept. of Microbiology and Immunology, University of North Dakota School of Medicine, Grand Forks: At low, noninducing temperature, *grpE* and/or *dnaK* mutations affect lysis of *E. coli* by ϕX174.

Balke, V., Nagaraja, V., Wall, L., Hattman, S., Dept. of Biology, University of Rochester, New York: Mutation and footprinting analysis of the phage Mu *mom* operon.

Barras, F.,[1] Marinus, M.G.,[2] [1]LCB, CNRS, Marseille, France; [2]Dept. of Pharmacology, University of Massachusetts Medical School, Worcester: Arrangement and evolution of Dam methylation sites (GATC) in the *E. coli* chromosome.

Blyn, L., Low, D., Dept. of Pathology, University of Utah, Salt Lake City: Analysis of a novel phase-variation switch in uropathogenic *E. coli*.

Bukau, B., Walker, G.C., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Deletion of the *dnaK* heat-shock gene of *E. coli* causes defects in chromosome distribution and plasmid maintenance.

Calendar, R.,[1] Erickson, J.W.,[2] Halling, C.,[1] Nolte, A.,[1] [1]Dept. of Molecular Biology, University of California, Berkeley; [2]Dept. of Bacteriology, University of Wisconsin, Madison: Deletion and insertion mutations in the *rpoH* gene of *E. coli* that produce functional σ^{32}.

Carlson, N.,[1] Little, J.,[1] Oberto, J.,[2] Weisberg, R.,[2] [1]Dept. of Biochemistry, University of Arizona, Tucson; [2]NCI, National Institutes of Health, Bethesda, Maryland: Characterization of specific DNA binding and RecA-mediated cleavage of the putative *cI* repressor from the λ-related phage, HK022.

Carty, M., Menzel, R., E.I. du Pont de Nemours, Experimental Station, Wilmington, Delaware: Relaxation (novobiocin)

activation of the *gyrA* promoter in an S-30 in vitro transcription-translation system—Evidence for regulatory factors.

Chandran, U., Hendrix, R., Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: Translation control of the bacteriophage λ tail gene *H*.

Chen, S.-M., Takiff, H., Patterson, T., Court, D., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Biochemical properties of the RNase III and ERA proteins; products of the *E. coli* RNase III operon.

Chon, Y., Gayda, R., Dept. of Microbiology, Louisiana State University and Agricultural Center, Baton Rouge: Localization of FtsA protein near the membrane septation sites in *E. coli* K-12.

Dailey, S.H., Finkelstein, M., Dept. of Molecular Biology, Biotechnology Division, Schering Corporation, Bloomfield, New Jersey: Plasmid stability in *E. coli*—A comparison of *E. coli* 294 and W3110.

Davagnino, J., Yorgey, P., Kolter, R., Harvard Medical School, Boston, Massachusetts: Production of a small unstable protein-toxin involves the action of three regulatory proteins that afford protection from proteolysis.

de la Cruz, N.B., Krebs, M.P., Reznikoff, W.S., Dept. of Biochemistry, University of Wisconsin, Madison: Partial purification and binding studies on the Tn*5* transposase.

DeLong, A.,[1] Syvanen, M.,[2] [1]Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts; [2]Dept. of Medical Microbiology and Immunology, University of California, Davis: Characterization of two Tn*5* mutants with *trans*-dominant defects in the inhibition of transposition.

Demirjian, D., Pagratis, N., Casadaban, M., Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois: Thermo-β-galactosidase—A new assayable marker.

Diaz, D.L.,[1] Rabin, B.A.,[1] Williams, K.R.,[2] Chase, J.W.,[1] [1]Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York; [2]Dept. of Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Connecticut: Structure and expression of the *E. coli xseB* gene, coding for the β subunit of exonuclease VII.

Dila, D., Raleigh, E.A., New England Biolabs, Beverly, Massachusetts: Genetic dissection of the methylcytosine-specific restriction system *mcrB* of *E. coli* K-12.

Driks, A.,[1] Greene, R.,[2] Schoenlein, P.,[3] Ely, B.,[3] Shapiro, L.,[2] DeRosier, D.,[1] [1]Graduate Program in Biology, Brandeis University, Waltham, Massachusetts; [2]Dept. of Microbiology, Columbia University, New York, New York; [3]Dept. of Biology, University of South Carolina, Columbia: Construction of the flagellum of *C. crescentus*.

Dul, E., Shatzman, A.R., Smith Kline and French Laboratories, King of Prussia, Pennsylvania: An unidentified λ function is required for expression of gene products encoded on plasmids containing a P_L transcription unit during SOS-mediated induction.

Eraso, J., Weinstock, G., Dept. of Biochemistry and Molecular Biology, University of Texas Medical School, Houston: Isolation of regulatory mutations in the *cea* gene.

Erickson, B.D., Burgess, R.R., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: RNA processing in the *rpsU-dnaG-rpoD* operon of *E. coli*.

Escalante-Semerena, J.C.,[1] Roth, J.R.,[2] [1]Dept. of Biochemistry, University of Wisconsin, Madison; [2]Dept. of Biology, University of Utah, Salt Lake City: *cobA*, a new genetic locus of *S. typhimurium* involved in B_{12} biosynthesis.

Ferrell, R., McIntosh, M., Dept. of Microbiology, University of Missouri School of Medicine, Columbia: Distribution of a repeated genetic element in porcine mycoplasmas suggests an extrachromosomal origin.

Frazier, M.W., Mosig, G., Dept. of Molecular Biology, Vanderbilt University, Nashville: A new phage T4 gene that prevents T4 late transcription in an *E. coli* mutant with a defective heat-shock σ_{32} factor.

Ganjam, K.,[1] Tuckman, M.,[1] Jacobs, W., Jr.,[1] Smith, C.,[2] Bloom, B.,[1] [1]Dept. of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, [2]Dept. of Human Genetics, Columbia University College of Physicians and Surgeons, New York, New York: Construction of physical maps of mycobacterial genomes.

Goodman, E.M.,[1] Greenebaum, B.,[1] Marron, M.T.,[2] [1]Biomedical Research Institute, University of Wisconsin, Parkside; [2]Office of Navy Research, Arlington, Virginia: Electromagnetic field effects on *E. coli*.

Greenberg, J.T., Monach, P., Demple, B., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Overlapping and distinct responses in *E. coli* to oxidative stresses generated by H_2O_2 or redox-cycling agents.

Grossman, T.H., Silverman, P.M., Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Inducible synthesis of functional F-pili in *E. coli* K-12.

Guo, H.-C., Roberts, J.W., Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Point mutational analysis of transcriptional pausing and initiation at the phage 82 late gene promoter.

SESSION 4 DNA MODIFICATION—ACTION OF IHF

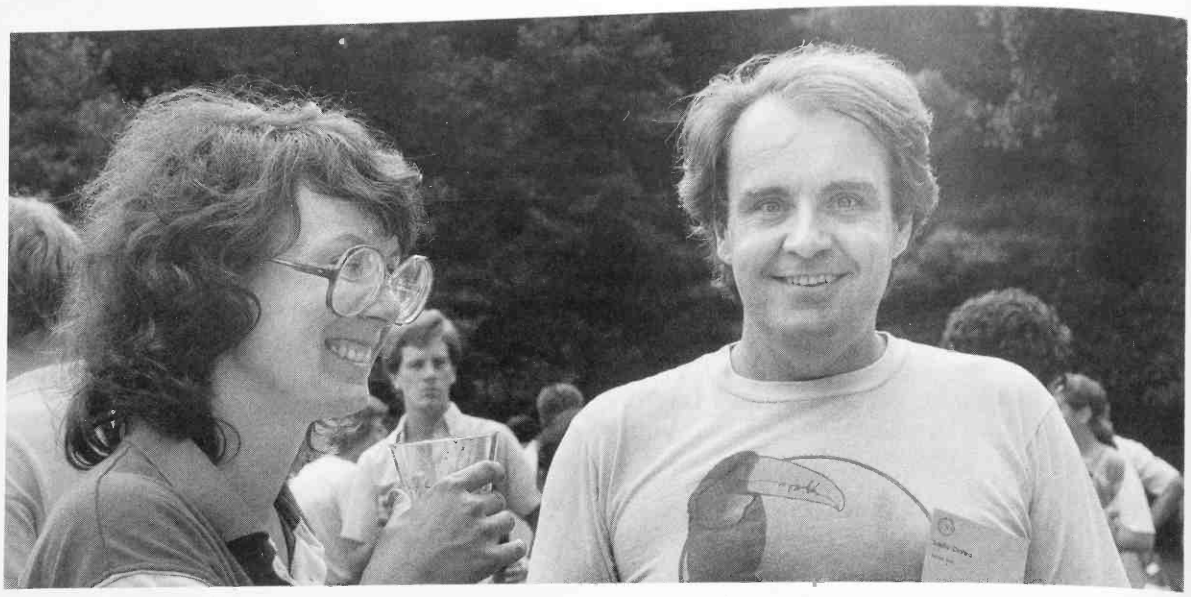
Heitman, J., Rockefeller University, New York, New York: Substrate recognition by the *Eco*RI endonuclease.

Kelleher, J.E., Daniel, A.S., Murray, N.E., Dept. of Molecular Biology, Edinburgh, Scotland: Defining structural domains of the *Eco*K methylase.

Sutherland, E., Raleigh, E.A., New England Biolabs, Beverly, Massachusetts: In vitro restriction of 5-methylcytosine-modified DNA by the *mcrB* system of *E. coli* K-12.

Miner, Z., Schlagman, S., Hattman, S., Dept. of Biology, University of Rochester, New York: Single-amino-acid changes that alter the sequence specificity of the T4 (Dam) DNA-adenine methyltransferase.

Wu, T.-H., Marinus, M.G., Dept. of Pharamacology,



M. Igo, K. Csonka

University of Massachusetts Medical School, Worcester: Spontaneous mutation and GATC sites in *E. coli*.

Bhagwat, A.S.,[1,2] Sohail, A.,[2,3] Lieb, M.,[4] [1]Dept. of Chemistry, Wayne State University, Detroit, Michigan; [2]Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; [3]University of the Punjab, Lahore, Pakistan; [4]University of Southern California School of Medicine, Los Angeles: A new gene involved in mismatch correction in *E. coli*.

Kosturko, L.D.,[1] Daub, E.,[2] Murialdo, H.,[2] [1]Dept. of Molecular Biology and Biochemistry, Wesleyan University, [2]Dept. of Medical Genetics, University of Toronto, Canada: Biochemistry of IHF/DNA interactions in λ DNA packaging.

Makris, J.C., Nordmann, P.L., Reznikoff, W.S., Dept. of Biochemistry, University of Wisconsin, Madison: 1. IHF is required for stimulation of IS*50* transposition in a *dam*[1] *host. 2. Inversion of the transposase gene with respect to the IS50* ends affects the level of transposition.

Greenstein, D., Horiuchi, K., Rockefeller University, New York, New York: Integration host factor interacts with the DNA replication enhancer of the filamentous phage fl.

van Rijn, P.A., Goosen, N., van de Putte, P., Dept. of Molecular Genetics, Leiden University, The Netherlands: Regulation of Mu transcription by IHF.

Kur, J., Hasan, N., Podhajska, A.J., Szybalski, W., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: In vitro transcription from the *b2-att* and P'_R promoter regions of coliphage λ —Repression by the *E. coli* integration host factor.

Tsui, P., Stevenson, B., Freundlich, M., Dept. of Biochemistry, State University of New York, Stony Brook: Specific binding of IHF to *ilv* promoter regions and effects on *ilvBN* attenuation.

SESSION 5 MEMBRANE PROTEINS

Boyd, D.,[1] Lee, C.A.,[2] Manoil, C.,[3] Beckwith, J.,[1] [1]Harvard Medical School, Cambridge, Massachusetts; [2]Stanford University Medical School, California; [3]University of Washington, Seattle: Determinants of membrane protein topology.

Gannon, P.,[1] Kumamoto, C.,[1,2] Depts. of [1]Physiology, [2]Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts: *seoC*-dependent export of maltose-binding protein requires a portion of the mature sequence.

Greenwood, J., Rowitch, D.H., Perham, R.N., Dept. of Biochemistry, University of Cambridge, England: Expression and site-directed mutagenesis of bacteriophage Pf1 coat protein gene in *E. coli*—Membrane insertion and processing.

Ball, T., Wasmuth, C., Benedik, M., Dept. of Biology, Texas A&M University, College Station: Regulation and excretion of *S. marcescens* nuclease.

Jakes, K.S.,[1] Davis, N.G.,[2] [1]Rockefeller University, New York, New York; [2]Institute for Molecular Biology, University of Oregon, Eugene: A hybrid toxin from phage f1 attachment protein and colicin E3 has altered cell receptor specificity.

Slauch, J.M., Silhavy, T.J., Dept. of Biology, Princeton University, New Jersey: The switch from OmpF to OmpC synthesis in *E. coli*.

Igo, M., Dept. of Molecular Biology, Princeton University, New Jersey: EnvZ, a regulatory protein involved in porin fluctuation, can be phosphorylated.

Misra, R., Benson, S.A., Dept. of Molecular Biology, Princeton University, New Jersey: Identification and characterization of a new porin protein, OmpG, of *E. coli* K-12.

Jacoby, G.H., Leidenix, M., Young, K.D., Dept. of Microbiology and Immunology, University of North Dakota School of Medicine, Grand Forks: Diverse fractionation techniques reveal an unequal distribution of *E. coli* penicillin-binding proteins among inner-membrane vesicles.

Gottesman, S., Clark, W., Maurizi, M., NCI, National Institutes of Health, Bethesda, Maryland: Genetics of proteolysis in *E. coli* —The Clp ATP-dependent protease.

DiRita, V.J., Miller, V.L., Mekalanos, J.J., Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Molecular basis of coordinate gene expression in *V. cholerae*.

SESSION 6 POSTER SESSION

Hamilton, E.P., Lee, N.L., Dept. of Biological Sciences, University of California, Santa Barbara: Fucose-induced binding of a mutant AraC protein to $ara I_2$ activates the *araBAD* operon promoter.

Heltzel, A., Totis, P., Summers, A.O., Dept. of Microbiology, University of Georgia, Athens: In vivo analysis of transcription initiation at the *mer* promoter of Tn*21*.

Hupp, T.R., Kaguni, J.M., Dept. of Biochemistry, Michigan State University, East Lansing, Michigan: Biochemical characterization of the *E. coli* DnaA5 protein.

Hwa, V., Salyers, A.A., Dept. of Microbiology, University of Illinois, Champaign-Urbana: Characterization and regulation of chondroitin sulfate utilization in *Bacteroides*.

Javad, T., Musso, R.E., Dept. of Biology, University of South Carolina, Columbia: Demonstration of the genes encoded by the insertion sequence IS*2* using *lacZ* fusions.

Kang, P.J., Craig, E.A., Dept. of Physiological Chemistry, University of Wisconsin, Madison: Cloning and identification of a new *E. coli* gene that suppresses the temperature-sensitive growth of a *dnaK* deletion mutant strain.

Kantorow, M., McKenney, K., Dept. of Genetics, George Washington University, and National Bureau of Standards, Gaithersburg, Maryland: Efficient site-directed mutagenesis and protein domain mapping using gene fusions.

Keller, J., Gerber, R., Del Tito, B.J., Sharr, D., Arcuri, E.J., Dept. of Biological Process Sciences, Smith Kline and French Laboratories, King of Prussia, Pennsylvania: Effect of mutations altering SOS regulation on nalidixic acid induction of heterologous protein synthesis in *E. coli*.

Kiino, D.R.,[1] Wilt, K.,[1] Rothman-Denes, L.B.,[1,2] Depts. of [1]Molecular Genetics and Cell Biology, [2]Biochemistry and Molecular Biology, University of Chicago, Illinois: At least four genes are involved in coliphage N4 adsorption.

Kim, J., Zwieb, C., Wu, C., Adhya, S., National Cancer Institute, NIH, Bethesda, Maryland: DNA bending by cAMP receptor protein; use of a DNA bending vector.

Lau, P.C.K., Condie, J.A., Biotechnology Research Institute, National Research Council of Canada, Montreal, Quebec: Colicin E9 plasmid contains an insertion sequence with features similar to those of the degenerate transposon, IS*101*.

Lazarus, A., Warren, F., Zabriskie, D., Shatzman, A., Smith Kline and French Laboratories, King of Prussia, Pennsylvania: Rate of thermal induction affects production of heterologous gene products from P_L vectors in *E. coli*.

Lim, D.F., Kuempel, P.L., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Deletion of *dif,* a *cis*-acting locus in the terminal region of the *E. coli* chromosome, induces SOS.

Liu, L., Berg, C.M., Dept. of Molecular and Cell Biology, University of Connecticut, Storrs: Mutagenization of dimeric plasmids.

Løbner-Olesen, A.,[1] Skarstad, K.,[2] Hansen, F.G.,[1] von Meyenburg, K.,[1] Boye, E.,[2] [1]Dept. of Microbiology, Technical University of Denmark, Copenhagen; [2]Dept. of Biophysics, Norwegian Radium Hospital, Oslow, Norway: The *DNAA* protein level determines the initiation mass of *E. coli* K-12.

McGovern, K., Beckwith, J., Dept. of Microbiology and Molecular Genetics, Harvard University School of Medicine, Boston, Massachusetts: Study of membrane localization of MalF, an integral membrane protein of *E. coli*.

Mecsas, J., Cowing, D.W., Gross, C.A., Dept. of Bacteriology, University of Wisconsin, Madison: Hydroxyl radical footprinting of $E\sigma^{32}$ promoter complexes at the *groE* promoter at different temperatures.

Michel, B., Zinder, N.D., Rockefeller University, New York, New York: F1 gene II protein translational repression by gene *V* protein.

Miller, J., Roy, C., Relman, D., Falkow, S., Dept. of Microbiology and Immunology, Stanford University, California: Analysis of *B. pertussis* virulence gene expression in *E. coli*.

Minnich, S.A.,[1] Ohta, N.,[2] Newton, A.,[2] [1]Dept. of Biology, Tulane University, New Orleans, Louisiana; [2]Dept. of Molecular Biology, Princeton University, New Jersey: Flagellin gene regulation in *C. crescentus*.

Miura-Masuda, A., Ikeda, H., Institute of Medical Science, University of Tokyo, Japan: Participation of DNA gyrase of *E. coli* in formation of spontaneous deletion in vivo.

Mullin, D.A.,[1] Van Way, S.M.,[1] Newton, A.,[2] [1]Dept. of Biology, Tulane University, New Orleans, Louisiana; [2]Dept. of Molecular Biology, Princeton University, New Jersey: Analysis of the *C. crescentus fla0* promoter using site-specific mutagenesis.

Musso, R., Hodam, T., Wilson, L., Dept. of Biology, University of South Carolina, Columbia: Tn5-derived kanamycin resistance cassettes for delivery of in-phase, frameshift, and translation termination mutations.

Ninfa, A.J., Mullin, D.A., Ramakrishnan, G., Newton, A.,

Dept. of Molecular Biology, Princeton University, New Jersey: *E. coli* σ^{54} RNA polymerase recognizes *C. crescentus flaN* and *flaK* flagellar gene promoters in vitro.

Numrych, T., Gardner, J., Dept. of Microbiology, University of Illinois, Urbana: Analysis of integration and excision of bacteriophage λ through mutagenesis of the Int arm-type binding sites.

Oeschger, M., Donze, D., Leitz, L., Dept. of Microbiology, Immunology, and Parasitology, Louisiana State University Medical Center, New Orleans: Antitermination regulation of RNA polymerase genes in *E. coli*.

Osuna, R., Schwacha, A., Bender, R.A., Dept. of Biology, University of Michigan, Ann Arbor: Deletion analysis of the *hutUH* operon regulatory region in *K. aerogenes*.

Plumbridge, J., IBPC, Paris, France: Structure and regulation of the *nag* regulon of *E. coli*.

Postle, K., Dept. of Microbiology, Washington State University, Pullman: Transcriptional regulation of the *E. coli tonB* gene.

Pruss, G.J., Dept. of Biology, University of South Carolina, Columbia: Divergent transcription units, translation, and plasmid DNA supercoiling.

Ramakrishnan, G., Newton, A., Dept. of Molecular Biology, Princeton University, New Jersey: Developmental regulation of flagellar genes in *C. crescentus*.

SESSION 7 TRANSCRIPTIONAL REGULATORY PROTEINS

Bushman, F.D., Ptashne, M., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Amino acid sequence requirements for transcriptional activation and DNA binding by λ repressor.

Orosz, L., Dallmann, G., Papp, P., Marincs, F., Dept. of Genetics, Attila József University, Szeged, Hungary: On the specificity of 16-3 repressor.

Takeda, Y.,[1] Sarai, A.,[2] Hausheer, F.H.,[1] [1]NCI-Frederick Cancer Research Facility, Frederick, [2]NCI, National Institutes of Health, Bethesda, Maryland: Recognition of the operator DNA sequence by Cro repressor.

Thliveris, A.T., Mount, D.W., University of Arizona, Tucson: Genetic analysis of the DNA-binding domain of LexA protein of *E. coli*.

Astromoff, A., Ptashne, M., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: λ Repressor cooperativity at a distance—Roles of operator sequence and protein flexibility.

Kim, J., Garges, S., Adhya, S., National Cancer Institute, NIH, Bethesda, Maryland: Mutation-induced allosteric changes in CRP.

Su, W.,[1] Adhya, S.,[2] Echols, H.,[1] [1]Dept. of Molecular Biology, University of California, Berkeley; [2]NCI, National Institutes of Health, Bethesda, Maryland: Formation of a DNA-wound nucleoprotein structure by RNA polymerase and Crp-cAMP at the P1 promoter of the *gal* operon.

Hendrickson, W., Flaherty, C., Yin, L., Molz, L., Dept. of Microbiology and Immunology, Penn State University, Hershey Medical Center, Pennsylvania: Regulation of the *E. coli araFG* promoter.

Yin, L., Hendrickson, W., Dept. of Microbiology and Immunology, Penn State University, Hershey Medical Center, Pennsylvania: Structure of the AraC protein-binding site.

Menon, K., Lee, N.L., Dept. of Biological Sciences, University of California, Santa Barbara: Activation of *ara* operons by truncated AraC proteins does not require inducer—Arabinose unmasks, rather than generates, a transcriptional activator.

Lee, N.L., Francklyn, C., Hamilton, E., Dept. of Biological Sciences, University of California, Santa Barbara: Activation of the *araBAD* operon promoter by AraC protein—Cooperative binding of *araC* to $araI_1$ and $araI_2$ sites.

SESSION 8 SIGMA FACTORS AND GLOBAL REGULATION

Lesley, S.A., Burgess, R.A., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Identification of a region in σ^{70} required for binding to core RNA polymerase.

Erickson, J.W., Gross, C.A., Dept. of Bacteriology, University of Wisconsin, Madison: Identification of a novel *E. coli* σ factor involved in high-temperature gene expression.

Kalman, S., Duncan, M.L., Thomas, S.M., Price, C.W., University of California, Davis: Regulation of the *sigB* gene encoding an alternative σ factor of *B. subtilis* RNA polymerase.

Daniels, D., Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: "Loss-of-specificity" mutation in an RNA polymerase σ factor defines a contact site with the –10 region of a cognate promoter.

Jaacks, K.J., Grossman, A.D., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Identification and characterization of genes controlled by *spoOH* (sigma-H) in *B. subtilis*.

Cutting, S., The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Coupling of late gene transcription to morphogenesis during sporulation in *B. subtilis*.

Keener, J., Popham, D.L., Kustu, S., Dept. of Microbiology and Immunology, University of California, Berkeley: Role of NTRC in initiation of transcription by σ^{54} holoenzyme and comparison with other prokaryotic regulatory systems.

Ninfa, A.J.,[1] Ninfa, E.G.,[1] Lupas, A.N.,[1] Stock, A.M.,[1] Magasanik, B.,[2] Stock, J.B.,[1] [1]Dept. of Molecular Biology, Princeton University, New Jersey; [2]Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Crosstalk between bacterial chemotaxis signal transduction proteins and the regulators of transcription of the Ntr regulon—Evidence that nitrogen assimilation and chemotaxis are controlled by a common phosphotransfer mechanism.

Best, E.A., Bender, R.A., Depts. of Microbiology and

Biology, University of Michigan, Ann Arbor: Nac—The "additional factor" required for Ntr control of *hut* in *Salmonella*.

Wilmes, M.R., Wanner, B.L., Dept. of Biological Sciences, Purdue University, West Lafayette, Indiana: Does "crosstalk" control the phosphate regulon in *phoR* mutants?

SESSION 9 POSTER SESSION

Rampersaud, A., Inouye, M., Dept. of Biochemistry, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway: Involvement of specific base pairs within the *ompF* and *ompC* promoter regions for DNA binding and transcriptional activation by the OmpR protein.

Rangwala, S.H., Fuchs, R.L., Drahos, D.J., Olins, P.O., Monsanto Company, St. Louis, Missouri: Use of the *E. coli recA* promoter for inducible expression in other gram-negative bacteria.

Ringquist, S.,[1] Smith, C.L.,[2] Depts. of [1]Genetics and Development, [2]Psychology and Microbiology, Columbia University, New York, New York: In vivo footprinting of the *E. coli* chromosome.

Ruckman, J.,[1] Hall, D.H.,[2] Parma, D.,[1] Gold, L.,[1] [1]Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder; [2]School of Applied Biology, Georgia Institute of Technology, Atlanta: A virus-encoded gene product is involved in specific nucleolytic cleavage of some T4 mRNAs.

Ryncarz, A.J., Lammers, P.J., Dept. of Chemistry and the Plant Genetic Engineering Laboratory, New Mexico State University, Las Cruces: A small inversion element resides within the 11-kb excision element that interrupts the *nifD* gene of *Anabaena* 7120.

Sampson, B.A., Misra, R., Benson, S.A., Dept. of Molecular Biology, Princeton University, New Jersey: Identification of a new gene of *E. coli* K-12 involved in outer membrane permeability.

Saporito, S.M.,[1] Smith-White, B.J.,[2] Cunningham, R.P.,[1] [1]State University of New York, Albany; [2]Upjohn Company, Kalamazoo, Michigan: Nucleotide sequence of the gene encoding exonuclease III of *E. coli*.

Schulz, V., Reznikoff, W.S., Dept. of Biochemistry, University of Wisconsin, Madison: In vitro secondary-structure analysis of mRNA from *lacZ* translation initiation mutants.

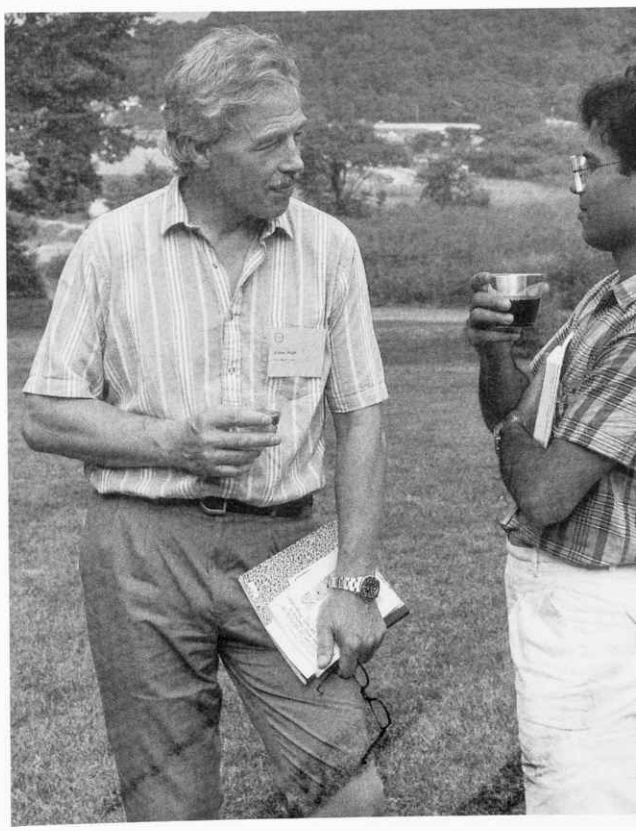
Schwartz, E., Welz, C., Rak, B., Institut für Biologie III, Universität Freiburg, Federal Republic of Germany: High-efficiency natural frameshifting in a new insertion element of *E. coli* K-12.

Schwedock, J., Fisher, R., Long, S., Dept. of Biological Sciences, Stanford University, California: Molecular and genetic studies of *Rhizobium meliloti* modulation genes.

Segall, A., Menzel, R., E.I. du Pont de Nemours, Experimental Station, Wilmington, Delaware: Regulation of DNA supercoiling—Insertion mutants that suppress double mutants defective in gyrase and topoisomerase I.

Semerjian, A.V., Fenton, A.C., Poteete, A.R., Dept. of Molecular Genetics and Microbiology, University of Massachusetts, Worcester: Identification of two phage P22 genes involved in recombination.

Connell, N., Genilloud, O., Seikhaus, D., Kolter, R., Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Point and deletion mutations that define an *E. coli* promoter regulated by growth phase.



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Shiba, K., Hama, C., Moriwaki, H., Asano, K., Mizobuchi, K., Dept. of Biophysics and Biochemistry, Faculty of Science, University of Tokyo, Japan: Studies on genes involved in replication and incompatibility of the Col Ib plasmid.

Singer, M., Gross, C., Dept. of Bacteriology, University of Wisconsin, Madison: A comprehensive mapping kit for *E. coli*.

Smith, K.A., Anderson, K.A., Salyers, A.A., Dept. of Microbiology, University of Illinois, Urbana: Characterization of a pullulanase and the starch utilization system of *Bacteroides thetaiotaomicron*.

Smith, L.D.,[1] Bertrand, K.P.,[2] [1]Dept. of Molecular Biology, Scripps Clinic and Research Foundation, La Jolla, California; [1]Biochemistry/Biophysics Program, Washington State University, Pullman: Mutations in the Tn*10 tet* repressor that interfere with induction—Location of the tetracycline-binding domain.

Snapper, S.B., Pane, L., Bloom, B.R., Jacobs, W.R., Jr., Albert Einstein College of Medicine, Bronx, New York: Expression of a selectable marker gene in mycobacteria using a temperate shuttle phasmid.

Speer, B.S., Salyers, A.A., University of Illinois, Urbana: Characterization of a gene that codes for a tetracycline-modifying enzyme.

Stout, V., Gottesman, S., NCI, National Institutes of Health, Bethesda, Maryland: Regulation of capsule synthesis by RcsA and RcsC.

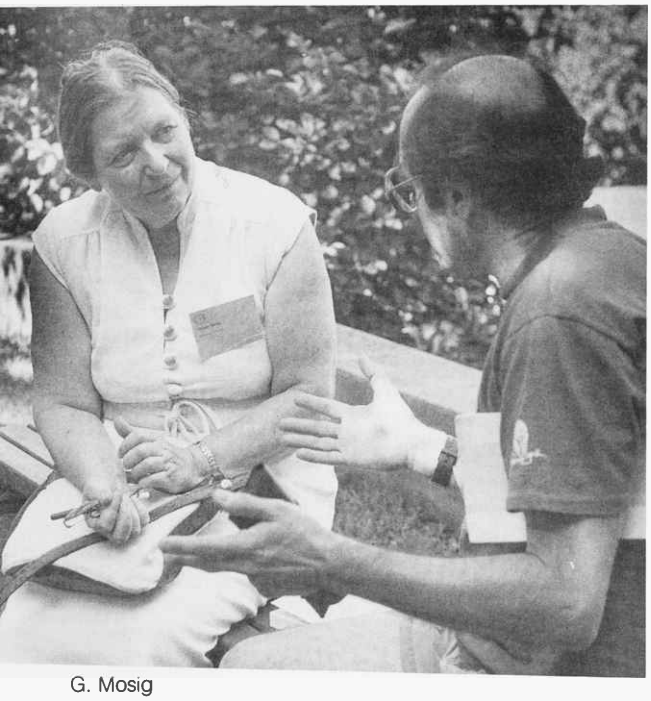
Sznyter, L.A., Brooks, J.E., New England Biolabs, Beverly, Massachusetts: Cloning and characterization of the *EagI* restriction-modification system.

Tilly, K., NCI, National Institutes of Health, Bethesda, Maryland: Participation of DnaJ protein in P1 plasmid maintenance.

Tuohy, T.,[1,2] O'Connor, M.,[2,3] Falahee, B.,[2,3] Hughes, D.,[1] Gesteland, R.,[2] Atkins, J.,[2,3] Thompson, S.,[1] [1]Dept. of Genetics, Trinity College, Dublin, Ireland; [2]Howard Hughes Medical Institute and Dept. of Human Genetics, University of Utah, Salt Lake City; [3]Dept. of Biochemistry, University College Cork, Ireland: Frameshift suppressors in *E. coli* and *S. typhimurium*.

Valenzuela, D., Ptashne, M., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Cooperativity "at a distance" by the P22 and 434 bacteriophage repressors.

Venkatesan, M.M., Buysse, J.M., Mills, J., Kopecko, D.J., Dept. of Bacterial Immunology, Walter Reed Army Institute of Research, Washington, D.C.: Characterization of *ipaBCDA* genes of *S. flexneri* associated with epithelial cell invasion and mapping of a regulatory element.



G. Mosig

Vargas, R., Menzel, R., E.I. du Pont de Nemours, Experimental Station, Wilmington, Delaware: Sequences 3′ to the start of transcription affect DNA relaxation (coumermycin) activation of gene expression—*lacZ* fusions behave differently than *galK* fusions.

Wanner, B.L., Boline, J.A., Metcalf, W., Dept. of Biological Sciences, Purdue University, West Lafayette, Indiana: Methane biogenesis from methylphosphonate by a cryptic gene(s) in *E. coli* K-12.

Watson, N., Palsrok, K., Olson, E., Dept. of Molecular Biology Research, Upjohn Company, Kalamazoo, Michigan: Mapping and characterization of plasmid mutations that increase bovine growth hormone gene expression in *E. coli*.

Wild, J., Gross, C.A., Dept. of Bacteriology, University of Wisconsin, Madison: *E. coli* mutants exhibiting increased synthesis of heat-shock proteins.

Xu, H., Dingwall, A., Bryan, R., Loewy, Z., Shapiro, L., Dept. of Microbiology, Columbia University, New York, New York: Temporal control of the *fla* gene network in *Caulobacter*—Positive and negative regulation.

Yajnik, V., Almond, N., Godson, G.N., New York University Medical Center, New York, New York: Antitermination in the *E. coli rpsU-dnaG-rpoD* operon.

Yamamoto, K.,[1] Takahashi, N.,[2] Yoshikura, H.,[1] Kobayashi, I.,[2] [1]Dept. of Bacteriology, Faculty of Medicine, University of Tokyo, [2]National Children's Medical Research Center, Japan: Plasmid homologous recombination—Origins of gene-conversion-type products in different pathways.

Yano, R., Yura, T., Institute for Virus Research, Kyoto University, Japan: *E. coli* mutations (*suhD*) causing reduced synthesis of ribosomal protein S15 can suppress an opal *rpoH* mutation.

SESSION 10 PHAGE REGULATION—OTHER TRANSCRIPTION SYSTEMS

Keener, J.,[1] Kustu, S.,[1] Halling, C.,[2] Dale, E.,[2] Morrison, T.,[2] Van Bokkelen, G.,[2] Calendar, R.,[2] Gebhardt, K.,[3] Lindqvist, B.H.,[3] Depts. of [1]Microbiology, [2]Molecular Biology, University of California, Berkeley; [3]Dept. of Biology, University of Oslo, Norway: *trans*-Activation by satellite phage P4.

Lee, T.-C., Christie, G.E., Dept. of Microbiology and Immunology, Virginia Commonwealth University, Richmond: Purification and characterization of the bacteriophage P2 *ogr* gene product.

Margolin, W., Howe, M.M., Dept. of Microbiology and Immunology, University of Tennessee, Memphis: In vitro activation of transcription from a bacteriophage Mu late promoter by Mu C protein.

Baek, N.-Y.,[1] Lindberg, G.K.,[2] Rothman-Denes, L.C.,[1,2] Depts. of [1]Molecular Genetics and Cell Biology, [2]Biochemistry and Molecular Biology, University of Chicago, Illinois: Regulation of N4 late transcription.

Glucksmann, A.,[1] Nichols, K.,[1] Rothman-Denes, L.B.,[1,2] Depts. of [1]Molecular Genetics and Cell Biology, [2]Biochemistry and Molecular Biology, University of Chicago, Illinois: N4 virion RNA polymerase-promoter interaction.

Deno, G., Schilke, B., Maloy, S., Dept. of Microbiology, University of Illinois, Urbana: Regulation of proline utilization by *S. typhimurium*—Expression of the *put* operon in vitro.

Hsu, L.M.,[1] Stellwagen, A.E.,[1] Giannini, J.K.,[1] Hattingh, S.E.,[1] Leung, C.,[1] Crosthwaite, J.C.,[2] [1]Program in Biochemistry, Mount Holyoke College, South Hadley, Massachusetts; [2]Dept. of Chemistry, University of North Carolina, Charlotte: Flanking sequence requirements of the *E. coli argT* promoter—A functional determination.

Menzel, R., E.I. du Pont de Nemours, Experimental Station, Wilmington, Delaware: Suppression of the temperature-sensitive character of a *gyrB*-defective strain by mutations affecting transcription.

French, S.L., Miller, O.L., Jr., Dept. of Biology, University of Virginia, Charlottesville: Transcriptional mapping of the *E. coli* chromosome by electron microscopy—*rrnC* to *rrnE*.

Aricò, B.,[1] Gross, R.,[1] Stibitz, S.,[2] Falkow, S.,[3] Rappuoli, R.,[1] [1]Sclavo Research Center, Siena, Italy; [2]Food and Drug Administration, Bethesda, Maryland; [3]Dept. of Medical Microbiology, University of Stanford, California: Regulation of the virulence factors in *Bordetella* spp.

Tartaglia, L.A., Storz, G., Jacobson, F.S., Ames, B.N., Dept. of Biochemistry, University of California, Berkeley: Regulation of a bacterial response to oxidative stress.

SESSION 11 SPECIAL SESSION

Ruvolo, P.P.,[1] Williams, K.R.,[2] Chase, J.W.,[1] [1]Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York; [2]Dept. of Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Connecticut: Biochemical and genetic characterization of conjugative plasmid SSBs from *E. coli*.

Panayotatos, N.,[1] Fontaine, A.,[2] Bäckman, S.,[1] [1]Michigan Biotechnology Institute, Lansing; [2]Institut Pasteur, Paris, France: A hybrid nuclease probe of DNA structure.

Koalick, E., Ronecker, H.J., Schnetz, K., Schwartz, E., Rak, B., Institut für Biologie, Universität Schänzlestr., Freiburg, Federal Republic of Germany: Analysis of mutations activating the cryptic ***bgl*** operon.

Gentry, D.R., Burgess, R.R., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: The gene encoding the ω subunit of RNA polymerase, *rpoZ,* is in the same operon as *spoT*.

Zhou, Y.N., Gross, C.A., Dept. of Bacteriology, University of Wisconsin, Madison: Examination of the heat-shock response in *E. coli* cells containing mutations affecting σ^{70} and σ^{32} reveals a translational regulatory mechanism.

Schaefer, M.R.,[1] Golden, S.S.,[2] Depts. of [1]Biochemistry, [2]Biology, Texas A&M University, College Station: Differential regulation of the *psbA* multigene family in the cyanobacterium *Synechococcus* sp. strain PCC 7942.

Denoya, C., Breidt, F., Dubnau, D., Dept. of Microbiology, Public Health Research Institute, New York, New York: Inducible and constitutive expression of erythromycin resistance mediated by *erm* determinants—Regulatory mechanisms involved.

King, J., Villafane, R., Fane, B., Haase-Pettingell, C., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Second-site suppressors of temperature-sensitive folding mutants in P22-infected *Salmonella*.

Mojumdar, M., Berget, B., Dept. of Biological Sciences, Carneigie Mellon University, Pittsburgh, Pennsylvania: Structural and functional roles of cysteine residues in the bacteriophage P22 tail protein.

Bartolomé, B., Jubete, Y., Fernandez-Moreno, M., Diaz-Aroca, E., Zabala, J.C., de la Cruz, F., Dept. de Biologie Molecular, Universidad de Cantabria, Santander, Spain: Genetic analysis of the transcriptional organization of the hemolysin genes of *E. coli*.

Cross, M., Koronakis, V., Hughes, C., Dept. of Pathology, Cambridge University, England: Transcript anti-termination dictates activation of *E. coli* hemolysin secretion genes.

Ishihama, A., Fujita, N., Ueshima, R., Nakayama, M., Kajitani, M., Dept. of Molecular Genetics, National Institute of Genetics, Shizuoka, Japan: Strengths and regulations of *E. coli* promoters.

SESSION 12 TRANSCRIPTION TERMINATION AND ANTITERMINATION

Hart, C.M., Roberts, J.W., Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Characterizing upstream sequence requirements for ϱ-dependent transcription termination.

Alifano, P.,[1] Nappo, A.G.,[1] Ciampi, M.S.,[2] [1]Dept. of Biology, University of Naples, [2]Institute of Genetics, University of Bari, Italy: Analysis of the *rho*-dependent transcription termination site responsible for polarity in the *hisG* gene of *S. typhimurium*.

Sparkowski, J., Das, A., Dept. of Microbiology, University of Connecticut, Farmington: Interaction of RNA polymerase with termination and antitermination factors—A recessive lethal, dominant suppressor mutation defining a critical site in the β subunit.

Court, D., Patterson, T., Wigle, T., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Transcription antitermination at *t*R1 mediated by λ function.

Oberto, J., Cam, K., Gottesman, M.E., Weisberg, R.A., NCI, National Institutes of Health, Bethesda, Maryland, and Columbia University Medical School, New York, New York: Unusual regulation of expression of genes in the P_L operon of lambdoid phage HK022.

Barik, S., Ghosh, B., Gradzielska, E., Lazinski, D., Whalen, W., Das, A., Dept. of Microbiology, University of Connecticut, Farmington: Biochemical and genetic dissection of a transcription antitermination apparatus.

Yarnell, W.S., Roberts, J.W., Dept. of Biochemistry, Cornell

D. Wulff, A. Poteete, G. Gussin, R. Calendar

University, Ithaca, New York: Q-Mediated antitermination of artificially paused complexes.

Kao, C., Bergsland, K., Snyder, L., Dept. of Microbiology, Michigan State University, East Lansing: The *gol* site of T4 bacteriophage—Antitermination with a difference?

Zhou, Y., McAllister, W.T., Dept. of Microbiology and Immunology, State University of New York Health Science Center Brooklyn: Termination of transcription by bacteriophage T7 RNA polymerase.

Linderoth, N.A., Calendar, R., Dept. of Molecular Biology, University of California, Berkeley: Cloning of the phage P4 *psu* gene and demonstration of polarity suppression activity.

Schnetz, K., Rak, B., Institut für Biologie III, Universität Schanzlestr., Freiburg, Federal Republic of Germany: Regulation of the β-glucoside (*bgl*) operon of *E. coli*.

Houman, F., Lopilato, J., Amster-Choder, O., Wright, A., Dept. of Molecular Biology, Tufts Medical School, Boston, Massachusetts: Antitermination in the *bgl* operon of *E. coli* K-12.

SESSION 13 POSTTRANSCRIPTIONAL EVENTS

Sarkar, N., Popowski, J., Shen, P., Taljanidisz, J., Karnik, P., Boston Biomedical Research Institute and Dept. of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Massachusetts: 3′-Terminal polyadenylation of mRNA in prokaryotes.

Goodrich, H.A.,[1] Gott, J.M.,[1] Xu, M.-Q.,[1] Scarlato, V.,[2,3] Shub, D.A.,[1] [1]Dept. of Biological Sciences, State University of New York, Albany; [2]Dept. of Biology, University of California, San Diego, La Jolla; [3]Institute of Genetics and Biophysics, Naples, Italy: A self-splicing group I intron in the DNA polymerase gene of the *B. subtilis* bacteriophage spo1.

Zeeh, A., Shub, D.A., Dept. of Biological Sciences, State University of New York, Albany: The product of the spliced phage T4 *sunY* gene is a processed protein.

Vogel, G., Toussaint, A., Higgins, P., Dept. of Biochemistry, University of Alabama, Birmingham Laboratory of Genetics, Free University of Brussels, Belgium: Regulation of Mu repressor.

Kameyama, L.,[1,2] Court, D.,[2] Guarneros, G.,[1] [1]Dept. of Genetics and Molecular Biology, CINVESTAV-IPN, Mexico City, Mexico; [2]NIC-Frederick Cancer Research Facility, Frederick, Maryland: Positive regulation of λ *N* gene expression by RNase III.

Krinke, L., Wulff, D.L., Dept. of Biological Sciences, State University of New York, Albany: OOP RNA initiates *cII-O* mRNA degradation through an RNase-III-dependent mechanism.

Bardwell, J.C.A., Takiff, H., Chen, S.-M., Court, D., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Regulation of the *rnc* operon.

Lee, S., Frost, L., Yanchar, N., Paranchych, W., Dept. of Biochemistry, University of Alberta, Edmonton, Canada: Regulation of the transfer operon of F plasmid by the FinOP system—FinO prevents degradation of the FinP antisense RNA.

Kornitzer, D., Altuvia, S., Teff, D., Oppenheim, A.B., Dept. of Molecular Genetics, Hebrew University-Hadassah Medical School, Jerusalem, Israel: A unique λ *cIII* mRNA structure is essential for the binding of mRNA to ribosomes.

Engelberg-Kulka, H., Miller, C., Benhar, I., Dept. of Molecular Biology, Hebrew University, Hadassah Medical School, Jerusalem, Israel: Translational error as a regulatory mechanism of gene expression—Natural frameshift suppression of the *trpR* gene.

Webster, K.R., Spicer, E.K., Yale University School of Medicine, New Haven, Connecticut: In vitro studies of T4 RegÅ protein-RNA interactions.

Carpousis, A.J.,[1] Mudd, E.A.,[1] Prentki, P.,[1] Belin, D.,[2] Krisch, H.M.,[1] Depts. of [1]Molecular Biology, [2]Pathology, University of Geneva, Switzerland: Maintenance of the capacity to make T4 single-stranded DNA-binding protein throughout infection by multiple promoters and mRNA processing.

Mouse Molecular Genetics

August 24—August 25

ARRANGED BY

Douglas Hanahan, Cold Spring Harbor Laboratory
Richard Palmiter, University of Washington, Seattle
Erwin Wagner, Institute of Molecular Pathology, Vienna

368 participants

The excitement in Mouse Molecular Genetics has been increasing dramatically in the last few years, and the August 1988 meeting at Cold Spring Harbor Laboratory revealed both the recent accomplishments and the promise of this blossoming field. The accelerating pace and new approaches for isolating developmentally interesting genes provide the grist, whereas transgenesis, the process of introducing foreign genes into the germ line of animals, provides the means of exploring their regulation and function. Techniques for identifying the *cis*-acting elements necessary for appropriate expression are well established; nevertheless, new levels of regulation are still being discovered and some of the elements lie long distances away from the genes they control. Once the *cis*-acting control elements are in hand, it is possible to use them to overexpress a particular gene in cells where it is normally expressed, or decrease its expression by using antisense constructs. It is also possible to use them to direct the expression of heterologous structural genes to specific cell types. Modifying gene expression in these ways often has profound effects on cell function and ultimately may affect the physiology of the animal, in some cases, mimicking human diseases. Applications of these techniques for studying complex diseases such as cancer or cellular interactions such as those that occur in the immune system were particularly evident at this meeting.

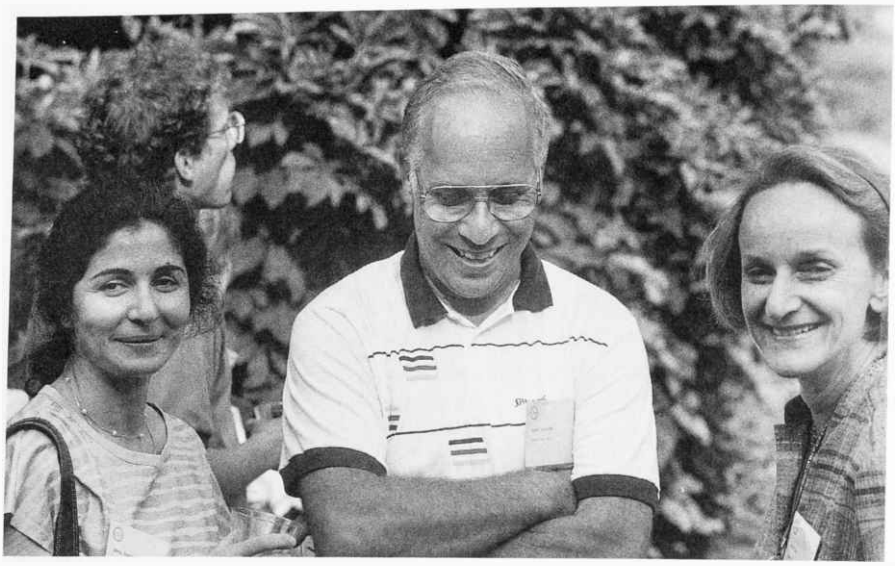
Improved methods of visualizing natural gene products, and for tagging cells with genetic markers, are revealing the complexity of gene expression patterns during development. By using retroviral vectors or by making chimeric mice, it is now possible to begin mapping cell lineages, an approach that may be especially rewarding in deciphering how the nervous system develops. Other genetic tricks allow the deletion of specific cell lineages by selective expression of toxins.

Introduction of foreign genes into the germ line of mice often produces new mutations that may have novel effects on development. As a consequence of the chromosomal tagging by the transgene, it is sometimes possible to isolate the flanking sequences and ultimately identify the gene that was disrupted. In a few, particularly gratifying cases, these new mutations map to the same locus as spontaneous or induced mutations that were identified previously.

Gene targeting, by homologous recombination or gene conversion, finally seems within reach and will add an essential tool to the currently available genetic approaches. Novel selection strategies for minimizing the number of nonhomologous integrants in embryonal stem cells as well as powerful screening approaches that allow one to identify the appropriate clones are in hand, and we can expect to soon learn the consequences of making gene disruptions in a variety of developmentally interesting genes.

This meeting clearly demonstrated that mouse molecular genetics has come of age, and we can anticipate a wealth of new information in the next few years that should provide invaluable insights into the developmental and physiological controls that operate in mammals.

This meeting was supported in part by funds from the National Science Foundation and the National Institute of Child Health and Human Development, a division of the National Institutes of Health.



M. Rassoulzadegan, H. Westphal, L. Pozzi

SESSION 1 GENE REGULATION

Chairman: **R. Palmiter,** University of Washington, Seattle

Hammer, R.E.,[1] Swift, G.H.,[2] Kruse, F.,[2] MacDonald, R.J.,[2] [1]Howard Hughes Medical Institute, Dept. of Cell Biology, [2]Dept. of Biochemistry, University of Texas Southwestern Medical Center, Dallas: Differential requirements for cell-specific enhancer domains in transfected cells and transgenic mice.

Grosschedl, R., Travis, A., Marx, M., Dept. of Microbiology and Immunology, University of California, San Francisco: Developmental regulation of immunoglobulin heavy-chain gene transcription in transgenic mice.

Grosveld, F., National Institute for Medical Research, London, England:Position-independent high-level expression of the human β-globin gene in transgenic mice.

Behringer, R.,[1] Ryan, T.,[2] Palmiter, R.,[3] Brinster, R.,[1] Townes, T.,[2] [1]School of Veterinary Medicine, University of Pennsylvania, Philadelphia; [2]Dept. of Biochemistry, University of Alabama, Birmingham; [3]Howard Hughes Medical Institute, University of Washington, Seattle: Regulation of the β-globin gene cluster during erythroid development in transgenic mice.

Johnson, J.E.,[1] Wilkie, T.M.,[1] Gartside, C.L.,[2] Wold, B.J.,[1] Hauschka, S.D.,[2] [1]Division of Biology, Caltech, Pasadena, California; [2]Dept. of Biochemistry, University of Washington, Seattle: Tissue-specific expression of muscle creatine kinase in transgenic mice.

Julien, J.-P.,[1] Beaudet, L.,[1] Tretjakoff, I.,[2] Peterson, A.,[2] [1]Institut du Cancer de Montreal, [2]Ludwig Institute for Cancer Research, Canada: Neuron-specific expression of a human neurofilament gene in transgenic mice.

Crenshaw, E.B. III,[1,2] Lira, S.A.,[2] Swanson, L.W.,[3,4] Rosenfeld, M.G.,[2,4] [1]Dept.of Biology, [2]Eukaryotic Regulatory Biology Program, University of California School of Medicine, [3]Neural Systems Laboratory, Salk Institute, [4]Howard Hughes Medical Institute, La Jolla: Both positive regulation and negative regulation are necessary for correct cell-type-specific expression of the rat prolactin gene.

Keller, S.A., Rosenberg, M.P., Osborn, L., Jones, J., Ting, C.-N., Meisler, M., Dept. of Human Genetics, University of Michigan, Ann Arbor: Tissue-specific expression of three amylase promoters in transgenic mice.

Small, J.A.,[1] Bieberich, C.,[2] Ghotbi, Z.,[1] Scangos, G.,[2] Clements, J.E.,[1,3] Depts. of [1]Neurology, [2]Biology, [3]Molecular Biology and Genetics, Johns Hopkins University, Baltimore, Maryland: A transgenic mouse model for expression of the visna virus LTR.

Platt, K.,[1] Ross, S.R.,[1] Min, H.Y.,[2] Spiegelman, B.M.,[2] [1]Dept. of Biochemistry, University of Illinois, Chicago; [2]Dana-Farber Cancer Institute and Dept. of Pharmacology, Harvard Medical School, Boston, Massachusetts: Regulation of an adipocyte gene by obesity.

Harris, S.,[1] McClenaghan, M.,[1] Simons, J.P.,[1] Whitelaw, C.B.A.,[1] Bessos, H.,[1] Prowse, C.,[1] Wilmut, I.,[1] Land, R.B.,[1] Bishop, J.O.,[2] Lathe, R.,[3] Clark, A.J.,[1] [1]AFRC—IAPGR (ERS) [2]Dept. of Genetics, University of Edinburgh, England; [3]LGME, CNR, Strasbourg, France: Expression of the ovine β-lactoglobulin gene in transgenic mice—Targeting the production of novel proteins to the mammary gland.

Strickland, S.,[1,2] Huarte, J.,[1] Belin, D.,[1] Vassalli, A.,[1] Rickles, R.,[2] Vassalli, J.-D.,[1] [1]University of Geneva Medical School, Switzerland; [2]Dept. of Pharmacology, State University of New York, Stony Brook: Use of antisense and chimeric RNA injection to study translational regulation of mRNA in mouse oocytes.

SESSION 2 ONCOGENESIS. I

Chairman: R. Dulbecco, Salk Institute, La Jolla

Nusse, R., Roelink, H., Rijsewijk, F., van de Heuvel, M., van de Vijver, M., Schuuring, E., van Deemter, L., Wagenaar, E., Division of Molecular Biology, Netherlands Cancer Institute, Amsterdam, The Netherlands: The *int* oncogenes in mouse mammary tumorigenesis and in embryogenesis.

Tsukamoto, A.,[1] Grosschedl, R.,[1] Parslow, T.,[2] Guzman, R.,[3] Varmus, H.,[1] Depts. of [1]Microbiology and Immunology, [2]Pathology, University of California, San Francisco, [3]Cancer Biology Research Laboratories, Berkeley: Transgenic mice expressing an MMTV-enhancer-driven ***int-1*** allele exhibit mammary gland hyperplasias and adenocarcinomas.

Berns, A., van Lohuizen, M., Verbeek, S., Saris, C., Domen, J., Krimpenfort, P., Breuer, M., Division of Molecular Genetics, The Netherlands Cancer Institute, Amsterdam: Identification of synergizing oncogenes in *pim-1* transgenic mice.

Andres, A.C., LeMeur, M., van der Valk, M.S., Schönenberger, C.A., Groner, B., Gerlinger, P., LGME, INSERM, Strasbourg, France, and Ludwig Institut, Bern, Switzerland: Ha-*ras* and c-*myc* oncogene expression distinctly interferes with differentiation and transformation of mammary epithelial cells in single and double transgenic mice.

Rüther, U., Wagner, E., European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: Consequences of c-*fos* expression in transgenic mice.

Boulter, C.A., Williams, R.L., Wagner, E.F., European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: Modulation of c-*src* expression in transgenic mice.

Lavigueur, A.,[1,2] Maltby, V.,[1] Mock, D.,[3] Brady, C.,[1] Rossant, J.,[1,2] [1]Mt. Sinai Hospital Research Institute, Depts. of [2]Medical Genetics, [3]Oral Pathology, University of Toronto, Canada: Induction of a broad spectrum of neoplasms in transgenic mice carrying the p53 oncogene.

Nussenzweig, M.C., Schmitt, E., Shaw, A., Sinn, E., Leder, P., Harvard Medical School and Howard Hughes Medical Institute, Boston, Massachusetts: Human IgM is an anti-oncogene in transgenic mice.

Dildrop, R., Moroy, T., Zimmermann, K., DePinho, R., Alt, F.W., Dept. of Biochemistry, Columbia University College of Physicians and Surgeons, New York, New York: Targeted expression of the N-*myc* and L-*myc* genes in the B-cell lineage of transgenic mice.

Yee, S.-P.,[1] Maltby, V.,[1] Mock, D.,[2] Rossant, J.,[1] Bernstein, A.,[1] Pawson, T.,[1] [1]Division of Molecular and Developmental Biology, Mt. Sinai Hospital Research Institute, [2]Dept. of Dentistry, University of Toronto, Canada: Neoplastic and cardiovascular disorders in transgenic mice expressing the v-*fps* protein-tyrosine kinase.

Suda, Y.,[1] Aizawa, S.,[2] Furuta, Y.,[2] Suzuki, M.,[2] Watanabe, N.,[2] Ikawa, Y.,[1] [1]Frontier Chromosome, [2]Laboratory of Molecular Oncology, Tsukuba Life Science Center, Riken, Japan: Malignant and erythroid-specific transformation in transgenic mice by the gp55 gene of Fr-SFFV.

SESSION 3 POSTER SESSION

Adrian, G.S., Yang, F., Reihl, R.M., Herbert, D.C., Weaker, F.J., Adrian, E.K., Robinson, L.K., Eddy, C.A., Pauerstein, C.J., Bowman, B.H., University of Texas Health Science Center, San Antonio, Texas: Developmental expression of the transferrin (*TF*) gene.

Brunkow, M.E., Yoo-Warren, H., Brannan, C.I., Tilghman, S.M., Dept. of Biology, Princeton University, New Jersey: Developmental regulation of the mouse H19 gene.

Chambers, J.C., Arnheiter, H., Lazzarini, R.A., NINCDS, National Institutes of Health, Bethesda, Maryland: Studies on tissue-specific expression by the myelin basic protein promoter in transgenic mice.

Chen, S.,[1,2] Andreason, G.L.,[1] Zhao, J.,[1] Landel, C.P.,[1] Evans, G.A.,[1] [1]Gene Expression and Cancer Biology Laboratories, Salk Institute for Biological Studies, La Jolla, [2]Dept. of Biology, University of California, San Diego: T-cell- and neuronal-cell-specific expression vector for transgenic mice.

Ikenaka, K.,[1] Kagawa, T.,[1] Mikoshiba, K.,[1,2] [1]Institute for Protein Research, Osaka University, [2]Dept. of Biological Regulation, National Institute for Basic Biology, Aichi,

Japan: Regulation of myelin proteolipid protein gene expression in normal and *jimpy* mutant mice.

Jami, J., Bucchini, D., Fromont-Racine, M., Pictet, R., INSERM, Institut Jacques-Monod, Paris, France: Cell-specific expression of the human insulin gene in transgenic mice.

Tremp, G.,[1] Daegelen, D.,[2] Cognet, M.,[1] Lone, Y.-C.,[1] Jami, J.,[2] Kahn, A.,[1] [1]Laboratoire de recherches en génétique et pathologie moléculaires, [2]Laboratoire de génétique physiologique, Institut Jacques Monod, Paris, France: Tissue-specific, hormonal, and nutritional control of the rat L-type pyruvate kinase gene transferred into transgenic mouse lines.

Lee, K.-F., Atiee, S.H., Henning, S.J., Rosen, J.M., Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas: Prolactin-regulated rat β-casein gene expression in transgenic mice.

Mehtali, M., Tomasetto, C., LeMeur, M., Gerlinger, P., Lathe, R., LGME-CNRS, INSERM, Strasbourg, France: Ubiquitous expression and methylation state of a housekeeping gene in transgenic mice.

Monteiro, M.,[1] Gearhart, J.,[2] Klaunberg, B.,[2] Cleveland, D.,[1] Depts. of [1]Biological Chemistry, [2]Physiology, Johns Hopkins University School of Medicine, Baltimore, Maryland: Construction of transgenic mice carrying the neurofilament NF-L gene from mouse.

Mullins, J.J., Sigmund, C., Kane-Haas, C., Wu, C., Pacholec, F., Gross, F., Roswell Park Memorial Institute, Buffalo, New York: Expression of renin genes in transgenic mice.

Pittius, C.,[1] Sankaran, L.,[1] Topper, Y.,[1] Westphal, H.,[2] Gordon, K.,[3] Hennighausen, L.,[1] [1]NIDDKD, [2]NICHHD, National Institutes of Health, Bethesda, Maryland; [3]Integrated Genetics, Framingham, Massachusetts: Regulation of the whey acidic protein gene and a hybrid gene containing the whey acidic protein gene promoter in transgenic mice.

Reshef, L.,[1] Nechushtan, H.,[1] Eisenberger, C.,[1] Cohen, H.,[1] Benvenisty, N.,[1] Shani, M.,[2] [1]Institute of Biochemistry, Hebrew University, Hadassah Medical School, Jerusalem, [2]Volcani Center, Rehovot, Israel: Tissues from different embryonal origin use separate *cis*-regulatory elements to confer expression of the phosphoenolpyruvate carboxykinase gene.

Roberts, B., Vltale, J., Capalucci, N., Gordon, K., Integrated Genetics, Framingham, Massachusetts: Expression of human genes in the mammary gland of transgenic mice.

Robinson, M.O.,[1] McCarrey, J.,[2] Simon, M.I.,[1] [1]Dept. of Biology, California Institute of Technology, Pasadena; [2]Division of Reproductive Biology, Johns Hopkins School of Public Health, Baltimore, Maryland: Testis-specific expression of phosphoglycerate-kinase-2-promoted fusion genes in transgenic mice.

Shani, M.,[1] Yaffe, D.,[2] Shinar, D.,[2] Einat, P.,[2] [1]ARO, Volcani Center, [2]Dept. of Cell Biology, Weizmann Institute of Science, Rehovot, Israel: Expression of muscle-specific genes introduced into multipotent embryonic stem cells and mouse germ line.

Koyama, M.,[1] Chesa, P.G.,[2] Rettig, W.J.,[2] Gordon, J.W.,[3] Silver, J.,[1] [1]Hospital for Joint Diseases, [2]Memorial Sloan-Kettering Cancer Center, [3]Mt. Sinai School of Medicine, New York, New York: Tissue- and species-specific regulation of the Thy-1 gene.

Vidal, M.A., Kollias, G., Morris, R., Grosveld, F., National Institute for Medical Research, London, England: Expression and function of Thy-1 using transgenic mice.

Yeung, C.-Y.,[1] Rauth, S.,[1] Ingolia, D.E.,[1] Ross, S.,[2] Depts. of [1]Genetics, [2]Biological Chemistry, University of Illinois College of Medicine, Chicago: Unusual tissue-specific gene expression directed by the murine adenosine deaminase gene promoter in transgenic mice.

Zaret, K.S., Milos, P.M., Stevens, K.A., Section of Biochemistry, Brown University, Providence, Rhode Island: *trans*-Acting factors causing hepatocyte-specific gene control.

Aizawa, S.,[1] Suda, Y.,[2] Furuta, Y.,[1] Suzuki, M.,[1] Ikawa, Y.,[1] [1]Laboratory of Molecular Oncology, [2]Frontier-Chromosome, RIKEN, Tsukuba, Japan: Cell-type-specific penetration of oncogenic genes.

Morello, D., Lavenu, A., Babinet, C., Unité de Génétique des Mammifères, Institut Pasteur, Paris, France: Biological consequences of human c-*myc* proto-oncogene expression in $H2^K$/human c-*myc* transgenic mice.

Dubois, N., Bennoun, M., Grimber, G., Allemand, I., Cavard, C., Chasse, J.-F., Kamoun, P., Briand, P., Laboratoire de Biochimie Génétique, Hôpital Necker, Paris, France: Hepatocarcinogenesis in trangenic mice carrying the antithrombine III–SV40 T antigen.

Choi, Y.,[1] Lee, I.,[2] Ross, S.R.,[1] [1]Dept. of Biological Chemistry, University of Illinois College of Medicine; [2]Dept. of Surgery and Pathology, St. Luke's-Rush Presbyterian School of Medicine, Chicago: A requirement for the SV40 small tumor antigen in tumorigenesis in transgenic mice.

DeMayo, J.L.,[1] Clift, S.M.,[1] Shen, R.-F.,[1] Slagle, B.L.,[2] Sepulveda, A.,[3] Finegold, M.L.,[3] Butel, J.S.,[2] Woo, S.L.C.,[1] Depts. of [1]Cell Biology, [2]Virology, [3]Pathology, Baylor College of Medicine, Houston, Texas: Tissue-specific expression of SV40 T antigen in trangenic mice directed by the regulatory elements of the human A1AT gene.

Dragani, T.A., Manenti, G., Sacchi, M.R.M., Della Porta, G., Division of Experimental Oncology, Istituto Nazionale Tumori, Milan, Italy: Identification of three genes underexpressed in murine hepatocellular tumors.

Efrat, S.,[1] Linde, S.,[2] Kofod, H.,[2] Spector, D.,[1] Delannoy, M.,[1] Grant, S.,[1] Hanahan, D.,[1] Baekkeskov, S.,[2] [1]Cold Spring Harbor Laboratory, New York; [2]Hagedorn Research Laboratory, Gentofte, Denmark: Establishment of pancreatic islet cells in culture via targeted oncogenesis in transgenic mice.

Feigenbaum, L.,[1] Hinrichs, S.H.,[2] Reynolds, R.K.,[1] Jay, G.,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Medical Pathology, University of California School of Medicine, Davis: JC virus and SV40 pathogenesis—Role of the transcriptional enhancers and transforming genes.

Grant, S.,[1] Seidman, I.,[2] Hanahan, D.,[1] Bautch, V.L.,[1] [1]Cold Spring Harbor Laboratory, [2]University Medical Center, New York: Altered tumor phenotype in transgenic mice containing two viral transgenes.

Harrington, M.A., Gonzales, F., Jones, P.A., University of

M. Rassoulzadegan, L. Pozzi

R. Palmiter

Southern California Comprehensive Cancer Center, Los Angeles: Effect of oncogenic transformation by chemicals and oncogenes on myogenic determination.

Heckl, K., Dony, C., Rüther, U., Wagner, E., European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: Expression of c-*fos* in transgenic mice during bone development.

Held, W.A., Mullins, J.J., Kuhn, N.J., Gallagher, J.F., Gu, D., Gross, K.W., Dept. of Molecular and Cellular Biology, Roswell Park Memorial Institute, Buffalo, New York: T-antigen expression and tumorigenesis in transgenic mice containing a mouse major urinary protein–SV40 T antigen hybrid gene.

Tremblay, P.J.,[1] Pothier, F.,[1] Hoang, T.,[1] Tremblay, G.,[2] Brownstein, S.,[2] Phillips, B.,[3] Jolicoeur, P.,[1] [1]Institut de Rescherches Clinques de Montréal, [2]McGill University, Montréal, Quebec, [3]Hospital for Sick Children, Toronto, Ontario, Canada: MMTV-Ha-*ras* as a transgene—Distinct effects in various tissues.

Kleinheinz, A.,[1] Gissmann, L.,[1] Turek, L.,[2] zur Hausen, H.,[1] [1]Institute of Virus Research, German Cancer Center, Heidelberg, Federal Republic of Germany; [2]Dept. of Pathology, Veterans Administration Center, Iowa City, Iowa: Oncogenic potential and tissue specificity of human papillomaviruses in transgenic mice.

Pavirani, A.,[1] Le Meur, M.,[1] Dalemans, W.,[2] Nakagawa, N.,[1] Nakagawa, T.,[1] Skern, T.,[1] Lathe, R.,[2] Gerlinger, P.,[2] Courtney, M.,[1] [1]Dept. of Molecular and Cellular Biology, Transgene S.A., [2]Laboratoire de Génétique Moléculaire des Eucaryotes, Institut de Chimie Biologique, Strasbourg, France: Lymphoid and liver cells as targets for oncogenesis and heterologous gene expression in transgenic mice.

Rassoulzadegan, M.,[1] Jensen, N.,[1] Guron, C.,[1] Pinçon-Raymond, M.,[2] Cuzin, F.,[1] [1]INSERM, Univesité de Nice, [2]INSERM, Paris, France: Non-tumoral pathological disorders correlated with expression of the gene encoding the large T antigen of polyomavirus in transgenic mice.

Stapleton, P., Takayama, Y., Symonds, G., Rowe, P.B., Childrens Medical Research Foundation, Camperdown, Australia: *myc*-mediated transformation—Secondary events involved in tumor progression.

Sarvetnick, N.,[1] Fox, H.,[2] Stewart, T.,[1] [1]Dept. of Developmental Biology, Genentech, South San Francisco, [2]Dept. of Pathology, University of California, San Francisco: An approach to elucidate the natural activities of interferon-γ.

Sawicki, J.A., Schmieg, F.I., Missera, M.A., Wistar Institute, Philadelphia, Pennsylvania: Effects of elevated c-*mos* expression on differentiation and tumorigenesis.

Sigmund, C.D., Mullins, J.J., Kim, U., Gross, K.W., Roswell Park Memorial Institute, Buffalo, New York: Transgenic mice containing renin SV40 T antigen gene fusions develop multiple neoplasias.

Steinhelper, M., Spector, D., Weinberg, P., Field, L., Cold Spring Harbor Laboratory, New York: Asymmetrical atrial hyperplasia induced by atrial natriuretic factor–T antigen gene expression.

Chen, J.,[1] Neilson, K.,[2] Jaffe, R.,[2] Van Dyke, T.,[1] [1]Dept. of Biological Sciences, University of Pittsburgh, [2]Dept. of Pathology, Childrens Hospital of Pittsburgh, Pennsylvania: The lymphotropic papovavirus early region induces neoplasia on the choroid plexus and lymphoid cells in transgenic mice.

Windle, J.,[1] Weiner, R.,[2] Mellon, P.,[1] [1]Salk Institute, La Jolla, [2]University of California, San Francisco: Anterior pituitary tumors in transgenic mice.

Wiseman, R.W.,[1] Stewart, B.C.,[2] Grenier, D.,[2] Miller, E.C.,[2] Miller, J.A.,[2] [1]NIEHS, Research Triangle Park, North Carolina; [2]University of Wisconsin, Madison: Characterization of Ha-*ras* mutations in chemically induced and spontaneous hepatomas of the B6C3F1 mouse.

Alpert, S.,[1] Hanahan, D.,[1] Teitelman, G.,[2] [1]Cold Spring Harbor Laboratory, [2]Dept. of Neurobiology, Cornell University Medical School, New York, New York: Transient expression of a hybrid insulin gene prior to neuronal commitment in transgenic mice.

Cazillis, M., Evans, M.J., Dept. of Genetics, University of Cambridge, England: In vivo tissue-specific expression of α-lactalbumin of guinea pig and β-casein of rat in the mouse.

Gossler, A., Darling, S., Rossant, J., Mt. Sinai Hospital

Research Institute, Toronto, Ontario, Canada: Identification of genomic DNA sequences developmentally regulated during mouse embryogenesis using embryonic stem cells.

Grez, M., Ziegler, M., Ostertag, W., Heinrich-Pette-Institut, Hamburg, Federal Republic of Germany: Transcriptional control in mouse embryonal carcinoma cells—Retroviruses as a model system.

Koller, B.H., Smithies, O., Dept. of Pathology, University of North Carolina, Chapel Hill: Targeted mutagenesis of β_2-microglobulin.

McNally, M., Lebkowski, J., Finch-Manzagol, S., Pletcher, A., Applied ImmunoSciences, Inc., Menlo Park, California: Isolation and characterization of mouse stem cells.

Robbins, P., Lehn, P., Mulligan, R., Whitehead Institute, Cambridge, Massachusetts: Regulation of retroviral transcription in embryonic carcinoma cells and in hematopoietic cells in vivo.

Rogers, M.B., Gudas, L.J., Dept. of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, and Dana Farber Cancer Institute, Boston, Massachusetts: Gene expression in visceral endoderm revealed by in situ hybridization to F9 embryoid bodies.

Sablitzky, F., Phillips, R.A., Hospital for Sick Children, Division of Haematology/Oncology, Toronto, Ontario, Canada: Selection of retroviral integration sites in transcriptionally active loci.

Snodgrass, R., Keller, G., Lineberger Cancer Research Center, University of North Carolina, Chapel Hill, and Institute for Immunology, Basel, Switzerland: Hematopoietic cell development from embryonic stem cell cultures.

Sumida, T., Müller, W., Yoshida, N., Rajewsky, K., Institute for Genetics, University of Cologne, Federal Republic of Germany: Chimeric hematopoietic system generated by using a pluripotential embryonic stem cell line.

Taketo, M., Bloom, M., Annarella, M., Jackson Laboratory, Bar Harbor, Maine: Enhancer and promoter traps in embryonal carcinoma cells by a recombinant transducing retrovirus.

Thompson, S., Melton, D.W., Dept. of Molecular Biology, Edinburgh University, Scotland: Use of homologous recombination, embryonic stem cells, and transgenic mice to investigate HPRT gene expression.

Van Zant, G., Holland, B.P., Eldridge, P.W., Dept. of Cell Biology and Anatomy, Texas Tech University Health Sciences Center, Lubbock: Lineage mapping of hematopoiesis through retroviral transduction of genes into stem cells of mid-gestation mouse embryos.

Weng, D.E.,[1] Morgan, R.A.,[2] Gearhart, J.D.,[1] [1]Dept. of Physiology, Johns Hopkins University School of Medicine, Baltimore, [2]NCI, National Institutes of Health, Bethesda, Maryland: Construction and characterization of a representative mouse blastocyst-specific cDNA library.

Westphal, K.-H.,[1] Brem, G.,[2] Müller, K.,[1] Büttner, R.,[1] Espen, J.,[1] [1]Laboratorium für molekulare Biologie-Genzentrum, Ludwig-Maximilians-Universität, [2]Institut für Tierzucht und Tierhygiene, München, Federal Republic of Germany: Thymic striated muscle cells down-regulate cellular oncogene expression and participate in normal embryogenesis with formation of functional muscle tissue.

Wiles, M.V., Basel Institute for Immunology, Switzerland: Mouse and human embryonic carcinoma cell differentiation.

Wilkemeyer, M., Ledley, F., Howard Hughes Medical Institute and Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas: Engineering mouse models for inborn errors of metabolism—Methylmalonic acidemia.

Beauchemin, N.,[1] Turbide, C.,[1] Bell, J.,[1] Fuks, A.,[1] Stanners, C.P.,[1,2] [1]McGill Cancer Centre, [2]Dept. of Biochemistry, McGill University, Montreal, Quebec, Canada: Characterization of murine carcinoembryonic antigen.

Fakharzadeh, S., Hoffman, E., George, D., Dept. of Human Genetics, Howard Hughes Medical Institute, University of Pennsylvania, Philadelphia: Molecular analysis of amplified DNA on double minutes in a tumorigenic derivative of mouse 3T3 cells.

Lin, C.S., Goldthwait, D.A., Samols, D., Dept. of Biochemistry, Case Western Reserve University, Cleveland, Ohio: Identification of Alu transposition in human lung carcinoma cells.

Maness, P.F., Dept. of Biochemistry, University of North Carolina, Chapel Hill: Developmental regulation of pp60-$^{c\text{-}src}$ and tyrosine phosphorylation of a 63–55-kD protein in nerve growth cones.

Ovitt, C.,[1] Jenuwein, T.,[2] Müller, R.,[3] [1]European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany; [2]Dept. of Microbiology and Immunology, University of California, San Francisco; [3]Institut für Molecular Biology and Tumor Research, Philipps-Universität, Marburg, Federal Republic of Germany: Identification of fos protein sequences specifying nuclear localization.

Paul, D.,[1] Kwon, B.S.,[2] Höhne, M.,[1] Kay, G.,[1] Tönjes, R.,[1] Hoffman, B.,[1] [1]Dept. of Cell Biology, Fraunhofer-Institute of Toxicology and Aerosol Research, Hannover, Federal Republic of Germany; [2]Molecular Genetics Laboratory, Guthrie Research Institute, Sayre, Pennsylvania: Establishment of SV40-immortalized normal and lethal albino deletion mutant mouse hepatocyte lines.

Wood, S.A., Rowe, P.B., Childrens Medical Research Foundation, Camperdown, Australia: Recombinant retrovirus in the development of transgenic mice.

SESSION 4 ONCOGENESIS. II

Chairman: **D. Hanahan,** Cold Spring Harbor Laboratory

Vogel, J.,[1] Hinrichs, S.H.,[2] Reynolds, R.K.,[1] Luciw, P.A.,[2] Jay, G.,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Medical Pathology, University of California, Davis: The HIV *tat* gene induces dermal lesions resembling Kaposi's sarcoma in transgenic mice.

Nerenberg, M.I.,[1] Wiley, C.A.,[2] Oldstone, M.B.A.,[1] [1]Research

Institute of Scripps Clinic, [2]Dept. of Neuropathology, University of California, San Diego, La Jolla: The HTLV-I *tat* gene induces tissue-specific disorders of proliferation and neoplasia in transgenic mice.

Howley, P.,[1] Lindren, V.,[1] Sippola-Thiele, M.,[1] Wetzel, E.,[2] Skowronski, J.,[2] Hanahan, D.,[2] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Cold Spring Harbor Laboratory, New York: Cell heritable stages of tumor progression in BPV-1 transgenic mice—Specific cytogenetic changes distinguish progression to fibrosarcomas.

Wilson, J.B.,[1] Stein, R.,[2] Levine, A.J.,[1] [1]Princeton University, New Jersey; [2]Merck Sharpe and Dohme, West Point, Pennsylvania: Neoplasia in transgenic mice harboring EBV latent genes.

Westphal, H., NICHHD, National Institutes of Health, Bethesda, Maryland: Targeting the eyes of transgenic mice.

Bautch, V.L.,[1] Grant, S.,[1] Seidman, I.,[2] Hassell, J.,[3] Hanahan, D.,[1] [1]Cold Spring Harbor Laboratory, [2]New York University Medical Center, New York; [3]McGill University, Montreal, Qeubec, Canada: Tumorigenesis promoted by polyomavirus large T antigen in transgenic mice.

Griep, A.E., Kuwabara, T., Westphal, H., NCI, National Institutes of Health, Bethesda, Maryland: Arrested development in the lens of transgenic mice expressing polyomavirus large T antigen.

Williams, R.L.,[1] Risau, W.,[2] Drexler, H.,[2] Wagner, E.F.,[1] [1]EMBL, Heidelberg, [2]Max-Planck-Institut für Psychiatrie, Martinseid, Federal Republic of Germany: Host-derived haemangiomas induced by endothelial cells expressing the polyoma middle T oncogene.

Baetge, E.E.,[1] Behringer, R.R.,[4] Messing, A.,[2] Sved, A.,[3] Brinster, R.L.,[4] Palmiter, R.D.,[1] [1]Howard Hughes Medical Institute, University of Washington, Seattle; [2]School of Veterinary Medicine, University of Wisconsin, Madison; [3]Dept. of Behavorial Neuroscience, University of Pittsburgh, [4]Laboratory of Reproductive Physiology, University of Pennsylvania, Philadelphia: Adrenal chromaffin tumors in transgenic mice.

Hammang, J.P.,[1] Behringer, R.R.,[2] Baetge, E.E.,[3] Palmiter, R.D.,[3] Brinster, R.L.,[2] Messing, A.,[1] [1]University of Wisconsin, Madison; [2]University of Pennsylvania, Philadelphia; [3]University of Washington, Seattle: Derivation of neuronal cell lines from retinal and adrenal tumors in PNMT-SV40 transgenic mice.

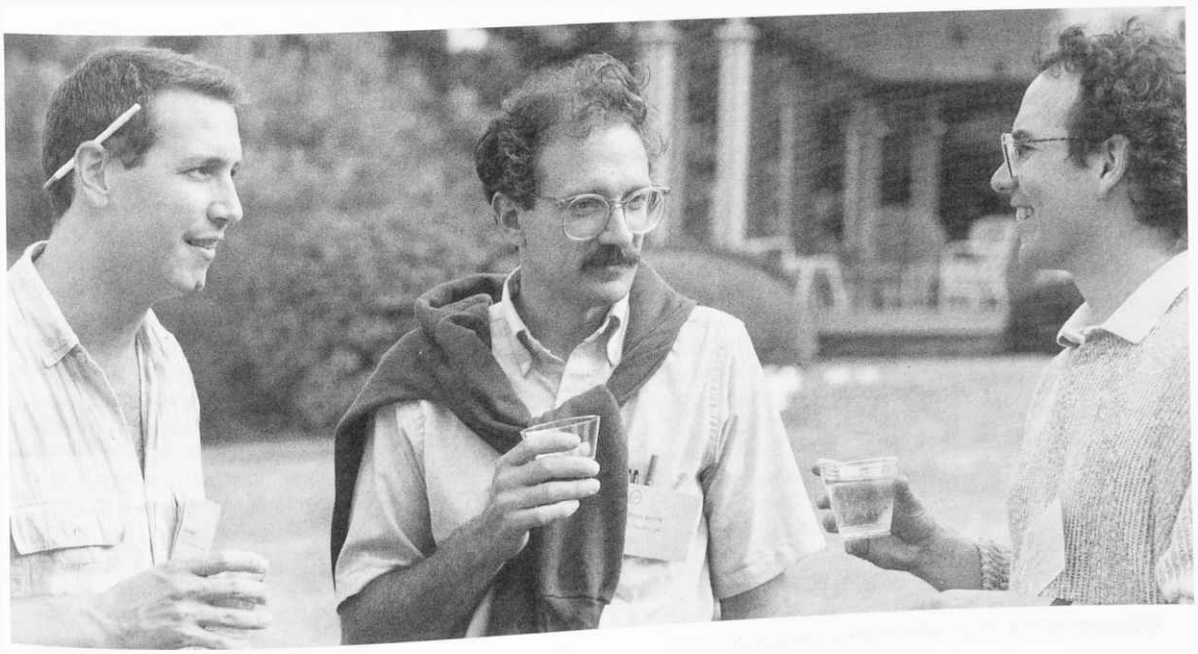
Messing, A.,[1] Sandgren, E.P.,[2] Paulson, D.,[2] Brinster, R.L.,[2] Palmiter, R.D.,[3] [1]University of Wisconsin School of Veterinary Medicine, Madison; [2]University of Pennsylvania School of Veterinary Medicine, Philadelphia; [3]Howard Hughes Medical Institute, University of Washington, Seattle: Myelination defects associated with expression of SV40 T antigen in Schwann cells and oligodendrocytes of transgenic mice.

SESSION 5 STEM CELLS

Chairman: A. McLaren, University College, London

Smithies, O., Dept. of Pathology, University of North Carolina, Chapel Hill: Targeted modification of genes in embryonic stem cells.

Capecchi, M.R., Thomas, K.R., Mansour, S., Kostic, D., Dept. of Biology, University of Utah, Salt Lake City: Site-directed mutagenesis by gene targeting in mouse-



J. McNeish, R. Woychik, T. Vogt

embryo-derived stem cells.

Skarnes, W., Rossant, J., Joyner, A., Mt. Sinai Hospital Research Institute, Toronto, Ontario, Canada: Production of mouse developmental mutants by insertion into genes active in embryonic stem cells.

Zimmer, A., Gruss, P., Dept. of Molecular Cell Biology, Max-Planck-Institute of Biophysical Chemical, Gottingen, Federal Republic of Germany: Targeted mutagenesis of the *Hox 1.1* gene by homologous recombination in embryonic stem cells.

Beier, D.R.,[1,3] Leder, P.,[1,3] Williams, D.A.,[2,3] Depts. of [1]Genetics, [2]Pediatrics, [3]Howard Hughes Medical Institute, Harvard Medical School, Boston, Massachusetts: Expression of a Fr-MLV LTR/c-*myc* gene in embryonic stem cells results in their differentiation.

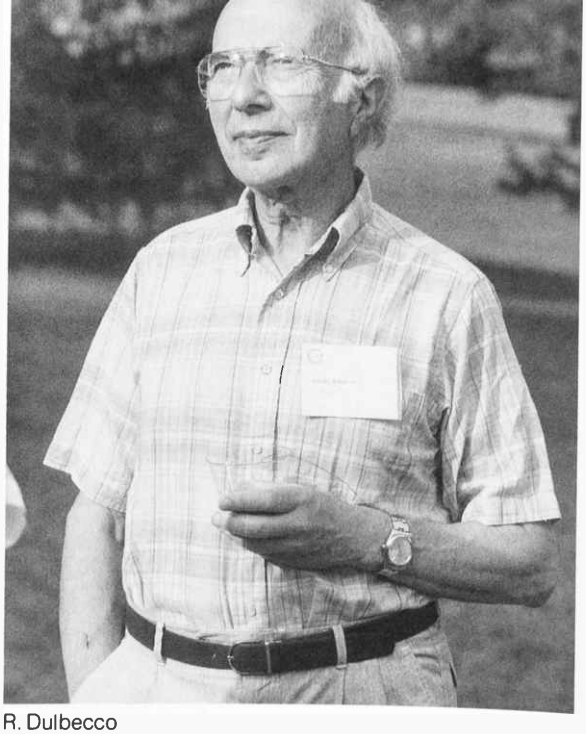
La Thangue, N.B., Dalianis, T., Murray, E.J., Rigby, P.W.J., National Institute for Medical Research, London, England: Regulation of transcription in murine embryonic stem cells.

Lovell-Badge, R.H.,[1] Robertson, E.J.,[2] [1]MRC Mammalian Development Unit, London, England; [2]Dept. of Human Genetics and Development, Columbia University, New York, New York: XY female mice, derived from embryonic stem cells, result from a mutation on the Y chromosome.

Adams, J.M., Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Victoria, Australia: Hematopoietic neoplasia and lineage commitment.

Keller, G.,[1] Wagner, E.,[2] [1]Basel Institute for Immunology, Switzerland; [2]European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: Altering hematopoiesis by retrovirus-mediated gene transfer.

Simonneau, M., Boisseau, S., Semonin, O., Poujeol, C., Laboratoire de Neurobiologie Cellulaire and Moléculaire, CNRS, Paris, France: Analysis of the early stages of mammalian neuronal differentiation using in vitro models and transgenic mice.



R. Dulbecco

Schmidt, G.H.,[1] Mead, R.,[2] [1]Dept. of Cell Biology, Fraunhofer Institute, Hannover, Federal Republic of Germany; [2]Dept. of Applied Statistics, The University, Reading, England: Interferences of developmental processes based on the analysis of mosaic transgenic mice.

SESSION 6 POSTER SESSION

Beddington, R.S.P., Hogan, A., Imperial Cancer Research Fund, Dept. of Zoology, Oxford, England: A transgenic cytoplasmic cell marker to study cell lineages during early organogenesis.

Al-Shawi, R.,[1] Burke, J.,[1] Jones, C.T.,[1] Kinnaird, J.,[1] MacIntosh, I.,[1] Simons, J.P.,[2] Bishop, J.O.,[1] [1]Dept. of Genetics, University of Edinburgh, [2]AFRC IAPGR-ERS, Scotland: Line-specific expression patterns of a compound foreign gene with a MUP promoter and an HSV thymidine kinase reporter in tissues of transgenic mice.

Chalifour, L., Gomes, M., Mes-Masson, A.-M., National Research Council, Biotechnology Research Institute, Montreal, Quebec, Canada: Inappropriate expression of oncomodulin is toxic to transgenic mice.

Chinsky, J.M., Ramamurthy, V., Knudsen, T.B., Kellems, R.E., [1]Dept. of Biochemistry, [2]Institute for Molecular Genetics, Baylor College of Medicine, Houston, Texas; [3]Dept. of Anatomy, East Tennessee State University, Johnson City: Developmental regulation of murine adenosine deaminase.

Chowdhury, K., Rohdewohld, H., Gruss, P., Dept. of Molecular Cell Biology, Max-Planck-Institute of Biophysical Chemistry, Gottingen, Federal Republic of Germany: Structure and expression of murine zinc-finger-containing genes.

Deschamps, J., de Graaff, W., Hameleers, M., de Laaf, R., Meijlink, F., Hubrecht Laboratory, Utrecht, The Netherlands: Homeobox-containing genes—Regulation of expression during mouse embryogenesis.

Dressler, G.R., Deutsch, U., Gruss, P., Max-Planck-Institute for Biophysical Chemistry, Goettingen, Federal Republic of Germany: Developmental expression of murine paired-box-containing genes.

Frohman, M.A., Martin, G.R., Dept. of Anatomy, University of California, San Francisco: Expression of the developing mouse embryo of the *En-1* gene protein.

Joyner, A., Davis, C., Auerbach, A., Skarnes, W., Rossant, J., Mt. Sinai Hospital Research Institute, Toronto, Ontario, Canada: Studies of the role of the mouse homeobox-containing gene, *En-2*, in development.

Keshet, E., Schiff, R., Itin, A., Motro, B., Dept. of Virology, Hebrew University-Hadassah Medical School, Jerusalem, Israel: Cell specificity and developmental regulation of murine retrovirus-like elements.

Mahon, K.A.,[1] Westphal, H.,[1] Gruss, P.,[2] [1]NICHD,

National Institutes of Health, Bethesda, Maryland: [2]Dept. of Molecular Biology, Max-Planck-Institute of Biophysical Chemistry, Gottingen, Federal Republic of Germany: Spatially restricted expression of homeobox gene *Hox 1.1* during mouse embryogenesis.

Murphy, S.P., Linney, E., Dept. of Microbiology and Immunology, Duke University, Durham, North Carolina: Differential expression of mouse homeobox *Hox 1.3* in F9 embryonal carcinoma cells.

Parada, L.F.,[1] Sassoon, D.,[2] [1]NCI-Frederick Cancer Research Facility, Frederick, Maryland; [2]Institut Pasteur, Paris, France: Expression of N-*myc* in the mouse embryo by in situ analysis.

Püschel, A.W., Balling, R., Gruss, P., Dept. of Molecular Cell Biology, Max-Planck-Institute of Biophysical Chemistry, Goettingen, Federal Republic of Germany: Regulation of *Hox 1.1* expression in mouse.

Reith, A.D., Cooper, A.R., Watson, C.J., Rigby, P.W.J., National Institute of Medical Research, London, England: GR1—A novel murine gene regulated during embryonal carcinoma cell differentiation and embryonic development.

Poirier, F., Chan, C.-T.J., Timmons, P.M., Rigby, P.W.J., National Institute for Medical Research, London, England: Isolation and characterization of genes regulated during mouse embryogenesis.

Rohdewohld, H., Chowdhury, K., Gruss, P., Dept. of Biophysical Chemistry, Göttingen, Federal Republic of Germany: Structure and expression of the murine zinc finger gene *mkr5*.

Sasaki, H., Hamada, T., Sakaki, Y., Research Laboratory for Genetic Information, Kyushu University, Fukuoka, Japan: Transgenic mice bearing a metallothionein-transthyretin fusion gene—Transgenic expression and genomic imprinting.

Schughart, K.,[1] Utset, M.F.,[2] Ruddle, F.H.,[1] Depts. of [1]Biology, [2]Human Genetics, Yale University, New Haven, Connecticut: Structure and expression of the murine homeobox-containing gene *Hox 2.2* and isolation of *Hox 3.3,* a homeobox-containing gene on chromosome 15.

Abel, K.J., Gross, K.W., Dept. of Molecular and Cellular Biology, Roswell Park Memorial Institute, Buffalo, New York: Analysis of the chromosomal environment of the murine renin loci by pulsed-field gel electrophoresis.

Barsh, G., Epstein, C.J., Dept. of Pediatrics, University of California, San Francisco: Physical and genetic characterization of a lethal recessive deletion at the mouse *agouti* locus.

Bullard, D., Schimenti, J., Dept. of Genetics, Case Western Reserve University, Cleveland, Ohio: Characterization of a candidate gene family for the mouse *t* complex responder locus.

Compton, J.G.,[1] Phillips, S.J.,[1] Ferrara, D.M.,[1] Crosby, J.L.,[1] Roop, D.,[2] Fuchs, E.,[3] Lalley, P.A.,[4] Martin, G.R.,[5] Nadeau, J.H.,[1] [1]Jackson Laboratory, Bar Harbor, Maine; [2]NCI, National Institutes of Health, Bethesda, Maryland; [3]University of Chicago, Illinois; [4]Institute for Medical Research of Bennington, Vermont; [5]University of San Francisco, California: Mouse keratin genes. Proximity to mutant loci on chromosomes 11 and 15 that affect the epidermis.

Lock, L.F., Gilbert, D.J., Swing, D.A., Jenkins, N.A., Copeland, N.G., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Extracellular virus infection leads to spontaneously acquired ecotropic germ-line proviruses in SWR/J-RF/J hybrid mice.

Miller, C.,[1] Carter, A.,[1] Brooks, J.,[1] Lovell-Badge, R.,[2] Brammer, W.,[1] [1]University of Leicester, [2]MRC Mammalian Development Unit, England: Renin gene expression in transgenic mice.

Munir, M.I., Rossiter, B.J.F., Caskey, C.T., Howard Hughes Medical Institute, Institute for Molecular Genetics, Baylor College of Medicine, Houston, Texas: Regional distribution of HPRT antisense RNA in a transgenic mouse brain.

Niswander, L., Yee, D., Magnuson, T., Dept. of Developmental Genetics, Case Western Reserve University, Cleveland, Ohio: The albino-deletion complex and early mouse development.

Ruppert, S., Boshart, M., Kelsey, G., Müller, G., Schütz, G., Institute of Cell and Tumor Biology, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Coordinate control of a set of liver-specific genes by two distinct *trans*-acting loci.

Seperack, P.K.,[1] Strobel, M.C.,[1] Moore, K.J.,[1] Kingsley, D.M.,[1] Mercer, J.A.,[1] Russell, L.B.,[2] Copeland, N.G.,[1] Jenkins, N.A.,[1] [1]NCI-Frederick Cancer Research Facility, Frederick, Maryland; [2]Oak Ridge National Laboratory, Tennessee: A retroviral insertion in the *dilute* locus provides molecular access to this region on mouse chromosome 9.

Silva, A.J., White, R., Dept. of Human Genetics and Howard Hughes Medical Institute, University of Utah, Salt Lake City: A novel genetic approach to study imprinting and methylation in mammals.

Stubbs, L.,[1] Poustka, A.,[2] Röhme, D.,[3] Russell, L.B.,[4] Lehrach, H.,[1] [1]Imperial Cancer Research Fund Laboratories, London, England: [2]Max-Planck-Institut für Medizinische Forschung, Federal Republic of Germany; [3]University of Lund, Sweden; [4]Biology Division, Oak Ridge National Laboratories, Tennessee: Approaching the mouse *Steel* locus from closely linked molecular markers.

Westaway, D., Carlson, G., Scott, M., Mirenda, C., Foster, D., Wälchli, M., Prusiner, S., Dept. of Neurology, University of California, San Francisco, and McLaughlin Research Institute, Great Falls, Minnesota: Molecular genetics of the mouse prion gene complex.

Borrelli, E.,[1] Heyman, R.,[1] Hsi, M.,[1] Sawchenko, P.,[2] Evans, R.M.,[1] Laboratories of [1]Gene Expression, [2]Developmental Neurobiology, Salk Institute, La Jolla, California: Inducible ablation of pituitary cells in mice expressing herpesvirus thymidine kinase.

Bohme, J., LeMeur, M., Gerlinger, P., Benoist, C., Mathis, D., Laboratoire de la Biologie Moleculaire des Eucaryotes, Strasbourg, France: Presence of the MHC class II complex on pancreatic cells in transgenic mice does not necessarily lead to diabetes.

Crenshaw, E.B. III,[1,4] Ryan, A.F.,[2,3] Rosenfeld, M.G.,[4,5] Depts. of [1]Biology, [2]Surgery/Otolaryngology, [3]Neurosciences, [4]Eukaryotic Regulatory Biology Program, University of California School of Medicine,

San Diego, [5]Howard Hughes Medical Institute, La Jolla: A behavioral and anatomical inner-ear mutant generated by insertional mutagenesis in transgenic mice.

Daitch, J.,[1] Selsing, E.,[1] Germain, R.,[2] Miller, J.,[3] [1]Rosenstiel Basic Medical Sciences Research Center and Dept. of Biology, Brandeis University, Waltham, Massachusetts; [2]NIAID, National Institutes of Health, Bethesda, Maryland; [3]University of Chicago, Illinois: Expression of allogeneic 1-A molecules on pancreatic β cells of transgenic mice causes diabetes.

Gridley, T., Gray, D., Soriano, P., Jaenisch, R., Whitehead Institute, Cambridge, Massachusetts: Analysis of *Mov 34*, a recessive lethal mutation induced by retroviral insertion.

Hino, O.,[1] Nomura, K.,[1] Ohtake, K.,[1] Kawaguchi, T.,[1] Sugano, H.,[1] Kitagawa, T.,[1] Saito, I.,[2] Rogler, C.E.,[3] Kimura, S.,[4] Yokoyama, M.,[4] Katsuki, M.,[4,5] [1]Cancer Institute, Dept. of Pathology, [2]National Institute of Health, Tokyo, Japan; [3]Albert Einstein College of Medicine, Bronx, New York; [4]Central Institute for Experimental Animal, [5]Tokai University, Kanagawa, Japan: Development of hepatitis virus transgenic mice.

Korn, R., Kemler, R., Max-Planck-Institut für Immunbiologie, Freiburg, Federal Republic of Germany: Female sterility of transgenic mice due to homozygosity of the transgene.

Kimura, M., Sato, M., Nozawa-Kimura, S., Yokoyama, M., Takahashi, R., Saitoh, S., Nomura, T., Katsuki, M., Dept. of DNA Biology, School of Medicine, Tokai University, and Central Institute for Experimental Animals, Japan: Restoration of hypomyelination by myelin basic protein mini-gene in transgenic *shiverer* mice.

Mark, W.H., Signorelli, K., Lacy, E., Sloan-Kettering Institute for Cancer Research, New York, New York: Molecular characterization of a peri-implantation insertional mutation.

McNeish, J.D.,[1] Sulik, K.K.,[2] Scott, W.J.,[1] Potter, S.S.,[1] [1]Childrens Hospital Research Foundation, Division of Basic Science Research, Cincinnati, Ohio; [2]Dept. of Cell Biology and Anatomy, University of North Carolina, Chapel Hill: Characterization of the insertional mutation, *legless*.

Popko, B.,[1] Puckett, C.,[2] Shine, D.,[3] Readhead, C.,[2] Hood, L.,[3] [1]Dept. of Biochemistry, University of North Carolina, Chapel Hill; [2]Division of Biology, California Institute of Technology, Pasadena; [3]Center for Biotechnology, Baylor College of Medicine, The Woodlands, Texas: Use of dysmyelinating mouse mutants and transgenic mice to elucidate the function of the myelin proteins in the myelination process.

Shawlot, W., Overbeek, P.A., Howard Hughes Medical Institute, Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas: Two different mutations of the *downless* gene in a single family of transgenic mice.

Weiher, H., Gray, D., Sharpe, A., Jaenisch, R., Whitehead Institute for Biomedical Research and Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Two new insertional mutants induced by retrovirus integration into the germ line of mice.

Widera, G.,[1] Pinkert, C.A.,[2] Lo, D.,[3] Burkly, L.C.,[4] Cowing, C.,[5] Flavell, R.A.,[4] Palmiter, R.D.,[6] Brinster, R.L.,[3] [1]Research Institute of Scripps Clinic, La Jolla, California; [2]University of Missouri, Columbia; [3]University of Pennsylvania, Philadelphia; [4]Biogen Research Corporation, Cambridge, Massachusetts; [5]Medical Biology Institute, La Jolla, California; [6]Howard Hughes Medical Institute, University of Washington, Seattle: Generation of specific I-E phenotypes in MHC class II Eα transgenic mice.

Wilkie, T.,[1] Braun, B.,[2] Palmiter, R.,[2] Muller, C.,[3] Hammer, B.,[4] [1]Biology Division, Caltech, Pasadena, California; [2]Howard Hughes Medical Institute, [3]Dept. of Obstetrics and Gynecology, University of Washington, Seattle; [4]Howard Hughes Medical Institute, University of Texas, Dallas: Deletion of the transgene restores fertility in MyK-103 male mice.

Brombacher, F., Köhler, G., Eibel, H., Max-Planck-Institut für Immunbiologie, Freiburg, Federal Republic of Germany: Expression of an anti-T-cell antibody gene in transgenic mice.

Ferrero, E., Goyert, S.M., Cellular and Molecular Biology Unit, Hospital for Joint Diseases, New York, New York: Isolation and characterization of a murine-macrophage-specific gene.

Hendrickson, E.A.,[1] Schatz, D.,[2] Weaver, D.,[1] [1]Dana-Farber Cancer Institute, Boston, [2]Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: The *scid* gene encodes a *trans*-acting factor that mediates the rejoining event of immunoglobulin gene rearrangement.

Holzschu, D., Roth, E., Gorman, K., Hinchman, C., Scalice, E., Daiss, J., Exploratory Sciences Division, Eastman Kodak Company, Rochester, New York: Construction of chimeric antibodies in vivo by targeted integration.

Lonberg, N.,[1] Littman, D.,[2] Lacy, E.,[1] [1]Memorial Sloan-Kettering Cancer Center, New York, New York; [2]University of California Medical School, San Francisco: Patterns of cell-type-specific expression of human CD8 in transgenic mice.

Obata, Y.,[1] Hamasima, N.,[1] Taguchi, O.,[1] Nishizuka, Y.,[1] Stockert, E.,[2] Old, L.J.,[2] Takahashi, T.,[1] [1]Aichi Cancer Center Research Institute, Nagoya, Japan; [2]Memorial Sloan-Kettering Cancer Center, New York, New York: Expression of *TL* genes in transgenic mice.

Kinloch, R., Roller, R., Fimiani, C., Wassarman, D., Wassarman, P., Dept. of Cell and Developmental Biology, Roche Institute of Molecular Biology, Nutley, New Jersey: Structure and expression of the mouse sperm receptor, ZP3.

Sarthy, V., Fu, M., Iberle, T., Dept. of Ophthalmology, University of Washington, Seattle: Induction of the glial intermediate protein gene in mice with retinal dystrophy.

Margison, G.P.,[1] Searle, P.,[2] O'Connor, P.J.,[1] [1]Paterson Institute for Cancer Research, Manchester, [2]Dept. of Cancer Studies, University of Birmingham, England: Production of transgenic mice containing a prokaryotic DNA alkyltransferase repair gene.

Maine, A.B., Ciejek-Baez, E., Dept. of Biochemistry, University of Rochester, New York: Regulation of aldolase-B gene expression in normal and lethal albino deletion mutant mice.

Jackson, M., Beaudet, A., O'Brien, W., Howard Hughes Medical Institute and Institute of Molecular Genetics, Baylor College of Medicine, Houston, Texas: Regulation

of argininosuccinate synthetase and argininosuccinate lyase in the mouse.

Fiering, S., Nolan, G.P., Chen, J., Herzenberg, L.A., Dept. of Genetics, Stanford University, California: Gene expression studies of the *E. coli* β-D-galactosidase gene in cell lines and transgenic mice using the fluorogenic viable cell FACS-FDG assay.

Eldridge, P.W., Van Zant, G., Dept. of Cell Biology and Anatomy, Texas Tech University Health Sciences Center, Lubbock: Detection by flow cytometry of viable mouse hematopoietic cells expressing retrovirally transduced β-galactosidase genes.

Edgell, M.H., Hardies, S.C., Casavant, C., Loeb, D., Shehee, R., Martin, S., Hutchison, C.A. III, Dept. of Microbiology, Program in Molecular Biology and Biotechnology, University of North Carolina, Chapel Hill: The β-globin locus, L1, and everything (actually the end of everything).

Hochgeschwender, U., Sutcliffe, J.G., Brennan, M.B., Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California: Molecular genetic approach to the developing cerebellum.

SESSION 7 DEVELOPMENTAL GENE EXPRESSION

Chairman: D. Solter, Wistar Institute

Rossant, J., Lescisin, K., Clarke, H., Prideaux, V., Varmuza, S., Mt. Sinai Hospital Research Institute, Toronto, Ontario, Canada: Trophoblast-specific gene expression and chromosomal imprinting.

Pourcel, C., Wu, X., Farza, H., Amar, L., Hadchouel, M., Institute Pasteur, Paris, France: Analysis of the sex-dependent imprinting of a chromosome-13 region using a transgene as a molecular probe.

Allen, N.D., Surani, M.A.H., Institute of Animal Physiology and Genetics Research, Cambridge, England: Position-dependent transgenes as probes in mouse developmental genetics and genomic imprinting.

DeLoia, J.,[1] Price, J.,[2] Solter, D.,[1] [1]Wistar Institute, Philadelphia, Pennsylvania; [2]Scripps Clinic, La Jolla, California: Use of a transgene to isolate an imprinted region in mice.

Renard, J.P., Richoux, V., Guenet, J.L., Babinet, C., Unite dé Génétique des Mammifères, Institut Pasteur, Paris, France: Action of the paternal genome on early development in the mouse—The DDK model.

McMahon, A.P.,[1] Wilkinson, D.G.,[2] [1]Roche Institute of Molecular Biology, Nutley, New Jersey; [2]National Institute for Medical Research, London, England: Expression of proto-oncogenes *int-1* and *int-2* during mouse development.

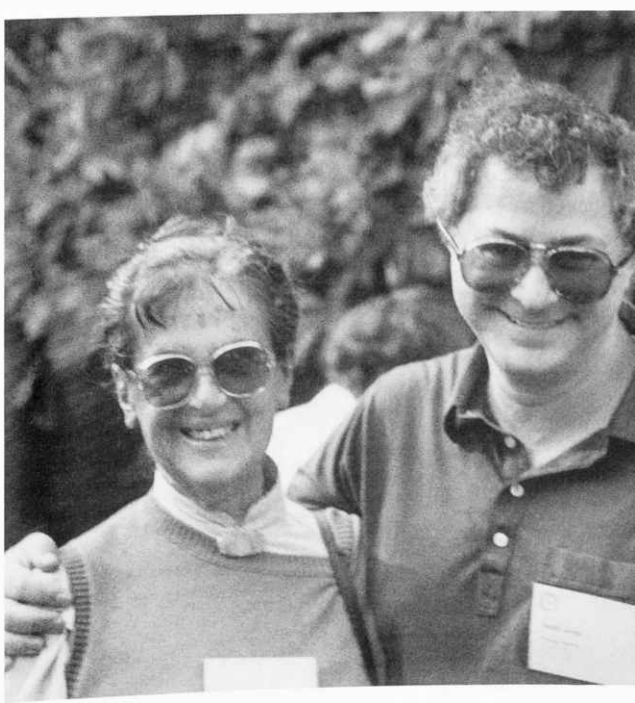
Martin, G.R.,[1] Goldfarb, M.,[2] Basilico, C.,[3] [1]Dept. of Anatomy, University of California School of Medicine, San Francisco; [2]Dept. of Biochemistry, Columbia University College of Physicians & Surgeons, [3]Dept. of Pathology, New York University School of Medicine, New York: Expression in the developing mouse embryo of a family of genes related to fibroblast growth factor.

Wolgemuth, D.J.,[1,2] Behringer, R.,[3] Mostoller, M.,[2] Brinster, R.L.,[3] Palmiter, R.D.,[4] [1]Dept. of Genetics and Development, [2]Center for Reproductive Sciences, Columbia University College of Physicians and Surgeons, New York, New York; [3]University of Pennsylvania School of Veterinary Medicine, Philadelphia; [4]Dept. of Biochemistry, Howard Hughes Medical Institute, University of Washington, Seattle: Expression of the mouse homeobox-containing gene *Hox 1.4* in transgenic mice.

Bierberich, C.J.,[1] Utset, M.,[1] Byrne, G.W.,[1] Awgulewitsch, A.,[2] Ruddle, F.H.,[1] [1]Dept. of Biology, Yale University, New Haven, Connecticut; [2]Dept. of Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston: Localized expression of the *Hox 3.1* gene and *Hox 3.1*/β-galactosidase fusion genes in mouse embryos.

Zakany, J., Tuggle, C.K., Patel, M.D., Nguyen-Huu, M.C., Depts. of Microbiology and Urology, Columbia University College of Physicians & Surgeons, New York, New York: Spatial and temporal regulation of homeobox genes in the embryonic central nervous system of transgenic mice.

Balling, R., Kessel, M., Gruss, P., Dept. of Molecular Cell Biology, Max-Plack-Institute for Biophysical Chemistry, Göttingen, Federal Republic of Germany: Over-expression of *Hox 1.1* in transgenic mice.



S. Waelsch, A. Levine

SESSION 8 IMMUNOLOGY

Chairman: K. Rajewsky, University of Koln

Storb, U.[1] Manz, J.,[1] Gollahon, K.,[1] Denis, K.,[2] Lo, D.,[2] Brinster, R.,[3] [1]Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois; [2]Howard Hughes Medical Institute and Dept. of Microbiology, University of California, Los Angeles; [3]Dept. of Veterinary Medicine, University of Pennsylvania, Philadelphia: Control of expression of immunoglobulin genes in transgenic mice.

Nemazee, D., Basel Institute of Immunology, Switzerland: A transgenic mouse model for B-cell tolerance.

Durdik, J., Gerstein, R.M., Rath, S., Nisonoff, A., Selsing, E., Dept. of Biology and Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, Massachusetts: Interchromosomal isotype switching by a microinjected Mu immunoglobulin heavy-chain gene in transgenic mice.

Hunziker, R.D., Margulies, D.H., NIAID, National Institutes of Health, Bethesda, Maryland: Molecular genetic and immunological characterization of mice transgenic for a polymorphic soluble class I antigen.

von Boehmer, H.,[1] Kishi, M.,[1] Uematsu, Y.,[1] Teh, H.S.,[1] Scott, B.,[1] Steinmetz, M.,[2] Blüthmann, H.,[2] Kisielow, P.,[1] [1]Basel Institute for Immunology, [2]Hoffmann-LaRoche & Co., Ltd, Basel, Switzerland: T cell repertoire selection in T cell receptor transgenic mice.

Fenton, R.,[1] Marrack, P.,[2] Kappler, J.,[2] Kanagowa, O.,[3] Seidman, J.,[1] [1]Dept. of Genetics, Harvard Medical School, Boston, Massachusetts; [2]Howard Hughes Medical Institute, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado; [3]Lilly Research Laboratory, La Jolla, California: Allelic exclusion of α/β and γ/δ T-cell antigen receptors in transgenic mice bearing a functional β-chain gene.

Fazekas de St. Groth, B.,[1] Berg, L.,[1] Ivars, F.,[1] Goodnow, C.,[2] Gilfillan, S.,[1] Garchon, H.-J.,[1] Erikson, J.,[1] Hedrick, S.,[3] Davis, M.,[1] [1]Dept. of Microbiology and Immunology, Stanford University, California; [2]Clinical Immunology Centre, University of Sydney, Australia; [3]Dept. of Biology, University of California, San Diego, La Jolla: Expression of rearranged T-cell-receptor α-chain transgenes affects T-cell differentiation pathways.

Brombacher, F., Kohler, G., Eibel, H., Max-Planck-Institut für Immunbiologie, Freibrug, Federal Republic of Germany: Expression of an anti-T-cell antibody gene in transgenic mice.

Skowronski, J., Alpert, S., Hanahan, D., Cold Spring Harbor Laboratory, New York: Factors modulating immunological nonresponsiveness and autoimmunity in insulin/T antigen transgenic mice.

Müller, W.,[1] Rüther, W.,[2] Vieira, P.,[1] Hombach, J.,[1] Rajewsky, K.,[1] Reth, M.,[1] [1]Institute for Genetics, University of Cologne, [2]EMBL, Heidelberg, Federal Republic of Germany: Block of B cell development in mice harbouring a transgene encoding membrane bound IGM.

SESSION 9 DISEASE MODELS AND INSERTIONAL MUTATIONS

Chairman: S. Waelsch, Albert Einstein College of Medicine

Vogt, T., Maas, R., Woychik, R., Zeller, R., Leder, P., Dept. of Genetics, Harvard Medical School, Boston, Massachusetts: The mouse limb deformity locus—Molecular analysis of a locus involved in pattern formation.

Shawlot, W., Overbeek, P.A., Howard Hughes Medical Institute, Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas: Insertional inactivation of the *downless* gene.

Costantini, F.D., Lee, J.J., Radice, G.R., Perry, W., Son, H.J., Dept. of Genetics and Development, Columbia University, New York, New York: Molecular analysis of embryonic lethal mutations.

Jaenisch, R.,[1] Choi, T.,[1] Gray, D.,[1] Gridley, T.,[1] Sharpe, A.,[1] Stacey, A.,[1] Weiher, H.,[1] Wu, H.,[1] Bateman, J.,[2] [1]Whitehead Institute for Biomedical Research and Dept. of Biology, Massachusetts Institute of Technology, Cambridge; [2]Research Foundation, Royal Childrens Hospital, Melbourne, Victoria, Australia: Mutations in transgenic mice.

Yamamura, K.,[1] Wakasugi, S.,[1] Inomoto, T.,[1] Yi, S.,[2] Naito, N.,[2] Iwanaga, T.,[1] Maeda, S.,[3] Takahashi, K.,[2] Shimada, K.,[3] [1]Institute for Medical Genetics, Depts. of [2]Pathology, [3]Biochemistry, Kumamoto University Medical School, Japan: A transgenic mouse model of familial amyloidotic polyneuropathy.

Dunn, A.R.,[1] Lang, R.A.,[1] Cuthbertson, R.A.,[2] Gonda, T.J.,[1] Metcalf, D.,[3] [1]Ludwig Institute for Cancer Research, Royal Melbourne Hospital, [2]Howard Florey Institute for Experimental Physiology and Medicine, University of Melbourne, [3]Walter and Eliza Hall of Medical Research, Royal Melbourne Hospital, Victoria, Australia: Transgenic mice aberrantly expressing murine GM-CSF develop accumulations of macrophages, blindness, and a fatal syndrome of tissue damage.

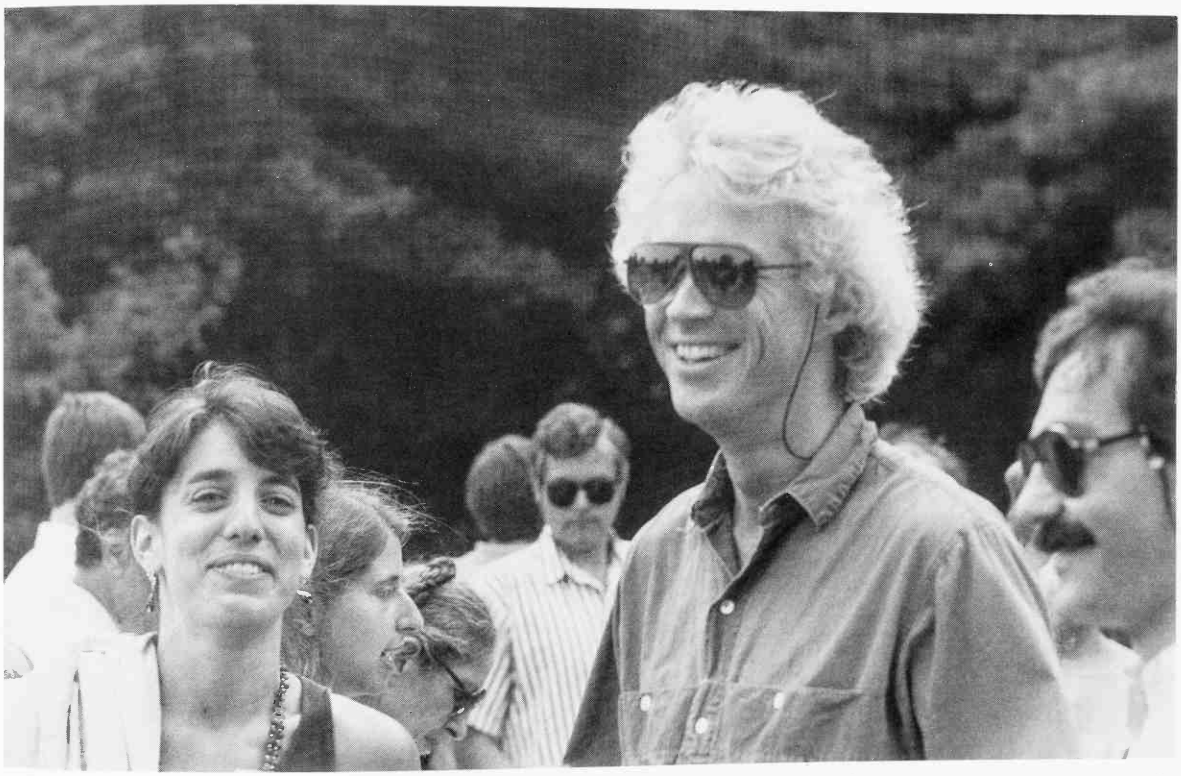
Sarvetnick, N., Stewart, T., Genentech, South San Francisco, California: Histological characterization of lesions within transgenic mice expressing interferon-δ and class II MHC antigens in pancreatic islets.

Roman, L.M.,[1] Simons, L.F.,[1] Hammer, R.E.,[1] Braciale, T.J.,[3] Braciale, V.L.,[3] Gething, M.-J.,[1,2] Sambrook, J.F.,[2] [1]Howard Hughes Medical Institute, [2]Dept. of Biochemistry, University of Texas Southwestern Medical Center, Dallas; [3]Dept. of Pathology, Washington University School of Medicine, St. Louis, Missouri: Expression of a foreign antigen, influenza hemagglutinin, on the surfaces of pancreatic β cells in transgenic mice.

Katsuki, M., Kimura, M., Sato, M., Kobayashi, K., Yokoyama, M., Nomura, T., Dept. of DNA Biology, School of Medicine, Tokai University, and Central

Institute for Experimental Animals, Japan: Conversion of mouse behavior by antisense DNA in transgenic mice.

Abramczuk, J.,[1] Leonard, J.,[1] Pezen, D.,[1] Rutledge, R.,[1] Hakim, E.,[1] Shearer, G.,[1] Frederickson, R.,[2] Notkins, A.,[1] Martin, M.,[1] [1]National Institutes of Health, Bethesda, Maryland; [2]University of Connecticut, Storrs: Production of HIV transgenic mice.



S. Alpert, D. Hanahan, G. Hollis

SESSION 10 GENETICS AND NEW TECHNIQUES

Chairman: **A. Levine,** Princeton University

Herrmann, B.G.,[1] Labeit, S.,[2] Poustka, A.,[2] Lehrach, H.,[3] [1]National Institute for Medical Research, London, England; [2]Max-Planck-Institut für Medizinische Forschung, Heidelberg, Federal Republic of Germany; [3]Imperial Cancer Research Fund Laboratories, London, England: Molecular cloning of a brachyury (T) locus candidate gene.

Cebra-Thomas, J., Brown, J., Pilder, S., Islam, S., Decker, C., Silver, L.M., Dept. of Molecular Biology, Princeton University, New Jersey: Transmission ratio distortion of the murine *t* haplotype.

Heyman, R.,[1,2] Borrelli, E.,[1,2] Lesley, J.,[1] Hyman, R.,[1] Evans, R.M.,[1,2] [1]Salk Institute, [2]Howard Hughes Medical Institute, San Diego, California: Directed expression of herpesvirus thymidine kinase allows for inducible cell destruction of the immune system in transgenic mice.

Landel, C.P.,[1] Zhao, J.,[1] Chen, S.,[1] Bok, D.,[2] Evans, G.A.,[1] [1]Gene Expression and Cancer Biology Laboratories, Salk Institute for Biological Studies, La Jolla, [2]Jules Stein Eye Institute, University of California, Los Angeles: Lens-specific expression of recombinant ricin induces developmental defects in the eyes of transgenic mice.

Rassoulzadegan, M., Leopold, P., Vailly, J., Blangy, A., Cuzin, F., INSET, Université de Nice, France: Episomal elements in transgenic mice.

Corden, J.L.,[1] Bartolomei, M.S.,[1] Overbeek, P.,[2] [1]Howard Hughes Medical Institute and Dept. of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Maryland; [2]Howard Hughes Medical Institute and Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas: Transgenic α-amanitin-resistant mice.

Byrne, G.W.,[1] O'Hare, P.,[2] Utset, M.,[1] Ruddle, F.H.,[1] [1]Dept. of Biology, Yale University, New Haven, Connecticut; [2]Marie Curie Research Institute, London, England: Multiplex gene regulation for the analysis of gene function in transgenic mice.

Rosenberg, M.P., Felder, B.K., Walton, E.M., Swing, D.A., Grammatikakis, N., Pavlakis, G.N., Jenkins, N.A., Copeland, N.G., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Molecular mechanisms of HTLV *trans*-activator gene action in transgenic mice.

Intermediates in Genetic Recombination

August 31—September 4

ARRANGED BY

Amar Klar, Cold Spring Harbor Laboratory
Raju Kucherlapati, University of Illinois
Charles Radding, Yale University
Jeffrey Strathern, NCI-Frederick Cancer Research Facility

313 participants

The meeting on Intermediates in Genetic Recombination attracted about 400 people and nearly 250 abstracts, divided into eight sessions. As implied in the title, the focus of the meeting was to understand the strand mechanics and the enzymatic functions involved in recombination. This question has been approached by a variety of mechanisms ranging from the classic genetic approach of monitoring the endproducts to determine the constraints placed on the intermediates to the physical techniques designed to capture transition states in the process. The eight sessions reflected this broad spectrum. Site-specific Recombination was chaired by David Sherrat. By changing the bases at the target sites, the sequence requirements for several systems have been defined. More importantly, some of these defective sites allow partial reactions to occur and hence trap intermediates for further analysis. Similar progress has been made in determining the essential parts of the enzymes involved in these processes. The session was noteworthy both for the recurrent themes and for the well-defined differences between the systems presented. The session on Retrotransposons, chaired by Steve Goff, focused on the integration step. This process is site-specific on the transposon but has little or no target specificity. The session on Biophysical Aspects of Recombination, chaired by Steve West, was dedicated to Dr. Paul Howard-Flanders. Most of the talks dealt with the characterization of *E. coli* proteins and DNA-protein complexes involved in homologous DNA recombination. Bill Holloman chaired the session on Enzymology of Recombination. This session continued the discussion of proteins, from several organisms, involved in strand exchange reactions and in the resolution of Holliday junctions. The systems that recognize and repair mismatched bases was the topic of a session chaired by Paul Modrich. These systems have been defined both genetically and biochemically. The talks included descriptions of a multiplicity of systems with specificity for different mismatches and the well-characterized methyl-directed mismatch repair system of *E. coli*. Homologous recombination between exogenously added DNA and the chromosomes of higher eukaryotic is becoming experimentally more accessible as a tool for making specific gene replacements. The session chaired by Nat Sternberg included several demonstrations of this technique as well as descriptions of homologous recombination events between chromosomal sequences. Homologous recombination in budding and fission yeast was covered in the session chaired by Tom Petes. This session included discussions of the nature of initiating events, the roles of gene products involved in recombination, and the physical demonstration of recombination intermediates. The final session was chaired by Susumu Tonegawa and dealt with the use of

recombination as a means of gene activation. Examples presented included the mating-type switching in yeast and the rearrangement of the genes for the immunoglobin proteins. The meeting provided an excellent opportunity for scientific exchange between experimentalists with different approaches. Those that work on substantially different systems often find inspiration both from the generalities that emerge and from the well-characterized distinctions.

This meeting was supported in part by the National Science Foundation.

Introduction - The Organizers

SESSION 1 SITE-SPECIFIC RECOMBINATION

Chairman: D. Sherratt, University of Glasgow

Bruist, M.F., Nash, H.A., Laboratory of Molecular Biology, National Institute of Mental Health, Bethesda, Maryland: A kinetic study of λ site-specific recognition.

Nunes-Duby, S.E., Landy, A., Division of Biology and Medicine, Brown University, Providence, Rhode Island: λ half attachment sites can serve as substrates for recombination and for an integrase-mediated ligation reaction.

Weinberg, R.L., Haskins, W.P., Cozzarelli, N.R., Dept. of Molecular Biology, University of California, Berkeley: Topological tests of the mechanisms of integration and excision by bacteriophage λ.

Ramaiah, N.,[1] Yagil, E.,[2] Kislev, N.,[1] Dolev, S.,[2] Weisberg, R.,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Tel Aviv University, Israel: Localization of the determinants of specificity of site-specific recombination.

Parsons, R.L., Evans, B.R., Crain, K., Jayaram, M., Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California: Intermediates in recombination revealed by step-arrest mutants of *Flp*.

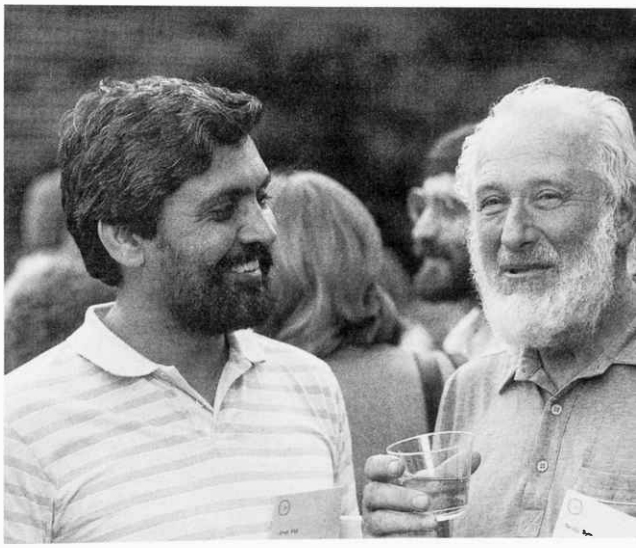
Umlauf, S., Qian, X.-H., Cox, M.M., Program of Biochemistry and Molecular Biology and Dept. of Biochemistry, University of Wisconsin, Madison: Mechanisms of site-specific recombination of the yeast 2μ plasmid—Studies of the role of DNA structure and cooperative binding of FLP protein.

Abremski, K., Wierzbicki, A., Hoess, R., Dept. of Central Research and Development, E.I. du Pont de Nemours & Co., Wilmington, Delaware: Studies on recombination intermediates generated during Cre-*lox* site-specific recombination.

Hatfull, G., Hughes, R., Sanderson, M., Freemont, P., Rice, P., Goldman, A., Steitz, T., Grindley, N., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: A structure-function analysis of the site-specific recombinase, resolvase, encoded by transposon γδ.

Bednarz, A.L., Stark, W.M., Boocock, M.R., Sherratt, D.J., Institute of Genetics, University of Glasgow, Scotland: Polarity of the Tn*3 res* and the directionality of strand exchange.

Kanaar, R.,[1] van de Putte, P.,[1] Cozzarelli, N.R.,[2] [2]Dept. of Biochemistry, University of Leiden, The Netherlands; [2]Dept. of Molecular Biology, University of California, Berkeley: Gin-mediated recombination of catenated and knotted DNA substrates—Implications for synaptic complex formation.



A. Klar, N. Symonds

Glasgow, A.C., Simon, M.I., Division of Biology, California Institute of Technology, Pasadena: Protein-DNA interactions and altered DNA structures in the *Salmonella* DNA inversion system.

Johnson, R.C.,[1] Ball, C.A.,[1] Bruist, M.F.,[2] [1]Dept. of Biological Chemistry, and the Molecular Biology Institute, University of California, Los Angeles; [2]Division of Biology, California Institute of Technology, Pasadena: Mechanism of strand exchange in Hin-mediated site-specific DNA inversion.

Benjamin, H., Kleckner, N., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Characterization of a complete intramolecular transposition reaction mediated by Tn*10* transposase.

Leung, P.C., Nallur, G.N., Harshey, R.M., Dept. of Molecular Biology, Research Institute of Scipps Clinic, La Jolla, California: Structure-function relationships in phage Mu transposase.

Adzuma, K., Mizuuchi, K., NIDDK, National Institutes of Health, Bethesda, Maryland: Action of Mu B ATPase in the Mu DNA strand-transfer reaction.

Bainton, R.J., Craig, N.L., Dept. of Microbiology and Immunology and Hooper Foundation, University of California, San Francisco: In vitro transposition of Tn*7*.

SESSION 2 RETROTRANSPOSITION

Chairman: S. Goff, Columbia University

Fujiwara, T., Craigie, R., NIDDK, National Institutes of Health, Bethesda, Maryland: Integration of exogenously added retroviral DNA in a cell-free reaction.

Bowerman, B., Brown, P.O., Bishop, M., Varmus, H.E., University of California, San Francisco: Biochemical and structural characterization of the nucleoprotein complex active in the integration of retroviral DNA.

Roth, M., Schwartzberg, P., Tanese, N., Goff, S.P., Dept. of Biochemistry and Molecular Biophysics, Columbia University College of Physicians & Surgeons, New York, New York: Characterization of mutants of the integration protein of Mo-MLV.

Levine, K., Steiner, B., Linial, M., Fred Hutchinson Cancer Research Center, Seattle, Washington: Molecular analysis of processed pseudogenes formed via retrofection.

Eichinger, D., Boeke, J., Dept. of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, Maryland: Cell-free transposition of the yeast transposable element Ty1.

Weinstock, K., Mastrangelo, M., Shafer, B., Garfinkel, D., Strathern, J., NCI-Frederick Cancer Research Facility, BRI-Basic Research Program, Frederick, Maryland: Insertions of Ty multimers at *HMLα*.

SESSION 3 BIOPHYSICAL: ASPECTS OF RECOMBINATION

Chairman: P. Howard-Flanders, Yale University

Bujalowski, W., Lohman, T.M., Dept. of Biochemistry and Biophysics, Texas A&M University, College Station: Negative cooperativity among single-stranded DNA-binding sites within individual *E. coli* SSB tetramers.

Kodadek, T., Stemke, K., Gan, D., Maine, R., Dept. of Chemistry and Clayton Foundation Biomedical Institute, University of Texas, Austin: The bacteriophage T4 UvsY protein—The first well-characterized accessory factor for homologous strand exchange.

Thaler, D.S., Sampson, E., Siddiqi, I., Rosenberg, S.M., Stahl, M.M., Stahl, F.W., Institute of Molecular Biology, University of Oregon, Eugene: RecBC‡ recombination of bacteriophage λ.

Lovett, S.T., Griffin IV, T.J., Kolodner, R.D., Dana-Farber Cancer Institute, Boston, Massachusetts: Purification and characterization of the RecF and RecJ proteins of *E. coli*.

Lindsley, J.E., Pugh, B.F., Kim, J.-I., Schutte, B.C., Cox, M.M., Dept. of Biochemistry, University of Wisconsin, Madison: Association and dissociation of RecA protein with duplex DNA.

Rao, B.J., Jwang, B., Flory, J., Radding, C.M., Dept. of Human Genetics, Yale University School of Medicine, New Haven, Connecticut: Defining the *E. coli* RecA-SSB strand-exchange machine—A reconstitution study.

Thresher, R., Griffith, J., Lineberger Cancer Research Center, University of North Carolina, Chapel Hill: Ethidium bromide and acridine orange cause RecA protein to form active complexes with duplex DNA.

Egelman, E.H., Yu, X., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Structure and dynamics of the *E. coli* RecA protein—Conformational changes and enzymatic function.

Conley, E.C., West, S.C., Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, England: RecA protein promotes homologous pairing between regions of duplex DNA.

Duckett, D.R.,[1] Murchie, A.I.H.,[1] Dickmann, S.,[2] von Kitzing, E.,[2] Kemper, B.,[3] Lilley, D.M.J.,[1] [1]Dept. of Biochemistry, University of Dundee, Scotland; [2]Max-Planck-Institut für Biophysikalischeme, Gottingen, Federal Republic of Germany; [3]Institut für Genetik, Universität zu Köln, Cologne, Federal Republic of Germany: The structure of the Holliday junction, and its resolution.

SESSION 4 ENZYMOLOGY OF RECOMBINATION

Chairman: B. Holloman, Cornell University Medical School

Kawasaki, K.,[1] Arai, N.,[2] Natori, M.,[2] Shibata, T.,[1] [1]Laboratory of Microbiology, RIKEN Institute, Saitama, [2]College of Agricultural and Veterinary Medicine, Nihon University, Tokyo, Japan: Homologous pairing and strand exchange promoted by proteins from fission yeast.

Sena, E.,[1] Fishel, R.,[2] Zarling, D.,[1] [1]Dept. of Molecular Biology, SRI International, Menlo Park, California; [2]NCI-Frederick Cancer Research Facility, Frederick, Maryland: Z-DNA affinity chromatography selects for yeast strand-transfer activity.

Sander, M., Lowenhaupt, K., Rich, A., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Properties of a *D. melanogaster* strand-transfer activity.

Heyer, W.-D., Kolodner, R.D., Dana-Farber Cancer Institute and Dept. of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts: Purification and characterization of a single-stranded DNA-binding protein from *S. cerevisiae* that stimulates the cognate mitotic strand-exchange protein.

Ganea, D.,[1] Frey, S.,[1] Boudos, C.,[1] Moore, P.,[2]

A. Skala, C. Radding

Kucherlapati, R.,[1] Depts. of [1]Genetics, [2]Microbiology and Immunology, University of Illinois, Chicago: Characterization of a human DNA-strand transferase.

Moore, S.P., Harris, C.J., Fishel, R., NCI-Frederick Cancer Research Facility, Frederick, Maryland: The human recombination strand-transfer process—Purification and characterization of the protein(s).

Hsieh, P., Camerini-Otero, C.S., Mills, F., Camerini-Otero, R.D., NIDDK, National Institutes of Health, Bethesda, Maryland: Recombinases can form DNA joint molecules in the absence of strand displacement.

Dykstra, C., Clark, A., Sugino, A., National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Identification of a gene encoding a meiotically inducible DNA-strand-transfer activity from *S. cerevisiae*.

West, S.C., Parsons, C.A., Picksley, S.M., Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, England: Resolution of recombination intermediates in vitro—Studies with model Holliday junctions.

Kemper, B.,[1] Kleff, S.,[2] Jensch, F.,[1] Pottmeyer, S.,[1] Seeman, N.,[3] Solaro, P.,[1] [1]Institut für Genetik, Köln, Federal Republic of Germany; [2]Dept. of Biochemistry, State University of New York, Stony Brook; [3]State University of New York, Albany: Resolution of recombinational intermediates by X-solvases, endonuclease VII from phage T4 and endonuclease Y3 from yeast *S. cerevisiae*.

Mueller, J.E.,[1] Kemper, B.,[2] Cunningham, R.P.,[1] Kallenbach, N.R.,[3] Seeman, N.C.,[1,3] [1]Dept. of Biology, State University of New York, Albany; [2]Institute of Genetics, University of Cologne, Federal Republic of Germany; [3]Dept. of Chemistry, New York University, New York: Cleavage preferences of T4 endonuclease VII for Holliday crossover analogs correlate with structural results in solution.

Kleff, S.,[1] Kemper, B.,[2] Sternglanz, R.,[1] [1]Dept. of Biochemistry, State University of New York, Stony Brook; [2]Institute of Genetics, Cologne, Federal Republic of Germany: Identification of a yeast mutant deficient in an endonuclease that cleaves Holliday junctions.

Holbeck, S.L.,[1] Taylor, A.F.,[1] Braedt, F.,[1] Smith, G.R.,[1,2] [1]Fred Hutchinson Cancer Research Center, [2]Dept. of Pathology, University of Washington, Seattle: Physical analysis of RecBCD enzyme action.

Kulkarni, S.K., Thaler, D.S., Shurvinton, C.E., Stahl, F.W., Institute of Molecular Biology, University of Oregon, Eugene: Stimulation of recombination by a palindrome on bacteriophage λ in a *recD* mutant.

Lu, B.C., Sakaguchi, K., Dept. of Molecular Biology and Genetics, University of Guelph, Ontario, Canada: Meiosis-specific deoxynucleases from the synchronous meiotic system of *Coprinus cinereus*.

SESSION 5 MISMATCH REPAIR

Chairman: P. Modrich, Duke University

Au, K.,[1] Clark, S.,[1] Grilley, M.,[1] Lahue, R.,[1] Su, S.-S.,[1] Thresher, R.,[2] Welsh, K.,[1] Griffith, J.,[2] Modrich, P.,[1] [1]Dept. of Biochemistry, Duke University Medical Center, [2]Lineberger Cancer Research Center, University

of North Carolina, Chapel Hill: Enzymology of methyl-directed DNA mismatch correction.

Claverys, J.-P., Méjean, V., Prudhomme, M., Centre de Recherche de Biochimie et de Génétique Cellulaires du CNRS, Université Paul Sabatier, Toulouse, France: DNA mismatch repair during transformation in *S. pneumoniae*.

Kramer, B., Kramer, W., Williamson, M.S., Fogel, S., Dept. of Genetics, University of California, Berkeley: DNA mismatch repair in *S. cerevisiae*—Analysis of substrate specificity and the effect of the *pms* mutations.

Hare, J.T., Taylor, J.H., Institute of Molecular Biophysics, Florida State University, Tallahassee: Bias in the selection of template strand in mismatch repair of vertebrate cells.

Brown, T.C.,[1] Jiricny, J.,[2] [1]MRC Radiobiology Unit, Harwell, England; [2]Friedrich Miescher Institute, Basel, Switzerland: Repair of mismatched bases in simian and human cells.

SESSION 6 RECOMBINATION IN HIGHER EUKARYOTES

Chairman: **N. Sternberg,** E.I. du Pont de Nemours & Co.

Campbell, C., Keown, W., Lowe, L., Kucherlapati, R., Dept. of Genetics, University of Illinois College of Medicine, Chicago: Gene modification by synthetic oligonucleotides in human cells.

Lin, F.-L.M., Sperle, K., Sternberg, N., Dept. of Central Research and Development, E.I. du Pont de Nemours & Co., Inc., Experimental Station, Wilmington, Delaware: Repair of double-stranded DNA breaks with a homolgous DNA fragment in gene transfer of mouse L cells.

Hu, W.S.,[1] Rio, D.,[2] Tjian, R.,[2] Shen, C.-K.J.,[1] [1]Dept. of Genetics, University of California, Davis; [2]Dept. of Biochemistry, University of California, Berkeley: *cis*-Controlling elements and *trans*-acting factor(s) of a homologous DNA recombination system in primate cells.

Colinas, R.J.,[1] Condit, R.C.,[2] Paoletti, E.,[3] [1]Albany Medical College, [2]State University of New York, Buffalo, [3]New York State Dept. of Health, Wadsworth Center for Labs and Research, Albany: Homologous recombination in vaccinia-infected cells requires a functional DNA polymerase.

Jasin, M., Berg, P., Dept. of Biochemistry, Stanford University School of Medicine, California: Selected homologous integrations in mammalian cells without target gene selection.

Scheerer, J., Adair, G., University of Texas M.D. Anderson Cancer Center, Smithville: Targeted correction of an *APRT* deletion mutation by homologous recombination with a fragment of the *APRT* gene.

Baker, M.D., Pennell, N., Bosnoyan, L., Shulman, M.J., Depts. of Immunology and Medical Genetics, University of Toronto, Canada: Homologous recombination of transferred and chromosomal immunoglobulin genes—Toward a mammalian *lac* operon.

Groden, J.,[1] Nakamura, Y.,[2] White, R.,[2] German, J.,[1] [1]Cornell University Graduate School of Medical Sciences, New York, New York; [2]University of Utah Medical Center, Salt Lake City: Homologous recombination in proliferating human cells.

Sauer, B., Henderson, N., Dept. of Central Research and Development, E.I. du Pont de Nemours and Co., Experimental Station, Wilmington, Delaware: Cre-mediated site-specific recombination in mammalian cells.

Heartlein, M.W.,[1] Latt, S.A.,[1,2] [1]Division of Genetics, Children's Hospital, Howard Hughes Medical Institute, Depts. of Pediatrics, [2]Genetics, Harvard Medical School, Boston, Massachusetts: Inverted duplication within and adjacent to heterologous selectable DNA is associated with DNA amplification in CHO cells.

SESSION 7 HOMOLOGOUS RECOMBINATION

Chairman: **T. Petes,** University of Chicago

Sun, H., Treco, D., Schultes, N., Szostak, J.W., Dept. of Molecular Biology, Massachusetts General Hospital, Boston: Double-strand breaks at an initiation site for meiotic recombination.

Cao, L., Alani, E., Kleckner, N., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Identification of a possible intermediate in meiotic chromosome synapsis and homologous recombination—A meiosis-specific, *RAD50, SPO11*-dependent DNA signal present between premeiotic DNA synthesis and maximal level of a heteroallelic recombinant.

Nag, D., Detloff, P., White, M., Petes, T.D., Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois: Non-Mendelian meiotic segregation at the ***HIS4*** locus of yeast.

Lichten, M.,[1] Nicolas, A.,[2] Schultes, N.P.,[3] Treco, D.,[3] Szoztak, J.W.,[3] Haber, J.E.,[4] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]University Paris, Orsay, France; [3]Dept. of Molecular Biology, Massachusetts General Hospital, Boston; [4]Rosenstiel Center, Brandeis University, Waltham, Massachusetts: Detection of heteroduplex DNA molecules among the products of yeast meiosis.

Rothstein, R., Rashba, E., Stollman, N., Ronne, H., Dept. of Genetics and Development, Columbia University College of Physicians & Surgeons, New York, New York: A genetic system to detect unselected mitotic recombination events between marked direct repeats.

Higgins, D., McGill, C., Strathern, J., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Recombinogenic nature of DNA lesions generated in vitro.

Klar, A.J.S., NCI-Frederick Cancer Research Facility, Frederick, Maryland: A cold spot of mitotic and meiotic

recombination in fission yeast inhibits meiotic recombination in flanking intervals.

Voelkel-Meiman, K., Xie, K., Roeder, S., Dept. of Biology, Yale University, New Haven, Connecticut: Studies on the mechanism of *HOT1*-promoted recombination events.

Hayden, M., Byers, B., Dept. of Genetics, University of Washington, Seattle: How much homology is needed for meiotic recombination?

Schuchert, P., Langsford, M., Grimm, C., Kaeslin, E., Kohli, J., Institute of General Microbiology, Bern, Switzerland: Analysis of recombination initiation in fission yeast.

Kramer, K.M., Haber, J.E., Brandeis University, Rosenstiel Research Center, Waltham, Massachusetts: Healing of double-strand breaks by de novo telomere formation.

Brunier, D.,[1] Peeters, B.,[2] Ehrlich, S.D.,[1] [1]Institut des Biotechnologies, INRS, Jouy en Josas, France; [2]Dept. of Genetics, University of Groningen, The Netherlands: Mechanisms of recombination between short homologous sequences.

Christman, M.F.,[1] Dietrich, F.S.,[1] Fink, G.R.,[1,2] [1]Whitehead Institute for Biomedical Research, Cambridge Center, [2]Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Mitotic recombination in the rDNA of *S. cerevisiae* is strongly suppressed through the combined action of DNA topoisomerases I and II.

Keil, R.L., McWilliams, A.D., Pindowski, C.L., Dept. of Biological Chemistry, Hershey Medical Center, Hershey, Pennsylvania: Mutations affecting rDNA recombination in *S. cerevisiae.*

Aguilera, A., Klein, H.L., Dept. of Biochemistry, New York University Medical Center, New York: Genetic and molecular analysis of recombination events enhanced by the hyper recombination mutant *hpr1*.

SESSION 8 GENE ACTIVATION BY RECOMBINATION

Chairman: **S. Tonegawa,** Massachusetts Institute of Technology

Klar, A.J.S., Cafferkey, R., NCI-Frederick Cancer Research Facility, Frederick, Maryland: The developmental fate of fission yeast cells is determined by the pattern of inheritance of parental and grandparental DNA strands.

Stavnezer, J., Nietupski, J., Lin, Y.-C., Radcliffe, G., Severinson, E., University of Massachusetts Medical School, Worcester: Immunoglobulin heavy-chain switching may be directed by prior induction of transcripts from constant-region genes.

Li, M., Halligan, B., Morzycka-Wroblewska, E., Desiderio, S., Howard Hughes Medical Institute and Dept. of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, Maryland: Identification and characterization of a protein that specifically binds the nonamer recombinational signals of immunoglobulin genes.

Lieber, M.R.,[1] Hesse, J.E.,[1] Lewis, S.,[1] Bosma, G.C.,[2] Mizuuchi, K.,[1] Bosma, M.J.,[2] Gellert, M.,[1] [1]NIDDK, National Institutes of Health, Bethesda, Maryland; [2]Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania: Severe combined immune deficiency (*scid*) mutation in mice—Joining of signal sequences but not coding segments in V(D)J recombination.

McCormack, W.T., Tjoelker, L., Carlson, L.M., Petryniak, B., Thompson, C.B., Howard Hughes Medical Institute, University of Michigan, Ann Arbor: Igλ V-J gene rearrangement in chicken bursal lymphocytes.

POSTER SESSION I

Site-specific Recombination

Hoess, R., Wierzbicki, A., Abremski, K., Dept. of Central Research and Development, E.I. du Pont de Nemours & Co., Inc., Wilmington, Delaware: Isolation of intermediates in the Cre-*lox* recombination pathway.

Sternberg, N., Dept. of Central Research and Development, Dept. of E.I. du Pont de Nemours and Co., Inc., Wilmington, Delaware: A new P1 site-specific recombination cloning system that permits the efficient isolation, amplification, and recovery of big DNA inserts.

Goodman, S.T., Yang, C.-C., Nash, H.A., NCI, National Institute of Mental Health, Bethesda, Maryland: IHF-DNA interactions—A binding and bending analysis.

Kitts, P.A., Nash, H.A., Laboratory of Molecular Biology, National Institute of Mental Health, Bethesda, Maryland: Bacteriophage λ site-specific recombination proceeds with a defined order of strand exchanges.

Amin, A.A., Sadowski, P.D., Dept. of Medical Genetics, University of Toronto, Ontario, Canada: Regions of the

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FLP protein required for site-specific DNA recognition.

Schwartz, C.J.E., Sadowski, P.D., Dept. of Medical Genetics, University of Toronto, Ontario, Canada: The FLP recombinase of 2μ circle of *S. cerevisiae* bends its DNA target.

Meyer-Leon, L., Huang, L.-C., Umlauf, S.W., Cox, M.M., Inman, R.B., Program in Cellular and Molecular Biology and Dept. of Biochemistry, University of Wisconsin, Madison: Intermediates and by-products in the FLP protein site-specific recombination reaction.

Musso, R.E., Black, T.A., Dept. of Biology, University of South Carolina, Columbia: IS*2*-encoded factors mediate excision of the bacterial insertion sequence IS*2*.

Waddell, C.S., Craig, N.L., Dept. of Biochemistry and Biophysics and the Hooper Foundation, University of California, San Francisco: Recognition of *att* Tn*7* target sequences by a Tn*7*-dependent DNA-binding activity.

Nissley, D., Fennewald, M., Dept. of Microbiology and Immunology, University of Health Sciences, Chicago Medical School, Illinois: Regions of the Tn*3* inverted repeat involved in transposase binding.

Huisman, O., Signon, L., Morisato, D., Kleckner, N., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Analysis of the ends of the transposon Tn*10*.

Benjamin, H., Kleckner, N., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Characterization of a complete intramolecular transposition reaction mediated by Tn*10* transposase.

Derbyshire, K.M., Kramer, M., Grindley, N.D.F., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Analysis of the *cis*-action of the IS*903* transposase.

Leung, P.C.,[1] Teplow, D.B.,[2] Harshey, R.M.,[1] [1]Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, [2]Division of Biology, California Institute of Technology, Pasadena: Mu B—ATP-binding, hydrolysis, and role in formation of the transposition intermediate.

Mizuuchi, M., Mizuuchi, K., NIDDK, National Institutes of Health, Bethesda, Maryland: Cloning and expression of partial polypeptides of the Mu A protein.

Abraham, Z., Symonds, N., School of Biological Sciences, University of Sussex, England: In vitro studies with the GAM protein of phage Mu.

Haffter, P., Bickle, T.A., Biocenter, Basal, Switzerland: Analysis of the topological specificity of the DNA invertase *cin* and the Tn*3* resolvase.

Gonzalez, T.N.,[1] Kanaar, R.,[2] Boles, T.C.,[1] Dungan, J.,[1] van de Putte, P.,[2] Cozzarelli, N.R.,[1] [1]Dept. of Molecular Biology, University of California, Berkeley; [2]Dept. of Biochemistry, State University of Leiden, The Netherlands: Synapsis of the recombination sites and the recombinational enhancer in the Mu Gin-DNA system.

Dungan, J.M., Cozzarelli, N.R., Dept. of Molecular Biology, University of California, Berkeley: Intermediates in the Tn*3* resolvase-mediated site-specific recombination.

Adams, D.E.,[1] Bliska, J.B.,[1] Sauer, B.,[2] Cozzarelli, N.R.,[1] [1]Dept. of Molecular Biology, University of California, Berkeley; [2]Dept. of Central Research and Development, E.I. du Pont de Nemours and Co., Inc., Experimental Station, Wilmington, Delaware: Cre-mediated plasmid recombination in *S. cerevisiae* and *E. coli*.

Sundström, L., Sköld, O., Swedberg, G., Rådström, P., Biomedical Center, Uppsala University, Sweden: Evidence for a site-specific recombination mechanism inserting antibiotic resistance genes into plasmids.

Lütticke, S., Saedler, H., Gierl, A., Max-Planck-Institut für Zuchtungsforschung, Köln, Federal Republic of Germany: Expression and characterization of a protein encoded by the En transposable element of *Z. mays*.

Plasterk, R.H.S., Netherlands Cancer Institute, Section of Chemical Carcinogenesis, Amsterdam: Transposition of the Tc1 element of the nematode *C. elegans*.

Shpakovski, G.V.,[1] Berlin, Y.A.,[2] [1]Institute of Bioorganic Chemistry, Minsk, [2]Shemyakin Institute of Bioorganic Chemistry, Moscow, Union of Soviet Socialists Republic: Regioselective recombinations as a class of illegitimate genomic rearrangements.

Klippel, A., Kahmann, R., Institut für Genbiologische Forschung Berlin, Federal Republic of Germany: Isolation and characterization of unusual Gin mutants.

Stewart, G.,[1] Stirling, C.,[1] Colloms, S.,[1] Szatmari, G.,[1] Summers, D.,[2] Sherratt, D.,[1] [1]Institute of Genetics, University of Glasgow, Scotland; [2]Dept. of Genetics, University of Cambridge, England: Characterization of *cis*- and *trans*-acting elements involved in ColE1 *cer* site-specific recombination.

Retrotransposition

Carroll, D., Knutzon, D.S., Garrett, J.E., Dept. of Biochemistry, University of Utah School of Medicine, Salt Lake City: Composite transposable elements from *Xenopus*.

Horak, I., Kröger, B., Baumruker, T., Goller, M., Institute of Virology and Immunobiology, University of Würzburg, Federal Republic of Germany: A sequence-specific single-stranded DNA-binding protein interacts with a recombination hot spot in mouse retrotransposons.

Huijser, P., Schulte, R., Saedler, H., Schwarz-Sommer, Z., Max-Planck-Institute für Zuchtungsforschung, Köln, Federal Republic of Germany: Is the Cin4 family of dispersed genomic sequences in maize capable of retrotransposition?

Horak, I., Edelmann, W., Institute of Virology and Immunobiology, University of Wurzburg, Federal Republic of Germany: Long terminal repeats of a mouse retrotransposon stimulate homologous recombination in vitro.

Biophysical Aspects of Recombination

Michel, B., D'Alençon, E., Ehrlich, S.D., Institute Jacques Monod, Paris, France: Deletion hot spots in chimeric *E. coli* plasmids.

Leach, D.,[1] Lindsey, J.,[1] Chalker, A.,[1] Okely, E.,[1] Lloyd, R.,[2] [1]Dept. of Molecular Biology, University of Edinburgh, Scotland; [2]Dept. of Genetics, University of Nottingham, England: Involvement of *E. coli* recombination in palindrome-mediated inviability.

Whoriskey, S.K., Miller, J.H., Molecular Biology Institute, University of California, Los Angeles: Identification of *E. coli* mutants with altered rates of spontaneous

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deletion formation.

Howard-Flanders, P., Akaboshi, E., Dept. of Molecular Biophysics, Yale University, New Haven, Connecticut: Differences in accessibility of DNA strands in RecA complexes as revealed by levels of protection against DNase I—Rates of forward and reverse polymerization.

Konforti, B.B., Davis, R.W., Dept. of Biochemistry, Stanford University, California: Homology at the 3′ end is preferred by RecA protein in formation of stable joint molecules.

Menetski, J.P., Kowalczykowski, S.C., Dept. of Molecular Biology, Northwestern University Medical School, Chicago, Illinois: Stable DNA heteroduplex formation by the *E. coli* RecA protein in the absence of ATP hydrolysis.

Lavery, P.E., Kowalczykowski, S.C., Dept. of Molecular Biology, Northwestern University Medical School, Chicago, Illinois: Biochemical basis of the temperature-inducible constitutive protease activity in the RecA441 protein of *E. coli*.

Lauder, S.D., Kowalczykowski, S.C., Dept. of Molecular Biology, Northwestern University Medical School, Chicago, Illinois: Asymmetry in the *E. coli* RecA protein-DNA filament.

Kowalczykowski, S.C., Krupp, R.A., Lauder, S.D., Lavery, P.E., Menetski, J.P., Dept. of Molecular Biology, Northwestern University Medical School, Chicago, Illinois: Biochemical properties of *E. coli* mutant RecA proteins and their relationship to biological function.

Harris, L.D., Griffith, J., Curriculum in Genetics and Lineberger Cancer Research Center, University of North Carolina, Chapel Hill: The UvsX protein of T4 bacteriophage catalyzes strand exchange and D-loop formation in vitro with the assistance of the UvsY and gene 32 proteins.

Rosenberg, S.M., [1,2] Sawitzke, J.A.,[1] [1]Institute of Molecular Biology, University of Oregon, Eugene; [2]Dept. of Biochemistry, University of Utah Medical School, Salt Lake City: Inhibition of *E. coli* Rec-mediated, Chi-stimulated recombination by RNase H.

Roman, L.J., Kowalczykowski, S.C., Dept. of Molecular Biology, Northwestern University of Medical School, Chicago, Illinois: Formation of heteroduplex DNA promoted by the combined activites of *E. coli* RecA and RecBCD proteins.

Siddiqi, I., Stahl, M.M., Stahl, F.W., Institute of Molecular Biology, University of Oregon, Eugene: Bacteriophage λ recombination by the RecF pathway of *E. coli*—Strand polarity of heteroduplex recombinants.

Yamamoto, K.,[1] Takahashi, N.,[2] Yoshikura, H.,[1] Kobayashi, I.,[2] [1]Dept. of Bacteriology, Faculty of Medicine, University of Tokyo, [2]National Children's Medical Research Center, Japan: Mechanism of homologous recombination in RecF pathway.

Schutte, B.C., Pugh, B.F., Bedale, W.A., Cox, M.M., Dept. of Biochemistry, University of Wisconsin, Madison: Extent of underwinding of duplex DNA resultling from (1) the direct binding of RecA protein in the presence of ATP and (2) the formation of a paranemic joint.

Honigberg, S.M., Muniyappa, K., Rould, E.A., Radding, C.M., Dept. of Human Genetics, Yale University School of Medicine, New Haven, Connecticut: Mechanics of unwinding and rewinding of helices in homologous recombination.

Donahue, C., Boles, T.C., Cozzarelli, N.R., University of California, Berkeley: Structure of multiply interlinked catenanes.

Boles, T.C.,[1] White, J.H.,[2] Cozzarelli, N.R.,[1] [1]Dept. of Molecular Biology, University of California, Berkeley; [2]Dept. of Mathematics, University of California, Los Angeles: Supercoiled DNA structure and implications for site-specific recombination.

Cohen, A., Nusbaum, A., Berger, I., Zilberstein, Z., Shalit, M., Dept. of Molecular Genetics, Hebrew University-Hadassah Medical School, Jerusalem, Israel: Plasmid double-strand ends—Origin and role in genetic recombination.

Johnston, B.H., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: The S1-sensitive structure of a $d(C\text{-}T)_n \cdot d(A\text{-}G)_n$ sequence in a plasmid probably involves triple-helical and single-strand regions.

McGovern, V.,[1] Hillyard, D.,[2] Higgins, P.,[1] [1]Dept. of Biochemistry, University of Alabama, Birmingham; [2]Howard Hughes Medical Institute, University of Utah, Salt Lake City: Protein organizing in supercoiled DNA.

Flanagan, P., Finn, K., Fennewald, M., Dept. of Microbiology and Immunology, University of Health Sciences, Chicago Medical School, Illinois: Analysis of inhibitors of resolvase.

Enzymology of Recombination

Surette, M.G., Chaconas, G., Dept. of Biochemistry, University of Western Ontario, London, Canada: A protein factor that substitutes for negative supercoils in the Mu DNA-strand-transfer reaction is *E. coli* IHF.

Takahashi, N., Kobayashi, I., Dept. of Infectious Diseases Research, National Children's Medical Research Center, National Children's Hospital, Tokyo, Japan: Genes affecting double-strand gap repair in *E. coli*.

Kobayashi, I., Takahashi, N., Dept. of Infectious Diseases Research, National Children's Medical Research Center, Tokyo, Japan: Double-strand cap repair through gene conversion by *E. coli* and λ.

Epstein, L.H., Munz, P.L., Young, C.S.H., Dept. of Microbiology, Columbia University, New York, New York: Evidence for ligation of noncognate DNA ends in extracts from mammalian cells.

Thode, S., Pfeiffer, P., Vielmetter, W., Institute for Genetics, University of Cologne, Federal Republic of Germany: Intermediates generated by in vitro joining of nonhomologous DNA double-strand termini.

Ikeda, H., Chiba, M., Shimizu, H., Fujimoto, A., Nashimoto, H., Honda, M., Institute of Medical Science, University of Tokyo, Japan: T4 DNA topoisomerase-mediated illegitimate recombination in vitro and in vivo.

Eisen, A., Camerini-Otero, R.D., NIDDK, National Institutes of Health, Bethesda, Maryland: Isolation and characterization of a recombinase from *Drosophila* embryos.

Mills, F.C., Brooker, J.S., Camerini-Otero, R.D., NIDDK, National Institutes of Health, Bethesda, Maryland: Human recombinase mediates strand exchange between immunoglobulin switch sites.

Jeyaseelan, R., Shanmugam, G., Madurai Kamaraj University School of Biological Sciences, India: Purification and characterization of an endonuclease from human placenta that cleaves cruciform structures and synthetic Holliday X junctions.

West, S.C., Elborough, K.M., Imperial Cancer Research Fund, Clare Hall, Laboratories, South Mimms, England: Detection of a protein from human cell extracts that binds synthetic Holliday junctions.

Dutreix, M., Moreau, P.L., Bailone, A., Devoret, R., GEMC, Enzymologie, Centre National de la Recherche Scientifique, Gif-sur-Yvette, France: A new RecA mutant whose recombination defect is reversed in a LexA (DEF) host.

Saldanha, R.,[1] Wenzlau, J.M.,[1] Butow, R.A.,[2] Perlman, P.S.,[1] [1]Dept. of Molecular Genetics, Ohio State University, Columbus; [2]Dept. of Biochemistry, University of Texas Southwestern Medical Center, Dallas: A latent intron-encoded maturase is also an endonuclease needed for intron mobility.

Palas, K.M., Kushner, S.R., Dept. of Genetics, University of Georgia, Athens: Biochemical analysis of the RecBCD enzyme of *E. coli*—Endonucleolytic activity is required for genetic recombination and DNA repair.

Mahajan, S.K., Mangoli, S.H., Molecular Biology and Agriculture Division, Bhabha Atomic Research Centre, Bombay, India: On the role of the RecBCD enzyme in recombination.

Murphy, K.C., Dept. of Molecular Genetics and Microbiology, University of Massachusetts, Worcester: Modification of RecBCD enzyme by phage P22 Abc protein.

Lowenhaupt, K., Sander, M., McCarthy, J., Rich, A., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Partial purification of a strand-transfer activity from *D. melanogaster* embryos.

Fasullo, M.T.,[1] Holloman, W.K.,[2] Rothstein, R.J.,[1] [1]Dept. of Genetics and Development, Columbia University, New York, [2]Dept. of Microbiology, Cornell University, New York, New York: Identification and characterization of Rec1-like proteins in the yeast *S. cerevisiae*.

Sugino, A., Hamatake, R., Dykstra, C., Clark, A., National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Presynapsis and synapsis of DNA promoted by the STPα and β single-stranded DNA-binding proteins of the yeast *S. cerevisiae*.

Lavoie, B.D., Chaconas, G., Dept. of Biochemistry, University of Western Ontario, London, Canada: Protein content and organization of synaptic complexes involved in the Mu DNA strand-transfer reaction in vitro.

Waldman, A.S., Liskay, R.M., Dept. of Therapeutic Radiology and Human Genetics, Yale University School of Medicine, New Haven, Connecticut: A Holliday-structure-resolving activity from human cells.

Zerbib, D.,[1] Gamas, P.,[1] Fayet, O.,[1] Jakowec, M.,[2] Prentki, P.,[2] Galas, D.,[2] Chandler, M.,[1] [1]CRBGC, Toulouse, France; [2]Dept. of Molecular Biology, University of Southern California, Los Angeles: Analysis of IS*1*-encoded proteins required for transposition.

Krauss, S.W., Randahl, H., Mosbaugh, D.W., Elliott, G.C., Syvaoja, J., Nishida, C., Linn, S., Dept. of Biochemistry, University of California, Berkeley: Enzymology of mammalian DNA polymerases that could participate in recombinational processes.

POSTER SESSION II

Mismatch Repair

Dowjat, A.K., Harris, C.J., Fishel, R., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Effect of mismatch repair and processing on the recombination of an adjacent gene in *E. coli*.

Smith, S.S., Kan, J.L.C., Baker, D.J., Dept. of Molecular

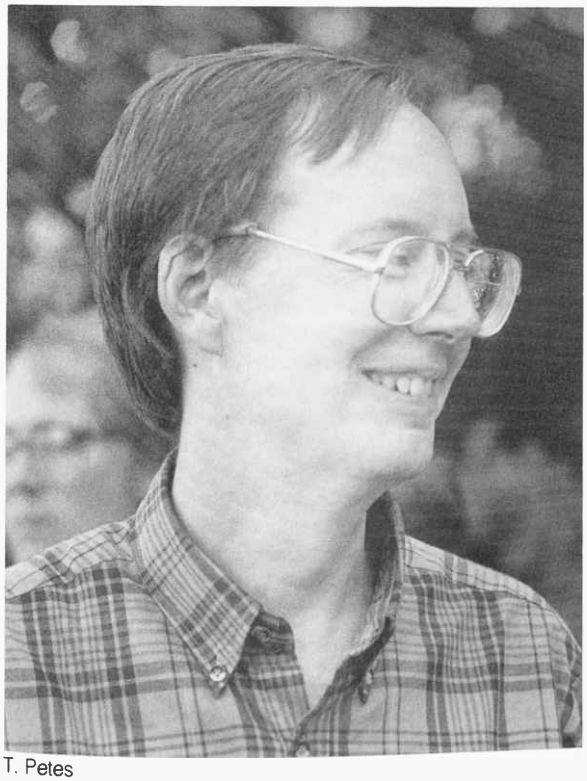
Surgery, City of Hope National Medical Center, Duarte, California: De novo methylation of cytosine in the formation of recombination intermediates.

Detloff, P., Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois: Recombination-directed repair—A general model of recombination.

Haber, L.T., Mankovich, J.A., Walker, G.C., Massachusetts Institute of Technology, Cambridge: Analysis of the *S. typhimurium mutS* and *mutL* genes required for mismatch repair.

Schäer, P., Kohli, J., Dept. of General Microbiology, University of Bern, Switzerland: A specific marker effect connected with mismatch repair in *S. pombe*—Sequence and genetic analysis.

Yashar, B.M., Modrich, P., Dept. of Biochemistry, Duke



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University Medical Center, Durham, North Carolina: In vitro characterization of VSP mismatch correction.

Heywood, L.A., Burke, J.F., University of Sussex, England: Analysis of mismatch repair in mammalian cells using a shuttle vector system.

Recombination in Higher Eukaryotes

Adair, G.,[1] Nairn, R.,[1] Wilson, J.,[2] Seidman, M.,[3] [1]University of Texas, M.D. Anderson Cancer Center, Sciences Park-Research/Division, Smithville; [2]Dept. of Biochemistry, Baylor College of Medicine, Houston, Texas; [3]Otsuka Pharmaceutical Co., Ltd., Rockville, Maryland: Targeted recombination at the endogenous ***APRT*** locus in CHO cells.

Bhattacharyya, N., Maher, V.M., McCormick, J.J., Carcinogenesis Laboratory, Michigan State University, East Lansing: Homologous recombination between duplicated thymidine kinase (*tk*) genes stably integrated within the genome of normally repairing and repair-deficient human cells.

Hellgren, D., Lambert, B., Dept. of Clinical Genetics, Karolinska Institute, Stockholm, Sweden: Induced recombination between duplicated *neo* genes stably integrated in the genome of CHO cells.

Wahls, W.P., Moore, P.D., Dept. of Microbiology and Immunology, University of Illinois, Chicago Medical Center: Human recombination hot spots—The effects of minisatellite and Z-DNA sequences.

Murnane, J.P., Yezzi, M.J., Young, B.R., Laboratory of Radiobiology and Environmental Health, University of California, San Francisco: Characterization of a hot spot for mitotic recombination in human cells.

Evans, D.H.,[1] McFadden, G.,[2] [1]Dept. of Molecular Biology and Genetics, University of Guelph, Ontario, [2]Dept. of Biochemistry, University of Alberta, Edmonton, Canada: Analysis of the mechanics of genetic recombination in poxvirus-infected cells.

Ball, A., Dept. of Microbiology, University of Alabama, Birmingham: Homologous recombination in vaccinia-virus-infected cells.

Konopka, A.K.,[1] Htun, H.,[2] Maizel, J.V., Jr.,[1] [1]NCI-National Institutes of Health, Frederick, Maryland; [2]Dept. of Physiological Chemistry, University of Wisconsin, Madison: Unusual DNA structures that may be involved in somatic cell illegitimate recombination.

Podgornaya, O., Perelygina, L., Tomilin, N., Institute of Cytology, Academy of Sciences, Leningrad, Union of Soviet Socialists Republic: Cooperative binding of human nuclear protein to Alu-family DNA repeat.

Tsujimura, T., Maher, V.M., McCormick, J.J., Carcinogenesis Laboratory, Michigan State University, East Lansing: A system for studying homologous recombination between repeated chromosomal sequences in human cell lines that differ in DNA repair capacity.

Nairn, R.S., Adair, G.M., Humphrey, R.M., University of Texas M.D. Anderson Cancer Center, Smithville: Influence of UV damage on homology-dependent intermolecular and targeted recombination events in CHO cells.

Maryon, E., Carroll, D., Dept. of Biochemistry, University of Utah, Salt Lake City: Homologous recombination of exogenous DNA in *X. laevis* oocytes.

Lin, F.-L.M., Sperle, K., Sternberg, N., Dept. of Central Research and Development, E.I. du Pont de Nemours & Co., Inc., Experimental Station, Wilmington, Delaware: Gene conversion is not an efficient mode of recombination in gene-transfer process in mammalian cells.

Keown, W., Campbell, C., Lowe, L., Kucherlapati, R., Dept. of Genetics, University of Illinois College of Medicine, Chicago: Correction of insertions and deletions by homologous recombination in mammalian cells.

Zheng, H., Wilson, J., Verna and Marrs McLean Dept. of Biochemistry, Baylor College of Medicine, Houston, Texas: Targeted recombination into amplified adenosine deaminase cell lines.

Shaw-White, J.R., Stringer, J.R., Dept. of Microbiology and Molecular Genetics, University of Cincinnati College of Medicine, Ohio: Homologous recombination in EBV-based episomes in human cells.

Homologous Recombination

Katz, K.S.,[1,2] Ratner, D.I.,[1] [1]Dept. of Biology, Amherst College, [2]Molecular and Cellular Biology Program, University of Massachusetts, Amherst: Homologous recombination and the repair of double-strand breaks during cotransformation of *D. discoideum*.

Strathern, J., Shafer, B., McGill, C., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Stimulation of recombination in yeast by gene II nicking enzyme of bacteriophage f1.

Thomas, B.J., Rothstein, R.J., Dept. of Genetics and Development, Columbia University College of Physicians & Surgeons, New York, New York: Elevated recombination rates in transcriptionally active DNA in *S. cerevisiae*.

Schultes, N.,[1] Treco, D.,[1] Nicolas, A.,[2] Szostak, J.,[1] [1]Dept. of Molecular Biology, Massachusetts General Hospital, Boston; [2]Laboratoire IMG, Université Paris, France: Bipolar gene conversion at the *ARG4* recombination initiation site in *S. cerevisiae*.

Schiestl, R.H., Prakash, S., Dept. of Biology, University of Rochester, New York: *RAD1*, an excision repair gene of *S. cerevisiae*, is involved in recombination.

Jinks-Robertson, S., Dept. of Biology, Emory University, Atlanta, Georgia: Effect of length of homology on heterochromosomal recombination in yeast.

Stewart, S., Roeder, S., Dept. of Biology, Yale University, New Haven, Connecticut: Effect of mutations in *HOT1*, a mitotic recombination hot spot, on recombination and transcription.

Louis, E.J., Haber, J.E., Rosenstiel Center, Brandeis University, Waltham, Massachusetts: Mitotic and meiotic recombination among Y′ repeats in *S. cerevisiae*.

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Borts, R.H.,[1] Leung, W.-Y.,[1] Williamson, M.,[2] Kramer, W.,[2] Fogel, S.,[2] Haber, J.E.,[1] [1]Rosenstiel Center, Brandeis University, Waltham, Massachusetts; [2]Dept. of Genetics, University of California, Berkeley: Effect of the *pms1-1* gene of *S. cerevisiae* on recombination in a genetic interval containing multiple heterozygosities.

Borts, R.H., Haber, J.E., Rosenstiel Center, Brandeis University, Waltham, Massachusetts: Position and tract length of conversions in a well-defined interval of *S. cerevisiae*.

Le Chevanton, L.,[1] Pukkila, P.J.,[2] [1]Laboratoire Interactions Moleculaires et Genomiques, Université Paris, France; [2]Dept. of Biology, University of North Carolina, Chapel, Hill: Targeted transformation in filamentous fungi.

Holloman, W.,[1,2] Bauchwitz, R.,[2] Fotheringham, S.,[2] Tsukuda, T.,[2] [1]Dept. of Microbiology, [2]Interdivisional Program in Molecular Biology, Cornell University Medical College, New York, New York: Molecular genetic analysis of recombination in *U. maydis*.

Bailone, A.,[1] Sommer, S.,[1] Bagdasarian, M.,[2] Devoret, R.,[1] [1]GEMC, Enzymologie, Centre National de la Recherche Scientifique, France; [2]Michigan Biotechnology Institute, Lansing: PsiB protein that prevents activation of RecA protein discriminates two recombination substrates in vivo.

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Rudin, N., Sugarman, E., Haber, J.E., Rosenstiel Center and Dept. of Biology, Brandeis University, Waltham, Massachusetts: *HO* endonuclease-induced recombination in yeast.

Esposito, M.S., Brown, J.T., Rudin, N., Lawrence Berkeley Laboratory, University of California, Berkeley: The *REC1* gene of *S. cerevisiae* is required for spontaneous mitotic gene conversion, intra- and intergenic recombination, genomic stability, repair of X-ray damage, and sporulation.

Rong, L., Palladino, A. Aguilera, A., Kein, H.L., Dept. of Biochemistry, New York University Medical Center, New York: Intrachromosomal hyper-gene conversion mutants of *S. cerevisiae*.

Klein, H.L., Dept. of Biochemistry, New York University Medical Center, New York: Different types of recombination events are controlled by the *RAD1* and *RAD52* genes of *S. cerevisiae*.

Menees, T., Roeder, S., Dept. of Biology, Yale University, New Haven, Connecticut: Isolation and characterization of *mei4-1*, a yeast mutant defective in meiotic recombination.

Dresser, M.E., Tiano, H.F., Giroux, C.N., National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Genetic control of chromosome synapsis in yeast—The *SPO11* gene but not the *RAD52* gene is required for formation of the synaptonemal complex.

Padmore, R., Kleckner, N., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: *rad50* mutants of *S. cerevisiae* are asynaptic in meiosis.

Ajimura, M.,[1] Kleckner, N.,[2] Ogawa, H.,[1] [1]Dept. of Biology, Faculty of Science, Osaka University, Japan; [2]Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Isolation and characterization of the mutants defective in meiotic recombination in *S. cerevisiae*.

Dornfeld, K.J., Livingston, D.M., Dept. of Biochemistry, University of Minnesota, Minneapolis: Fusion of the *RAD52* gene to the *GAL1* promoter permits controlled induction of plasmid recombination in yeast.

Hoekstra, M.F., Mull, E.E., Heffron, F., Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California: Genetic functions interacting with a site-specific double-strand break.

Singer, J.D., Nickoloff, J.A., Hoekstra, M.F., Heffron, F., Dept. of Molecular Biology, Scripps Clinic and Research Foundation, La Jolla, California: Physical and genetic analysis of double-strand break-induced recombinational repair in yeast.

F. Stahl

Kaback, D.B.,[1] de Jonge, P.,[2] Steensma, H.Y.,[2] [1]Dept. of Microbiology and Molecular Genetics, University of Medicine and Dentistry, New Jersey Medical School, Newark; [2]Dept. of Cell Biology and Genetics, Leiden University, The Netherlands: Analysis of meiotic recombination on chromosome 1 from *S. cerevisiae*.

Symington, L.,[1] Petes, T.,[2] [1]Institute for Cancer Research and Dept. of Microbiology, University of Columbia Medical School, New York, New York; [2]Dept. of Biology, University of North Carolina, Chapel Hill: Analysis of meiotic recombination events on yeast chromosome III.

Sutton, P.R., Liebman, S.W., Laboratory for Cell, Molecular, and Developmental Biology, University of Illinois, Chicago: Deletions of the *CYC1-OSM1* region in yeast due to conversion by two distinct repetitive sequence families.

Hollingsworth, N., Goetsch, L., Byers, R., Dept. of Genetics, University of Washington, Seattle: Role of the *HOP1* protein in meiotic pairing in *S. cerevisiae*.

McGill, C., Shafer, B., Strathern, J., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Synthetic locus for recombination studies in yeast.

Szankasi, P., Ponticelli, A.S., Smith, G.R., Fred Hutchinson Cancer Research Center, Seattle, Washington: Enzymes and sites promoting meiotic recombination in *S. pombe*.

Kuzminov, A.V., Mazin, A.V., Dianov, G.L., Salganik, R.I., Institute of Cytology and Genetics, Siberian Branch of the Union of Soviet Socialists Republic Academy of Sciences, Novosibirsk: Direct-repeat-mediated recombination in *E. coli* plasmids.

Bennett, C.B., Resnick, M.A., National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Consequences of unrepaired double-strand breaks in yeast.

Gene Activation by Recombination

White, C.I., Connolly, B.M., Haber, J.E., Rosenstiel Center, Brandeis University, Waltham, Massachusetts: A putative intermediate in GAL-HO-induced mating-type switching in *S. cerevisiae*.

McBroom, L.D.B., Sadowski, P.D., Dept. of Medical Genetics, University of Toronto, Ontario, Canada: Formation of specific protein-DNA complexes near the Y/Z junction of *S. cerevisiae* mating-type locus.

Waters, S.H., Stavnezer, J., Dept. of Molecular and General Genetics, University of Massachusetts Medical School, Worcester: Nuclear B-cell-specific immunoglobulin switch sequence-binding activity.

Katzenberg, D.R., Weinreb, A., Tilley, S.A., Birshtein, B.K., Dept. of Cell Biology, Albert Einstein College of Medicine, Bronx, New York: Molecular analysis of an unequal sister chromatid exchange site in a mouse myeloma cell line.

Lewis, S., Hesse, J., Mizuuchi, K., Gellert, M., NIDDK, National Institutes of Health, Bethesda, Maryland: Unusual strand exchanges in V(D)J recombination.

Bakkeren, G., Koukolíková-Nicola, Z., Grimsley, N., Hohn, B., Friedrich Miescher-Institut, Basel, Switzerland: Recovery of *A. tumefaciens* T-DNA molecules from whole plants early after their transfer.

The Molecular Diagnostics of Human Cancer

September 7—September 11

ARRANGED BY

Mark E. Furth, Regeneron, New York
M.F. Greaves, Institute of Cancer Research, London

232 participants

The 1988 Cancer Cells meeting, "The Molecular Diagnostics of Human Cancer", focused on the medical implications of recent discoveries concerning the genetic basis of neoplastic disease. This international meeting brought together more than

232 scientists and physicians who share overlapping interests in fundamental cancer biology and in the potential clinical ramifications of the detection of specific molecular abnormalities in cancer cells. The active participation of individuals with complementary expertise in basic and clinical research proved stimulating and productive, and the focus on studies directly concerning human disease marked a new venture in the Laboratory's series of Cancer Cells meetings. This theme was highlighted in six "clinical overviews" designed to help educate molecular biologists in medical aspects of cancer, as well as to define areas in which improved diagnostic assays might have significant clinical impact. The program of research talks centered principally on the role of oncogenes and their protein products in human tumors and emphasized the utility of activated oncogenes as cancer markers. The oncogene proteins serve a wide variety of regulatory functions and include nuclear proteins involved in the control of DNA replication and gene expression, growth factors and their cell-surface receptors, and elements of signal transduction pathways, such as protein kinases and GTP-binding proteins. Assays either for the overproduction of particular oncogene proteins, often associated with gene amplification, or for qualitative abnormalities in oncogenes and their protein products have proven valuable in the specific diagnosis and/or prediction of prognosis of certain human cancers. Perhaps more importantly, insights into the genetic basis of cancer may guide the development of improved forms of therapy. The program included sessions on nuclear oncogene proteins, growth factors, growth factor receptors, and the products of the *ras, arc,* and *raf* oncogenes. Other sessions focused on chromosomal rearrangements known or likely to be associated with oncogene activation, such as the "Philadelphia chromosome," characteristic of chronic myelogenous leukemia, in which a translocation activates the *abl* gene. The meeting provided an exceptionally good forum for the critical discussion of very recent data on the prognostic implications of particular oncogene alterations in a number of cancers, notably pediatric tumors such as neuroblastoma, the myelogenous leukemias, and carcinomas of the colon, bladder, pancreas, and breast. Other timely presentations focused on the importance of gene loss in some of the major human cancers and on the identification and potential function of "anti-oncogenes." Additional sessions concerned several topics closely related to the main subject of the meeting, including the genetic basis for the resistance of tumor cells to chemotherapeutic drugs and the role of DNA tumor virus oncogenes in some human cancers.

This meeting was funded in part by the National Cancer Institute, a division of the National Institutes of Health.

SESSION 1 NUCLEAR ONCOGENE PROTEINS AS CANCER MARKERS

Chairman: **M. Israel,** National Cancer Institute

Clinical Overview: *M. Israel*

Dalla-Favera, R., Dept. of Pathology and Kaplan Cancer Center, New York University, New York: *myc* and *ras* oncogene activation in lymphoid malignancies—Detection, frequency, and possible biological roles.

Schwab, M., Deutsches Krebsforschungszentrum, Institut für Experimentelle Pathologie, Heidelberg, Federal Republic of Germany: Amplification of cellular oncogenes in tumor progression.

Alt, F., Yancopoulos, G.D., Collum, R., Dildrop, R., Oltz, E., Kaplan, K., DePinho, R., Depts. of Biochemistry and Microbiology, Columbia University College of Physicians & Surgeons, New York: Molecular probes for the characterization of lymphoid and neural tumors.

Israel, M.A., Cooper, M., Helman, L., El Badry, O., Thiele, C.,

M. Furth, M. Greaves

NCI, National Institutes of Health, Bethesda, Maryland: Developmentally regulated, lineage-associated markers as guideposts for cancer therapy.

Ahuja, H., Cline, M.J., Dept. of Medicine, University of California, Los Angeles: Alterations of the p53 gene and blast crisis of CML.

Benchimol, S., Ontario Cancer Institute and Dept. of Medical Biophysics, University of Toronto, Ontario, Canada: Expression of P53 protein in leukemia.

Jenkins, J.R., Sturzbecher, H.-W., Brain, R., Grimaldi, M., Maimets, T., Rudge, K., Court, W., Addison, C., Cell Proliferation Laboratory, Marie Curie Cancer Research Institute, Oxted, England: Identification and analysis of human p53 mutants that are transdominant modulators of DNA replication in vivo.

SESSION 2 MOLECULAR ASSAYS FOR CHROMOSOMAL REARRANGEMENTS I. LEUKEMIAS AND LYMPHOMAS

Chairman: M.F. Greaves, Institute of Cancer Research, London

Clinical Overview: B. Clarkson, Memorial Sloan-Kettering Cancer Center

Clark, S.,[1] Crist, W.,[4] Champlin, R.,[2] Najfeld, V.,[5] Witte, O.,[1,3] [1]Dept. of Microbiology and Molecular Biology Institute, [2]Division of Hematology/Oncology, Dept. of Medicine, [3]Howard Hughes Medical Institute, University of California, Los Angeles; [4]Dept. of Hematology/Oncology, St. Jude Children's Research Hospital, Memphis, Tennessee; [5]Tumor Cytogenetics, Mt. Sinai Medical Center, New York, New York: Ph-chromosome-positive human leukemias and the BCR-ABL oncogene.

Kurzrock, R.,[1,2] Shtalrid, M.,[1] Kantarjian, H.,[2] Gutterman, J.,[1] Talpaz, M.,[1,2] Depts. of [1]Clinical Immunology and Biological Therapy, [2]Hematology, M.D. Anderson Cancer Center, Houston, Texas: Correlation of molecular and clinical characteristics in CML patients.

Grosveld, G.,[1] Hermans, A.,[1] von Lindern, M.,[1] van Baal, S.,[1] Meijer, D.,[1] Selleri, L.,[1] van der Plas, D.,[1] Wiedemann, L.,[2] Groffen, J.,[3] Heisterkamp, N.,[3] Bootsma, D.,[1] [1]Dept. of Cell Biology and Genetics, Erasmus University, Rotterdam, The Netherlands; [2]Leukemia Research Fund Center, London, England; [3]Childrens Hospital, University of Southern California, Los Angeles: *bcr-abl* oncogene activation in CML and Ph-positive ALL.

Wiedemann, L., van der Feltz, M., Gow, J., Shivji, M., Allen, P., Leukemia Research Fund Center, Institute of Cancer Research, London, England: Characterization of BCR-ABL translocation products in Ph-positive leukemias.

Chaganti, R.S.K., Memorial Sloan-Kettering Cancer Center, New York, New York: Molecular approaches to diagnostic and prognostic evaluation of lymphoma and CML.

Lee, M., Cabanillas, F., Freireich, E., Trujillo, J., Stass, S., M.D. Anderson Cancer Center, University of Texas, Houston: Detection of minimal residual circulating cells carrying the t(14;18) by DNA sequence amplification (PCR).

Yunis, J., Dept. of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis: Prognostic significance of *bcl-2* oncogene rearrangement in follicular and diffuse large-cell and mixed-cell lymphoma.

Cleary, M.L., Ngan, B.-Y., Chen-Levy, Z., Nourse, J., Dept.

of Pathology, Stanford University, California: BCL-2 proto-oncogenic protein associated with t(14;18) translocations—Biochemical properties and expression in non-Hodgkin's lymphomas.

Tycko, B.,[1] Palmer, J.D.,[1] Smith, S.D.,[2] Sklar, J.,[1] Depts. of [1]Pathology, [2]Pediatrics, Stanford University School of Medicine, California: PCR amplification of rearranged antigen receptor genes using junction-specific oligonucleotides—Possible application to detection of minimal residual disease in ALL.

SESSION 3 POSTER SESSION

Adnane, J.,[1] Simon, M.P.,[1] Gaudray, P.,[1] De Lapeyrière, O.,[2] Ayraud, N.,[1] Jeanteur, P.,[3] Theillet, C.,[3] [1]LGMCH, Nice, [2]INSERM, Marseille, [3]US CNRS, Montpellier, France: Proto-oncogene amplification in human breast carcinomas.

Anderson, A.E., Schneider, N.R., Allen, G.J., Ranganathan, R., Burns, J., Jhanwar, S.C., Cunningham, I., O'Reilly, R.J., Chaganti, R.S.K., Memorial Sloan-Kettering Cancer Center, New York, New York: Detection of posttransplant minimal disease CML by *bcr* rearrangement analysis.

Aubin, R.A., Fourney, R.M., Mirzayans, R., Dietrich, K.D., Paterson, M.C., Molecular Genetics and Carcinogenesis Laboratory, W.W. Cross Cancer Institute, Edmonton, Alberta, Canada: Analysis of proto-oncogene structure and expression in subjects belonging to cancer-prone kindreds.

Barnett, T., Elting, J., Kretschmer, A., Goebel, S., Hart, J., Nothdurft, M.A., Austen, D., Kamarck, M., Molecular Diagnostics, Inc., and Molecular Therapeutics, Inc., West Haven, Connecticut: Carcinoembryonic antigens—A highly conserved family of human tumor antigens with novel structural features.

Birg, F.,[1] Torrès, H.,[1] Maroc, N.,[1] Razanajaona, D.,[1] Fäy, C.,[1] Courcoul, M.A.,[1] Lavezzi, C.,[1] Dubreuil, P.,[1] Pébusque, M.J.,[1] Tabilio, A.,[2] Guilbert, L.,[3] Mannoni, P.,[1] [1]INSERM, Institut Paoli-Calmettes, Marseille, France; [2]University of Perugia, Italy; [3]University of Alberta, Edmonton, Canada: Hematopoietic growth factors and human AML.

Carney, W.P., Hamer, P.J., Shea, D.E., Pullano, T.G., Oncogene Research Group, Dept. of Medical Products, E.I. du Pont, North Billerica, Massachusetts: Novel monoclonal antibodies for the specific detection of the Ha-*ras* and N-*ras* p21s.

Carrino, J.J.,[1] Liebowitz, D.,[2] Westbrook, C.A.,[1] [1]Dept. of Medicine, University of Chicago, Illinois; [2]Dept. of Medicine, Columbia University, New York, New York: PCR method for the detection of BCR-ABL fusion mRNA and identification of alternative splicing in CML and Ph[1]-positive ALL.

Condon, M.R., Ganguly, S., Doucette, L., Chaganti, R.S.K., Memorial Sloan-Kettering Cancer Center, New York, New York: A new breakpoint cluster in the *bcl-2* gene that translocates to the immunoglobulin heavy-chain switch region in t(14;18).

Crossen, P.E.,[1] Atkinson, C.H.,[2] [1]Cytogenetic and Molecular Oconology Unit, [2]Dept. of Oncology, Christchurch Hospital, New Zealand: Histiocytic lymphoma with germ-line heavy-chain immunoglobulin genes but rearranged κ light-chain and TCR-β genes.

R. Burt

Daya-Grosjean, L.,[1] Suarez, H.G.,[1] Schlaifer, D.,[1] Nardeux, P.,[1] Renault, G.,[1] Bos, J.L.,[2] Sarasin, A.,[1] [1]Institut de Recherches Scientifiques sur le Cancer, Villejuif, France; [2]Dept. of Medical Biochemistry, Sylvius Laboratories, Leiden, Holland: Modified oncogenes in skin tumors from a repair-deficient syndrome, xeroderma pigmentosum.

Dmitrovsky, E.,[1] Moy, D.,[1] Griffin, O.,[3] Samaniego, F.,[3] Reuter, V.,[2] Bosl, G.,[1] Chaganti, R.,[3] Depts. of [1]Medicine, [2]Pathology, [3]Laboratory of Cancer Genetics and Cytogenetics, Memorial Sloan-Kettering Cancer Center, New York, New York: Expression of the N-*myc* oncogene in human germ cancer.

Duigou, G.J.,[1] Babiss, L.E.,[2] Iman, D.S.,[3] Shay, J.W.,[3] Fisher, P.B.,[1] [1]Columbia University College of Physicians & Surgeons, [2]Rockefeller University, New York, New York; [3]University of Texas Southwestern Medical Center, Dallas: Somatic cell hybrids between normal rat embryo fibroblasts and adenovirus-transformed rat embryo cells result in a suppression of the tumorigenic-progression phenotype.

Durnam, D.M., Anders, K.R., Bryant, E.M., Thomas, E.D., Fred Hutchinson Cancer Research Center, Seattle, Washington: Use of a Y-chromosome-specific in situ hybridization assay in assessing the efficacy of bone marrow transplantation.

Farr, C.J.,[1] Saiki, R.K.,[2] McCormick, F.,[3] Pragnell, I.B.,[4] Marshall, C.J.,[1] [1]Institute of Cancer Research, Chester Beatty Laboratories, London, England; Depts. of [2]Human Genetics, [3]Molecular Biology, Cetus Corporation, Emeryville, California; [4]Beatson Institute for

Cancer Research, Glasgow, Scotland: PCR analysis of *ras* gene mutations in AML.

Ferre, F., Garduno, F., Peter, J.B., Cytometrics Inc., Division of Speciality Laboratories, Inc., San Diego, California: Detection of BCR-ABL RNA on crude cell extract by a modified PCR.

Filatov, L., Mamayeva, S., Tomilin, N., Institute of Cytology, Academy of Sciences, Leningrad, Union of Soviet Socialists Republic: New clusters of Alu-family DNA repeats detected in chromosomes 3, 8, and 14 of acute leukemia patients.

Flamm, S.L., Wellstein, A., Kern, F., Lippman, M.E., Gelmann, E.P., NCI, National Institutes of Health, Bethesda, Maryland: Expression of FGF peptides in normal and malignant human mammary epithelial cells.

Goldstein, L.J.,[1] Galski, H.,[1] Fojo, A.,[1] Willingham, M.,[1] Lai, S.,[1] Gazdar, A.,[1] Pirker, R.,[2] Green, A.,[3] Crist, W.,[3] Grant, C.,[4] Kovach, J.,[4] Lieber, M.,[4] Gottesman, M.M.,[1] Pastan, I.,[1] [1]NCI, Bethesda, Maryland; [2]University of Vienna, Austria; [3]St. Jude Children's Research Hospital, Division of Hematology-Oncology, Memphis, Tennessee; [4]Mayo Clinic, Rochester, Minnesota: Intrinsic and acquired expression of a multidrug-resistant gene in human tumors.

Hailat, N., Palutke, M., Kithier, K., Dept. of Pathology, Wayne State University School of Medicine, Detroit, Michigan: Expression and functional studies of CD2 antigen in a chronic T-cell leukemia.

Halaban, R.,[1] Langdon, R.,[1] McGuire, J.,[1] Baird, A.,[3] Ghosh, S.,[1] Dotto, P.,[2] Depts. of [1]Dermatology, [2]Pathology, Yale University School of Medicine, New Haven, Connecticut; [3]Dept. of Neuroendocrinology, Salk Institute, La Jolla, California: Basic FGF, a paracrine and autocrine growth factor for normal and malignant melanocytes, respectively.

Hedge, P.J., Rider, S.H., Moore, G., Yagle, M., Sheer, D., Solomon, E., Imperial Cancer Research Fund, London, England: A chromosome-17-specific repeat mapping to the region of the acute promyelocytic leukemia breakpoint.

Hiorns, L.R.,[1] Kerr, I.B.,[2] Cotter, F.E.,[1] Young, B.D.,[1] [1]Dept. of Medical Oncology, St. Bartholomew's Hospital, [2]Director's Laboratory, Imperial Cancer Research Fund, London, England: Incidence and clinicopathological significance of *ras* family oncogene mutations in a series of human leukemias and preleukemic disorders.

Janssen, J.W.G., Lyons, J., Buschle, M., Bartram, C.R., Dept. of Pediatrics, University of Ulm, Federal Republic of Germany: Application of the PCR technique in the detection of minimal residual disease in Ph[1]-positive CML and *RAS* point mutations in hematopoietic neoplasias.

Johnson, B.E.,[1] Peng, J.,[2] Naylor, S.,[3] Zbar, B.,[4] Brauch, H.,[4] Gazdar, A.F.,[1] [1]NCI, Navy Medical Oncology Branch, [2]Medicine Branch, Bethesda, Maryland; [3]Dept. of Cellular and Structural Biology, University of Texas Health Sciences Center, San Antonio; [4]NCI-Frederick Cancer Research Facility, Frederick, Maryland: The short arm of chromosome 3 is not lost in extrapulmonary small-cell cancer.

Kasid, Y.,[1] Weichselbaum, R.R.,[2] Beckett, M.,[2] Dunphy, E.,[2] Mroczkowski, Z.,[1] Dritschilo, A.,[1] Merlino, G.T.,[3] [1]Dept. of Radiation Medicine, Georgetown University, Washington, D.C.; [2]University of Chicago Center for Radiation Therapy, Illinois; [3]NCI, National Institutes of Health, Bethesda, Maryland: Gene amplification and aberrant transcription of the EGFR in human laryngeal squamous carcinoma cells.

Kasid, U.,[1] Pfeifer, A.,[2] Weichselbaum, R.R.,[3] Dritschilo, A.,[1] Mark, G.,[4] [1]Dept. of Radiation Medicine, Georgetown University, Washington, D.C.; [2]NCI, National Institutes of Health, Bethesda, Maryland; [3]University of Chicago Center for Radiation Therapy, Illinois; [4]Dept. of Cellular and Molecular Biology, Merck, Sharp & Dohme Research Laboratories, Rahway, New Jersey: *RAF*-oncogene-mediated regulation of three radiation-resistant head and neck cancers.

SESSION 4 GENE DELETIONS AND ANTI-ONCOGENES AS CANCER MARKERS

Chairman: B.A.J. Ponder, Institute of Cancer Research, Sutton

Ponder, B.A.J.,[1] Mathew, C.G.P.,[1] Smith, B.,[1] Marcus, E.,[1] Landsvater, R.,[2] de Meerman, G.J.,[2] Lips, C.J.M.,[3] Geerdink, R.A.,[3] Nakamura, Y.,[4] Buys, C.H.C.M.,[2] [1]Institute of Cancer Research, Sutton, Surrey, England; [2]State University of Groningen, [3]State University of Utrecht, The Netherlands; [4]Howard Hughes Medical Institute, Salt Lake City, Utah: Allele loss in multiple endocrine neoplasia type 2.

Yandell, D.W., McGee, T.L., Campbell, T.A., Dayton, S., Dryja, T.P., Dept. of Ophthalmology, Harvard Medical School and Massachusetts Eye and Ear Infirmary, Boston: Direct genomic sequencing of alleles at the human retinoblastoma susceptibility locus to detect intragenic disease-causing mutations.

Reissmann, P.,[1] Lee, W.H.,[2] Simon, M.,[3] Slamon, D.,[1] [1]Dept. of Medicine, University of California School of Medicine, Los Angeles; [2]University of Chicago, Pritzger School of Medicine, Illinois: Studies of the retinoblastoma gene in human sarcomas.

Dyson, N.,[1] Buchkovich, J.,[1] Whyte, P.,[1] Horowitz, J.M.,[2] Weinberg, R.A.,[2] Harlow, E.,[1] [1]Cold Spring Harbor Laboratory, New York; [2]Whitehead Institute for Biomedical Research, and Massachusetts Institute for Biology, Cambridge: Transforming proteins of several DNA tumor viruses interact with the retinoblastoma gene product.

Kerr, I.B.,[1] Murday, V.A.,[1] Hiorns, L.,[2] Bussey, H.J.R.,[3] Bodmer, W.F.,[1] [1]Director's Laboratory, [2]Dept. of Medical Oncology, Imperial Cancer Research Fund, St. Bartholomew's Hospital, [3]St. Mark's Hospital, London, England: Incidence of Ki-*ras* mutation and chromosome-5 allele loss in a short series of colorectal carcinomas arising in cases of familial adenomatous polyposis.

Law, D.J.,[1] Olschwang, S.,[2] Monpezat, J.-P.,[2] Lefrancois, D.,[3] Jagelman, D.,[4] Petrilli, N.J.,[5] Thomas, G.,[2] Feinberg, A.P.,[1] [1]Howard Hughes Medical Institute and Depts. of Internal Medicine and Human Genetics, University of Michigan Medical School, Ann Arbor; Labortoire de [2]Genetique Moleculaire des Tumeurs, [3]Structure et Mutagenese Chromosomiques, Institut Curie, Paris, France; [4]Dept. of Colorectal Surgery, Cleveland Clinic, Fort Lauderdale, Florida; [5]Surgical Developmental Oncology, Roswell Park Memorial Institute, Buffalo, New York: Multiple nonsyntenic allelic losses in human colorectal carcinomas.

Housman, D.E.,[1] Rose, A.,[1] Jones, C.,[2] Igo, C.[1] Glaser, T.,[1] Csll, K.,[1] [1]Massachusetts Institute for Technology, Cambridge; [2]Eleanore Roosevelt Institute for Cancer Research, Denver, Colorado: Genetic analysis of the WILM tumor region of chromosome 11.

Pierotti, M.A.,[1] Radice, P.,[1] Lacerenza, S.,[1] Mondini, P.,[1] Radice, M.T.,[1] Pilotti, S.,[2] Della Porta, G.,[1] [1]Divisioni di Oncologie Sperimental [2]Anatomia Patologica, Istituto Nazionale Tumor, Milano, Italy: Loss of 11p heterozygosity in human tumors of the urogenital tract.

Rapp, U.R., Sithanandam, G., Beck, T.W., Brennscheidt, U., Minna, J.D., Zbar, B., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Loss of heterozygosity at the c-*raf*-1 locus and analysis of c-*raf* protein in small-cell lung carcinoma.

SESSION 5 CELL-SURFACE RECEPTOR PROTEINS AS CANCER MARKERS: EPIDERMAL FACTOR RECEPTOR AND *NEU/HER*-2 PROTEIN

Chairman: **J. Mendelsohn,** Memorial Sloan-Kettering Cancer Center

Clinical Overview: J. Mendelsohn

Hendler, F., Shump-Sui, A., Nanu, L., Richards, C.S., Ozanne, B., University of Louisville, Kentucky; University of Texas Health Science Center, Dallas: Increased EGF R1 binding predicts a poor survival in squamous tumors.

Harris, A.L., Nicholson, S., Sainsbury, R., Neal, D., Smith, S., Dept. of Clinical Oncology, Newcastle General Hospital, England: EGFR—A marker of early relapse in breast cancer and tumor stage progression in bladder cancer.

Mendelsohn, J., Memorial Sloan-Kettering Cancer Center and Cornell University Medical College, New York, New York: Potential clinical applications of anti-EGFR monoclonal antibodies.

Bruce, J.N., Duigou, G.J., Fisher, P.B., Columbia University College of Physicians & Surgeons, New York, New York: Expression of the EGFR in primary brain tumors.

Slamon, D.J., University of California School of Medicine, Los Angeles: Studies of the potential role of the N-*myc* and *HER-2/neu* proto-oncogenes in specific human malignancies.

van de Vijver, M.J., Peterse, J.L., Mooi, W.J., Wisman, P., Lomans, J., Nusse, R., Division of Molecular Biology and Dept. of Pathology, The Netherlands Cancer Institute, Amsterdam: Overexpression of the *neu* (or c-*erbB*-2 or *HER-2*) protein is very frequent in comedo-type ductal carcinoma in situ, but not of prognostic value in stage II breast cancer.

Gullick, W.J.,[1] Venter, D.J.,[2] Tuzi, N.,[1] Quirke, P.,[3] [1]ICRF, Oncology Group, Hammersmith Hospital, London, [2]Ludwig Institute for Cancer Research, London, [3]Dept. of Pathology, University of Leeds, England: Elevated expression of the c-*erbB*-2 proto-oncogene in human breast and stomach tumors as an indicator of prognosis.

Ali, I.U.,[1] Lideraeau, R.,[2] Callahan, R.,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Center, Rene Hugeunin, St. Cloud, France: Prognostic significance of genetic alterations in human breast neoplasia.

Fourney, R.M.,[1] Dietrich, K.D.,[1] Danyluk, J.,[1] Jamil, N.,[1] Paterson, A.H.G.,[1] Lees, A.W.,[1] Krause, B.,[1] McEwan, A.,[1] Lukka, H.,[1] Hanson, J.,[1] McBlain, W.H.,[2] Willan, B.,[1] Slamon, D.J.,[3] Paterson, M.C.,[1] [1]Cross Cancer Institute, Misericordia Hospital, [2]Dept. of Endocrinology, University of Alberta, Edmonton, Canada; [3]Dept. of Pathology, University of California, Los Angeles: Use of *HER-2/neu* oncogene amplification as a prognostic factor in node-negative breast cancer.

Guérin, M.,[1] Gabillot, M.,[1] Mathieu, M.C.,[2] Travagli, J.P.,[3] Riou, G.,[1] Laboratorie de [1]Pharmacologie Clinique et Moléculaire, [2]d'Anatomie Pathologique, [3]Dépt. de Chirurgie, Institut Gustave Roussy, Villejuif, France: Expression of c-*erbB*-2 and EGFR genes in inflammatory and noninflammatory breast cancers—Association with cancers of poor prognosis.

SESSION 6 POSTER SESSION

Kawasaki, E.S.,[1] Clark, S.S.,[2] Coyne, M.Y.,[1] Smith, S.D.,[3] Champlin, R.,[4] Witte, O.N.,[2] McCormick, F.P.,[1] [1]Dept. of Molecular Biology, Cetus Corporation, Emeryville, [2]Dept. of Microbiology and Molecular Biology Institute, University of California, Los Angeles, [3]Dept. of Pediatrics, Children's Hospital at Stanford, Palo Alto, [4]Dept. of Medicine, Division of Hematology/Oncology, University of California Center for the Health Sciences, Los Angeles: Molecular analysis of leukemias by amplification and detection of leukemia-specific mRNA sequences.

Khokhar, M.T., Section of Human Genetics, Institute of Cancer Research, Belmont, Surrey, England: Detection of the B and T lymphocytes in a patient with B-cell ALL after bone marrow transplantation.

Larsson, C.,[1] Skogseid, B.,[2] Oberg, K.,[3] Nakamura, Y.,[3] Nordenskjöld, M.,[1] [1]Dept. of Clinical Genetics, Karolinsk Hospital, Stockholm, [2]Dept. of Internal Medicine,

[3]Ludwig Institute for Cancer Research, University Hospital, Uppsala, Sweden; [4]Howard Hughes Medical Institute, University of Utah Medical Center, Salt Lake City: Multiple endocrine neoplasia type-1 gene maps to chromosome 11 and is lost in insulinoma.

Leitzel, K.,[1] Bryce, W.,[2] Tomita, J.,[2] Mandarino, G.,[2] Thomason, A.,[3] Tribby, I.,[2] Devare, S.,[2] Billingsley, M.,[1] Harvey, H.,[1] Bartholomew, M.,[1] Lipton, A.,[1] [1]M.S. Hershey Medical Center, Pennsylvania; [2]Abbott Laboratories, Inc., North Chicago, Illinois; [3]Amgen, Thousand Oaks, California: Elevated plasma PDGF levels in cancer patients.

Marino, P.A., Gottesman, M.M., Pastan, I., NCI, National Institutes of Health, Bethesda, Maryland: Regulation of the multidrug resistance gene (*MDR1*) in regenerating rat liver.

Matsushime, H., Shibuya, M., Dept. of Genetics, Institute of Medical Science, University of Tokyo, Japan: Examination of tissue-specific expression and cDNA cloning of rat c-*ros*-1.

Moser, A.R., Dove, W.F., Pitot, H.C., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: *ACE*—A dominant mutation predisposing to spontaneous intestinal adenocarcinogenesis in the mouse.

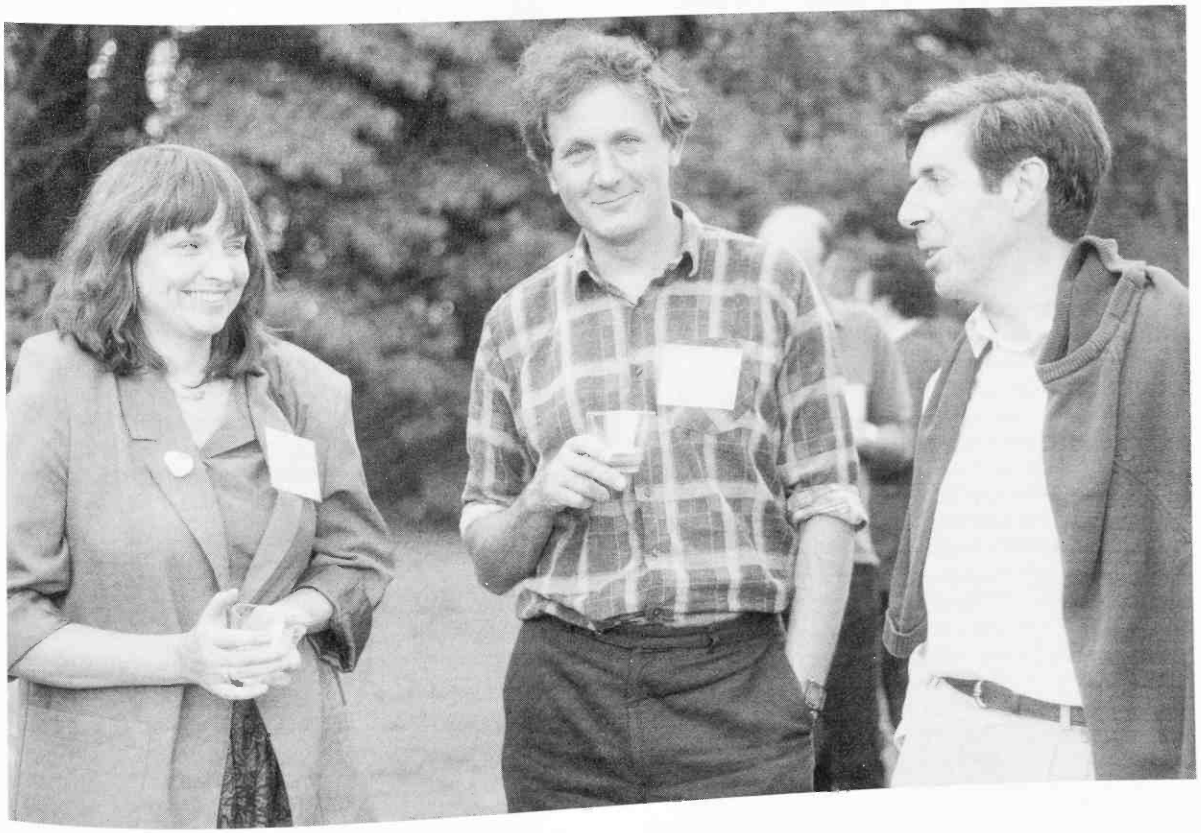
Murday, V.A.,[1] Bussey, H.J.R.,[2] Levitt, S.,[4] Jones, T.,[3] Sheer, D.,[3] Bodmer, W.F.,[1] Slack, J.,[5] [1]Director's Laboratory, [2]St. Marks' Hospital, [3]Human cytogenetics, Imperial Cancer Research Fund, London, England; [4]Cancer Foundation of Western Australia, Perth; [5]Royal Free Medical School, London, England: Clinical application of linked RFLPs in the diagnosis and management of patients with familial adenomatous polyposis.

Nagao, M., Sakai, R., Ochiai, M., Ishikawa, F., Ikeda, I., Sugimura, T., Carcinogenesis Division, National Cancer Center Research Institute, Tokyo, Japan: Identification of a transforming-activity-suppressing sequence in the c-*raf* oncogene.

Neri, A.,[1] Baldini, L.,[3] Ferrero, D.,[4] Knowles, D.M.,[2] McCormick, F.,[5] Dalla-Favera, R.,[1] [1]Dept. of Pathology, New York University Medical Center, [2]Dept. of Pathology, Columbia University College of Physicians & Surgeons, New York, New York; [3]Istituto di Scienze Mediche, University of Milan, [4]Cattedra di Ematologie, University of Turin, Italy; [5]Cetus Corporation, Emeryville, California: Analysis of *ras* oncogene mutation in lymphoid neoplasms—Differences among tumor subtypes.

Nistér, M.,[1] Claesson-Welsh, L.,[2] Hammacher, A.,[2] Heldin, C.-H.,[2] Westermark, B.,[1] [1]Dept. of Pathology, University Hospital, [2]Ludwig Institute for Cancer Research, Biomedical Center, Uppsala, Sweden: Characterization of the PDGF-A-type receptor on human clonal glioma cells.

Obata, Y.,[1] Takahashi, T.,[2] Hida, T.,[1] Ueda, R.,[1] Watanabe, H.,[1] Ariyoshi, Y.,[1] Sugiura, T.,[1] Takahashi, T.,[1] [1]Aichi Cancer Center, [2]Nagoya University School of Medicine, Japan: Expression and amplification of *myc* gene family in small-cell lung cancer and its relation to biological characteristics.



L. Wiedemann, B.A.J. Ponder, B. Young

Paterlini, P.,[1,3] Garreau, F.,[1] Zarski, J.P.,[1] Cariani, E.,[1] Lasserre, C.,[1] Franco, D.,[2] Pisi, E.,[3] Brechot, C.,[1] [1]INSERM, Necker, Paris, [2]Hopital Louise-Michel, [3]Istituto di Clinca Medica, Bologna, Italy: Loss of heterozygosity at chromosome-11p loci in human adult primary liver cancers and benign liver tumors.

Peiper, S.C.,[1] Ashmun, R.,[1] Downing, J.,[1] Lemmons, R.,[2] Look, A.T.,[1] [1]Dept. of Tumor Cell Biology, St. Jude Hospital, Memphis, Tennessee; [2]Dept. of Pediatrics, University of Utah, Salt Lake City: Molecular cloning and chromosomal assignment of gene encoding CD33 myeloid antigen.

Reeve, A.E.,[1] Sih, S.A.,[2] Raizis, A.M.,[1] Feinberg, A.P.,[2] [1]Molecular Carcinogenesis Laboratory, Dept. of Biochemistry, University of Otago, Dunedin, New Zealand; [2]Howard Hughes Medical Institute, Depts. of Internal Medicine and Human Genetics, University of Michigan Medical School, Ann Arbor: Loss of chromosome-11 alleles in sporadic Wilms' tumor does not involve chromosome band 11p13.

Reisman, D., Greenberg, M., Rotter, V., Dept. of Cell Biology, Weizmann Institute of Science, Rehovot, Israel: The human p53 gene contains two promoters, one of which maps to intron 1 and whose expression is induced during differentiation of HL-60 cells.

Richter, H.,[1,2] Krolewski, J.J.,[1] Watkins, P.,[4] Knowles, D.M.,[3] Dalla- Favera, R.,[1] Depts. of [1]Pathology, [2]Medicine, New York University Medical Center, [3]Dept. of Pathology, Columbia University College of Physicians & Surgeons, New York, New York; [4]Integrated Genetics, Framingham, Massachusetts: Analysis of oncogene expression in lymphoid malignancies using a novel multigene expression assay.

Riou, G.,[1] Barrois, M.,[1] Sheng, Z.M.,[1] Duvillard, P.,[2] Lhomme, C.,[3] [1]Laboratoire de Pharmacologie Clinique et Moléculaire, [2]Service d'Histopathologie, [3]Service de Gynécologie, Institut Gustave Roussy, Villejuif, France: Somatic deletions and mutations of c-Ha-*ras* gene in human cervical cancers.

Ron, D.,[1] Graziani, G.,[1] Srivastava, S.,[2] Aaronson, S.A.,[1] Eva, A.,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]V. Lombardi Cancer Center, Georgetown University Medical School, Washington, D.C.: Amino-terminal truncation of the human *dbl* proto-oncogene product significantly enhances its transforming activity.

Sakamoto, H.,[1] Odagiri, H.,[1] Hattori, Y.,[1] Miyagawa, K.,[1] Yoshida, T.,[1] Nakatani, H.,[2] Sugimura, T.,[1] Terada, M.,[1] [1]Genetics Division, National Cancer Center Research Institute, Tokyo, [2]Dept. of Pathology, Hiroshima University School of Medicine, Japan: Amplified gene from stomach cancer, *sam,* belongs to one of the tyrosine kinase receptor genes.

Schuitemaker, H.,[1] Kuppen, P.,[1] Van 't Veer, L.,[1] Den Englese, L.,[2] Schrier, P.I.,[1] [1]Dept. of Clinical Onocology, University Hospital, Leiden, [2]The Netherlands Cancer Institute, Amsterdam: Mechanism of resistance of a human ovarian cancer cell line against cisplatin.

Schwartz, H.S.,[1] Jenkins, R.B.,[2] Moses, H.L.,[1] [1]Vanderbilt University, Nashville, Tennessee; [2]Mayo CLinic, Rochester, Minnesota: Telomeric translocations and growth factor analysis in giant-cell tumor of bone.

Selleri, L.,[1] von Lindern, M.,[1] Hermans, A.,[1] Meijer, D.,[1] Torelli, G.,[2] Grosveld, G.,[1] [1]Dept. of Cell Biology and Genetics, Erasmus University, Rotterdam, The Netherlands; [2]Institute of Internal Medicine and Hematology, University of Modena, Italy: Deletion of all or part of the *bcr* central sequences from the chimeric BCR-ABL mRNA still results in Ph-positive CML.

Senger, D.R.,[1] Perruzzi, C.A.,[1] Gracey, C.F.,[1] Papadopoulos, A.,[1] Tenen, D.G.,[2] Depts. of [1]Pathology, [2]Medicine, Beth Israel Hospital and Harvard Medical School and Charles A. Dana Research Institute, Boston, Massachusetts: Secreted phosphoproteins associated with neoplastic transformation in human cells.

Seth, A., Watson, D., Blair, D., Papas, T., NCI-Frederick Cancer Research Facility, Frederick, Maryland: The c-*ets*-2 proto-oncogene has transforming activity when overexpressed in NIH-3T3 cells.

Solin, T., Henttu, P., Vihko, P., Biocenter and Dept. of Clinical Chemistry, University of Oulu, Finland: Hybridization analysis of prostate mRNAs using cDNAs for human prostatic acid phosphatase and prostate-specific antigen as probes.

Stapleton, P., Takayama, Y., Rowe, P.B., Symonds, G., Children's Medical Research Foundation, Camperdown, Australia: c-*raf* activation as a secondary event in tumor progression.

Stern, R.,[1] Dollbaum, C.,[2] Decker, M.,[1] Longaker, M.,[3] Depts. of [1]Pathology, [2]Medicine, [3]Surgery, University of California School of Medicine, San Francisco: A glycoprotein present in fetal and breast cancer patient sera stimulates synthesis of hyaluronic acid.

Stern, R.,[1] Smith, H.S.,[2] [1]Dept. of Pathology, University of California, San Francisco, [2]Peralta Cancer Research Institute, Oakland: Hyaluronic acid accumulation in response to growth factors distinguishes normal fibroblasts from tumor-derived fibroblasts.

Strebhardt, K., Hradetzky, D., Holtrich, U., Rübsamen-Waigmann, H., Chemotherapeutisches Forschungsinstitut, Georg-Speyer-Haus, Frankfurt, Federal Republic of Germany: A member of the *src* family, c-*tkl*, is expressed in human T lymphocytes.

Takeya, T., Kato, J., Sato, M., Institute for Chemical Research, Kyoto University, Japan: Novel activated form of $pp60^{c\text{-}src}$.

Tamm, J., Derynck, R., Dept. of Developmental Biology, Genentech, Inc., South San Francisco, California: Sequences responsible for maintaining TGF-β in a latent (inactive) form.

Tazartes, O., Muraca, R., Sismondi, P., Giai, M., Bottero, A., Dati, C., De Bortoli, M., Saglio, G., Sezione di Biochimica, Dip. d Medicine e Oncologie Sperimentale, Biologia Animale, Scienze Biomediche e Oncologie Umana e Cattedra, Ginecologie e Ostetricia, dell'Universita' di Torino, Italy: c-*erbB*-2 amplification in human breast carcinomas.

Tenhunen, J., Syvänen, A.-C., Laaksonen, M., Eloranta, J., Söderlund, H., Orion Corporation Ltd., Orion Genetic Engineering Laboratory, Helsinki, Finland: Detection of N-*myc* oncogene mRNA level in tumor cell line by affinity-based hybrid collection.

Teyssier, J.R.,[1] Couillin, P.,[2] Benard, J.,[3] [1]INSERM, Reims, [2]INSERM, Paris, [3]Institut Gustave Roussy, Villejuif, France: Loss of the 11p13-encoded surface antigen MIC4 in vincristine-resistant human carcinoma cells with an acquired del(11)(p13) and a reversed nontumorigenic *mdr* phenotype.

Thomas, H.G., Bordoni, R., Richmond, A., Dept. of Medicine, Veterans Administration Medical Center and Emory University, Atlanta, Georgia: Characterization of the cellular response to melanoma growth stimulatory activity.

Trimpe, K.L.,[1] Dombkowski, D.M.,[1] Hamer, P.J.,[1] McKenzie, S.,[2] Rabin, H.,[1] Carney, W.P.,[1] [1]Medical Products Dept., E.I. du Pont de Nemours and Company, Inc., North Billerica, [2]Applied BioTechnology, Cambridge, Massachusetts: Flow cytometric analysis of c-*erbB*-2 and *ras* oncogene products in human breast carcinoma cells.

van de Vijver, M.J., Peterse, J.L., Mooi, W.J., Lomans, J., Wisman, P., van de Bersselaar, R., Bos, J.L., Nusse, R., Division of Molecular Biology and Dept. of Pathology, The Netherlands Cancer Institute, Amsterdam: Oncogene activation in human breast cancer.

Versteeg, R., Peltenburg, L., Plomp, A., Krüse, M., Schrier, P.I., Dept. of Clinical Oncology, University Hospital, Leiden, The Netherlands: c-*myc* regulates class I HLA expression in human melanoma—A message to the immune system?

Wang, W.-P.,[1] Lehtoma, K.,[2] Varban, M.L.,[2] Sandberg, P.,[3] Bunnag, P.,[2] Chiu, I.-M.,[1,2,3] [1]Dept. of Molecular Genetics, [2]Comprehensive Cancer Center, [3]Dept. of Internal Medicine, Ohio State University, Columbus: Cloning and expression of cDNA and genomic DNA coding for human heparin-binding growth factor-1.

Watson, D.K., Pribyl, L.J., Papas, T.S., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Molecular comparison of *ets* and *ets*-related genes.

Weston, A.,[1] Willey, J.C.,[1] Modali, R.,[1] Sugimura, H.,[1] Light, B.,[1] Haugen, A.,[1] Resau, J.,[2] McDowell, E.,[2] Trump, B.F.,[2] Mann, D.L.,[1] Harris, C.C.,[1] [1]NCI, National Institutes of Health, Bethesda, [2]Dept. of Pathology, University of Maryland, Baltimore: Loss of heterozygosity in human non-small-cell bronchogenic carcinoma.

Williams, M.E., Lee, J.T., Innes, D.J., Depts. of Internal Medicine and Pathology and Diagnostic Molecular Genetics Laboratory, University of Virginia Medical Center, Charlottesville: Sequential rearrangement of *bcl-2* and c-*myc* proto-oncogenes in tumor DNA from a patient with non-Hodgkin's lymphoma.

Yin, S.,[1] Carney, W.,[2] Lam, T.,[1] Marks, P.,[1] McKenzie, S.,[1] Mobtaker, H.,[1] Panicali, D.,[1] Zolnay, S.,[1] [1]Applied bioTechnology, Cambridge, [2]E.I. du Pont de Nemours and Company, North Billerica, Massachusetts: Generation of nucleic acid probes and antibodies specific to the human *neu* oncogene and its products.

Yokoyama, K., Gachelin, G., Tsukuba Life Science Center, RIKEN, Japan: Antisense-RNA-induced gene suppression of endogenous *myc* proto-oncogene expression—Its application to molecular diagnostics.

Young, B.D.,[1] Cotter, F.,[1] Tuszynski, A.,[1] Zucca, E.,[2] Lister, T.A.,[1] [1]Imperial Cancer Research Fund, London, England; [2]Oncologia Medica, Ospedal San Giovanni, Bellinzona, Switzerland: Detection and quantitation of the translocation t(14;18) by enzymatic amplification.

Yuasa, Y., Tokyo Medical and Dental University School of Medicine, Japan: Transforming genes in familial polyposis coli patient's cells detected by a tumorigenicity assay.

Yunis, J.,[1] Bos, J.,[2] [1]Dept. of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis; [2]Laboratory for Molecular Carcinogenesis, Sylvius Laboratoreis, Leiden, The Netherlands: Preponderance of N-*ras* mutation in myelodysplastic syndrome with monocytic features and poor prognosis.

Zehnbauer, B., Griffin, C., Burns, W., Santos, G., Johns Hopkins Oncology Center, Baltimore, Maryland: Recombinant DNA analyses in allogeneic bone marrow transplantation.

SESSION 7 GROWTH FACTORS EXPRESSED BY CANCER CELLS AS TUMOR MARKERS

Chairman: **B. Ozanne,** University of Texas Health Science Center, Dallas

Fantl, V.,[1] Brookes, S.,[2] Smith, R.,[1] Casey, G.,[2,4] Barnes, D.,[3] Dickson, C.,[1] Peters, G.,[2] [1]Viral Carcinogenesis Laboratory, [2]Molecular Oncology Laboratory, [3]Clinical Oncology Unit, Imperial Cancer Research Fund, London, England; [4]Dept. of Microbiology and Molecular Genetics, University of California, Irvine: Characterization of the proto-oncogene *int-2* and its potential for the diagnosis of human breast cancers.

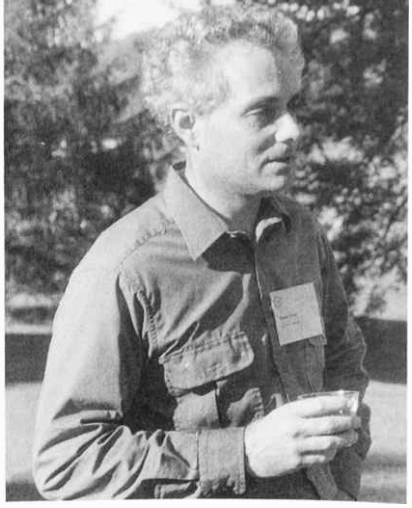
Derynck, R.,[1] Lindquist, P.B.,[1] Brachmann, R.,[1] Bringman, T.S.,[1] Wilcox, J.N.,[1] Pittelkow, M.,[2] Elder, J.T.,[3] Voorhees, J.J.,[3] Moses, H.L.,[4] Coffey, R.J.,[4] [1]Genentech, Inc., South San Francisco, California; [2]Mayo Clinic/ Foundation, Rochester, Minnesota; [3]University of Michigan, Ann Arbor; [4]Vanderbilt University School of Medicine, Nashville, Tennessee; Endogenous expression of TGF-α.

Terada, M., Miyagawa, K., Yoshida, T., Sakamoto, H., Odagiri, H., Sugimura, T., Genetics Division, National Cancer Center Research Institute, Tokyo, Japan: Transforming growth factor gene, *hst-1*.

Theillet, C.,[1] Le Roy, X.,[2] De Lapeyrière, O.,[3] Grosgeorges, J.,[4] Adnane, J.,[4] Raynaud, S.D.,[4] Simoni-Lafontaine, J.,[1] Goldfarb, M.,[5] Escot, C.,[2] Birnbaum, D.,[3] Gaudray, P.,[4] [1]CNRS, Centre Paul Lamargue, [2]INSERM, Montpellier, [3]INSERM, Marseille, [4]LGMCH, Nice, France; [5]Columbia University College of Physicians & Surgeons, New York, New York: Amplification of FGF-related genes in human tumors.

Stewart, A.F., Burtis, W.J., Mangin, M., Ikeda, K.,

B. Ozanne, G. Peters, A. Harris



T. Broker

Insogna, K.L., Broadus, A.E., West Haven Veterans Administration Medical Center and Yale University School of Medicine, New Haven, Connecticut: Purification and molecular cloning of a novel parathyroid hormone-like protein responsible for humoral hypercalcemia of malignancy.

Aaronson, S., NCI, National Institutes of Health, Bethesda, Maryland: Overexpression of growth factors or their receptors in human malignancies.

Willkins, R.J.,[1] Molenaar, A.J.,[1] Ohlsson, R.,[3] Reeve, A.E.,[1] Yun, K.,[2] Becroft, D.M.O.,[4] Depts. of [1]Biochemistry, [2]Pathology, University of Otago Medical School, Dunedin, New Zealand; [3]Centrum for Bioteknik, Karolinska Institutet, Huddinge, Sweden; [3]Princess Mary Laboratory, Auckland Hospital, New Zealand: Wilms' tumorigenesis, insulin-like growth factor II gene expression and blocked differentiation.

Ashmun, R.A.,[1,2] Look, A.T.,[1,2] Roussel, M.F.,[1] Roberts, W.M.,[2] Ohtsuka, M.,[1] Sherr, C.J.,[1] Depts. of [1]Tumor Cell Biology, [2]Hematology/Oncology, St. Jude Children's Research Hospital, Memphis, Tennessee: Monoclonal antibodies to the human c-*fms* gene product (CSF-1 receptor) detect cell-surface receptors on human myeloid leukemic blasts.

Kacinski, B.M.,[1] Bloodgood, R.S.,[1] Carter, D.,[1] Yang-Feng, T.,[1] Yee, L.D.,[1,5] Wang, K.-I.,[1] Brown, E.L.,[2] Wong, G.G.,[2] Clark, S.C.,[2] Alderman, E.M.,[2] Stanley, E.R.,[3] Eng, M.,[1] Donahue, J.,[1] Foellmer, H.,[1] Oemar, B.,[3] Ariza, A.,[1] Gerald, W.,[1] Jones, M.,[1] Schwartz, P.E.,[1] Chambers, J.T.,[1] Chambers, S.K.,[1] Kohorn, E.I.,[1] Rohrschneider, L.R.,[4] Rothwell, V.,[4] [1]Yale University School of Medicine, New Haven, Connecticut; [2]Genetics Institute, Cambridge, Massachusetts; [3]Albert Einstein College of Medicine, Bronx, New York; [4]Fred Hutchinson Cancer Research Center, Seattle, Washington; [5]Oregon Health Sciences University, Portland: M-CSF (CSF-1), its receptor, the *FMS* protein, and other lymphohematopoietic factors and receptors involved in macrophage activation (IL-3, G-IFN, GM-CSF) play important roles in producing the proliferative and invasive characteristics of human ovarian, endometrial, and other adenocarcinomas in vivo and in vitro.

SESSION 8 ACTIVATED ONCOGENE PROTEINS AS CANCER MARKERS: *RAS, SCR,* AND *RAF*

Chairman: M. Furth, Regeneron

Clinical Overview: N. Rosen, National Cancer Institute

Perucho, M.,[1] Forrester, K.,[1] Almoguera, C.,[1] Kahn, S.,[1] Lama, C.,[1] Shibata, D.,[2] Arnheim, N.,[3] Grizzle, W.E.,[4] [1]Dept. of Biochemistry, State University of New York, Stony Brook; [2]Dept. of Pathology, University of Southern California Medical Center, [3]Dept. of Biological Sciences, University of Southern California, Los Angeles; [4]Dept. of Pathology, University of Alabama, Birmingham: c-Ki-*ras* mutational activation in human carcinomas.

Carter, G., Hughes, D., Clark, R., Jacobs, A., Padua, R.A., Dept. of Hematology, University of Wales College of Medicine, Cardiff, Scotland: *RAS* mutations detected by PCR and specific oligonucleotide hybridization in preleukemia and in remission samples following cytotoxic treatment for lymphoma.

Kumar, R., Barbacid, M., NCI-Frederick Cancer Research Facility, Frederick, Maryland: A diagnostic procedure for the rapid detection of *ras* oncogene mutations at the single-cell level.

Rabin, H.,[1] Carney, W.,[1] Trimpe, K.,[1] Pullano, T.,[1] Panicali, D.,[2] [1]Du Pont Medical Products Dept., North Billerica, [2]Applied Biotechnology, Inc., Cambridge, Massachusetts: Expression of *ras* and *neu* oncogene proteins as determined by monoclonal antibodies.

Rosen, N., Ross, F., Sartor, O., Bostick, F., Veillette, A., Bolen, J., NCI, National Institutes of Health, Bethesda, Maryland: Altered expression of c-*src*-related proto-oncogenes in human colon cancer.

Frackelton, A.R., Jr., Huhn, R.D., Dept. of Medicine, Roger Williams General Hospital and Brown University, Providence, Rhode Island: Phosphotyrosyl proteins isolated from cell lines and peripheral blood leukocytes derived from individuals with CML.

Pfeifer, A.M.A.,[1] Kasid, U.,[4] Tsokos, M.G.,[2] Kessler, D.K.,[3] Weischselbaum, R.R.,[5] Thorgeirsson, S.S.,[3] Dritschilo, A.,[4] Mark, G.E.,[6] Laboratories of [1]Human Carcinogenesis, [2]Pathology, [3]Experimental Carcinogenesis, NCI, National Institutes of Health, Bethesda, Maryland; [4]Dept. of Radiology Medicine, Georgetown University Medical Center, Washington, D.C.; [5]Dept. of Radiology Oncology, University of Chicago, Illinois; [6]Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey: Implication of the c-*raf*-1 proto-oncogene in neoplastic transformation in vivo and in vitro.

SESSION 9 CHROMOSOMAL REARRANGEMENTS AS CANCER MARKERS II. SOLID TUMORS: MULTIDRUG RESISTANCE

Chairman: **R.S.K. Chaganti,** Memorial Sloan-Kettering Cancer Center

Clinical Overview: *W.M. Linehan,* National Cancer Institute

Linehan, W.M.,[1] Anglard, P.,[1] Brauch, H.,[2] Robertson, C.,[1] Sargent, E.,[1] Gomella, L.,[1] Wade, T.,[1] Tory, K.,[2] Lerman, M.,[2] Kasid, A.,[1] Zbar, B.,[2] [1]NCI-Frederick Cancer Research Facility, Frederick, Maryland: Human renal cell carcinoma—Genetic changes important in tumor origin and tumor progression and characterization of growth factor expression.

Chandrasekharappa, S.C., Westbrook, C.A., LeBeau, M.M., University of Chicago Medical School, Illinois: Molecular analysis of the DEL (5Q) myeloid leukemias.

Drabkin, H.,[1,2] Smith, D.,[3] Jones, C.,[2] Gemmill, R.,[4] [1]University of Colorado Medical Center, [2]Eleanor Roosevelt Institute for Cancer Research, Denver, Colorado; [3]Wayne State University School of Medicine, Detroit, Michigan; [4]Southwest Biomedical Research Institute, Scottsdale, Arizona: Toward a physical map of human chromosome 3 and molecular analysis of disease-related rearrangements.

Sharma, S.,[1] Birchmeier, C.,[1] Bolger, G.,[1] Rabin, M.,[2] Rodgers, L.,[1] O'Neill, K.,[1] Riggs, M.,[1] Wigler, M.,[1] [1]Cold Spring Harbor Laboratory, New York; [2]Dept. of Pediatrics, University of Miami Medical School, Florida; *ROS1* gene products in human glioblastoma cell lines—Analysis of a potentially activating rearrangement in *ROS1*.

Reeves, B.R.,[1] Smith, S.,[1] Fisher, C.,[2] Westbury, C.,[2] Gusterson, B.A.,[1] Warren, W.A.,[1] Martin, C.A.,[1] Knight, J.,[1] Chan, A.M.-L.,[1] Cooper, C.S.,[1] [1]Institute of Cancer Research, [2]Royal Marsden Hospital, London, England: Characterization of the X;18 translocation present in human synovial sarcomas.

Gottesman, M.M., Goldstein, L.J., Willingham, M.C., Pastan, I., NCI, National Institutes of Health, Bethesda, Maryland: Molecular biology of a human multidrug transporter.

Roninson, I.B.,[1] Patel, M.C.,[1] Noonan, K.E.,[1] Chen, C.-J.,[1] Choi, K.,[1] Chin, J.E.,[1] Kaplan, R.,[1] Soffie, R.,[1] Lee, I.,[1] Coon, J.S.,[2] [1]Dept. of Genetics, University of Illinois College of Medicine, [2]Dept. of Pathology, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois: Molecular mechanism and diagnostics of multidrug resistance in human tumor cells.

Cordon-Cardo, C., O'Brien, J.P., Casals, D., Bertino, J.R., Memorial Sloan-Kettering Cancer Center, New York: Immunoanatomic and immuno-pathologic expression of multidrug resistance gene product.

SESSION 10 DNA TUMOR VIRUS ONCOGENES AS CANCER MARKERS

Chairman: **D. Lowy,** National Cancer Institute

Clinical Overview: *D. Lowy*

Aurelian, L.,[1] Terzano, P.,[2] Smith, C.C.,[1] Chung, T.,[1] Shamsuddin, A.,[1] Costa, S.,[2] Orlandi, C.,[2] [1]University of Maryland, Baltimore; [2]University of Bologna, Italy: Amino-terminal epitope of HSV-2 ICP10 protein as a molecular diagnostic marker for cervical intraepithelial neoplasia.

Guiot, M.-C.P.,[1] Cavenee, W.K.,[1] Banks, L.,[2] Crawford, L.,[2] Arseneau, J.,[3] Matlashewski, G.,[3] [1]Ludwig Institute for Cancer Research, Montreal, Canada; [2]Imperial Cancer Research Fund, London, England; [3]McGill University, Montreal, Canada: Detection of HPV-16 early proteins in premalignant cervical leasions using monoclonal antibodies.

Broker, T.R.,[1] Stoler, M.H.,[1] Whitbeck, A.,[1] Rhodes, C.,[1] Wolinsky, S.M.,[2] Chow, L.T.,[1] [1]Dept. of Pathology and Biochemistry, University of Rochester School of Medicine, New York; [2]Infectious Disease Unit, Northwestern University Medical School, Chicago, Illinois: In situ analyses of gene expression in preinvasive and invasive cervical neoplasia.

Manos, M.,[1] Ting, Y.,[1] Lewis, A.,[1] Wolinsky, S.,[2] Broker, T.,[3] Wright, D.,[1] [1]Dept. of Molecular Biology, Cetus Corporation, Emeryville, California; [2]Dept. of Medicine, Northwestern Medical School, Chicago, Illinois; [3]Dept. of Biochemistry, University of Rochester, New York: Detection and typing of genital HPVs using the PCR.

Ferre, R., Garduno, F., Peter, J.B., Cytometrics Inc., Division of Speciality Laboratories, Inc., San Diego, California: Detection of HPV types 6/11, 16, and 18 using the PCR.

Modern Approaches to New Vaccines Including Prevention of AIDS

September 14—September 18

ARRANGED BY

Robert Chanock, National Institutes of Health
Harold S. Ginsberg, Columbia University
Richard A. Lerner, Research Institute of Scripps Clinic
Fred Brown, Wellcome Biotechnology Ltd.

320 participants

The annual meeting on Modern Approaches to New Vaccines, held in September 1988, maintained the level of excellence established during the preceding five conferences. Attendance was 15% greater than that of the previous year. There was also a significant increase in the number of high-quality abstracts. As a consequence, we arranged for additional papers to be presented at the meeting by scheduling additional 5-minute talks. Nevertheless, enthusiasm remained high and audience participation during the discussion periods was both spirited and extensive.

The program included sessions on Immunology, Parasitology, and Bacteriology, three sessions on Virology, and three sessions on AIDS. More than 70 posters were also on display at the Poster session.

This meeting was supported in part by the Rockefeller Foundation.

SESSION 1 IMMUNOLOGY

Chairman: **R. Lerner,** Research Institute of Scripps Clinic

Janda, K.D.,[1] Schloeder, D.,[1] Benkovic, S.,[2] Lerner, R.A.,[1] [1]Dept. of Molecular Biology, Research Institute of Scripps Clinic; [2]Dept. of Chemistry, Pennsylvania State University, University Park: Induction of an antibody that catalyzes hydrolysis of an amide bond.

Hogle, J., Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California: Structural basis for serotype specificity of polioviruses.

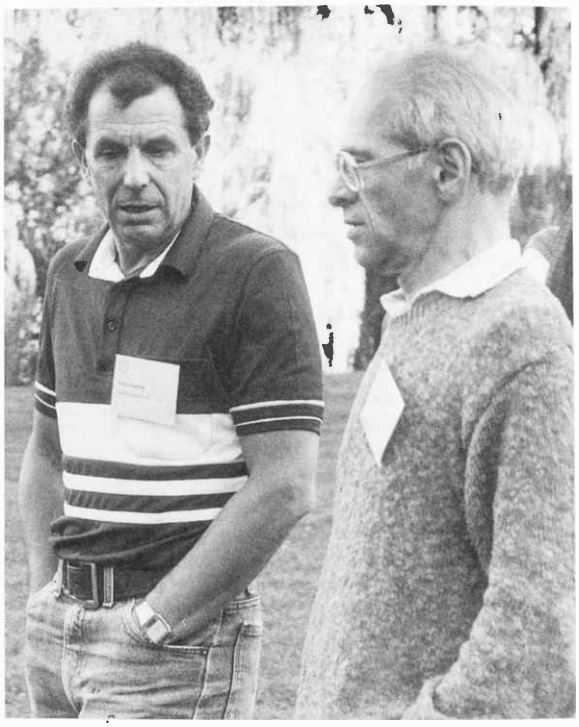
Berzofsky, J.A., NCI, National Institutes of Health, Bethesda, Maryland: Immunodominance of T-cell epitopes—Applications to vaccine design.

Satterthwait, A., Lerner, R.A., Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California: Structure and immunogenicity of conformationally restricted peptides.

Tam, J., Rockefeller University, New York, New York: Multiple antigen peptide system as a novel design for peptide-based vaccines and immunoasseys.

Heath, A.W., Haque, N.A., de Souza, J.B., Playfair, J.H.L., Dept. of Immunology, UCMSM, London, England: Interferon-γ as an effective immunological adjuvant.

Milich, D.,[1] Hughes, J.,[1] Jones, J.,[1] McLachlan, A.,[1] Stahl, S.,[3] Wingfield, P.,[3] Thornton, B.,[2] [1]Scripps Clinic and Research Foundation, [2]Biotechnology Center, Inc., La Jolla, California; [3]Glaxo Institute for Molecular Biology, Geneva, Switzerland: Characterization of the



D. Rowlands, A.R. Neurath

HBV nucleocapsid (HBcAg) as an immunologic carrier moiety.

Stuart, D., Rowlands, D., Fox, G., Fry, E., Acharya, R., Brown, F., Oxford University and Wellcome Biotechnology Ltd., Beckenham, Kent, England: Three-dimensional structure of FMDV.

van Eden, W.,[1] van der Zee, R.,[2] Meloen, R.H.,[3] Noordzij, A.,[1] van Embden, J.D.A.,[2] Hensen, E.J.,[1] [1]Dept. of Infectious Diseases and Immunology, University of Utrecht; [2]Laboratory of Bacteriology, National Institute of Public Health and Environmental Hygiene, Bilthoven, [3]Central Veterinary Institute, Lelystad, The Netherlands: A modified "pepscan" method for the rapid identification and characterization of T-cell epitopes in protein antigens.

SESSION 2 AIDS. I

Chairman: **H.S. Ginsberg,** Columbia University

Siliciano, R.,[1] Berman, P.,[2] Gregory, T.,[2] Reinherz, E.,[3] [1]Johns Hopkins University School of Medicine, Baltimore, Maryland; [2]Genentech, Inc., South San Francisco, California; [3]Harvard Medical School, Boston, Massachusetts: Analysis of host-HIV interactions in AIDS with anti-gp120 human T-cell clones—Effect on HIV genomic heterogeneity and a mechanism for cell depletion.

Lee, T.-H., Suy, W.-J., Chou, M.-J., Huang, J.-J., Essex, M., Dept. of Cancer Biology, Harvard University School of Public Health, Boston, Massachusetts: Immunodominant domain of HIV-1 gp120—A possible obstacle for vaccine development.

Lamarre, D.,[1] Capon, D.,[2] Sekaly, R.P.,[1] [1]Molecular Immunology, Clinical Research Institute of Montreal, Canada; [2]Genentech, San Francisco, California: Recombinant gp120 will inhibit the functional interaction between CD4 and human MHC class II antigens.

Sadaie, M.R.,[1] Cullen, B.R.,[2] Wong-Staal, F.,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Howard Hughes Medical Institute and Dept. of Medicine, Duke University Medical Center, Durham, North Carolina: HIV-1 Rev protein displays a *trans*-repressor role that inhibits virus replication—Implications for viral latency.

Ahmad, N., Venkatesan, S., NIAID, National Institutes of Health, Bethesda, Maryland: Functional interactions of HIV-1 *trans*-regulatory proteins Rev and Nef.

Partin, K., Kräusslich, H., Bradley, J., Handler, C., Wimmer, E., Carter, C., Dept. of Microbiology, State University of New York, Stony Brook: Substrate determinants of the HIV proteinase.

Burger, H.,[1] Eilbott, D.,[1] Peress, N.,[1] La Neve, D.,[1] Orenstein, J.,[2] Gendelman, H.,[3] Seidman, R.,[1] Weiser, B.,[1] [1]State University of New York, Stony Brook; [2]George Washington University, [3]Walter Reed Army Institute of Research, Washington, D.C.: HIV expression and replication in macrophages in the spinal cords of AIDS patients with myelopathy.

Maury, W., Potts, B., Rabson, A.B., NCI, National Institutes of Health, Bethesda, Maryland: Infection of human placental tissue by HIV-1.

McPhee, D.A.,[1] Kemp, B.E.,[2] Stapleton, D.I.,[2] Cumming, S.A.,[1] Pavuk, N.C.,[1] Doherty, R.R.,[1] [1]McFarlane Burnet Center for Medical Research, Fairfield, [2]St. Vincent's Institute for Medical Research, Fitzroy, Victoria, Australia: Putative contact prototopes for HIV-1 envelope proteins gp120/gp41—Antiviral action of synthetic peptide analogs.

Chanda, P.K., Bhat, B.M., Mason, B.B., Morin, J.E., Molnar-Kimber, K.L., Natuk, R.J., Dheer, S.K., Mizutani, S., Lubeck, M.D., Davis, R.A., Hung, P.P., Biotechnology and Microbiology Division, Wyeth-Ayerst Research, Philadelphia, Pennsylvania: Expression of HIV envelope glycoproteins by a nondefective adenovirus vector.

Arenzana-Seisdedos, F.,[1] Israël, N.,[1] Bachelerie, F.,[1] Hazan, U.,[1] Dautry, F.,[2] Virelizier, J.L.,[1] [1]Laboratoire d'Immunologie Virale, Institut Pasteur, Paris, [2]Laboratoire d'Oncologie Moleculaire, Institut Gustave Roussy, Villejuif, France: *trans*-Activation of the HIV-LTR by cotransfection of an Ha-*ras* expression vector in human cells.

SESSION 3 VIROLOGY. I

Chairman: **R. Chanock,** NIAID, National Institutes of Health

Heinz, F.X., Mandl, C., Guirakhoo, F., Holzmann, H., Kunz, C., Institute of Virology, University of Vienna, Austria: A structural and antigenic model of the tick-borne encephalitis virus envelope protein E.

Lai, C.-J.,[1] Zhang, Y.-M.,[1] Falgout, B.,[1] Bray, M.,[1] Chanock, R.,[1] Eckels, K.H.,[2] [1]NIAID, National Institutes of Health, Bethesda, Maryland; [2]Walter Reed Army Institute of Research, Washington, D.C.: Use of dengue virus structural proteins and nonstructural protein NS1 produced by recombinant baculovirus for immunization against dengue virus infection.

Bray, M., Falgout, B., Zhao, B., Chanock, R., Lai, C.-J., NIAID, National Institutes of Health, Bethesda, Maryland: Mice immunized with recombinant vaccinia virus expressing dengue structural proteins and/or nonstructural protein NS1 are protected against fatal dengue encephalitis.

Rothman, A.,[1] Kurane, I.,[1] Zhang, Y.-M.,[2] Lai, C.J.,[2] Ennis, F.A.,[1] Dept. of Medicine, University of Massachusetts Medical Center, Worcester; [2]NIAID, National Institutes of Health, Bethesda, Maryland: Recombinant baculovirus containing dengue 4E and NS1 antigens stimulates specific memory T cells.

Kurane, I., Ennis, F.A., Dept. of Medicine, University of

Massachusetts Medical Center, Worcester: Human T-cell responses to dengue viruses at a clonal level.

Roehrig, J., Hunt, A., Johnson, J., Bolin, R., Mathews, J., U.S. Centers for Disease Control, Fort Collins, Colorado: Synthetic peptide vaccine strategy for inducing flavivirus immunity.

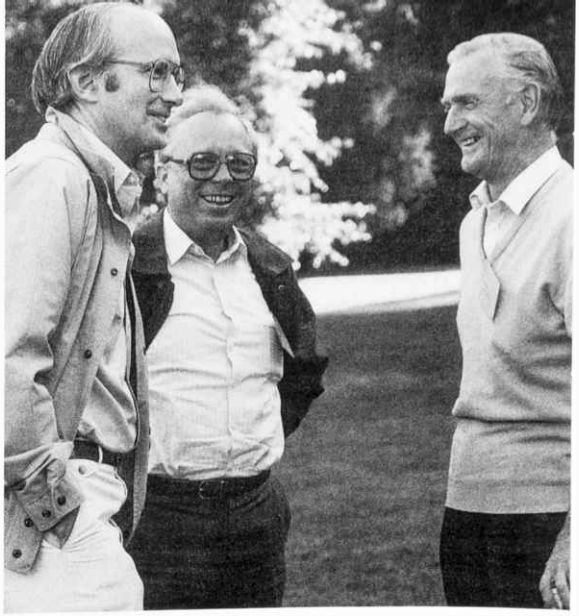
Ott, G., Van Nest, G., Gervase, B., Carlson, J., Goldbeck, C., Ng, P., Sanchez-Pescador, L., Burke, R.L., Chiron Corporation, Emeryville, California: Development of an HSV subunit vaccine.

Molnar-Kimber, K.L.,[1] Haigwood, N.L.,[2] Najarian, R.,[2] Jarocki-Witek, V.,[1] Dheer, S.K.,[1] Stauffer, B.,[1] Mizutani, S.,[1] Conley, A.J.,[1] Davis, A.R.,[1] Hung, P.P.,[1] [1]Biotechnology and Microbiology Division, Wyeth-Ayerst Research, Philadelphia, Pennsylvania; [2]Chiron Corp., Emeryville, California: Characterization of the adenovirus-4 E3 region for the development of live recombinant vaccines.

Kotwal, G.J.,[1] Buller, R.M.L.,[1] Kapikian, A.Z.,[1] Stephens, E.,[2] Compans, R.W.,[2] Moss, B.,[1] [1]NIAID, National Institutes of Health, Bethesda, Maryland: [2]Dept. of Microbiology, University of Alabama, Birmingham: Analysis of recombinant vaccinia virions for presence of foreign proteins in the envelope.

Yilma, T.,[1] Hsu, D.,[1] Jones, L.,[1] Owens, S.,[1] Grubman, M.,[2] Mebus, C.,[2] Yamanaka, M.,[3] Dale, B.,[3] [1]University of California, Davis; [2]Plum Island Animal Disease Laboratory, Greenport, New York; [3]California Biotechnology, Inc., Mountain View: Protection of cattle against rinderpest with vaccinia virus recombinants expressing the HA or F gene of rinderpest virus.

Tsukiyama, K.,[1,2] Yoshikawa, Y.,[1] Kamata, H.,[1] Yamanouchi, K.,[1] Asano, K.,[2] Maruyama, T.,[2] Funahashi, S.,[2] Sugimoto, M.,[2] Shida, H.,[3] [1]Institute of Medical Science, University of Tokyo, [2]Fundamental Research Laboratory, Kogyo, [3]Virus Research Institute, Kyoto University, Japan: Development of recombinant rinderpest vaccine.



S. Lemon, G. Siegl, F. Brown

Hunt, L.A.,[1] Brown, D.W.,[2] Robinson, H.L.,[2] Naeve, C.W.,[3] Webster, R.G.,[4] [1]Dept. of Microbiology and Immunology, University of Louisville School of Medicine, Kentucky; [2]Dept. of Pathology, University of Massachusetts Medical Center, Worcester; [3]Division of Virology, St. Jude Children's Research Hospital, Memphis, Tennessee: Avian-retrovirus-expressed hemagglutinin protects against lethal influenza infection.

SESSION 4 PARASITOLOGY

Chairman: M. Good, National Institutes of Health

Scott, P.,[1] Natovitz, P.,[1] Coffman, R.L.,[2] Pearce, E.,[1] Sher, A.,[1] [1]NIAID, National Institutes of Health, Bethesda, Maryland; [2]DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, California: Different T-helper subsets transfer protective immunity or exacerbation in cutaneous leishmaniasis.

Cesbron-Delauw, M.F.,[1] Guy, B.,[2] Pierce, R.J.,[1] Torpier, G.,[1] Lenzen, G.,[2] Cesbron, J.Y.,[1] Fourmaux, M.P.,[1] Leite, P.,[1] Darcy, F.,[1] Lecocq, J.P.,[2] Capron, A.,[1] [1]CIBP, INSERM, CNRS, Institut Pasteur, Lille, [2]Transgene SA, Strasbourg, France: Molecular characterization of a major secreted immunogen of *T. gondii*.

Kumar, S.,[1] Miller, L.H.,[1] Quakyi, I.A.,[1] Keister, D.B.,[1] Houghten, R.A.,[2] Maloy, W.L.,[1] Moss, B.,[1] Berzofsky, J.A.,[1] Good, M.F.,[1] [1]NIAID, National Institutes of Health, Bethesda, Maryland; [2]Scripps Clinic and Research Foundation, La Jolla, California: *P. falciparum* sporozoites induce circumsporozoite protein-specific CTL, and the epitope is identified in a polymorphic region of the molecule.

Sadoff, J.C.,[1] Ballou, W.R.,[1] Barrom, L.S.,[1] Ou, J.,[1] Young, J.F.,[2] [1]WRAIR, Washington, D.C.; [2]Smith Kline & French Laboratories, Swedeland, Pennsylvania: Oral *S. typhimurium* circumsporozoite recombinant vaccines protect against malaria.

de la Cruz, V.F., Maloy, W.L., Miller, L.H., Good, M.F., McCutchan, T.F., NIAID, National Institutes of Health, Bethesda, Maryland: Polymorphism in T-cell determinants from the circumsporozoite protein of *P. falciparum* results in lack of cross-reactivity of sensitized T cells.

Russo, D., Sundy, J., Weidanz, W., Malaria Research Group, Hahnemann University, Philadelphia, Pennsylvania: Identification and characterization of T epitopes residing within recombinant and synthetic peptides derived from the circumsporozoite protein of *P. falciparum*.

Kaslow, D., Quakyi, I., Syin, C., Raum, M., Keister, D., Coligan, J., McCutchan, T., Miller, L., NCI, National Institutes of Health, Bethesda, Maryland: Molecular structure of a vaccine candidate from the sexual stage of human malaria—Implications of minimal antigenic variation on vaccine development of Pfs25.

Saul, A.,[1] Jones, G.,[1] Gale, J.,[1] Lord, R.,[1] Edmundson, H.,[1]

Epping, R.,[1] Kara, U.,[1] Pye, D.,[2] Geysen, H.M.,[2] [1]Queensland Institute of Medical Research, Brisbane; [2]Commonwealth Serum Laboratories, Parksville, Victoria, Australia: Development of a peptide vaccine for the asexual stage of *P. falciparum*.

Smythe, J.,[1] Coppel, R.,[1] Brown, G.,[1] Ramasamy, R.,[2] Kemp, D.,[1] Anders, R.,[1] [1]Walter and Eliza Hall Institute, Melbourne, [2]Queensland Institute of Medical Research, Brisbane, Australia: Selection and characterization of *P. falciparum* membrane antigens as candidate vaccine molecules.

Romero, P.,[1] Tam, J.P.,[2] Schlesinger, J.,[1] Nussenzweig, V.,[1] Nussenzweig, R.S.,[1] Zavala, F.,[1] [1]New York University Medical Center, [2]Rockefeller University, New York, New York: Identification of multiple T cell epitopes within the circumsporozoite (cs) protein of *Plasmodium berghei*.

SESSION 5 AIDS. II

Chairman: **E. Norrby,** Karolinska Institutet

Nara, P.,[1] Dunlop, N.,[1] Kessler, J.,[1] Fischinger, P.,[2] [1]NCI-Frederick Cancer Research Facility, Frederick, Maryland; [2]DHHS Public Health Services, Washington, D.C.: Characterization of HIV-1 neutralization—Detailed kinetic analysis of antisera from infected humans, chimpanzees, and gp120-vaccinated animals.

Prince, A.M., Saunders, A., Pascual, D., Andrus, L., Bianco, C., Lindsley F. Kimball Research Institute, New York Blood Bank, New York, New York: Why do high-titer neutralizing antibodies not protect against HIV?

Takeda, A.,[1] Tuazon, C.U.,[2] Ennis, F.A., [1]University of Massachusetts Medical School, Worcester; [2]George Washington University School of Medicine, Washington, D.C.: Antibody-enhanced infection by HIV-1 via Fc-receptor-mediated entry.

Weiner, D.B.,[1] Williams, W.V.,[1] Hoxie, J.A.,[1] Berzofsky, J.A.,[2] Greene, M.I.,[1] [1]University of Pennsylvania, Philadelphia; [2]NCI, National Institutes of Health, Bethesda, Maryland: Non-CD4 molecules of human T cells important in gp120-gp41 T-cell interactions.

Wahren, B.,[1] Rosen, J.,[4] Sandström, E.,[2] Mathiesen, T.,[1] Modrow, S.,[5] Wigzell, H.,[3] [1]National Bacteriological Laboratory, [2]Södersjukhuset, [3]Karolinska Institutet, Stockholm, Sweden; [4]Johnson and Johnson Biotechnology Center, La Jolla, California; [5]Max von Pettenkofer Institute, Munich, Federal Republic of Germany: HIV-1 peptide sequences inducing a proliferation response in lymphocytes from infected persons.

Letvin, N.L., Tsubota, H., Harvard Medical School, New England Regional Primate Research Center, Southborough, Massachusetts: The CD8 molecule is required for T-lymphocyte inhibition of AIDS virus replication.

Sawyer, L.,[1] Katzenstein, D.,[1] Hendry, M.,[1] Zeger, S.,[2] Boone, E.,[1] Vujcic, L.,[1] Williams, C.,[1] Quinnan, G.,[1] Multicenter AIDS Cohort Study, [1]CBER, NCI, National Institutes of Health, Bethesda, [2]Johns Hopkins University, Baltimore, Maryland: Different roles of ADCC and neutralizing antibodies in HIV infection.

Tyler, D.S., Nastala, C.L., Lyerly, H.K., Matthews, T.J., Bolognesi, D.P., Weinhold, K.J., Depts. of Surgery, Microbiology, and Immunology, Duke University Medical Center, Durham, North Carolina: Anti-HIV cytotoxicity—Role of anti-gp120 cytophilic arming antibodies in therapeutic and vaccine strategies.

Ljunggren, K.,[1,2] Albert, E.M.J.,[1,3] Nagy, K.,[1] Jondal, M.,[2] Fenyö, E.M.,[1] Norrby, E.,[1] Depts. of [1]Virology, [2]Immunology, Karolinska Institutet, [3]Dept. of Virology, National Bacteriological Laboratory, Stockholm, Sweden: ADCC detects strain-specific differences among HIV-1 and HIV-2 isolates.

Parks, E.,[1] Norrby, E.,[2] Johnson, P.,[3] [1]Johnson and Johnson Biotechnology Center, La Jolla, California; [2]Karolinska Institutet, Stockholm, Sweden; [3]Georgetown University, Rockville, Maryland: Site-directed serology of HIV-2 infection using human and simian synthetic peptides.

Hewlett, I.K.,[1] Gregg, R.A.,[1] Hawthorne, C.A.,[1] Mayner, R.E.,[1] Ou, C.Y.,[2] Schochetman, G.,[2] Schumacher, R.T.,[3] Epstein, J.S.,[1] [1]Division of Blood and Blood Products, Food and Drug Administration, Bethesda, Maryland; [2]Centers for Disease Control, Atlanta, Georgia; [3]Boston Biomedica Inc., Mansfield, Massachusetts: Detection of HIV-1-specific DNA and RNA in plasma by polymerase chain reaction.

Rylatt, D.,[1] Kemp, B.,[2] Bundesen, P.,[1] McPhee, D.,[3] Doherty, R.,[3] Hillyard, C.,[1] [1]AGEN Biomedical Ltd., Brisbane, [2]St. Vincent's Institute for Medical Research, Melbourne, [3]Fairfield Hospital, Melbourne, Australia: Rapid whole-blood immunoassay for HIV-1 antibody.

SESSION 6 POSTER SESSION

Ahmad, N., Venkatesan, S., NIAID, National Institutes of Health, Bethesda, Maryland: Mechanism of HIV-1 Rev protein function.

Ahmad, N., Venkatesan, S., NIAID, National Institutes of Health, Bethesda, Maryland: The Nef (B orf) product of HIV-1 is a transcriptional repressor of HIV-1 LTR.

Allison, A.C., Byars, N.E., Dept. of Immunology, Institute of Biological Sciences, Syntex Research, Palo Alto, California: Development and applications of a stable adjuvant formulation.

Appel, J., Pinilla, C., Houghten, R.A., Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California: Efficacy of completely synthetic branched multiple-copy peptide polymers as immunogens.

Arya, S.C., Greater Kailash-II, New Delhi, India: Ensuring field stability of new vaccines through their prior accelerated degradation testing.

Ball, J.M.,[1] Payne, S.L.,[1] Issle, C.J.,[2] Fontenot, J.D.,[1]

Montelaro, R.C.,[1] Depts. of [1]Biochemistry, [2]Veterinary Science and Veterinary Microbiology and Parasitology, Louisiana State University, Baton Rouge: Localization of EIAV glycoprotein epitopes using recombinant and synthetic peptide methodologies.

Bennett, D.D.,[1] Kashi, K.,[1] Lavangie, D.C.,[3] McMahon, P.,[3] Wright, S.E.,[1,2] [1]Viral Oncology Laboratory, Veterans Administration Medical Center, [2]Depts. of Internal Medicine and Biochemistry, Texas Tech University School of Medicine, Armarillo; [3]AgriTech Systems, Inc., Portland, Maine: Mechanism of recombinant avian retrovirus RAV-O-A1 protection against ASV-A-induced sarcoma.

Brake, D., Lyons, B., Rosenberg, M., Debouck, C., Smith Kline & French Laboratories, King of Prussia, Pennsylvania: Characterization of HIV-1 *tat* regulatory function in mammalian cells.

Browning, M.J., Petrarca, M.A., Diamond, D.C., Reiss, C.S., Dept. of Pathology, Harvard Medical School and Division of Pediatric Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts: Functional characteristics of class II MHC-restricted responses to VSV in H-2^d mice.

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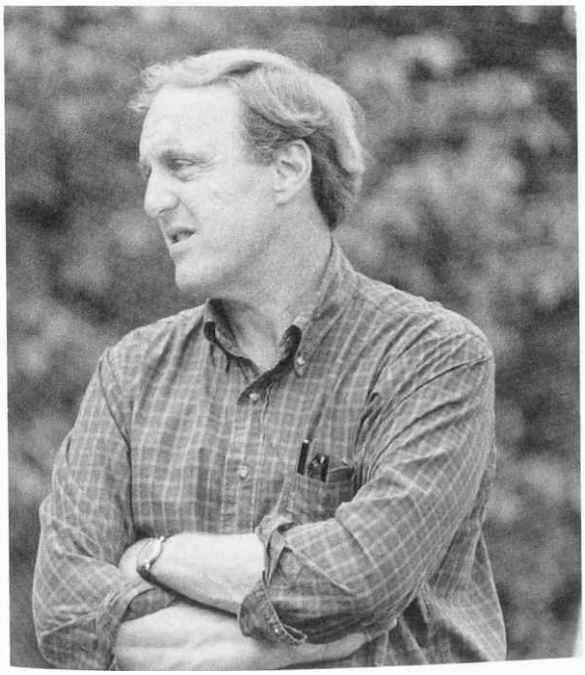

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N. Letvin

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SESSION 7 VIROLOGY. II

Chairman: C.-J. Lai, NIAID, National Institutes of Health

Murray, M.G.,[1] Bradley, J.,[1] Yang, X.-F.,[1] Murdin, A.,[1] Wimmer, E.,[1] Moss, E.G.,[2] Racaniello, V.R.,[2] [1]Dept. of Microbiology, State University of New York, Stony Brook, [2]Dept. of Microbiology, Columbia University College of Physicians & Surgeons, New York, New York: Poliovirus host range is determined by a short amino acid sequence in neutralization antigenic site I.

Lemon, S.M., Ping, L.-H., Murphy, P., Day, S.P., Jansen, R.W., University of North Carolina, Chapel Hill: Identification of an immunodominant antigenic site of HAV.

Emerson, S., Rosenblum, B., Feinstone, S., Purcell, R., NIAID, National Institutes of Health, Bethesda, Maryland: Identification of the HAV genes involved in adaptation to tissue-culture growth and attenuation.

Russell, S.M., Trowbridge, M., Appleyard, G., Speller, S.A., Clarke, B.E., Vadolas, J., Francis, M.J., Sangar, D.V., Rowlands, D.J., Brown, F., Wellcome Biotech, Kent, England: Mapping of neutralization epitopes of human rhinovirus type 2 with monoclonal antibodies.

Francis, M.J.,[1] Hastings, G.Z.,[1] Peat, N.,[2] Campbell, R.O.,[1] Rowlands, D.J.,[1] Brown, F.,[1] [1]Wellcome Biotech, Kent, [2]Dept. of Zoology, University College, London, England: T-cell help for B-cell antibody production to rhinovirus peptides.

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SESSION 8 AIDS. III

Chairman: N. Letvin, Harvard Medical School

Ho, D.D., Li, X.L., Moudgil, T., Gurney, M., University of California Medical School, Los Angeles; University of Chicago, Illinois: A region in the second conserved domain of gp120 is important for antibody neutralization of HIV-1.

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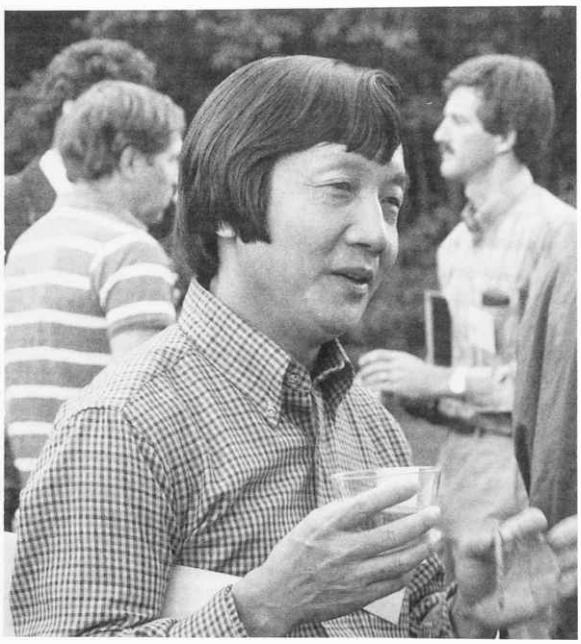
Hosmalin, A.M.,[1] Nara, P.L.,[2] Zweig, M.,[2] Cease, K.B.,[1] Gard, E.A.,[3] Markham, P.D.,[3] Daniel, M.D.,[4] Desrosiers, R.C.,[4] Berzofsky, J.A.,[1] [1]NCI, National Institutes of Health, Bethesda, [2]NCI, Frederick Cancer Research Facility, Frederick, Maryland; [3]Bionetics Research, Rockville, Maryland; [4]New England Primate Research Center, Southborough, Massachusetts: Enhancement of an antibody response to the envelope glycoprotein of HIV-1 by priming with helper-T-cell epitope peptides.



C.-J. Lai

Kieny, M.P.,[1] Lathe, R.,[2] Rivière, Y.,[3] Girard, M.,[4] Montagnier, L.,[3] Lecocq, J.P.,[1] [1]Transgene S.A., [2]LGME-CNRS, INSERM, Strasbourg, [3]Institut Pasteur, Paris, [4]Pasteur Vaccins, Marnes-La-Coquette, France: Removal of the cleavage site improves the immunogenicity of the HIV envelope protein.

Barrett, N.,[1] Mitterer, A.,[1] Eible, J.,[1] Eibl, M.,[1] Moss, B.,[2] Dorner, F.,[1] [1]Immuno AG, Vienna, Austria; [2]National Institutes of Health, Bethesda, Maryland: Large-scale production, purification, and immunological analysis of vaccinia-recombinant-derived HIV-1 gp160.

Pyle, S.,[1] Bess, J., Jr.,[1] Morein, B.,[2] Lerche, N.,[3] Kelliher, J.,[4] Nara, P.,[1] Arthur, L.,[1] [1]NCI-Frederick Cancer Research Facility, Frederick, Maryland; [2]Dept. of Virology, Faculty of Veterinary Medicine, Biomedicum, Uppsala, Sweden; [3]California Primate Research Center, University of California, Davis; [4]Primate Research Institute, Holloman Air Force Base, Alamogordo, New Mexico: Primate immunizations with HIV-1 gp120 ISCOMs.

Lüke, W.,[1] Schneider, J.,[1] Schreiner, D.,[1] Hayami, H.,[2] Kelliher, J.,[3] Hunsmann, G.,[1] [1]German Primate Centre, Göttingen, Federal Republic of Germany; [2]Institute of Medical Science, Tokyo, Japan; [3]Primate Research Institute, Alamogordo, New Mexico: Vaccination of rhesus monkeys with micelles of the external SIV_{AGM}TYO-7 glycoprotein gp130.

Fultz, P.N.,[1] Steimer, K.,[3] Mawle, A.,[2] McClure, H.,[1] Horaist, C.,[1] Dina, D.,[3] [1]Yerkes Primate Research Center, Emory University, [2]Division of Host Factors, Centers for Disease Control, Atlanta, Georgia; [3]Chiron Corporation, Emeryville, California: Postinfection immunization of HIV-1-infected chimpanzees with recombinant HIV-1 *env* and *gag* antigens.

Sutjipto, S.,[1] Carlson, J.,[2] Jennings, M.,[2] Luciw, P.,[2] McGraw, T.,[1] Pedersen, N.,[1] Marx, P.,[1] Gardner, M.,[2] [1]California Primate Research Center, [2]Dept. of Medical Pathology, University of California, Davis: SIV-infected macaques—A model for pre- and postexposure immunization.

Goudsmit, J.,[1] Bakker, M.,[1] Smit, L.,[1] Meloen, R.,[2] [1]Human Retrovirus Laboratory, AMC, Amsterdam, [2]Central

B.R. Murphy, P. Chanock, M. Good

Veterinary Institute, Lelystad, The Netherlands: Immunization with strain-specific and chimeric nonapeptides of an HIV-1 neutralization epitope results in antibodies with shared and cross-reactivity.

SESSION 9 VIROLOGY. III

Chairman: **F. Brown,** Wellcome Biotechnology Ltd.

Klavinskis, L.S., Oldstone, M.B.A., Whitton, J.L., Dept. of Immunology, Research Institute of Scripps Clinic, La Jolla, California: Evidence that vaccines can be engineered to elicit cytotoxic T lymphocytes and protect against viral infection.

Cannon, M.J., Openshaw, P.J.M., Askonas, B.A., National Institute for Medical Research, London, England: Lethal pulmonary disease in RS-virus-infected mice following transfer of cytotoxic T cells.

Murphy, B.R.,[1] Olmsted, R.A.,[1] Collins, P.L.,[1] Chanock, R.M.,[1] Prince, G.A.,[2] [1]NCI, National Institutes of Health, Bethesda, [2]Johns Hopkins University, Baltimore, Maryland: Passive transfer of RS virus antiserum suppresses the immune response to the RS virus fusion (F) and large (G) glyco-proteins expressed by recombinant vaccinia viruses.

Hsu, M.-C.,[1,2] Harbison, M.,[3] Reinhard, G.,[3] Grosz, H.,[3] Davis, K.,[1] Laboratories of [1]Virology, [2]Animal Research, Rockefeller University, New York, New York; [3]Dept. of Oncology and Virology, Hoffmann-La Roche Research Center, Nutley, New Jersey: Protection of mice from wild-type Sendai virus infection by protease activation mutants.

Dalrymple, J.M.,[1] Kakach, L.T.,[2] Collette, M.S.,[2] [1]U.S. Army Medical Research Institute of Infectious Diseases, Virology Division, Fort Detrick, Frederick, Maryland; [2]Molecular Genetics, Inc., Minnetonka, Minnesota: Mapping protective determinants of Rift Valley fever virus using recombinant vaccinia viruses.

Esposito, J., Novembre, F., Knight, J., Brown, D., Shaddock, J., Chandler, F., Baer, G., Centers for Disease Control, Atlanta, Georgia: Oral immunization of animals with raccoon poxvirus expressing rabies virus genes.

Versteeg, J.P.M.,[1] Langeveld, S.A.,[1] de Vries, P.,[2] van Binnendijk, R.S.,[2] Voorma, H.O.,[1] Weisbeek, P.J.,[1] Osterhaus, A.,[2] UytdeHaag, F.G.C.M.,[2] [1]Institute of Molecular Biology and Medical Biotechnology, University of Utrecht, [2]National Institute of Public Health and Environmental Hygiene, Bilthoven, The Netherlands: Epitope mapping of the fusion protein of measles virus.

Nicholas, J.A., Mitchell, M.A., Levely, M.E., Kinner, J.H., Rubino, K.L., Smith, C.W., Upjohn Company, Kalamazoo, Michigan: A synthetic peptide stimulates T-helper cells and antibody reactive with RS virus.

Vennema, H.,[1] de Groot, R.,[1] Harbour, D.,[2] Dalderup, M.,[1] Horzinek, M.C.,[1] Spaan, W.,[1] [1]Institute of Virology, Utrecht, The Netherlands; [2]Dept. of Veterinary Medicine, Bristol, England: Early death after challenge with feline infectious peritonitis virus of kittens immunized with a recombinant vaccinia virus expressing the FIPV spike protein.

Tannock, G.A., Arvidson, Y., Faculty of Medicine, University of Newcastle, New South Wales, Australia: A model for determining immunogenic relationships between strains of avian infectious bronchitis virus.

SESSION 10 BACTERIOLOGY

Chairman: **B. Murphy,** NIAID, National Institutes of Health

Bartley, T.D.,[1] Whiteley, D.W.,[1] Mar, V.L.,[1] Burns, D.L.,[2] Burnette, W.N.,[1] [1]Amgen Inc., Thousand Oaks, California; [2]CBA, Food and Drug Administration, Bethesda, Maryland: Recombinant pertussis toxoid—In vitro formation of holotoxin from recombinant S1 subunits and natural B oligomer.

Burnette, W.N.,[1] Cieplak, W.,[2] Mar, V.L.,[1] Kaljot, K.T.,[2] Sato, H.,[3] Keith, J.M.,[2] [1]Amgen Inc., Thousand Oaks, California; [2]National Institutes of Health, Rocky Mountain Laboratories, Hamilton, Montana; [3]National Institutes of Health, Tokyo, Japan: Recombinant pertussis toxin—Enzymatic inactivation of the S1 subunit by a site-specific mutation that conserves its protective epitope.

Francotte, M.,[1] Feron, C.,[1] Capiau, C.,[2] Locht, C.,[1] De Wilde, M.,[1] [1]Dept. of [1]Molecular and Cellular Biology, [2]Human Vaccine Development, Smith Kline-RIT, Rixensart, Belgium: Production and characterization of monoclonal antibodies specific to the different subunits of pertussis toxin.

Francotte, M.,[1] Capiau, C.,[2] Locht, C.,[1] Dept. of [1]Molecular and Cellular Biology, [2]Human Vaccine Development, Smith Kline-RIT, Rixensart, Belgium: Identification of the S2 subunit of pertussis toxin as the haptoglobin-binding subunit.

Shahin, R.D., Simmons, M., Manclark, C.R., Federal Drug Administration, Bethesda, Maryland: Immunization with the B oligomer of pertussis toxin protects against lethal aerosol challenge with *B. pertussis*.

Schmidt, M.A., Schmidt, W., Zentrum für Molekulare Biologie Heidelberg, Federal Republic of Germany: Antibodies against synthetic peptides of the pertussis toxin S2 subunit—Cross-reaction and inhibition of receptor binding.

Zealey, G., Loosmore, S., Radika, K., Yacoob, R., Cockle, S., Boux, H., Chong, P., Klein, M., Connaught Research Institute, Ontario, Canada: Construction of *B. pertussis* strains that secrete toxin analogs.

Brooks, E., Faulds, D., Codon, South San Francisco, California: The *M. hyponeumoniae* 74.5-kD antigen elicits neutralizing antibodies and shares sequence similarity with heat-shock proteins.

Anilionis, A., Deich, R.A., Fulginiti, J.P., Quinn-Dey, T., Seid, R.C., Wilhelm, S., Praxis Biologics, Inc., Rochester, New York: Biologic activity of antisera raised against a recombinant form of the *H. influenzae* 15,000-dalton outer-membrane lipoprotein, PCP, expressed in *E. coli*.

Majarian, W.R.,[1] Kasper, S.J.,[1] Brey, R.N. III,[2] Depts. of [1]Immunology, [2]Molecular Biology, Praxis Biologics, Inc., Rochester, New York: Expression of heterologous epitopes as recombinant flagella on the surface of attenuated *Salmonella*.

Clements, J.D., Tulane University School of Medicine, New Orleans, Louisiana: Use of attenuated mutants of *Salmonella* as carriers for delivery of heterologous antigens to the secretory immune system.

Summary: **B. Murphy**

Ribosome Synthesis

September 21—September 25

ARRANGED BY

James D. Friesen, University of Toronto
Lasse Lindahl, University of Rochester
Edward Morgan, Roswell Park Memorial Institute
Jonathan R. Wagner, Albert Einstein College of Medicine
Janice M. Zengel, University of Rochester

288 participants

This meeting was the first to focus on all aspects of ribosome synthesis, from transcription of ribosomal RNA and protein genes to the assembly of ribosomes. Another hallmark of the meeting was bringing together researchers working on both prokaryotic and eukaryotic organisms. This is a particularly useful strategy in the study of ribosomes, an organelle that exhibits so many features conserved throughout evolution.

The meeting was dedicated to Professor Ole Maaløe, one of the founding

fathers of the study of the regulation of ribosome synthesis. The scientific program offered about 225 reports, of which 77 were given with slides and the remainder with posters. These presentations demonstrated how far we have come in elucidating intricate molecular details of some regulatory mechanisms, and yet how far we must go before even the most important regulatory circuits are fully understood. In some areas, such as the regulation of rRNA and ribosomal protein synthesis in bacteria and yeast, at least some of the major regulatory pathways have been identified, and the focus is now on understanding the interactions of the regulators affecting transcription and translation. In other areas, like the eukaryotic RNA polymerases and perhaps in particular ribosome assembly, many participating components have yet to be identified and investigated.

This meeting was supported in part by Accurate Chemical and Scientific Corp., Beckman Instruments, Inc., E.I. du Pont de Nemours & Company, Merck and Co., Inc., Mettler Instrument Corporation, MilliGen, Promega Corp., United States Biochemical Corporation, VWR Scientific, and Worthington Biochemical Corp. Grants were also received from the National Institute of General Medical Sciences and the National Institute of Child Health and Human Development, divisions of the National Institutes of Health.

SESSION 1 RIBOSOMES: FROM GENE TO FUNCTION

Chairman: E. Blackburn, University of California, Berkeley

Ribosomes Through the Ages

Noller, H.F., Moazed, D., Robertson, J.M., Allen, P.N., Powers, T., Stern, S., Thimann Laboratories, University of California, Santa Cruz: Functional map of rRNA.

Brimacombe, R., Greuer, B., Mitchell, P., Osswald, M., Schüler, D., Stade, K., Stiege, W., Wiener, L., Max-Planck-Institut für Molekulare Genetik, Abt. Wittmann, Berlin, Federal Republic of Germany: Three-dimensional structure of *E. coli* 16S and 23S rRNAs.

Cunningham, P., Nègre, D., Nurse, K., Weitzmann, C., Ofengand, J., Roche Institute of Molecular Biology, Roche Research Center, Nutley, New Jersey: Point mutations in 16S RNA that affect protein biosynthesis.

Dennis, P.P.,[1] Shimmin, L.,[1] Newton, C.,[1] Yee, J.,[1] Ramirez, C.,[2] Matheson, A.,[2] [1]Dept. of Biochemistry, University of British Columbia, [2]University of Victoria, Canada: Evolutionary comparison of the L10 and L12 equivalent genes and proteins from archaebacteria, eubacteria, and eukaryotes.

Maintenance of Ribosomal RNA Genes

Dover, G.A., Hancock, J.M., Dept. of Genetics, University of Cambridge, England: Molecular coevolutionary paradoxes in the rDNA multigene family in *Drosophila* and other eukaryotic nuclear genomes.

Yu, G.-L., Blackburn, E.H., Dept. of Molecular Biology, University of California, Berkeley: Selectively replicating rDNA forms in *T. thermophila* transformed with circular rDNA plasmids.

Endow, S., Komma, D., Glass, S., Soler-Niedziela, L., Yamamoto, A., Dept. of Microbiology and Immunology, Duke University, Durham, North Carolina: Magnification—Ribosomal gene increase by induced sister chromatid exchange.

Sweeney, R., Yao, M.-C., Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington: In vivo analysis of insertion mutations in rRNA genes of *Tetrahymena*.

SESSION 2 TRANSCRIPTION OF RIBOSOMAL RNA: PROKARYOTES

Chairman: J. Friesen, University of Toronto

Nomura, M., Cole, J.R., Yamagishi, M., Threadgill, G.J., Dept. of Biological Chemistry, University of California, Irvine: Feedback regulation of rRNA synthesis in *E. coli*.

Bremer, H., Baracchini, E., Hernandez, V., Tedin, K., Biology Programs, University of Texas, Dallas: Control of RNA synthesis in bacteria by ppGpp.

Sarubbi, E.,[1] Rudd, K.E.,[1] Xiao, H.,[1] Glaser, G.,[2] Cashel, M.,[1] [1]NICHD, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Cellular Biochemistry, Hadassah Medical School, Jerusalem, Israel: Use of *spoT* mutants in ppGppase to relate steady-state ppGpp levels, growth rates, and *rnaA* RNA operon promoter activities in *E. coli*.

Gourse, R.L.,[1] Dickson, R.R.,[2] Gaal, T.,[1] Newlands, J.T.,[1]

[1]Dept. of Bacteriology, University of Wisconsin, Madison; [2]Dept. of Genetics, University of Georgia, Athens: Growth-rate regulation and upstream activation of rRNA transcription—Genetic and biochemical studies of the RNA polymerase–rRNA promoter interaction.

Morgan, E., Gaudino, R., Roswell Park Memorial Institute, Buffalo, New York: Transcriptional events involving Box A sequences in precursor-specific regions of *E. coli* rRNA operons.

Berg, K., Squires, C.L., Squires, C., Dept. of Biological Sciences, Columbia University, New York, New York: Transcription antitermination in rRNA operons.

Horwitz, R., Greenblatt, J., Banting and Best Dept. of Medical Research and Dept. of Medical Genetics, University of Toronto, Ontario, Canada: Transcriptional elongation complexes synthesizing *E. coli* rRNA contain the *E. coli* elongation factors NusA, NusB, and NusG.

Lukacsovich, T., Csiszar, K., Venetianer, P., Institute of Biochemistry, Biological Research Center, Szeged, Hungary: Regulation of rRNA synthesis in *E. coli* by sequences upstream and downstream from the *rrnB* P_2 promoter.

M. Nomura

SESSION 3 POSTER SESSION

Amils, R.,[1] Sanchez, E.,[1] Londei, P.,[2] [1]Centro de Biología Molecular, Madrid, Spain; [2]Dipt. di Biopatologia Umana, Universita di Roma, Policlinico Umberto, [3]Viale Regina Elena, Italy: Total reconstitution of 70S ribosomes from a halophilic archaebacteria under intracellular ionic conditions.

Amils, R.,[1] Sanz, J.L.,[1] Marín, I.,[1] Ramirez, L.,[1] Abad, J.P.,[2] Smith, C.,[3] [1]Centro de Biología Molecular, Madrid, Spain; Depts. of [2]Genetics and Development, [3]Microbiology and Psychiatry, Columbia University College of Physicians & Surgeons, New York, New York: Variable rRNA gene copies in extreme halophilic archaebacteria.

Arevalo, S., Zinker, S., Fernandez-Tomas, C., Dept. de Genetica y Biologia Molecular, Centro de Investigacion y de Estudios Avanzados del IPN, Mexico: Biosynthesis of ribosomal proteins in poliovirus-infected HeLa cells.

Baronas-Lowell, D.M., Warner, J.R., Dept. of Cell Biology, Albert Einstein College of Medicine, Bronx, New York: Analysis of duplicated ribosomal protein gene function in *S. cerevisiae*.

Bauer, B.F.,[1] Rowley, K.,[1] Holmes, W.M.,[1] Moore, K.,[2] Artz, S.,[2] [1]Dept. of Microbiology, Medical College of Virginia, Richmond; [2]Dept. of Bacteriology, University of California, Davis: Additional factors may be required for in vitro ppGpp inhibition of a tRNA operon from *E. coli*.

Behrens, S.,[1] Zacharias, M.,[1] Eberle, J.,[1] Szymkowiak, C.,[1] Wagner, R.,[2] [1]Max-Planck-Institut für Molekulare Genetik, Berlin, [2]Institut für Physikalische Biologie, Universität Dusseldorf, Federal Republic of Germany: Effects of mutations in regulatory regions of the *rrnB* operon from *E. coli* on rRNA synthesis and cell growth.

Benavente, R., Scheer, U., Institute of Zoology, University of Würzburg, Federal Republic of Germany: Microinjection of nucleolar antibodies as a tool for the study of nucleolar structure and function.

Bennett-Guerrero, E., Byahatti, S., Santer, M., Dept. of Biology, Haverford College, Pennsylvania: Site-specific mutation in the central domain of 16S rRNA of *E. coli*.

Björnsson, A., Isaksson, L.A., Dept. of Microbiology, Biomedical Center, Uppsala University, Sweden: Test system for measurement of translational efficiency in vivo.

Bonham-Smith, P.C.,[1] Bourque, D.P.,[1,2] Depts. of [1]Biochemistry, [2]Molecular and Cellular Biology, University of Arizona, Tucson: Translation of chloroplast-encoded mRNA—Initiation signals and potential feedback regulation of ribosomal protein synthesis.

Brown, S., Fred Hutchinson Cancer Research Center, Seattle, Washington: Suppressor analysis of 4.5S RNA in *E. coli*.

Bujard, H., Brunner, M., Deuschle, U., Knaus, R., Lanzer, M., Peschke, U., Zentrüm fur Molekulare Biologie, Universität Heidelberg, Federal Republic of Germany: Sequences determining the functional program of *E. coli* promoters.

Burgin, A.B.,[1] Parados, K.,[2] Lane, D.J.,[2] Pace, N.R.,[1] [1]Dept. of Biology, Indiana University, Bloomington; [2]Gene-Trak Systems, Framingham, Massachusetts: Excision of intron-like elements from *Salmonella* 23S rRNA precursors.

Campbell, G.P., Whitelaw, P.F., Palmer, R.M., Hesketh, J.E., Muscle Biochemistry Group, Rowett Research Institute, Aberdeen, Scotland: Indomethacin inhibits the stimulation of rRNA synthesis by insulin in L6 myoblasts.

Canonaco, M.A.,[1] Pon, C.L.,[1] Gualerzi, C.O.,[1,2] [1]Max-Planck-Institut für Molekulare Genetik, Berlin, Federal Republic of Germany; [2]Dept. of Cell Biology, University of Camerino, Italy: Ribosome-mRNA interaction during translational initiation. The initiation factors affect an alternative occupancy of a dual ribosomal-binding site by mRNA.

Chang, J.-H., Dumbar, T.S., Olson, M.O.J., Dept. of Biochemistry, University of Mississippi Medical Center,

Jackson: cDNAs and deduced primary structures of two forms of nucleolar protein B23.

Cigan, A.M., Donahue, T.F., Dept. of Molecular Biology and Biochemistry, Northwestern University Medical School, Chicago, Illinois: Initiation factor eIF-2 and $tRNA^{met}$ function in directing the scanning ribosome to the eukaryotic translational start site.

Clark, M.W., Campbell, J., Abelson, J., Division of Biology, California Institute of Technology, Pasadena: SSB-1 of yeast is a nucleolar-specific silver-binding protein and is most likely involved in pre-ribosome assembly.

Cooperman, B.S., Buck, M.A., Olah, T.A., Dept. of Chemistry, University of Pennsylvania, Philadelphia: 30S reconstitution revisited—Quantitation of the protein content of reconstituted 30S subunits.

Cummings, H.S., Hershey, J.W.B., Dept. of Biological Chemistry, University of California School of Medicine, Davis: Structure and expression of the *infA* region of the *E. coli* chromosome coding for protein synthesis initiation factor IF-1.

de Boer, H.A., Hui, A.S., Genentech, Inc., South San Francisco, California: Mutagenesis at the mRNA decoding site in the 16S rRNA using the specialized ribosome system in *E. coli*.

De Stasio, E.A., Dahlberg, A.E., Brown University, Providence, Rhode Island: Mutagenesis of a base-paired site in *E. coli* 16S rRNA confers aminoglycoside resistance and affects bacterial growth.

Dever, T.E.,[1] Smit-McBride, Z.,[2] Conroy, S.C.,[1] Hershey, J.W.B.,[2] Merrick, W.C.,[1] [1]Dept. of Biochemistry, Case Western Reserve University School of Medicine, Cleveland, Ohio; [2]Dept. of Biological Chemistry, University of California School of Medicine, Davis: Sequence analysis of eukaryotic protein synthesis factors.

Dholakia, J.N., Wahba, A.J., Dept. of Biochemistry, University of Mississippi Medical Center, Jackson: Mechanism and regulation of the guanine nucleotide exchange reaction in eukaryotic polypeptide initiation.

Diaz, J.-J.,[1] Gandrillon, O.,[2] Samarut, J.,[2] Madjar, J.-J.,[1] [1]Laboratoire de Biologie Moleculaire et Cellulaire, Faculte de Medecine Alexis Carrel, [2]Laboratoire de Biologie Moleculaire et Cellulaire, Ecole Normal Superieure, Lyon, France: Increase in ribosomal protein S6 phosphorylation is due to v-*erbB* transforming activity and not to v-*erbA* mitogenic activity in AEV-infected chicken embryo fibroblasts.

Douthwaite, S.,[1] Noller, H.F.,[2] [1]Dept. of Molecular Biology, Odense University, Denmark; [2]Thimann Laboratories, University of California, Santa Cruz: Structural alterations in domain II of 23S RNA and their effects on ribosome function.

Egebjerg, J., Garrett, R.A., Institute of Chemistry, Aarhus, Denmark: The GTPase domain of the ribosome—Structure-function relationships of the 23S RNA region associated with protein L11 and the L10 $(L12)_4$ pentameric complex from *E. coli*.

Ehrenberg, M., Bilgin, N., Claesens, F., Kurland, C.G., Institute of Molecular Biology, Biomedical Center, Uppsala, Sweden: Kinetic studies of S12 mutants—Causes of drug dependence.

El Hag, G.A., Thomas, F.J.P., Bourque, D.P., Depts. of Biochemistry and Molecular and Cellular Biology, University of Arizona, Tucson: Isolation and sequence of cDNA for tobacco chloroplast ribosomal proteins L7/L12 and L24.

Endo, Y., Chan, Y.L., Wool, I.G., Dept. of Biochemistry and Molecular Biology, University of Chicago, Illinois: Use of α-sarcin and ricin to study a functionally important rRNA domain.

Fleming, G.,[1] Belhumeur, P.,[2] Skup, D.,[2] Fried, H.,[1] [1]Dept. of Biochemistry, University of North Carolina, Chapel Hill; [2]Institut du Cancer de Montreal, Quebec, Canada: Functional substitution of yeast ribosomal protein L29 by mouse ribosomal protein L27′ in yeast ribosomes.

Franceschi, F., Schnier, J., Nierhaus, K.H., MPI für Molekulare Genetik, Berlin, Federal Republic of Germany: Principles of the assembly of *E. coli* ribosomes—Role of protein L15 in late assembly reactions.

Gerbi, S.A., Jeppesen, C., Stebbins-Boaz, B., Savino, R., Division of Biology and Medicine, Brown University, Providence, Rhode Island: *Xenopus* U3 snRNA.

Giese, K., Phua, S.H., Subramanian, A.R., Max-Planck-Institut für Molekulare Genetik, Abt. Wittmann, Berlin, Federal Republic of Germany: Chloroplast ribosomal proteins L12 and L13 are encoded in the nucleus. Isolation of their cDNA clones from a λgt11 library using oligonucleotides and antibodies.

Glass, R.E., Ralphs, N., Rowland, G., Queens Medical Centre, Nottingham, England: A revised structure-function map of the β subunit of *E. coli* RNA polymerase.

Gonzalez, I.L., Sylvester, J.E., Dept. of Pathology, Hahnemann University, Philadelphia, Pennsylvania: Human rRNA—Variation and evolution.

Gregory, S., Morgan, E., Roswell Park Memorial Institute, Buffalo, New York: Isolation of a recessive, conditionally dominant antibiotic resistance mutation in a 16S rRNA gene of *E. coli*.

Gudkov, A.T., Bubunenko, M.G., Venyaminov, S.Y., Institute of Protein Research, Moscow, Union of Soviet Socialists Republic: Role of ribosomal proteins and rRNA in the structure and function of ribosomes.

Ganoza, M.C., Banting and Best Dept. of Medical Research, University of Toronto, Ontario, Canada: Reconstruction of translation—Possible role of three novel non-ribosomal proteins.

Ganoza, M.C., Margolin-Brzezinski, D., Patel, S., Park, K.-S., Banting and Best Dept. of Medical Research, University of Toronto, Ontario, Canada: Regulation of gene expression at the translational level.

Hariharan, N., Perry, R.P., Institute for Cancer Research, Fox Chase, Philadelphia, Pennsylvania: Common design features of mouse ribosomal protein promoters.

Hartmann, R.K., Specht, T., Kröger, B., Schultze, S., Erdmann, V.A., Institut für Biochemie, Freie Universität, Berlin, Federal Republic of Germany: Local and transcriptional decoupling of 16S rRNA genes from 23S/5S rRNA genes in the thermophilic eubacterium *T. thermophilus*.

Henderson, S.L.,[1] Wejksnora, P.J.,[2] Tower, J.,[1] Sollner-Webb, B.,[1] [1]Dept. of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland; [2]Dept. of Biological Sciences, University of

Wisconsin, Milwaukee: An RNA Pol I promoter located in the CHO and mouse rDNA spacer—Functional analysis and factor requirements.

Hill, W.E., Pond, D., Saunders, J., Tassanakajohn, A., Dept. of Chemistry, University of Montana, Missoula: rRNA structural changes occurring upon activation of the 30S ribosomal subunit.

Huang, S., Hershey, J.W.B., Dept. of Biological Chemistry, University of California School of Medicine, Davis: Glucocorticoids specifically inhibit initiation factor gene expression in P1798 lymphosarcoma cells.

Hümbelin, M.,[1] Safer, B.,[2] Chiorini, J.A.,[2] Hershey, J.W.B.,[1] Cohen, R.B.,[2] [1]Dept. of Biological Chemistry, University of California School of Medicine, Davis; [2]NHLBI, National Institutes of Health, Bethesda, Maryland: Isolation and characterization of the promoter and flanking regions of the gene for the human protein synthesis initiation factor eIF-2α.

Isaksson, L.A., Faxén, M., Kirsebom, L.A., Dept. of Microbiology, Biomedical Center, Uppsala, Sweden: Efficiency of suppressor tRNAs in vivo is not correlated with error formation and proofreading efficiency in vitro.

Ishihama, A., Fujita, N., Nomura, T., Ueshima, R., Nakayama, M., Dept. of Molecular Genetics, National Institute of Genetics, Mishima, Japan: Regulatory factors involved in transcription-translation coupling circuits in *E. coli*.

Ju, Q., Warner, J.R., Dept. of Cell Biology, Albert Einstein College of Medicine, Bronx, New York: Regulation of ribosome synthesis in *S. cerevisiae*.

Kawakami, K., Itoh, K., Nakamura, Y., Dept. of Tumor Biology, Institute of Medical Science, University of Tokyo, Japan: Cloning of the *Salmonella* RF2 gene and its structural relationship to the *E. coli* RF2 gene.

Kelley-Geraghty, D.C., All-Robyn, J.A., Liebman, S.W., University of Illinois, Chicago: Cloning of the *SUP46* omnipotent suppressor in *S. cerevisiae*.

Khanna-Gupta, A., Ware, V.C., Dept. of Biology and Center for Molecular Bioscience and Biotechnology, Lehigh University, Bethlehem, Pennsylvania: Nucleocytoplasmic transport of ribosomes in a eukaryotic system—Evidence for a facilitated transport mechanism.

Kruppa, J., Fischer, D., Bauche, A., Minzlaff, U., Dept. of Molecular Biology, Hamburg University, Federal Republic of Germany: Polyclonal antibodies against an octapeptide of ribosomal protein S6 containing the phosphorylation site for cAMP-dependent protein kinase.

Labella, T., Braaten, D.C., Little, R.D., Dickson, K.R., Schlessinger, D., Washington University School of Medicine, St. Louis, Missouri: Genomic organization of human rDNAs.

Labhart, P., Lucchini, R., Reeder, R.H., Fred Hutchinson Cancer Research Center, Seattle, Washington: Tests of the "hand-over" model for ribosomal gene transcription in *Xenopus*.

Langer, C., Kreader, C., Weaver, W., Heckman, J., Chemistry Dept., Indiana University, Bloomington; Nuclear genes for cytoplasmic and mitochondrial ribosomal proteins in *N. crassa*.

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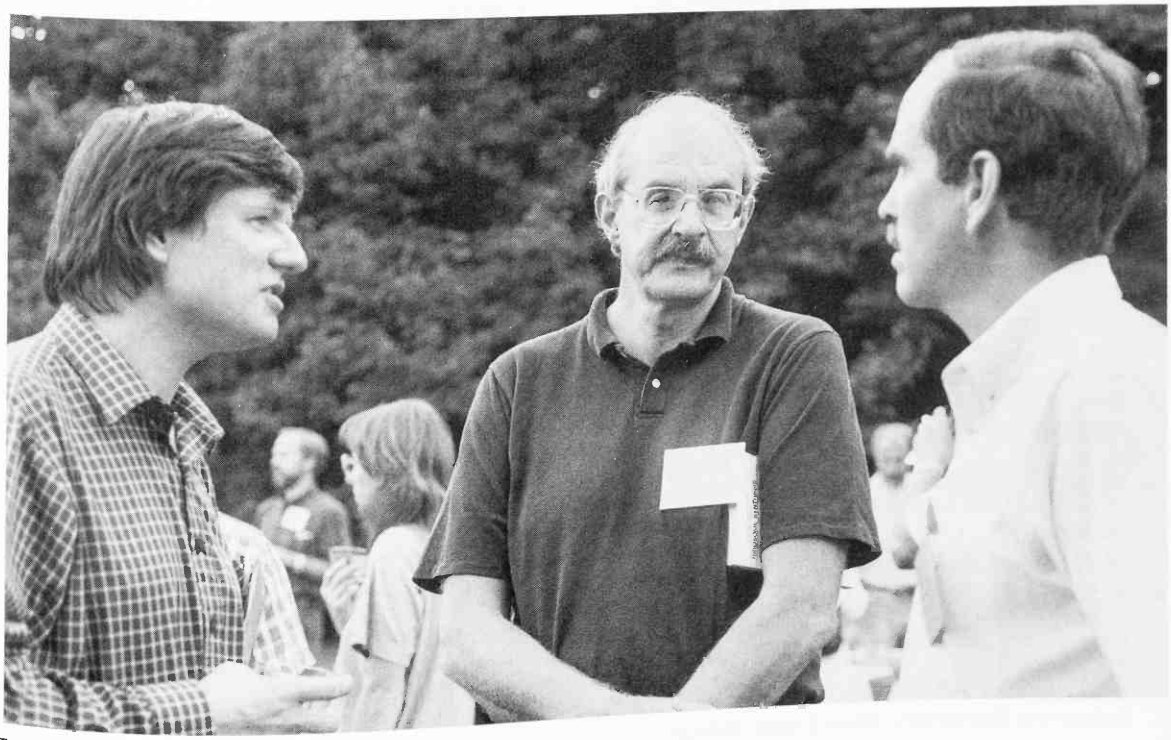
SESSION 4 TRANSCRIPTION OF RIBOSOMAL RNA: EUKARYOTES

Chairman: **R. Reeder,** Fred Hutchinson Cancer Research Center

Reeder, R.H.,[1] McStay, B.,[1] Walker, P.,[1] Schultz, M.,[1] Kristensen, K.,[2] Westegaard, O.,[2] [1]Fred Hutchinson Cancer Research Center, Seattle, Washington; [2]Aarhus University, Denmark: Regulation of *Xenopus* ribosomal gene transcription.

Moss, T., Read, C., Firek, S., Guimond, A., Larose, A.-M., Centre de Recherche en Cancerologie de l'Universite Laval, Quebec, Canada, and Biophysic Laboratories, Portsmouth Polytechnic, England: rRNA synthesis—Its promotion and enhancement.



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Paule, M.R., Bateman, E., Hoffman, L., Iida, C., Kownin, P., Kubaska, W., Risi, P., Zwick, M., Dept. of Biochemistry and Cellular and Molecular Biology Program, Colorado State University, Fort Collins: Initiation and regulation mechanisms of rRNA synthesis in eukaryotes.

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Johnson, S.P., Warner, J.R., Dept. of Cell Biology, Albert Einstein College of Medicine, Bronx, New York: Enhancer function in a mini-rDNA repeat of *S. cerevisiae*.

Sollner-Webb, B., Pape, L., Henderson, S., Ryan, K., Porretta, R., Mougey, E., Paalman, M., Tower, J., Dept. of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland: rDNA transcription in mouse and frog.

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C. Portier. M. Bolotin-Fukuhara

Bell, S.P., Learned, R.M., Jantzen, M., Tjian, R.T., Howard Hughes Medical Institute, Dept. of Biochemistry, University of California, Berkeley: Interactions between Pol I transcription factors SL1 and UBF1.

SESSION 5 SYNTHESIS OF PROTEINS AND FACTORS: EUKARYOTES

Chairman: J.R. Warner, Albert Einstein College of Medicine

Perry, R.P., Atchison, M.L., Chung, S., Hariharan, N., Kelley, D., Moura-Neto, R., Institute for Cancer Research, Fox Chase, Philadelphia, Pennsylvania: A comparative study of mouse ribosomal protein promoters.

Flusser, G., Ginzburg, V., Meyuhas, O., Dept. of Developmental Biochemistry, Institute of Biochemistry, Hebrew University-Hadassah Medical School, Jerusalem, Israel: Glucocorticoids induce transcription of ribosomal protein genes in rat liver.

Rhoads, D.D., Chen, I.T., Maki, C., Montgomery, D., VanSlyke, B., Roufa, D.J., Division of Biology, Kansas State University, Manhattan: Molecular and somatic genetics of mammalian *RPS14*.

Bartel, B., Finley, D., Varshavsky, A., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Three of the yeast ubiquitin genes encode ribosomal proteins.

Moritz, M., Tsay, Y.-F., Woolford, J., Dept. of Biological Sciences, Carnegie Mellon University, Pittsburgh, Pennsylvania: *RPL16*$^-$ mutants indicate that ribosomal protein L16 is necessary for 60S subunit assembly in *S. cerevisiae*.

Eng, F.J., Johnson, S.P., Warner, J.R., Depts. of Cell Biology and Biochemistry, Albert Einstein College of Medicine, Bronx, New York: Mechanism of the autogenous regulation of mRNA splicing by a yeast ribosomal protein.

Planta, R.J., Biochemisch Laboratorium, Vrije Universiteit, Amsterdam, The Netherlands: Control of ribosome biogenesis in yeast.

Beccari, E., Carnevali, F., La Porta, C., Dept. of Genetics and Molecular Biology, University of Rome, Italy: Interaction of nuclear factors with an upstream sequence of a *X. laevis* ribosomal protein gene promoter.

Bozzoni, I., Caffarelli, E., Ciafré, S., Fragapane, P., Lucioli, A., Presutti, C., Dipt. di Genetica e Biologie Molecolare, Universita di Roma, Italy: Regulation of expression of the *X. laevis* L1 ribosomal protein gene and evolutionary analysis of its yeast counterpart.

Tyler, B.M., Harrison, K., Shi, Y., Research School of Biological Sciences, Australian National University: The ribo box—A transcriptional element common to rRNA and protein genes in *N. crassa*.

Kristiansen, K., Dreisig, H., Andreasen, P.H., Larsen, L.K., Nørgaard, P., Rosendahl, G., Dept. of Molecular Biology, Odense University, Denmark: The structure of *Tetrahymena* ribosomal protein genes and the regulation of their expression.

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Möller, W., Maessen, G.D.F., Janssen, G.M.C., Amons, R., Dept. of Medical Biochemistry, Sylvius Laboratories, Leiden, The Netherlands: Studies on EF-1 and ribosomes of *Artemia*.

Morrow, B.E., Warner, J.R., Dept. of Cell Biology, Albert Einstein College of Medicine, Bronx, New York: Characterization of rRNA enhancer-binding protein 1 in *S. cerevisiae*.

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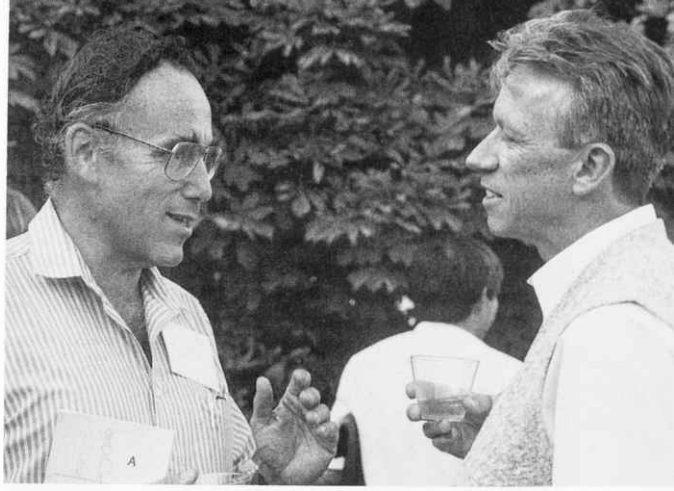

R. Perry, M. Paule

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Yamagishi, M., Nomura, M., Dept. of Biological Chemistry, University of California, Irvine: Synthesis of rRNA and ribosomal proteins in *S. pombe.*

Zacharias, M.,[1] Göringer, H.U.,[1] Wagner, R.,[2] [1]Max-Planck-Institut für Molekulare Genetik, Abt. Wittmann, Berlin, [1]Institut für Physikalische Biologie, Universität Düsseldorf, Federal Republic of Germany: Characterization of an *E. coli rrnB*-P_2-promoter mutation and a putative internal promoter.

Zhou, D.X., Bisanz-Seyer, C., Mache, R., CNRS, Université Joseph, Fourier, Grenoble, France: Presence of a large pool of one ribosomal protein in the stroma of chloroplast that is homologous to one domain of the *E. coli* S1 ribosomal protein.

Workshop: *Transcription by RNA Polymerase I*

SESSION 7 SYNTHESIS OF PROTEINS AND FACTORS: PROKARYOTES

Chairman: **M. Nomura,** University of California, Irvine

Mattheakis, L., Vu, L., Nomura, M., Dept. of Biological Chemistry, University of California, Irvine: Retroregulation of the synthesis of ribosomal proteins L14, L24, and S12 by feedback repressor ribosomal proteins in *E. coli.*

J. Warner, W. Merrick

Climie, S., Scime, A., Harrington, T., Friesen, J.D., Dept. of Medical Genetics, University of Toronto and Hospital for Sick Children, Ontario, Canada: Genetic and structural studies of the mRNA target site and the regulator protein in the *rplJL* operon of *E. coli*.

Zengel, J.M., Archer, R.H., Lindahl, L., Dept. of Biology, University of Rochester, New York: Attenuation control of the *E. coli* S10 ribosomal protein operon.

Lindahl, L., Archer, R.H., McCormick, J.R., Zengel, J.M., Dept. of Biology, University of Rochester, New York: Translational coupling in the *E. coli* S10 ribosomal protein operon.

Moine, H., Romby, P., Ebel, J.P., Ehresmann, B., Ehresmann, C., Institut de Biologie Moléculaire et Cellulaire, CNRS, Strasbourg, France: mRNA structure and gene regulation at the translational level in *E. coli*— The case of threonine:tRNA^{Thr} ligase.

Springer, M., Graffe, M., Dondon, J., Grunberg-Manago, M., IBPC, Paris, France: A specific link between translational repression and informational suppression in *E. coli*.

Draper, D.E., Tang, C.K., Dept. of Chemistry, Johns Hopkins University, Baltimore, Maryland: A pseudoknot structure in the α mRNA is required for translational repression by S4.

Bosch, L., Vijgenboom, E., van Delft, J.H.M., Nilsson, L., Dept. of Biochemistry, Leiden University, The Netherlands: Elongation factor EF-Tu binds to the upstream activator region of the tRNA-*tufB* operon.

Wikström, P.M., Bystrom, A.S., Bjork, G.R., Dept. of Microbiology, University of Umeå, Sweden: Nonautogenous and discoordinate translational control of the *trmD* operon of *E. coli*.

Rasmussen, M.D., Pedersen, S., University Institute of Microbiology, Copenhagen, Denmark: Regulation of the *rpsA* gene in *E. coli*.

SESSION 8 RIBOSOMES FROM TWO GENOMES: ORGANELLAR RIBOSOMES

Chairman: R. Butow, University of Texas Southwestern Medical Center

O'Brien, T., Denslow, N., Piatyszek, M., Graak, H., Bryant, M., Gillevet, P., Dept. of Biochemistry and Molecular Biology, University of Florida, Gainesville: Synthesis, assembly, and organization of proteins in bovine mitochondrial ribosomes.

Bolotin-Fukuhara, M., Daignan-Fornier, B., Laboratoire de Génétique Moléculaire, Orsay, France: Mutational analysis of structure-function relationship in the large rRNA of yeast mitochondria.

Johnson, S., Dang, H., Ellis, S., Dept. of Biochemistry, University of Louisville, Kentucky: Import of mitochondrial ribosomal proteins into yeast mitochondria.

Fearon, K., Partaledis, J., Mason, T., Dept. of Biochemistry and Program in Molecular and Cellular Biology, University of Massachusetts, Amherst: Structure and regulation of nuclear genes in *S. cerevisiae* that specify mitochondrial ribosomal proteins.

Harris, E.H., Liu, X.-Q., Boynton, J.E., Gillham, N.W., Depts. of Botany and Zoology, Duke University, Durham, North Carolina: Involvement of chloroplast genes in biogenesis of chloroplast ribosomes in *C. reinhardtii*.

Romby, P.,[1] Westhof, E.,[1] Mache, R.,[2] Ebel, J.P.,[1] Ehresmann, C.,[1] Ehresmann, B.,[1] [1]Institut de Biologie Moléculaire et Cellulaire, CNRS, Strasbourg; [2]Laboratoire de Biologie Végétale, Université de Grenoble, France: RNA structure and protein recognition. II. Chloroplastic 5S rRNA from spinach and interaction with protein L12.

Gray, M.W.,[1] Boer, P.H.,[1] Boulanger, J.,[2] Heinonen, T.Y.K.,[1] Lemieux, C.,[2] Schnare, M.N.,[1] Turmel, M.,[2] [1]Dept. of Biochemistry, Dalhousie University, Halifax, Nova Scotia; [2]Dept. de biochimie, Université Laval, Québec, Canada: Discontinuous rRNAs in mitochondria and chloroplasts.

Butow, R.A., Dept. of Biochemistry, University of Texas Southwestern Medical Center, Dallas: Interactions between mitochondria and the nucleus in yeast— Influence of the mitochondrial genotype on novel transcription of the nuclear rDNA repeat.

SESSION 9 RIBOSOME ASSEMBLY AND THE NUCLEOLUS

Chairman: D. Schlessinger, Washington University

Functional Organization of the Nucleolus

Scheer, U., Dabauvalle, M.-C., Institute of Zoology, University of Würzburg, Federal Republic of Germany: Functional organization of the nucleolus.

Amalric, F., Belenguer, P., Bouche, G., Bourbon, H., Bugler, B., Caizergues-Ferrer, M., Erard, M., Ghisolfi, L., Lapeyre, B., Centre de Biochimie et Génétique Cellulaires, CNRS, Toulouse, France: Role of nucleolin in expression and packaging of pre-rRNA.

Schmidt-Zachmann, M.S., Peters, J.M., Franke, W.W., Institute of Cell and Tumor Biology, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Protein N038/B23—Molecular characterization of a major nucleolus-associated member of the nucleoplasmin family.

Assembly Interactions

Gregory, R.J., King, S.R., Wower, I., Zimmermann, R.A., University of Massachusetts, Amherst: Structural and regulatory properties of S8-RNA interactions in *E. coli.*

Nishi, K.,[1] Morel-Deville, F.,[2] Hershey, J.W.B.,[2] Leighton, T.,[3] Schnier, J.,[3] [1]Max-Planck-Institut für Molekulare Genetik, Abt. Wittmann, Berlin, Federal Republic of Germany; [2]Dept. of Biological Chemistry, University of California, Davis; [3]Dept. of Microbiology and Immunology, University of California, Berkeley: Identification of an EIF-4A-like protein involved in ribosomal 50S subunit assembly in *E. coli.*

Hadjiolov, A.A.,[1] Dabeva, M.D.,[1] Hadjiolova, K.V.,[1] Dimova, R.N.,[2] Gajdardjieva, K.C.,[1] [1]Institute of Cell Biology and Morphology, [2]Regeneration Research Laboratory, Bulgarian Academy of Science, Sofia: Ribosome assembly and processing in rat hepatocytes upon D-galactosamine transcription block.

Sequence Requirements for Processing

Musters, W., Klootwijk, J., Planta, R.J., Biochemisch Laboratorium, Vrije Universiteit, Amsterdam, The Netherlands: Mutational analysis of yeast rDNA.

Kass, S.,[1] Craig, N.,[2] Sollner-Webb, B.,[1] [1]Dept. of Biological Chemistry, Johns Hopkins School of Medicine, [2]Dept. of Biological Science, University of Maryland, Baltimore: Sequences required for processing of mouse rRNA.

Eichler, D.C., Shumard, C.M., Dept. of Biochemistry, University of South Florida College of Medicine, Tampa: Limited cleavages of mouse pre-rRNA by a nucleolar endoribonuclease include the early +650 processing site.

Maroney, P.A.,[1] Hannon, G.,[1] Branch, A.,[2] Robertson, H.D.,[2] Nilsen, T.W.,[1] [1]Dept. of Molecular Biology and Microbiology, Case Western Reserve University School of Medicine, Cleveland, Ohio; [2]Rockefeller University, New York, New York: Accurate processing of human pre-rRNA in vitro.

Intracellular Transport

Underwood, M., Bataille, N., Fried, H., Dept. of Biochemistry, University of North Carolina, Chapel Hill: Intracellular trafficking of ribosomal components.

Khanna-Gupta, A., Ware, V.C., Dept. of Biology and Center for Molecular Bioscience and Biotechnology, Lehigh University, Bethelehem, Pennsylvania: Inhibition of ribosome nucleocytoplasmic transport using a synthetic DNA oligomer complementary to a conserved region in eukaryotic 28S rRNA.

Allison, L.A., Bakken, A.H., Dept. of Zoology, University of Washington, Seattle: Nucleocytoplasmic transport of stored 5S RNA during ribosome biogenesis in *X. laevis* oocytes.

D. Schlessinger, L. Lindahl

SESSION 10 RIBOSOMES AND DEVELOPMENT

Chairman: F. Amaldi, Universitá dé Roma "Tor Vergata"

McCutchan, T.F., Waters, A.P., NIAID, National Institutes of Health, Bethesda, Maryland: *Plasmodium* species have stage-dependent ribosomes.

Mariottini, P., Bagni, C., Amaldi, F., Dipt. di Biologia, Universitá di Roma, Italy: The 5′-untranslated region is involved in the translational control of mRNA for ribosomal protein S19 in *Xenopus* development.

Wormington, M., Keiper, B., O'Keefe, R., Varnum, S., Rosenstiel Center, Brandeis University, Waltham, Massachusetts: Translational control of ribosomal protein synthesis during oocyte maturation and the early development of *X. laevis.*

Steel, L.F., Jacobson, A., Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester: Posttranscriptional regulation of ribosomal protein gene expression during *Dictyostelium* development.

Patel, R., Tamate, H., Hongo, S., Riedl, A., Jacobs-Lorena, M., Dept. of Genetics, Case Western Reserve University, Cleveland, Ohio: Regulation of ribosomal protein mRNA translation during early development of *Drosophila.*

Pellegrini, M., Vallett, S.M., Weber, H.W., Dept. of Molecular Biology, University of Southern California, Los Angeles: Changes in rRNA synthesis in mitotic and nonmitotic *Drosophila* cells.

Bowman, L.H., Sun, C., Hammond, M.L., Dept. of Biology, University of South Carolina, Columbia: In vitro and in vivo studies of mouse ribosomal protein mRNA translation.

Thompson, E.A., Mahajan, P.B., Dept. of Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston: Hormonal regulation of transcription of rDNA.

Webb, M.L., Cavender, J.F., Jacob, S.T., Dept. of Pharmacology and Cell and Molecular Biology Center, Pennsylvania State University College of Medicine, Hershey: Regulation of ribosomal gene transcription by glucocorticoids.

Cell and Molecular Neurobiology of *Aplysia*

September 28—October 2

ARRANGED BY

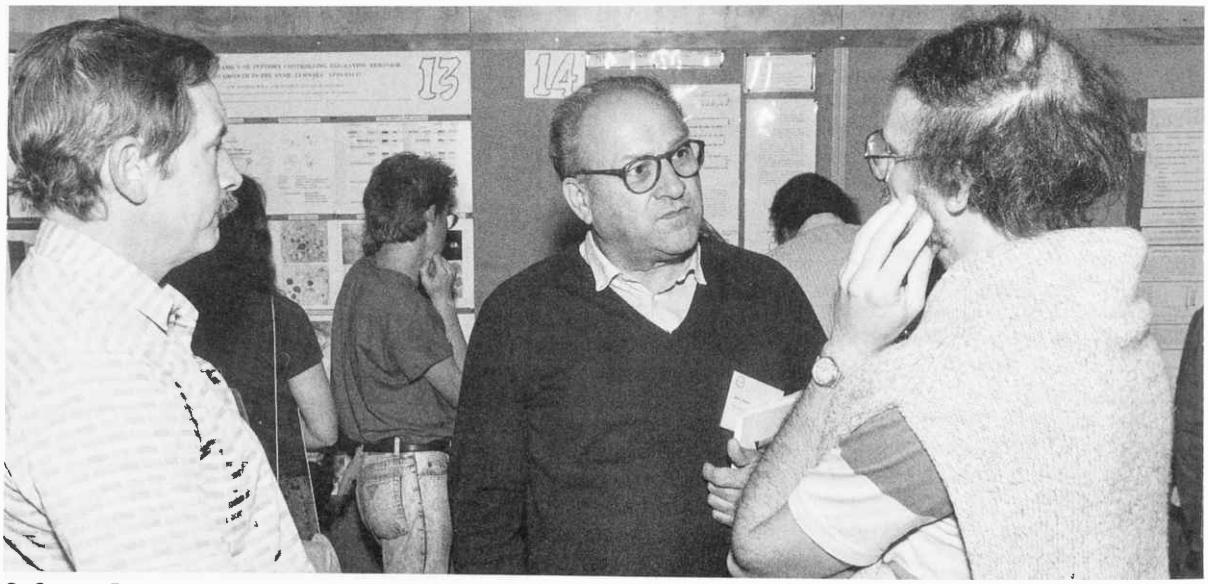
Hersch M. Gerschenfeld, Ecole Normale Supérieure
Leonard Kaczmarek, Yale University Medical School
Eric Kandel, Columbia University College of Physicians & Surgeons
Richard Scheller, Stanford University
Felix Strumwasser, Marine Biological Laboratory

111 participants

The first Cold Spring Harbor Laboratory meeting on Cell and Molecular Neurobiology of *Aplysia* featured presentations by an international group of speakers on a wide variety of topics, in which *Aplysia* has proven capable of providing new and general insights into neuroscience. These included (1) the use of giant growth cones of identified cells to study the dynamics of growth cone motility and intracellular organelle rearrangement using video-enhanced microscopy; (2) imaging of intracellular Ca^{++} fluxes in response to neuronal stimulation; (3) mechanisms for second messenger control of ion channel function and transmitter and hormone release; (4) molecular and cellular control of peptide production and processing; (5) analyses of complex neural circuitry and the control of animal behavior; (6) molecular studies of circadian rhythms; (7) cellular and molecular mechanisms for learning, and memory; and (8) neural development. It was exciting to hear presentations of such a high level, on a wide variety of topics within the context of a single organism, and in a small, intimate setting.

The meeting was funded in part by the National Science Foundation.

Welcoming Remarks: Eric R. Kandel



G. Cottrell, E. Roubos, J. Joosse

SESSION 1 CELL IMAGING AND NEURONAL GROWTH

Chairman: S.J. Smith, Yale School of Medicine

Goldberg, D.J., Burmeister, D.W., Dept. of Pharmacology and Center for Neurobiology and Behavior, Columbia University, New York, New York: How axons grow—Insights from high-resolution video microscopic observations of *Aplysia* growth cones.

Forscher, P., Smith, S.J., Howard Hughes Medical Institute, Yale School of Medicine, New Haven, Connecticut: Dynamic actin-microtubule interactions in regulation of neuronal growth cone structure and function.

Smith, S.J., Forscher, P., Howard Hughes Medical Institute, and Section of Molecular Neurobiology, Yale University School of Medicine, New Haven, Connecticut: Actions of cAMP on microtubules and actin filaments in growth cones of cultured bag cell neurons of *Aplysia*.

Guthrie, P.C., Kater, S.B., Dept. of Anatomy and Neurobiology, Colorado State University, Fort Collins: Control of growth cone behavior by classic integrative mechanisms—Neurotransmitters, electrical activity, and, ultimately, intracellular calcium.

Connor, J.A., AT&T Bell Laboratories, Murray Hill, New Jersey: Digital imaging of regional calcium changes in isolated *Aplysia* neurons.

Schacher, S., Center for Neurobiology and Behavior, Columbia University College of Physicians & Surgeons, New York, New York: Synapse formation and synapse specificity.

SESSION 2 POSTER SESSION

Single Channels and Channel Modulation

Smit, A.B., Geraerts, W.P.M., Dept. of Biology, Vrije Universiteit, Amsterdam, The Netherlands: Organization and expression of an insulin-related peptide gene family in growth-controlling neurons of *L. stagnalis*.

Kits, K.S., Lodder, J.C., Dept. of Biology, Vrije Universiteit, Amsterdam, The Netherlands: Modulation of Ca currents in growth hormone cells producing insulin-like peptides in *L. stagnalis*.

Keicher, E., Nicaise, G., Laboratoire de Cytologie Expérimentale, Université de Nice, France: Granule-containing glial cells in *Aplysia*—Are they involved in calcium regulation of the perineuronal extracellular spaces?

Brézina, V., Howard Hughes Medical Institute, Center for Neurobiology and Behavior, Columbia University, New York, New York: Toluene, benzene, and other hydrophobic solvents activate "S"(-like) K current in *Aplysia* neurons.

Peptides, Small Molecule Transmitters, and Synaptic Transmission

Berry, R.W., Dept. of Cell Biology and Anatomy, Northwestern University, Chicago, Illinois: Regulation of pro-ELH biosynthesis requires RNA synthesis.

Loechner, K., Azhderian, E., Kaczmarek, L.K., Dept. of Pharmacology, Yale University School of Medicine, New Haven, Connecticut: Release and action of autoactive peptides in bag cell neurons.

Roubos, E.W., van Heumen, W.R.A., Slootstra, J., Dept. of Biology Vrije Universiteit, Amsterdam, The Netherlands: Cellular dynamics of peptides controlling egg-laying behavior and growth in the snail *L. stagnalis*.

Linacre, A.M.T., Kellett, E., Burke, J.F., Benjamin, P.R., University of Sussex School of Biology, Falmer, Brighton, England: Structure of a gene encoding FMRF amide-like peptides in *L. stagnalis*.

Miller, M.W., Cropper, E.C., Alevizos, A., Tenenbaum, R., Kupfermann, I., Weiss, K.R., Center for Neurobiology and Behavior, Columbia University, New York, New York: Distribution of buccalin-like immunoreactivity in the central nervous system and peripheral tissues of *Aplysia*.

Cropper, E.C., Tenenbaum, R., Vllim, F.S., Kupfermann, I., Weiss, K.R., Center for Neurology and Behavior, Columbia University, New York, New York: Parabuccalin—A novel neuropeptide localized to cholinergic buccal motor neurons B15 and B16 of *Aplysia*.

Hooper, S.L., Weiss, K.R., Kupfermann, I., Center for Neurobiology and Behavior, Columbia University, New York, New York: Gut stimulation evokes a slow IPSP in histaminergic neuron C2, mediated by CCK-gastrin-like immunoreactive buccal neuron, B18.

Shapiro, E.,[1] Kretz, R.,[2] [1]Howard Hughes Medical Institute, Center for Neurobiology and Behavior, New York, New York; [2]Dept. of Anatomy and Special Embryology, University of Fribourg, Switzerland: Effects of membrane potential and activity pattern on presynaptic function.

Poulain, B., Fossier, P., Baux, G., Tauc, L., Laboratoire de Neurobiologie Cellulaire et Moléculaire, CNRS, France: Evidence for a modulation of acetylcholine release by autoreceptors at a central synapse of *Aplysia*.

Poulain, B.,[1] Tauc, L.,[1] Maisey, E.A.,[2] Wadsworth, J.D.F.,[2] Dolly, J.O.,[2] [1]Laboratoire de Neurobiologie Cellulaire et Moléculaire, CNRS, France; [2]Dept. of Biochemistry, Imperial College, London, England: *Aplysia* as model for the study of the mechanism of inhibition of cholinergic transmission by botulinum neurotoxin.

Hellmich, M.R.,[1] Navarro, J.V.,[2] Strumwasser, F.,[1] [1]Marine Biological Laboratory, Woods Hole, [2]Dept. of Physiology, Boston University School of Medicine, Massachusetts: Bacteria-toxin-catalyzed ADP-ribosylation of membrane and soluble components in tissues of *A. californica*.

Fredman, S.M.,[1] Rieke, G.A.,[2] Depts. of [1]Physiology, [2]Anatomy, Meharry Medical College, Nashville, Tennessee: Behavioral, physiological, and morphological effects of kainic acid in *Aplysia*.

SESSION 3 SINGLE CHANNELS AND CHANNEL MODULATION

Chairman: H. Gerschenfeld, Ecole Normale Superieure

Gerschenfeld, H.M., Hammond, C., Harris-Warrick, R., Paupardin-Tritsch, D., Laboratoire de Neurobiologie, Ecole Normale Supérieure, Paris, France: Recent studies on the neurotransmitter-induced regulation of voltage-dependent Ca current in snail neurons.

Volterra, A., Buttner, N., Siegelbaum, S.A., Center for Neurobiology and Behavior, Howard Hughes Medical Institute, Dept. of Pharmacology, Columbia University College of Physicians & Surgeons, New York, New York: Role of two G proteins in the up- and down-modulation of the S K^+ **channel.**

Brézina, V., Howard Hughes Medical Institute, Center for Neurobiology and Behavior, Columbia University, New York, New York: *Aplysia* neurons possess a family of G-protein-linked neurotransmitter receptors mediating both activation of "S"(-like) K current and suppression of Ca current.

Kehoe, J.S., Laboratoire de Neurobiologie, Ecole Normale Supérieure, Paris, France: cAMP-mediated synaptic responses in the medial neurons of *A. californica*.

Kaczmarek, L.M., Depts. of Pharmacology and Molecular and Cellular Physiology, Yale University School of Medicine, New Haven, Connecticut: Regulation of excitability in peptide-secreting bag cell neurons of *Aplysia*.

Levitan, I.B., Carrow, G.M., Lin, S., Dept. of Biochemistry, Brandeis University, Waltham, Massachusetts: Specificity and plasticity of synaptic connections between cultured *Aplysia* neurons.

SESSION 4 PEPTIDES, SMALL MOLECULE TRANSMITTERS, AND SYNAPTIC TRANSMISSION

Chairman: R. Scheller, Stanford University

Fossier, P., Baux, G., Tauc, L., Laboratoire de Neurobiologie Cellulaire et Moleculaire, CNRS, Paris, France: G proteins control quantal evoked acetylcholine release at a neuro-neuronal synapse in *Aplysia*.

Cottrell, G.A., Dept. of Biology and Preclinical Medicine, University of St. Andrews, Fife, Scotland: Multiple selective actions of molluscan peptides of the FMRFamide-series (FM/LRFamide and XDPFLRFamide) and their biological significance.

Joosse, J., Dept. of Biology, Vrije Universiteit, Amsterdam, The Netherlands: Central and peripheral expression of genes coding for (neuro-)peptides involved in control systems of reproduction, growth, energy metabolism, and ionic regulation.

Scheller, R., Fisher, J., Sossin, W., Newcomb, R., Dept. of Biological Sciences, Stanford University, California: Proteolytic processing and packaging of peptides from the *Aplysia* egg-laying hormone precursor.

Blankenship, J.E., Nagle, G., Painter, S., Kruger, T., Choate, J., Shope, S., Marine Biomedical Institute, Galveston, Texas: Roles of identified peptides in *Aplysia* reproduction.

Mayeri, E., Jansen, R., Brown, R.O., Dept. of Physiology, University of California, San Francisco: Diversity in the sets of currents modulated by egg-laying hormone and bag cell peptide in various target neurons of *Aplysia*.

Lloyd, P.E., Pearson, W.L., Connolly, C.M., Dept. of Pharmacology and Physiological Science and Communication on Neurobiology, University of Chicago, Illinois: Pedal peptide—A major peptide transmitter in *Aplysia*.

SESSION 5 POSTER SESSION

Circadian Rhythms

Khalsa, S.B.S., Block, G.D., Dept. of Biology, University of Virginia, Charlottesville: Role of calcium in phase shifting the ocular circadian pacemaker of the opisthobranch *Bulla*.

Ralph, M.R., Block, G.D., Dept. of Biology, University of Virginia, Charlottesville: cAMP and intracellular calcium regulation of circadian rhythms in *Bulla*.

Strack, S.,[1] Jacklet, J.,[1] Siwicki, K.,[2] Roshbash, M.,[1] Hall, J.,[2] [1]Dept. of Biology and Neurobiology Research Center, State University of New York, Albany; [2]Dept. of Biology, Brandeis University, Waltham, Massachusetts: Immunological evidence for a protein in the nervous system of *Aplysia* and *Bulla* similar to the *period* protein in *Drosophila*.

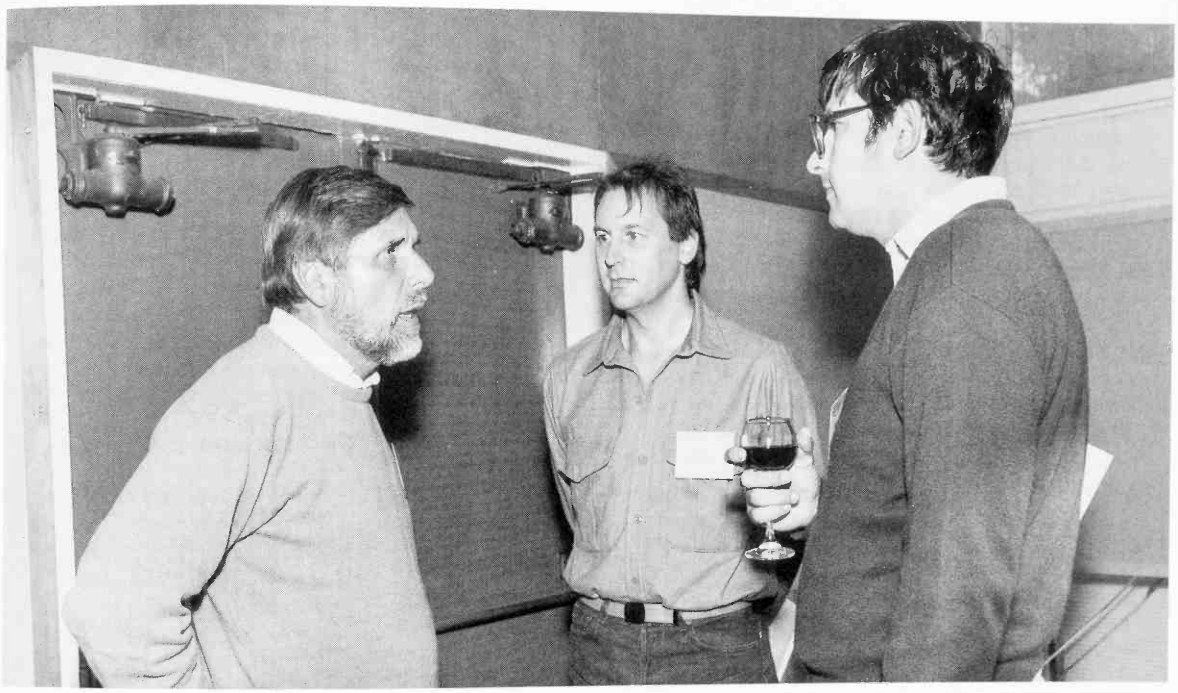
Neural Circuitry and Behavior

Koester, J., Center for Neurobiology and Behavior, New York State Psychiatric Institute and Columbia University, New York, New York: Modulation of *Aplysia* renal pore activity by L10, the LUQ cells, and an unidentified peripheral motor neuron.

Alevizos, A.,[1,2] Weiss, K.R.,[1] Koester, J.,[1] [1]Center for Neurobiology and Behavior, New York State Psychiatric Institute, [2]Dept. of Physiology and Cellular Biophysics, Columbia University, New York, New York: Modulation of respiratory pumping by cell R15.

Teyke, T., Weiss, K.R., Kupfermann, I., Center for Neurobiology and Behavior, Columbia University, New York, New York: Kinetics of the head-turning response of *Aplysia*.

Rosen, S.C., Miller, M.W., Weiss, K.R., Kupfermann, I.,



F. Strumwasser, B. Rothman, D. Glick

Center for Neurobiology and Behavior, Columbia University, New York, New York: Neural control of the initiation and maintenance of biting behavior in *Aplysia*.

Ter Maat, A., Ferguson, G.P., Pinsker, H.M., Marine Biomedical Institute, University of Texas, Galveston: *Aplysia* egg-laying behaviors—Neuronal and hormonal control in intact and freely behaving animals.

Ter Maat, A., Ferguson, G.P., Jansen, R.F., Pieneman, A.W., Dept. of Biology, Free University, Amsterdam, The Netherlands: Egg-laying behaviors in *L. stagnalis*.

Lukowiak, K., Higgins, A., Cawthorpe, D.R.L., Martinez-Padron, M., Neuroscience Research Group, Faculty of Medicine, University of Calgary, Alberta, Canada: Adaptive gill behaviors in *Aplysia*—Peptidergic modulation.

Leonard, J.L., Dept. of Zoology, University of Oklahoma, Norman: Six identified motor neurons in search of a function.

Fiore, L., Geppetti, L., Universita de Pisa, Italy: Cross-correlation-based evaluations of bidirectional impulse transmission in *Aplysia* cerebrobuccal connective.

Koike, H., Umitsu, Y., Matsumoto, H., Dept. of Neurophysiology, Tokyo Metropolitan Institute for Neurosciences, Japan: A nerve-contractor motoneuron in *Aplysia*.

Learning

Cook, D.G., Kuenzi, F.M., Carew, T.J., Depts. of Biology and Psychology, Yale University, New Haven, Connecticut: Operant conditioning of identified muscles and motor neurons in *Aplysia*.

Cleary, L.J.,[1] Hammer, M.,[1,2] Byrne, J.H.,[1] [1]Dept. of Neurobiology and Anatomy, University of Texas Medical School, Houston; [2]Institut für Neurobiologie, Frei Universität, Berlin, Federal Republic of Germany: Serotonin broadens action potentials in both somata and axons of pleural sensory neurons in *Aplysia*.

Marcus, E.A., Wright, W.G., Carew, T.J., Depts. of Biology and Psychology, Yale University, New Haven, Connecticut: Behavioral and cellular dissociation of multiple components of nonassociative learning in *Aplysia*.

Sweatt, J.D., Kandel, E.R., Columbia University College of Physicians & Surgeons and Howard Hughes Medical Institute, New York, New York: Serotonin (5-HT) causes a persistent increase in protein phosphorylation that is transcription-dependent—A molecular mechanism contributing to long-term sensitization in *Aplysia* sensory neurons.

Wager-Smith, K.,[1] Kandel, E.R.,[1,2] Sweatt, J.D.,[1,2] [1]Columbia University, [2]Howard Hughes Medical Institute, New York, New York: Subcellular location and partial amino acid sequence of proteins that are phosphorylated in *Aplysia* sensory neurons in response to serotonin.

Barzilai, A., Kennedy, T.E., Sweatt, J.D., Kandel, E.R., Columbia University College of Physicians & Surgeons and Howard Hughes Medical Institute, New York, New York: Serotonin (5-HT) induces changes in protein synthesis during the acquisition phase of long-term facilitation in the sensory neurons of *Aplysia*.

Kennedy, T.E., Kandel, E.R., Knapp, M., Sweatt, J.D., Columbia University College of Physicians & Surgeons and Howard Hughes Medical Institute, New York, New York: Characterization of a protein whose net rate of synthesis increases in association with long-term sensitization in *Aplysia*.

Zhao, B.,[1] Dugan, D.,[1,2] Kandel, R.E.,[1,2] Knapp, M.,[1] Columbia University College of Physicians & Surgeons, [2]Howard Hughes Medical Institute, New York, New York: Molecular cloning of highly abundant polyadenylated RNAs from *Aplysia* nervous system.

Baxter, D.A., Buonomano, D., Corcos, S., Patel, S., Byrne, J.H., Dept. of Neurobiology and Anatomy, University of Texas Medical School, Houston: Small networks of adaptive elements that reflect the properties of neurons in *Aplysia* exhibit higher-order features of classic conditioning.

Development of Synaptic Plasticity and Learning

Buriani, A., Savage, M.J., Goldberg, D.J., Dept. of Pharmacology and Center for Neurobiology and Behavior, Columbia University, New York, New York: Axotomy induces early changes in the incorporation of [^{35}S]methionine into proteins in the nucleus of the *Aplysia* neuron R2.

Glanzman, D.L.,[1,2] Schacher, S.,[2] Kandel, E.R.,[1,2] [1]Howard Hughes Medical Institute, [2]Columbia University College of Physicians & Surgeons, New York, New York: Morphology of *Aplysia* sensory neurons in dissociated cell culture alone and with target motor neurons—Correlates of synaptic specificity and evidence for synaptic competition.

Peretz, B., Dept. of Physiology and Biophysics, University of Kentucky Medical Center, Lexington: Age effects on long-term neuron function and its behavioral expressions in adult *Aplysia*.

SESSION 6 CIRCADIAN RHYTHM

Chairman: F. Strumwasser, Marine Biological Laboratory

Strumwasser, F., Marine Biological Laboratory, Woods Hole, Massachusetts: Two model neuropeptidergic systems controlling different behaviors in *Aplysia*.

Jacklet, J.,[1] Siwicki, K.,[2] Rosbash, M.,[2] Hall, J.,[2] [1]Dept. of Biology and Neurobiology Research Center, State University of New York, Albany; [2]Dept. of Biology, Brandeis University, Waltham, Massachusetts: Circadian pacemaker neurons in *Aplysia* and *Bulla* eyes—Membrane properties and *period*-like protein immunoreactivity.

Block, G.D., Khalsa, S.B.S., Ralph, M.R., McMahon, D.G., Dept. of Biology, University of Virginia, Charlottesville: Cellular analysis of phase and period regulation of a molluscan ocular circadian pacemaker.

Eskin, A., Yeung, S.J., Raju, U., Dept. of Biology, University of Houston, Texas: Identification of components of circadian timers in the eye of *Aplysia*.

SESSION 7 NEURAL CIRCUITRY AND BEHAVIOR

Chairman: J. Koester, Columbia University

Koester, J.,[1] Alevizos, A.,[1,2] Weiss, K.R.,[1] [1]Center for Neurobiology and Behavior, New York State Psychiatric Institute, [2]Dept. of Physiology and Cellular Biophysics, Columbia University, New York, New York: Generation and modulation of respiratory pumping.

Getting, P.A., Frost, W.N., Dept. of Physiology and Biophysics, University of Iowa, Iowa City: "Self-tuning" within a neural circuit—Are neural networks dynamically organized?

Gardner, D., Dept. of Physiology, Cornell University Medical College, New York, New York: Synaptic variability in 3- and 4-cell neural networks.

Spira, M.E., Segev, I., Werman, R., Yarom, Y., Dept. of Neurobiology, Hebrew University, Jerusalem, Israel: Experimental and theoretical analysis of the electrotonic structure of cultured *Aplysia* neurons.

Cohen, L., Hoepp, H.-P., Wu, J.-Y., Xiao, C., Zecevic, D., London, J., Dept. of Physiology, Yale University School of Medicine, New Haven, Connecticut: Optical measurement of neuron activity during the gill withdrawal reflex in *Aplysia*.

Kirk, M.D., Plummer, M.R., Cirenza, P.F., Rathouz, M.M., Church, P.J., Dept. of Biology, Boston University, Massachusetts: Premotor neurons B41 and B42 of the buccal ganglia—Morphology, bursting properties, synaptic connections, and responses to SCPB.

Kupfermann, I., Cropper, B.C., Rosen, S.C., Weiss, K.R., Center for Neurobiology and Behavior, Columbia University, New York, New York: Control and modulation of feeding behavior in *Aplysia*.

SESSION 8 LEARNING

Chairman: J. Byrne, University of Texas, Houston

Bailey, C.H., Chen, M., Center for Neurobiology and Behavior, Columbia University College of Physicians & Surgeons and New York State Psychiatric Institute, New York: Morphological basis of long-term memory in *Aplysia*.

Byrne, J.H., Scholz, K.P., Eskin, A., Dept. of Neurobiology and Anatomy, University of Texas Medical School, Houston; Dept. of Biology, University of Houston, Texas: Steps toward an understanding of the biochemical and biophysical basis of long-term sensitization.

Kandel, E.R., Barzilai, A., Kennedy, T., Sweatt, D., Columbia University College of Physicians & Surgeons, New York,

New York: Molecular similarities and differences between long-term and short-term memory for sensitization—Transcriptional function for modulatory transmitters important for learning.

Schwartz, J.H., Howard Hughes Medical Institute, Columbia University College of Physicians & Surgeons, New York, New York: Multiple second-messenger systems modulate facilatory and inhibitory processes in *Aplysia* sensory neurons.

Abrams, T.W., Goldsmith, B., Bolton, M., Dept. of Biology and Institute of Neurological Sciences, University of Pennsylvania, Philadelphia: Studies of additional mechanisms of stimulus convergence during activity-dependent facilitation in *Aplysia* sensory neurons.

Walters, E.T., Dept. of Physiology and Cell Biology, University of Texas Medical School, Houston: Functional significance of distributed cellular memory in circuits controlling defensive behavior of *Aplysia*.

Crow, T., Forrester, J., Dept. of Neurobiology and Anatomy, University of Texas Medical School, Houston: Possible role for protein kinase C in light–5-HT-induced enhancement of generator potentials in *Hermissenda* B photoreceptors.

SESSION 9 DEVELOPMENT OF SYNAPTIC PLASTICITY AND LEARNING

Chairman: T.J. Carew, Yale University

Carew, T.J., Depts. of Psychology and Biology, Yale University, New Haven, Connecticut: Developmental emergence of different forms of learning in *Aplysia.*

Sharma, J.S., Wiederhold, M.L., Division of Otorhinolaryngology, University of Texas Health Science Center, San Antonio: Development of the statocyst in *Aplysia.*

Bidwell, J.P., Howard Hughes Medical Institute, Woods Hole, Massachusetts: Artificial seawater and *A. californica.*

Chiel, H.J.,[1] Tank, D.W.,[2] [1]Dept. of Biology, Case Western Reserve University, Cleveland, Ohio; [2]Dept. of Molecular Biophysics Research, AT&T Bell Laboratories, Murray Hill, New Jersey: Analysis of presynaptic inhibition using *Aplysia* neurons in culture.

Zucker, R.S., Dept. of Physiology/Anatomy, University of California, Berkeley: Calcium-activated currents in *Aplysia* neurons studied with "caged calcium" chelators.

DesGroseillers, L., Dept. of Biochemistry, University of Montreal, Canada: Molecular cloning of the genes encoding a LUQ-specific neuropeptide and a neutral endopeptidase-like enzyme.

E. Kandel, D. Gardner


BANBURY
CENTER

BANBURY CENTER DIRECTOR'S REPORT

Sixteen meetings, with over 500 participants, were held at the Banbury Center in 1988. The topics reflect the increasing diversity of Banbury Center meetings, with subjects drawn from research in "basic" science, biotechnology, human diseases, and the neurosciences.

HIV and AIDS

Discussions of AIDS featured strongly during the year. Research on the human immunodeficiency virus (HIV), the causative agent of AIDS, has shown that complex interactions between virus and host-cell factors govern expression of HIV genes. Controversial data have been accumulating about these interactions, and the **Control of HIV Gene Expression** meeting was one of the highlights of the Banbury year. Needless to say, the meeting did not resolve all of the issues, but at least the relevant parties met and an attempt was made to untangle the confusion of the names of the HIV genes. This came to fruition later in the year with the publication in *Nature* of a proposal for a standardized nomenclature.

Molecular Genetics and Human Inherited Diseases

Two meetings dealt with the molecular biology and genetics of human disorders. The **Genetic Approaches in Schizophrenia** meeting was particularly exciting as it brought together expert geneticists and psychiatrists who have been studying the genetics of schizophrenia. The aim of the meeting was to determine if the armamentarium of molecular genetics that has been applied so successfully in other human inherited disorders can be applied to schizophrenia. However, it became clear during the course of the meeting that so little is known about the underlying biology of schizophrenia, and its definitive diagnosis is so difficult, that more patient and family studies are required.

The **Molecular Biology of Alzheimer's Disease** meeting showed that research on this disease is at a more satisfactory stage. This meeting included a remarkable group of studies ranging from neuroanatomical genetics, through protein chemistry, to molecular genetics. In a particularly exciting session, it was shown that β-amyloid protein is not a constituent of the paired helical filaments that accumulate in the tangles and plaques that are characteristic of Alzheimer's disease. In addition to evaluating data, meeting participants discussed the ethics of performing early autopsies to obtain brain samples from patients. The overwhelming consensus of the participants was that these procedures are ethical and provide invaluable data.

Technical Developments in Molecular Biology

The Banbury Center is noted for meetings dealing with the technical aspects of molecular biology, and another meeting in the **Viral Vectors** "series" was held in March. Not suprisingly, the topic of retroviruses as vectors dominated the meeting, but there were presentations dealing with small DNA viruses such as bovine papillomavirus and vectors for expression such as the insect baculoviruses.

During the past two years, a new technique called the polymerase chain reaction (PCR) has been sweeping through molecular biology laboratories. The technique enables very large quantities of specific DNA sequences to be generated from very small starting amounts of DNA. Perkin-Elmer Cetus sponsored the **Polymerase Chain Reaction** meeting, which brought a group of the world's leading exponents of the technique to the Banbury Center to discuss the latest, novel uses of the technique. PCR applications are very diverse and it became clear during the meeting that they are limited only by the ingenuity of the research scientist!

Another meeting dealing with the molecular biology techniques was that on **DNA Technology and Forensic Science**. Participants included forensic scientists, population geneticists, representatives of law enforcement agencies, prosecution and defense attorneys, ethicists, and civil libertarians. There were many energetic exchanges on the whole range of problems inherent in the introduction of DNA evidence into the legal system. The meeting set out these issues in a clear and forthright way for further debate in the legal and forensic communities.

Linkage analysis using restriction-fragment-length polymorphisms (RFLPs) has revolutionized human genetics, and the meeting on **Molecular Markers and Their Application to Problems in Plant Genetics** showed that RFLPs are likely to have a similar impact in plant genetics. Detailed RFLP maps are being prepared for a number of agriculturally important crops, and RFLPs linked with quantitative trait loci should improve the efficiency of breeding programs.

Topics in Basic Research

Ubiquitin is a small, highly conserved protein that, as its name implies, seems to take part in many processes in the cell. **The Ubiquitin System** was the subject of a meeting in Spring 1988, at which the molecular genetics of ubiquitin and its structure were discussed, as well as its role in protein degradation.

One of the most popular meetings of the year was **Cell Cycle Control in Eukaryotes**. An interesting feature of this meeting was the wide range of organisms that has been selected for study of cell-cycle control. The techniques of modern molecular biology provide tools for getting to the basic features of cell-cycle control, for example, by selecting genes that are activated or proteins that are synthesized when cells are stimulated to enter mitosis.

Sloan Foundation Workshops

The Workshops for science journalists and Congressional staff workers continue to be very successful in introducing the two groups to important scientific issues. The **AIDS Update** for the Congressional staff was, not surprisingly, very popular. The speakers included the leading people in the field, covering the molecular biology of the human immunodeficiency virus, epidemiology, and drug treatments. One of the highlights of the meeting was a discussion of the social impact of AIDS.

The continuing interest of the Press in molecular biology, especially in relation to human health, was evident from the 20 journalists who came to the workshop on the **Impact of DNA Technology in Medicine** in March. The topics of the meeting included DNA diagnosis, gene therapy, and AIDS. At least three of the journalists wrote stories using the meeting as background, including a front-page story in the *Wall Street Journal*.

The Baring Brothers/CSHL Conference

For the third year, the Banbury Center was host to a meeting for senior business executives, this year organized in conjunction with Baring Brothers. The meeting attracted a record number of participants, including executives from companies in our Corporate Sponsor Program. The subject was **Manipulating the Immune Response**. Once again an outstanding group of scientists came, and they covered such topics as cytokines, catalytic antibodies, and *scid* mice for analysis of immune function. One afternoon was spent in the DNA Learning Center, where a group of industry's leaders enthusiasically tackled an experiment.

Other Meetings

We have continued to make the Center available to other groups when appropriate. The deans of the Associated Medical Schools of New York came for a study

weekend, reviewing once more the problems they face operating in a city like New York. In April, the Klingenstein Fund held a review of the neuroscience research supported by the Fund. In May, the Cold Spring Harbor School District brought parent leaders to the Banbury Center to discuss AIDS education. This fascinating meeting provided a perspective on AIDS that was very different from perspectives provided by the technical meeting on HIV gene expression. Here, a small community was trying to come to grips with the complex and diverse responses of society to this disease. We have also continued to host seminars for the local community of Lloyd Harbor. These are presentations by members of nonprofit organizations in the village. The 1988–1989 meetings season began in October, when I spoke on genes and cancer, and in December, Don Nilson of Friends World College spoke on differences between American and Japanese cultures.

Funding

The Corporate Sponsor Program once again underpinned the meetings program, providing funding for five meetings, and support for the meeting on **Cytoskeletal Proteins in Tumor Diagnosis** came from the James McDonnell Foundation. In addition, companies and federal sources provided $120,000 in contributions for the support of other meetings. Particularly noteworthy was the meeting on **Polymerase Chain Reaction**, sponsored entirely by Perkin-Elmer Cetus. A number of companies, including five Japanese companies, underwrote the costs of another very successful **Therapeutic Peptides** meeting. As a result, we were very pleased to welcome a larger than usual number of Japanese scientists to the meeting. This proved to be one of the special features of this meeting, and I hope that we will be able to increase Japanese participation in other meetings. However, this cannot be done without support because of the high costs of travel between Japan and the United States.

A number of plant biotechnology companies contributed to the cost of the plant molecular markers meeting, and many of these companies have expressed an interest in supporting a similar meeting in 1989. Five biotechnology companies helped to underwrite the meeting on the **Control of Gene Expression in HIV**, and the success of the meeting was instrumental in obtaining a contract from the National Institute for Allergic and Infectious Diseases to hold two meetings on HIV/AIDS in 1989. I hope that this will be extended for a three-year period. Three companies applying "DNA fingerprinting," together with one of the National Institutes, contributed to the **DNA Technology and Forensic Science** meeting. Unusually for Cold Spring Harbor Laboratory, this was *not* one of the National Institutes of Health, but the National Institute of Justice!

Full details of support for Banbury Center meetings are listed under "Grants and Contributions." I am very encouraged by the positive responses of companies to requests for support for meetings, although my goal is to try to obtain some longer-term funding for meetings in specific areas. These responses show that we are holding meetings that deal with exciting scientific research and that also have wider implications for society.

Funding for Congressional and Science Journalist Workshops

The Sloan Foundation began to support the Banbury Center program as long ago as August 1978. That the Foundation has given us funding for ten consecutive years is an indication of the success and importance of this workshop series. The present grant will fund two further meetings to be held in 1989, but I am very pleased to report that the Sloan Foundation has approved a further three years of funding for this program. One feature that we are going to introduce into the next series of meetings is a laboratory session at the DNA Learning Center. We hope that doing even a simple experiment using restriction endonucleases and running gels will give the participants a better appreciation of molecular biology research.

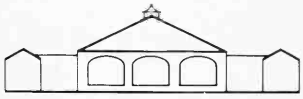
Banbury Center Publications

These publications are now the responsibility of Cold Spring Harbor Laboratory Press, but I want to mention that a more flexible policy regarding the style of books has been implemented and their publication has been speeded up. Meetings may be published in the **Current Communications in Molecular Biology**

series, the **Banbury Report** series, or as individual volumes. The **Control of Gene Expression in HIV** meeting was the first to be published as a special volume, and it appeared within five months of the meeting. We expect the books based on the **Polymerase Chain Reaction** and the **DNA Technology and Forensic Science** meetings to be firsts in their fields.

The Banbury Center Logo

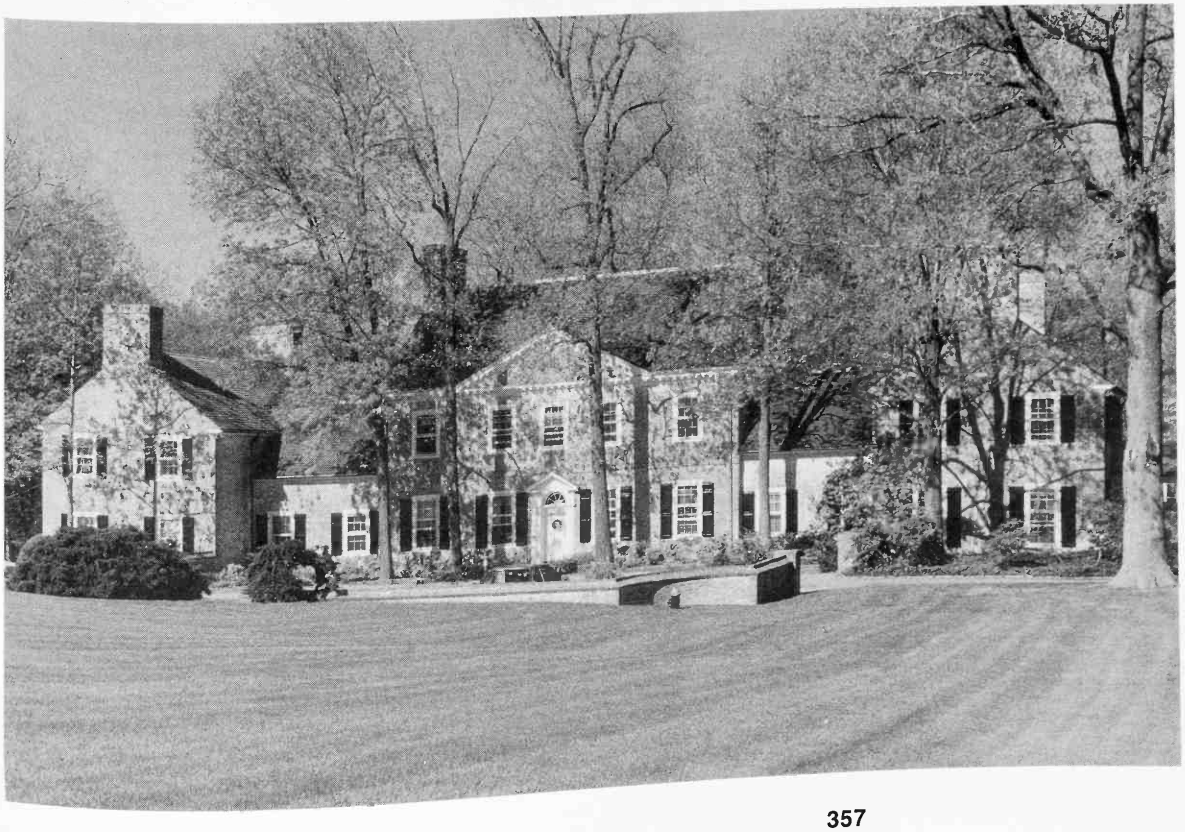
To make the activities of the Center known more widely, we have designed a logo, a stylized view of the Conference Center, that will appear on the title page of our publications.



Looking Forward to 1989

I remarked in last year's Annual Report that the Banbury Center program would become more diverse and deal with an increasing number of topics in the areas of biotechnology, human diseases, and the social impact of modern biology. This change in emphasis was evident in the 1988 program and will continue in 1989. There will be meetings dealing with "basic" research (recessive oncogenes; early development in *Drosophila* and mouse; molecular biology and evolution), environmental issues (germ-line mutations), biotechnology (genetic engineering of livestock), and social issues (alcoholism; scientific misconduct).

Robertson House provides housing and dining accommodations at Banbury Center



Conclusion

I have been at the Banbury Center for just over one year, and I am finding it to be as enjoyable and fascinating as I had expected. I was enthusiastic about the aims of the Banbury Center before I arrived, and my experience with the variety of topics and the enthusiasm of participants demonstrates that the Banbury Center is a unique resource for exchanging scientific information. Bea Toliver, the Center's administrative assistant, together with Barbara Fischer and Eleanor Sidorenko, and Katya Davey at Robertson House, worked hard, often under considerable pressure, to ensure that our program was implemented smoothly. All the indications are that 1989 at the Banbury Center will be as successful, exciting, and innovative as 1988.

Jan Witkowski

Publications

Caskey, C.T., R.A. Gibbs, J.A. Witkowski, and J.F. Hejtmancik. 1988. Diagnosis of human inheritable defects by recombinant DNA. *Phil. Trans. R. Soc. B* **319:** 353–360.

Hejtmancik, J.F., J.A. Witkowski, S. Gunnel, S. Davis, L. Baumbach, and C.T. Caskey. 1988. Prenatal and carrier detection of Duchenne muscular dystrophy using recombinant DNA technology. In *Nucleic acid probes in diagnosis of human genetic diseases.* (ed. A.M. Willey), pp. 83–100. Alan R. Liss Inc., New York.

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. Complementary cDNA probes for the Duchenne muscular dystrophy locus demonstrate a previously undetectable deletion in a patient with dystrophic myopathy, glycerol kinase deficiency and congenital adrenal hypoplasia. *J. Clin. Invest.* (in press).

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.* (in press).

Witkowski, J.A. 1988. The molecular genetics of Duchenne muscular dystrophy: The beginning of the end? *Trends Genet.* **4:** 27–30.

Witkowski, J.A. 1988. The discovery of "split" genes: A scientific revolution. *Trends Biochem. Sci.* **13:** 110–113.

Witkowski, J.A. 1988. Fifty years on molecular biology's hall of fame. *Trends Biotechnol.* **6:** 234–243.

Witkowski, J.A. 1989. Huxley in the laboratory: Embracing inquisitiveness and widespread curiosity. In *Julian Huxley—Biologist and statesman of science* (ed. A. van Helden). (In press.)

Witkowski, J.A. 1989. Milestones in the development of DNA technology. In *DNA: From the crime lab. to the courtroom.* American Chemical Society. (In press.)

Witkowski, J.A. 1989. Dystrophin and Duchenne muscular dystrophy. *J. Child Neurol.* (in press).

Witkowski, J.A. and C.T. Caskey. 1988. Duchenne muscular dystrophy—DNA diagnosis in practice. *Curr. Neurol.* **8:** 1–36.

MEETINGS

Congressional Workshop on AIDS

January 28–January 30

ARRANGED BY

J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory, New York

SESSION 1

J.D. Watson, Cold Spring Harbor Laboratory, New York: Introduction.
I. Chen, University of California, Los Angeles: Molecular biology of HIV.
H. Jaffe, Centers for Disease Control, Atlanta, Georgia: Epidemiology of AIDS.
A. Stanley, Los Alamos National Laboratory, New Mexico: The future of the AIDS epidemic—Computer modeling.

SESSION 2

G.B. Scott, University of Miami School of Medicine, Florida: Pediatric aspects of HIV infections.
S. Broder, National Cancer Institute, Bethesda, Maryland: Combating HIV. I. Drug therapy.
J. Petricciani, U.S. Public Health Service, Washington, D.C.: Combating HIV. II. Vaccines.

SESSION 3

R. Stall, Rutgers University, New Brunswick, New Jersey: Combating HIV. III. Education and behavior.
M. F. Silverman, American Foundation for Research on AIDS, Los Angeles, California: Social aspects of the AIDS epidemic.

The Control of HIV Gene Expression

February 28–March 2

ARRANGED BY

R. Franza, Cold Spring Harbor Laboratory, New York
B.R. Cullen, Duke University Medical Center, Durham, North Carolina
F. Wong-Staal, National Cancer Institute, Bethesda, Maryland

SESSION 1: HIV *TRANS*-ACTING ELEMENTS

F. Wong-Staal, National Cancer Institute, Bethesda, Maryland: Mutagenesis of the *tat* and *trs* genes of an infectious HIV genome.

G.N. Pavlakis, NCI-Frederick Cancer Research Facility, Maryland: HIV regulation by viral *trans*-activators.

C.A. Rosen, Roche Institute of Molecular Biology, Nutley, New Jersey: Regulation of HIV gene expression by the *art* protein.

S. Venkatesan, National Institutes of Health, Rockville, Maryland: Properties of *tat* and 3′ *orf* mutants of HIV.

D. Capon, Genentech, Inc., South San Francisco, California: Regulation of HIV gene expression by the HIV-1 *tat* gene product.

B.R. Cullen, Duke University Medical Center, Durham, North Carolina: HIV *tat* gene function.

B.M. Peterlin, University of California, San Francisco: HIV-1 activation and *trans*-activation by the *tat* gene product.

A.P. Rice, Cold Spring Harbor Laboratory, New York: The use of adenovirus vectors to analyze HIV gene expression.

M. Rosenberg, Smith Kline & French Laboratories, King of Prussia, Pennsylvania: HIV *trans*-activation phenomenon and protease function.

E. Holland, Stanford University School of Medicine, California: Mutations in the *tar* region of HIV-1.

L. Montagnier, Institut Pasteur, Paris, France: Diversity and gene function of the human immunodeficiency viruses.

M. Emerman, Institut Pasteur, Paris, France: HIV-2 *tat*.

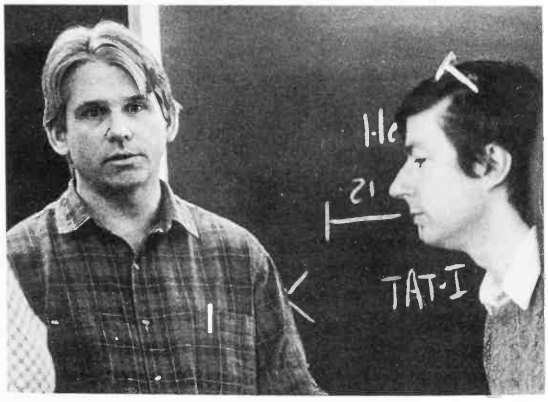
W. Haseltine, Dana-Farber Cancer Institute, Cambridge, Massachusetts: Regulation of replication of HIV-1.

R. Gallo, L. Montagnier

J. Brady

J. Clements

G. Pavlakis, B. Felber



B. Franza, T. Curran

SESSION 2: CELLULAR FACTORS INVOLVED IN RETROVIRAL GENE EXPRESSION

P.A. Baeuerle, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: Activation of NF-κB.

R. Franza, Cold Spring Harbor Laboratory, New York: Study of cellular proteins that interact with the HIV long terminal repeat.

T. Curran, Roche Institute of Molecular Biology, Nutley, New Jersey: *fos* and gene regulation.

W.C. Greene, Duke University Medical Center, Durham, North Carolina: HIV-1 and T-cell activation.

K.A. Jones, Salk Institute, San Diego, California: Analysis of the cellular transcription complex at the HIV promoters.

M.A. Norcross, U.S. Food and Drug Administration, Bethesda, Maryland: Characterization of HIV-1 enhancer-binding proteins.

R.G. Roeder, Rockefeller University, New York: Eukaryotic transcription factors and mechanisms.

J. Kadonaga, University of California, Berkeley: Promoter-selective activation of transcription by Sp1.

H.E. Varmus, University of California, San Francisco: Signals for the expression of the HIV *pol* gene by ribosomal frameshifting.

J.D. Mosca, Johns Hopkins Oncology Center, Baltimore, Maryland: Herpesvirus *trans*-activation—Role of HIV-1 *tat* in RNA stability.

SESSION 3: REGULATION OF GENE EXPRESSION IN RELATED RETROVIRUSES

I.S.Y. Chen, University of California, Los Angeles: Pathogenesis of HTLV/HIV.

J. Brady, National Institutes of Health, Bethesda, Maryland: HTLV-I gene regulation.

B. Felber, NCI-Frederick Cancer Research Facility, Maryland: Regulation of viral and cellular promoters by the transcriptional activator of HTLV-I.

J.E. Clements, Johns Hopkins Hospital, Baltimore, Maryland: *trans*-Activation of visna virus—A neurotropic lentivirus of sheep.

F. Wong-Staal, National Cancer Institute, Bethesda, Maryland: Meeting summary.

Journalists' Workshop on "The Impact of DNA Technology in Medicine"

March 6–March 8

ARRANGED BY

J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory, New York

SESSION 1: THE "NEW" GENETICS

J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory, New York: Introduction.

P. Ward, Institute for Molecular Genetics, Baylor College of Medicine, Houston, Texas: Duchenne muscular dystrophy—DNA diagnosis in practice.

J. Gitschier, Howard Hughes Medical Institute, University of California, San Francisco: Hemophilia—State-of-the-art DNA diagnosis.

M. Furth, Oncogene Science Inc., Manhasset, New York: Cancer diagnosis.

SESSION 2: GENE THERAPY AND DNA DIAGNOSIS

F. Ledley, Howard Hughes Medical Institute, Baylor College of Medicine, Houston, Texas: Gene therapy—Reality and future promise.

J. Sninsky, Cetus Corporation, Emeryville, California: DNA probes in the diagnosis of acquired diseases.

D.J. Green, Cellmark Diagnostics, Germantown, Maryland: DNA "fingerprinting"—What it is and what it does.

SESSION 3: SOCIAL AND ETHICAL CONSEQUENCES

R. Myers, Boston University Medical Center, Massachusetts: DNA diagnosis in Huntington's chorea.

L. Andrews, American Bar Foundation, Chicago, Illinois: Genetic testing—Protecting the individual.

Viral Vectors

March 13–March 16

ARRANGED BY

Y. Gluzman, Lederle Laboratories, Pearl River, New York
S.H. Hughes, NCI-Frederick Cancer Research Facility, Maryland

SESSION 1

B. Moss, National Institutes of Health, Bethesda, Maryland: Vaccinia virus and vaccinia virus/baceriophage T7 hybrid vectors.

B. Roizman, University of Chicago, Illinois: Genetic engineering of herpes simplex viruses for use as vaccines and vectors.

L. Post, Upjohn Company, Kalamazoo, Michigan: Pseudorabies virus—A possible vector for vaccines in livestock animals.

E.S. Mocarski, Stanford University School of Medicine, California: Recombinant cytomegalovirus-based expression vectors.

B. Mason, Wyeth Laboratories, Inc., Philadelphia, Pennsylvania: Recombinant adenovirus as a vaccine.

K.L. Berkner, ZymoGenetics, Seattle, Washington: Efficiency of translation of polycistronic messages in uninfected and adenovirus-infected cells.

SESSION 2

E.A. Dzierzak, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: In vivo expression of a normal cellular human β-globin gene transduced via retroviral infection of murine bone marrow.

E. Gilboa, Memorial Sloan-Kettering Cancer Center, New York, New York: Retroviral gene transfer—Applications to human therapy.

A.D. Miller, Fred Hutchinson Cancer Research Center, Seattle, Washington: Retrovirus-mediated gene transfer into skin fibroblasts.

J. Ellis. Mt. Sinai Hospital Research Institute. Toronto. Canada: Gene targeting with retroviral vectors.

R. Cone, Cold Spring Harbor Laboratory. New York: Establishment of differentiated cell lines using retroviral vectors.

SESSION 3

D. DiMaio, Yale University School of Medicine, New Haven, Connecticut: Bovine papilloma genetics—Implications for vector design.

S. Goodbourn, Imperial Cancer Research Fund Laboratories, London, England: Use of a bovine papillomavirus vector to study gene expression.

W. Hammerschmidt, McArdle Laboratory for Cancer Research, Madison, Wisconsin: Viral vectors derived from Epstein-Barr virus.

R.F. Margolskee, Roche Institute of Molecular Biology,

Nutley, New Jersey: Epstein-Barr virus shuttle vectors for stable episomal replication of cDNA expression libraries in human cells.

M. Manos, Cetus Corporation, Emeryville, California: Use of an SV40/adenovirus recombinant in an inducible mammalian expression system.

R. Kaufman, Genetics Institute, Cambridge, Massachusetts: The role of eIF-2 α phosphorylation in translational control in transfected and adenovirus-infected cells.

N. Muzyczka, State University of New York, Stony Brook: The genetics of adeno-associated virus.

SESSION 4

S.H. Hughes, NCI-Frederick Cancer Research Facility, Maryland: Retroviral vectors and adaptors.

J.M. Coffin, Tufts University School of Medicine, Boston, Massachusetts: Effect of antisense RNA on retrovirus replication.

M. Linial, Fred Hutchinson Cancer Research Center, Seattle, Washington: Retrofection—Reverse transcription and integration of nonretroviral RNAs after viral infection.

R. Dornburg, University of Wisconsin, Madison: A retroviral vector system to study the formation of cDNA genes.

A.J. Kingsman, University of Oxford, England: Exploitation of a retrotransposon to produce polyvalent particulate proteins.

H. Piwnica-Worms, Dana-Farber Cancer Institute, Boston, Massachusetts: Interactions between $pp^{60c\text{-}src}$ and the middle T antigen of polyomavirus in insect cells.

SESSION 5

P. Ahlquist, University of Wisconsin, Madison: Plant RNA virus gene expression vectors.

D.M. Bisaro, Ohio State University, Columbus: Genetic analysis of tomato golden mosaic virus.

J. Futterer, Friedrich-Miescher-Institut, Basel, Switzerland: Transient expression from CaMV signals in plant protoplasts.

S. Schlesinger, Washington University School of Medicine, St. Louis, Missouri: Development of Sindbis virus and defective-interfering RNAs as expression vectors.

M.D. Summers, Texas A&M University, College Station: Baculovirus-directed foreign gene expression.

N.C. Jones, Imperial Cancer Research Fund Laboratories, London, England: Overproduction of E1A, human EGF receptor, and protein kinase proteins in the baculovirus expression system—Functional characterization of purified proteins.

Cell Cycle Control in Eukaryotes

March 20–March 23

ARRANGED BY

D. Beach, Cold Spring Harbor Laboratory, New York
C. Basilico, New York University Medical Center, New York
J. Newport, University of California, San Diego, La Jolla

SESSION 1

S. Reed, Research Institute of Scripps Clinic, La Jolla, California: Control of cell division in *S. cerevisiae*.

F. Cross, Fred Hutchinson Cancer Research Center, Seattle, Washington: Size control in *S. cerevisiae*.

K. Matsumoto, DNA Research Institute, Palo Alto. California: Cell cycle control within the G_1 phase of *S. cerevisiae*.

C. Basilico, New York University Medical Center, New York: Cloning of cell cycle genes.

N. Heintz, Rockefeller University, New York, New York: Factors controlling histone gene expression during the cell cycle.

J. Roberts, Fred Hutchinson Cancer Research Center, Seattle, Washington: Regulation of DNA replication.

T. Roberts, Dana-Farber Cancer Institute, Boston, Massachusetts: Oncogenes and signal transduction.

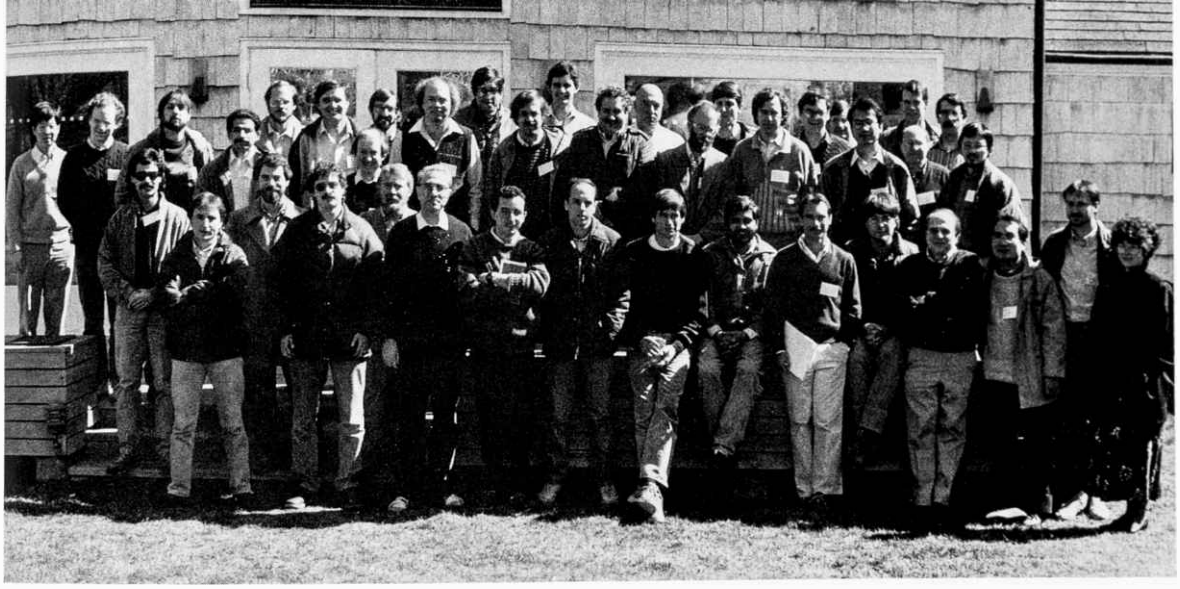
SESSION 2

H.L. Ozer, Hunter College, CUNY, New York, New York: Studies with mammalian cell mutants temperature-sensitive for cell and viral DNA synthesis.

B. Stillman, Cold Spring Harbor Laboratory, New York: Cellular proteins required for multiple stages of DNA replication.

W. Earnshaw, Johns Hopkins University School of Medicine, Baltimore, Maryland: Synthesis, stability, and modification of DNA topoisomerase II across the eukaryotic cell cycle.

R. Laskey, CRC Molecular Embryology Group, Cambridge, England: Control of DNA replication in *Xenopus* egg extracts.



D.M. Glover, Imperial College of Science and Technology, London, England: Mitosis in *Drosophila*.

P. O'Farrell, University of California, San Francisco: Programming spatial patterns of gene expression and cell division times in early *Drosophila* embryos.

T. Nishimoto, Kyushu University, Maidashi, Fukuoka, Japan: Identification of human RCCI protein and the possible involvement of ubiquitin for onset of chromosome condensation.

SESSION 3

D. Beach, Cold Spring Harbor Laboratory, New York: Control of mitosis by the *cdc2* protein kinase in fission yeast and human cells.

N.R. Morris, UMDNJ-Robert Wood Johnson Medical School, Piscataway, New Jersey: Regulation of mitosis in *Aspergillus nidulans*.

L.H. Hartwell, University of Washington, Seattle: *RAD9* controls the G_2 transition in *S. cerevisiae*.

T. Hunt, University of Cambridge, England: Role of cyclin synthesis and destruction in meiotic and mitotic cell cycles in eggs and oocytes.

J. Ruderman, Duke University, Durham, North Carolina: Mitotic cyclins and the cell cycle in early embryos.

W. Dunphy, University of California, San Diego: A yeast *cdc* gene product regulates mitotic conversion in *Xenopus* egg extracts.

J.L. Maller, University of Colorado School of Medicine, Denver: Purification and characterization of maturation-promoting factor from *Xenopus* eggs.

D. Chelsky, E.I. duPont de Nemours & Company, Wilmington, Delaware: Lamin B methylation and assembly.

Session 4

J.S. Hyams, University College, London, England: The fission yeast cytoskeleton and cell-cycle control.

B. Byers, University of Washington, Seattle: Regulation of the spindle pole body in budding yeast.

M. Yanagida, Kyoto University, Japan: Genetic control of mitotic anaphase—Association and dissociation of sister chromatids in cell cycle.

W.Z. Cande, University of California, Berkeley, California: Regulation of anaphase spindle elongation in vitro.

D. Vandre, Southern Methodist University, Dallas, Texas: Phosphorylation state of microtubule organizing centers—Regulation of activity during mitosis.

K. Hennessy, Massachusetts Institute of Technology, Cambridge, Massachusetts: Characterization of new cell cycle mutants.

S. Jentsch, Massachusetts Institute of Technology, Cambridge, Massachusetts: Ubiquitin-ligation system of *S. cerevisiae*.

C.D. Stiles, Dana-Farber Cancer Institute, Boston, Massachusetts: The role of PDGF-inducible genes in the mitogenic response of fibroblast cells.

D. Nathans, Johns Hopkins University School of Medicine, Baltimore, Maryland: The genomic response to growth factors.

R. Franza, Cold Spring Harbor Laboratory, New York: Fos complex interacts with control elements that contain an AP1 site.

R. Bravo, European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: Complexity of the genetic early response to growth factors in mouse fibroblasts.

R. Baserga, Temple University School of Medicine, Philadelphia, Pennsylvania: Expression of growth-regulated genes.

E. Harlow, Cold Spring Harbor Laboratory, New York: Protein complexes between dominant and recessive oncoproteins.

E. Ziff, New York University Medical Center, New York: Gene regulation by growth factors and oncogenes.

The Ubiquitin System

March 27–March 30

ARRANGED BY

M.J. Schlesinger, Washington University School of Medicine, St. Louis, Missouri
A. Hershko, Technion-Israel Institute of Technology, Haifa, Israel

SESSION 1: STRUCTURE, CHEMISTRY, BIOSYNTHESIS OF UBIQUITIN

Chairperson: I.A. Rose, Fox Chase Cancer Center, Philadelphia, Pennsylvania

W.J. Cook, University of Alabama, Birmingham: Crystal structure of ubiquitin.

D. Ecker, Smith Kline & French Laboratories, King of Prussia, Pennsylvania: Structures and functional activities of site-specific mutants of ubiquitin.

N. Agell, Washington University School of Medicine, St. Louis, Missouri: In vitro biosynthesis of ubiquitin-containing proteins.

V.A. Fried, St. Jude Children's Research Hospital, Memphis, Tennessee: The intrinsic proteolytic activity of ubiquitin.

T.R. Butt, Smith Kline & French Laboratories, King of Prussia, Pennsylvania: In vivo and in vitro activities of "engineered" ubiquitin conjugates.

K.D. Wilkinson, Emory University, Atlanta, Georgia: Structure and enzymology of ubiquitin-dependent systems.

SESSION 2: UBIQUITIN AND PROTEIN TURNOVER

Chairperson: M. Rechsteiner, University of Utah School of Medicine, Salt Lake City

A. Hershko, Technion-Israel Institute of Technology, Haifa: Selectivity of ubiquitin protein ligase system.

A. Ciechanover, Technion-Israel Institute of Technology, Haifa: Role of arginyl-tRNA-protein transferase in recognition of substrates of the ubiquitin system.

A. Varshavsky, Massachusetts Institute of Technology, Cambridge: The degradation signal in a short-lived protein.

I.A. Rose, Fox Chase Cancer Center, Philadelphia, Pennsylvania: Role of ubiquitin hydrolases in protein breakdown.

D.K. Gonda, Massachusetts Institute of Technology, Cambridge, Massachusetts: The N-end rule in a mammalian cell-free system.

V. Chau, Wayne State University School of Medicine, Detroit, Michigan: Is polyubiquitin the recognition signal?

C.M. Pickart, State University of New York, Buffalo: Mechanisms of inhibition by arsenite of ubiquitin-dependent proteolysis.

SESSION 3: UBIQUITIN GENES AND EXPRESSION

Chairperson: M.J. Schlesinger, Washington University School of Medicine, St. Louis, Missouri

D. Finley, Massachusetts Institute of Technology, Cambridge: Functional analysis of the yeast ubiquitin genes.
R.T. Baker, John Curtin School of Medical Research, Canberra, Australia: Structure and expression of the human ubiquitin gene family.
P.K. Lund, University of North Carolina, Chapel Hill: Expression of human ubiquitin genes.
H.L. Ennis, Roche Institute of Molecular Biology, Nutley, New Jersey: Structure of *Dictyostelium discoideum* ubiquitin genes and their regulation during development.
A. Muller-Taubenberger, Max-Planck-Institute for Biochemistry, Martinsried, Federal Republic of Germany: Extended ubiquitin in *Dictyostelium*.
K. Gausing, University of Aarhus, Denmark: Structure and expression of ubiquitin genes in plants.
J.T. Lis, Cornell University, Ithaca, New York: Characterization of ubiquitin gene structure and expression in *Drosophila*.

SESSION 4: UBIQUITIN IN CELLULAR STRUCTURES

Chairperson: A. Varshavsky, Massachusetts Institute of Technology, Cambridge

W.M. Bonner, National Cancer Institute, Bethesda, Maryland: Metabolism of ubiquitinated histone 2A.
J.R. Davie, University of Manitoba, Winnipeg, Canada: Ubiquitinated histones—H2B is preferentially located in transcriptionally active chromatin.
A.L. Haas, Medical College of Wisconsin, Milwaukee: Ubiquitin pools in skeletal muscle.
E. Fryberg, Johns Hopkins University, Baltimore, Maryland: An actin-ubiquitin conjugate in insect flight muscle.
M. van de Rijn, Stanford University School of Medicine, California: Biosynthesis of Mel-14.

SESSION 5: PROTEOLYSIS

Chairperson: A. Ciechanover, Technion-Israel Institute of Technology, Haifa

R.D. Vierstra, University of Wisconsin, Madison: Ubiquitin proteolytic pathway in higher plants.
M. Rechsteiner, University of Utah School of Medicine, Salt Lake City: Ubiquitin/ATP-dependent proteases.
R.G. Kulka, Hebrew University of Jerusalem, Israel: Ubiquitin conjugation patterns in ubiquitin system mutants.
A.L. Goldberg, Harvard Medical School, Boston, Massachusetts: ATP-dependent proteases.
J.F. Dice, Tufts University School of Medicine, Boston, Massachusetts: Lysosomal pathways of protein degradation.
G.N. DeMartino, University of Texas, Dallas: Ubiquitin-mediated and ubiquitin-independent pathways of intracellular proteolysis.
M.R. Maurizi, National Cancer Institute, Bethesda, Maryland: Regulatory functions of ATP-dependent proteases in *E. coli*.

Genetic Approaches to Schizophrenia

April 17–April 20

ARRANGED BY

L. Delisi, State University of New York, Stony Brook
F. Henn, State University of New York, Stony Brook
D. Housman, Massachusetts Institute of Technology, Cambridge
H. Pardes, New York State Psychiatric Institute, New York

SESSION 1: CLINICAL ISSUES RELEVANT TO THE GENETICS OF SCHIZOPHRENIA

Chairperson: H. Pardes, New York State Psychiatric Institute, New York

J.D. Watson, Cold Spring Harbor Laboratory, New York: Introduction.
F. Henn, State University of New York, Stony Brook: The clinical nature of the disease process—Problems of diagnosis and heterogeneity.
Discussion

SESSION 2: CLINICAL GENETICS OF SCHIZOPHRENIA

Chairperson: H. Pardes, New York State Psychiatric Institute, New York

K.S. Kendler, Medical College of Virginia, Virginia Commonwealth University, Richmond: Is there a genetic component in schizophrenia?
Discussion

SESSION 3: REVIEW OF CURRENT FAMILY AND RFLP STUDIES

Chairperson: **F. Henn,** State University of New York, Stony Brook

T. Bishop, University of Utah, Salt Lake City: Applying molecular genetic strategies to the study of schizophrenia—What is needed.

Discussion

SESSION 4: IS A MOLECULAR GENETICS OF SCHIZOPHRENIA POSSIBLE?

Chairperson: **F.S. Collins,** University of Michigan Medical School, Ann Arbor

Overviews: (1) from a psychiatrist; (2) from a linkage expert; (3) from a molecular biologist.

Discussion

SESSION 5: WHAT ARE THE BIOLOGICAL PHENOTYPES OF SCHIZOPHRENIA?

Overviews on various topics in neuroanatomy and neurochemistry.

Discussion

The Molecular Biology of Alzheimer's Disease

April 24–April 27

ARRANGED BY

C.F. Finch, University of Southern California, Los Angeles
P. Davies, Albert Einstein College of Medicine, Bronx, New York

SESSION 1: NEUROCHEMISTRY AND NEUROANATOMY

D.M. Bowen, Institute of Neurology, London, England: Absence of both hypometabolism and widespread loss of pyramidal neurones antemortem?

P. Davies, Albert Einstein College of Medicine, Bronx, New York: Further studies of A68.

C.E. Finch, University of Southern California, Los Angeles: Cloning for mRNAs that have regionally selective alterations in Alzheimer's disease.

F. Hefti, University of Miami, Florida: Nerve growth factor reestablishes several cholinergic pathways—Implications for Alzheimer's disease.

R. Reeves, Johns Hopkins University School of Medicine, Baltimore, Maryland: An animal model for studies of Down's syndrome and Alzheimer's disease.

S.I. Rapoport, National Institute on Aging, Bethesda, Maryland: Is Alzheimer's a phylogenetic disease?

P.D. Coleman, University of Rochester Medical Center, New York: Growth-associated protein (GAP-43) in Alzheimer's disease.

F.H. Gage, University of California, San Diego: Effects of NGF on cholinergic neurons in the central nervous system.

P.L. McGeer, University of British Columbia, Vancouver, Canada: Immune system response in Alzheimer's disease.

J. Rogers, Institute for Biogerontology Research, Sun City, Arizona: Neuroimmunology of Alzheimer's disease.

G.A. Higgins, University of Rochester Medical Center, New York: In situ hybridization of amyloid-β-protein mRNA transcripts in the hippocampal formation in Alzheimer's disease.

C.A. Miller, University of Southern California School of Medicine, Los Angeles: Neuronal specificity in Alzheimer's disease.

J.H. Morrison, Research Institute of Scripps Clinic, LaJolla, California: The cellular, laminar, and regional distribution of neurofilament protein and amyloid-β-protein mRNA in neocortex—Implications for Alzheimer's disease pathology.

D.L. Price, Johns Hopkins University School of Medicine, Baltimore, Maryland: Alzheimer's disease and animal models.

SESSION 2: MOLECULAR BIOLOGY OF PLAQUES AND TANGLES

K. Beyreuther, University of Heidelberg, Federal Republic of Germany: Alzheimer's disease and the amyloid gene product.

K. Goldgaber, National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, Maryland: The amyloid-β-protein precursor gene encodes a family of secreted polyprotein—A hypothesis.

S.A. Johnson, University of Southern California, Los Angeles: β-Amyloid gene expression and cellular localization in Alzheimer's disease brain.

C.L. Masters, University of Heidelberg, Federal Republic of Germany: Molecular basis of amyloidosis in Alzheimer's disease.

B. Muller-Hill, Institut fur Genetik der Universitat, Koln, Federal Republic of Germany: The precursor of Alzheimer's disease A4 protein.

R.L. Neve, The Children's Hospital, Boston, Massachusetts: Expression of Alzheimer amyloid precursor messenger RNAs in the developing adult brain.

D.J. Selkoe, Harvard Medical School, Brigham and Women's Hospital, Boston, Massachusetts: β-Amyloid precursor proteins—Regional CNS processing and comparison of PHF-related proteins.

R. Tanzi, Massachusetts General Hospital, Boston: Molecular genetics analysis of the APP gene.

H.M. Wisniewski, Institute for Basic Research in Developmental Disabilities, Staten Island, New York: β-Peptide precursor protein producing and processing cells.

A. Klug, C.M. Wischik, and Michael Goedert, MRC Laboratory of Molecular Biology, Cambridge, England: Structure and biochemistry of the Alzheimer tangle.

G. Dean, University of Cincinnati College of Medicine, Ohio: Untangling the insoluble—A characterization of Alzheimer's paired helical filaments.

SESSION 3: GENETICS

A.D. Roses, Duke University Medical Center, Durham, North Carolina: Linkage in late-onset Alzheimer's disease.

P.H. St. George-Hyslop, Massachusetts General Hospital, Boston: Molecular genetics of sporadic and familial Alzheimer's disease.

T.D. Bird, Seattle VA Medical Center, Washington: The clinical and neuropathological spectrum of familial Alzheimer's disease in 24 kindreds.

G.D. Schellenberg, University of Washington School of Medicine: Evidence for phenotypic heterogeneity in familial Alzheimer's disease.

R.C. Mohs, Veterans Administration Medical Center, Bronx, New York: Familial aggregation of Alzheimer's disease—Implications for genetic models.

S.B. Prusiner, University of California, San Francisco, School of Medicine: The formation of brain amyloids; molecular genetics of neurodegeneration—The prion model.

Refining Ocular Motor Models through Simulation—Workshop on Computational Neuroscience

July 5–July 9

ARRANGED BY

L. Optican, National Eye Institute, Bethesda, Maryland
S. Hockfield, Yale University, New Haven, Connecticut

SESSION 1: COMPUTER SYSTEMS

L. Optican, National Eye Institute, Bethesda, Maryland

SESSION 2: EYE PLANT

H.P. Goldstein, Wills Eye Hospital, Philadelphia, Pennsylvania
J.D. Enderle, North Dakota State University, Fargo

SESSION 3: VOR

T. Raphan, Brooklyn College, New York
H.L. Galiana, McGill University, Montreal, Quebec, Canada
T.C. Hain, Johns Hopkins Hospital, Baltimore, Maryland

SESSION 4: SACCADES

A.J. van Opstal, University of Nijmegen, The Netherlands
K. Hepp, Eidgenovvische Techn Hock, Zurich, Switzerland
C.A. Scudder, Washington University School of Medicine, St. Louis, Missouri
P. Inchingolo, University of Trieste, Italy
D. Tweed, The University of Western Ontario, London, Canada
S. Grossberg, Boston University, Massachusetts
D.L. Sparks, University of Alabama, Birmingham
D.M. Waitzman, National Eye Institute, Bethesda, Maryland

SESSION 5: PURSUIT

S.G. Lisberger, University of California School of Medicine, San Francisco
D.A. Robinson, Johns Hopkins University, Baltimore, Maryland
R. Krauzlis, University of California School of Medicine, San Francisco
J.R. Carl, National Eye Institute, Bethesda, Maryland
E.L. Keller, Smith-Kettlewell Institute for Visual Science, San Francisco, California
R.H. Wurtz, National Eye Institute, Bethesda, Maryland
L.E. Mays, University of Alabama, Birmingham
F.A. Miles, National Eye Institute, Bethesda, Maryland

Cytoskeletal Proteins in Tumor Diagnosis

October 6–October 9

ARRANGED BY

M. Osborn, Max-Planck-Institute for Biophysical Chemistry, Goettingen, Federal Republic of Germany
K. Weber, Max-Planck-Institute for Biophysical Chemistry, Goettingen, Federal Republic of Germany

SESSION 1: NEURAL AND NEUROENDOCRINE MARKERS

Chairperson: M. Osborn, Max-Planck-Institute for Biophysical Chemistry, Goettingen, Federal Republic of Germany

N.J. Cowan, New York University, New York: Regulation of expression of the genes encoding neurofilament and glial filament proteins.

L.A. Sternberger, University of Maryland, Baltimore: Neurofilament phosphorylation—Reactive and degenerative.

J.Q. Trojanowski, University of Pennsylvania, Philadelphia: Diagnostic problems in neuropathology—An overview of recent efforts to address diagnostic and prognostic problems with monoclonal antibodies to neurofilaments.

M.L. Shelanski, Columbia University College of Physicians & Surgeons, New York, New York: Peripherin and other markers of neural differentiation.

V.E. Gould, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois: Neuroendocrine and nerve sheath tumors.

A.F. Gazdar, Naval Hospital, Bethesda, Maryland: Differentiation and molecular biology of lung cancer.

SESSION 2: DIFFERENTIATION MARKERS IN THE MESENCHYME AND ITS TUMORS

Chairperson: G. Gabbiani, University of Geneva, Switzerland

M. Altmannsberger, University of Giessen, Federal Republic of Germany: Distinction of small round-cell tumors of children with special emphasis on neuroblastomas and rhabdomyosarcomas.

G. Gabbiani, University of Geneva, Switzerland: Actin isoform identification in the diagnosis of soft tissue tumors and of nonmalignant smooth muscle proliferation.

A.M. Gown, University of Washington, Seattle: Anti-actin antibodies—Use in diagnosis.

D. Helfman, Cold Spring Harbor Laboratory, New York: Regulation of expression of marker molecules of myogenesis.

J.S. Morrow, Yale University, New Haven, Connecticut: Spectrins and the cortical cytoskeleton—Tissue specificity.

K. Gatter, John Radcliffe Hospital, Oxford, England: Value of cytoskeletal markers in diagnosis of lymphomas and other tumors.

A.M. Vogel, St. Louis University, Missouri: Melanocyte-specific cytoplasmic antigens.

C.C. Kumar, Schering Corporation, Bloomfield, New Jersey: Regulation of smooth-muscle-specific myosin light-chain-2 isoforms by oncogenes and by tumor-promoting agents.

SESSION 3: DIFFERENTIATION MARKERS: SWITCHES DURING DEVELOPMENT AND USES IN CYTOLOGY AND IN TUMOR DIAGNOSIS

Chairperson: L.A. Sternberger, University of Maryland, Baltimore

L.G. Koss, Montefiore Medical Center and Albert Einstein College of Medicine, Bronx, New York: Diagnostic cytology and cell markers—Some practical considerations.

M. Osborn, Max-Planck-Institute for Biophysical Chemistry, Goettingen, Federal Republic of Germany: IF typing in cytology.

B. Cunningham, Rockefeller University, New York, New York: Cell adhesion molecules.

P. Cowin, New York University, New York: Molecular markers of adhering junctions.

I. Virtanen, University of Helsinki, Finland: Changes of expression of intermediate filaments during development and in culture.

I. Damjanov, Jefferson Medical College, Philadelphia, Pennsylvania: Cytoskeletal and lectin markers of embryonal carcinomas and teratocarcinomas.

D. Louvard, Pasteur Institut, Paris, France: The use of villin for histopathological and serological diagnosis of digestive tumors.

SESSION 4: EPITHELIA AND CARCINOMAS I

Chairperson: T.-T. Sun, New York University, New York

W.W. Franke, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Molecular biological and histological aspects of expression of cytokeratins and desmosomal proteins.

B. Lane, Imperial Cancer Research Fund, Herts, England: Differential expression of keratins as seen by monoclonal antibodies.

J.G. Rheinwald, Dana-Farber Cancer Institute, Boston, Massachusetts: Keratin 19 expression as a marker of premalignancy in oral epithelium.

R. Moll, University of Mainz, Germany: Cytoskeletal markers in the classification of carcinomas and their metastases.

R.B. Nagle, University of Arizona, Tucson: The study of intermediate filaments as an adjuvant to pathological diagnosis.

H. Battifora, City of Hope National Medical Center, Duarte, California: Fixatives and proteases, their effect in the demonstration of intermediate filaments by immunohistochemistry.

M. Miettinen, University of Helsinki, Finland: Intermediate filaments in sarcomas—New findings suggest complex patterns of expression.

SESSION 5: EPITHELIA AND CARCINOMAS II

Chairperson: **W.W. Franke,** German Cancer Research Center, Heidelberg, Federal Republic of Germany

E.V. Fuchs, University of Chicago, Illinois: Regulation of keratin gene expression in human epithelial cells.

T.-T. Sun, New York University, New York: Pathways of keratinocyte differentiation.

S.P. Banks-Schlegel, National Heart, Lung and Blood Institute, Bethesda, Maryland: Keratin proteins and involucrin—Diagnostic aids in neoplasis.

H. Kahn, Women's College Hospital, Toronto, Canada: Keratin patterns in epithelial tumors.

D.R. Roop, National Institutes of Health, Bethesda, Maryland: The use of monospecific keratin antisera to monitor different stages of carcinogenesis.

F. Ramaekers, University Hospital, Nijmegen, The Netherlands: The use of monoclonal antibodies to cytokeratins in the characterization of epithelial lesions with special emphasis on their application in flow cytometry.

G. Riethmuller, University of Munich, Federal Republic of Germany: Oncogene expression and tumorigenesis of early disseminated cancer cells from human bone marrow—Identification of single tumor cells with anti-cytokeratin antibodies.

The Pancreatic β Cell: Development, Cell and Molecular Biology, and Immunopathology

October 16–October 19

ARRANGED BY

G. Cahill, Howard Hughes Medical Institute, Bethesda, Maryland
D. Hanahan, Cold Spring Harbor Laboratory, New York
H.O. McDevitt, Stanford University School of Medicine, California

SESSION 1: BIOLOGY AND MOLECULAR BIOLOGY OF THE β CELL

Chairperson: G. Cahill, Howard Hughes Medical Institute, Bethesda, Maryland

D. Steiner, University of Chicago, Illinois: Cellular and molecular biology of the β cell.

W. Rutter, University of California, San Francisco: Insulin genes and receptors.

R. Stein, Vanderbilt University, Nashville, Tennessee: Insulin gene regulation—The role of positive and negative transcription factors in pancreatic β-cell-specific expression.

M.J. Tsai, Baylor College of Medicine, Houston, Texas: Regulation of the rat insulin II gene expression—*cis*- and *trans*-acting factors.

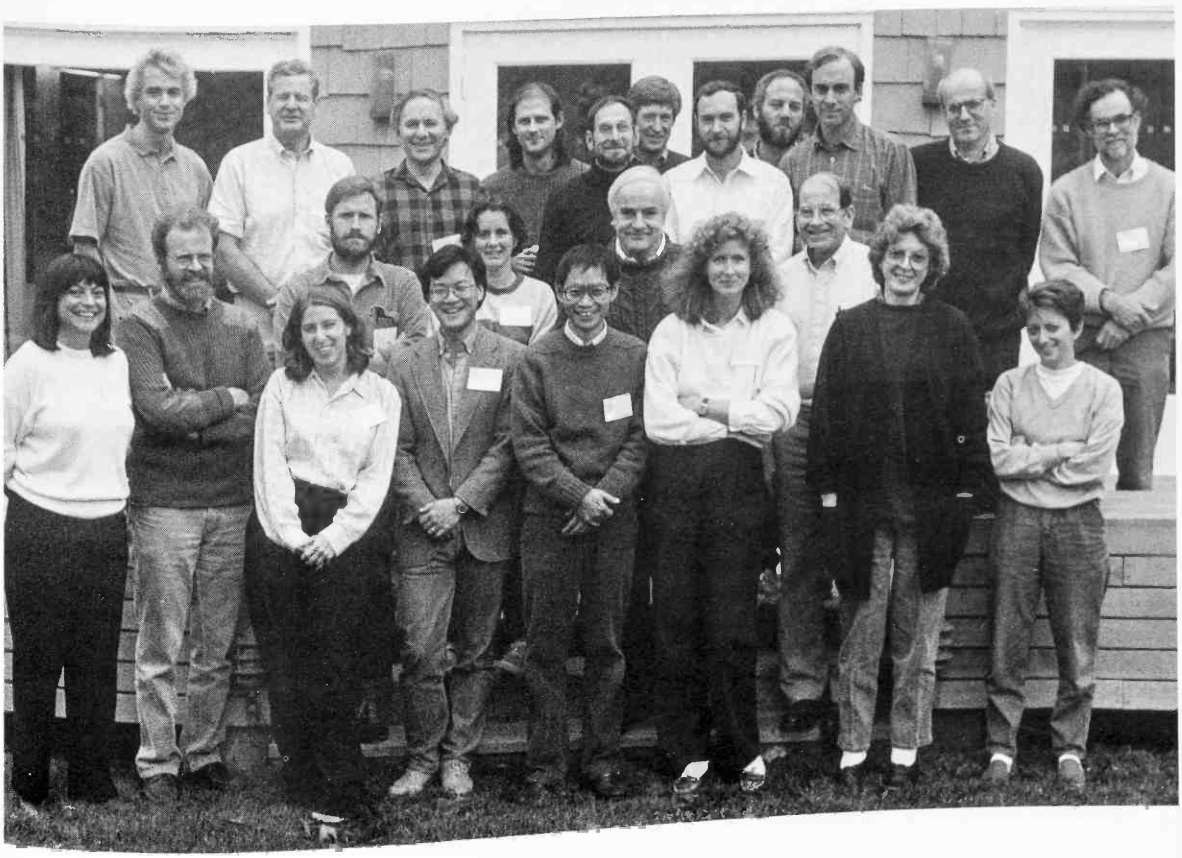
G.I. Bell, University of Chicago, Illinois: Characterization of proteins expressed in the β cell—A molecular analysis.

G. Teitelman, New York Hospital–Cornell University Medical Center, New York: Expression of neural antigens by pancreatic β cells—Developmental implications.

SESSION 2: INSULIN-DEPENDENT DIABETES

Chairperson: J. Kappler, University of Colorado Health Science Center, Denver

G. Cahill, Howard Hughes Medical Institute, Bethesda, Maryland, and Ronald Kahn, Joslin Diabetes Center, Boston, Massachusetts: The nature of diabetes and its physiologic defects.



S. Baekkeskov, Hagedorn University, Gentofte, Denmark: Characterization of the 64K autoantigen in diabetes.
H.O. McDevitt, Stanford University School of Medicine, California: Role of class II MHC molecules in type I diabetes.
E.H. Leiter, The Jackson Laboratory, Bar Harbor, Maine: Genetic control of interferon-γ-induced class-I-like genes in NOD islets and macrophages.
A. Like, University of Massachusetts Medical School, Worcester: Reconstitution studies in the BB/Wor model of spontaneous autoimmune diabetes.

SESSION 3: IMMUNOLOGICAL TOLERANCE

Chairperson: N.A. Mitchison, University of London, England

N.A. Mitchison, University of London, England: General perspectives on tolerance.
J. Kappler, University of Colorado Health Science Center, Denver: Shaping of the T-cell repertoire by tolerance.
J. Sprent, Scripps Clinic, La Jolla, California: T-cell selection in the thymus.
D. Hanahan, Cold Spring Harbor Laboratory, New York: Tolerance or autoimmunity to a transgenic β-cell antigen.
D. Lo, University of Pennsylvania School of Veterinary Medicine, Philadelphia: Transgenic mice with specific expression of class II MHC on β cells—Antigen-presenting function and tolerance induction.
General discussion: Mechanisms of tolerance.

SESSION 4: AUTOIMMUNITY

Chairperson: P. Marrack, Howard Hughes Medical Institute Research Laboratories, Denver, Colorado

N. Sarvetnick, Genentech, Inc., South San Francisco, California: Interferon-γ-induced diabetes in transgenic mice.
J. Sambrook, University of Texas Southwestern Medical Center, Dallas: Expression of a foreign antigen, influenza virus hemagglutinin, on the surfaces of pancreatic β cells in transgenic mice—A model for autoimmune diabetes.
J.F. Bach, Institut National de la Sante et de la Recherche Medicale, Paris, France: MHC-based immunomanipulation of anti-β-cell autoimmunity in NOD mice.
C.A. Janeway, Jr., Yale University School of Medicine, New Haven, Connecticut: Approaches to the analysis of autoantigens.

SESSION 5: TRANSGENIC DIABETES/HORMONE SECRETION

Chairperson: H.O. McDevitt, Stanford University School of Medicine, California

L. Harrison, Walter & Eliza Hall Institute of Medical Research, Melbourne, Australia: Mechanisms of β-cell destruction in type I diabetes—Immune and nonimmune.
D. Mathis, Institut de Chimie Biologique, Faculte de Medecine, Strasbourg, France: Expression of MHC class II molecules on β cells is not a sufficient condition for insulin-dependent diabetes.
N. Sarvetnick, Genentech, Inc., South San Francisco, California: EM analysis of class II expression in β cells of transgenic mice.
S. Efrat, Cold Spring Harbor Laboratory, New York: A new model for diabetes in transgenic mice.
P. Epstein, Baylor College of Medicine, Houston, Texas: Overexpression of calmodulin in β cells of transgenic mice.
R. Kelly, University of California, San Francisco: Hormone secretion of endocrine cells.
L. Villa-Komaroff, The Children's Hospital, Boston, Massachusetts: Mutations of insulin, effects on processing and secretion.

Therapeutic Peptides and Proteins: Formulation, Delivery, and Targeting

October 23–October 26

ARRANGED BY

D.T. Liu, U.S. Food and Drug Adminstration, Bethesda, Maryland
D. Marshak, Cold Spring Harbor Laboratory, New York

SESSION 1: FORMULATIONS

Chairperson: Z. Shaked, CODON, South San Francisco, California

Z. Shaked, CODON, South San Francisco, California: Formulation of pharmaceutical proteins.
A.P. MacKenzie, University of Washington, Seattle: Freeze-drying of peptide and protein-containing sytems.

R. Pearlman, Genentech, Inc., South San Francisco, California: Formulation strategies for recombinant proteins—Growth hormone and tissue-type plasminogen activator.

S. Hershenson, Cetus Corporation, Emeryville, California: Formulation of interferon-β-scr17 (Betascron^{TM}), a hydrophobic protein, using a nonionic surfactant.

SESSION 2: ROUTES FOR DELIVERY

Chairperson: S.S. Davis, University of Nottingham, England

S.S. Davis, University of Nottingham, England: Oral administration of peptides.

S. Muranishi, Kyoto Pharmaceutical University, Japan: Biopharmaceutical aspects of enhanced-transmembrane delivery of peptides and proteins.

L. Illum, University of Nottingham, England: Nasal delivery of peptides and proteins.

J.P. Longenecker, California Biotechnology Inc., Mountain View, California: Nasal delivery of proteins for systemic use.

SESSION 3: PHARMACOKINETICS

Chairperson: L.Z. Benet, University of California, San Francisco

L.Z. Benet, University of California, San Francisco: Pharmacokinetics of peptides and proteins—Boundaries of formulation, delivery, and targeting.

S. Poole, National Institute for Biological Standards and Control, Herts, England: Pharmacokinetics and tissue targeting.

M.J. Browne, Beecham Pharmaceuticals Research Division, Surrey, England: Slow in vivo clearance of novel tissue-type plasminogen activator species and hybrid enzymes.

A.M. Breckenridge, University of Liverpool, England: Therapeutic peptides—A clinical pharmacologist's views.

SESSION 4: REGULATORY ASPECTS I

Chairperson: D.T. Liu, U.S. Food and Drug Administration, Bethesda, Maryland

S. Sobel, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Rockville, Maryland: U.S. perspectives on drug regulation.

T. Hayakawa, National Institute of Hygienic Sciences, Tokyo, Japan: Preclinical study groups for biotechnology drugs as an aid in the development of regulatory policies.

SESSION 5: BRAIN PEPTIDES

Chairperson: N. Sherwood, University of Victoria, British Columbia, Canada

L.L. Rubin, Athena Neurosciences, Inc., San Carlos, California: Cell biology of the blood-brain barrier.

J.E. Rivier, Salk Institute, La Jolla, California: Pharmacology of selected hypothalamic releasing factors.

N. Sherwood, University of Victoria, British Columbia, Canada: Formulation and delivery of gonadotrophin-releasing hormones and their analogs for control of reproduction in fish.

SESSION 6: CONTROLLED DELIVERY

Chairperson: R. Langer, Massachusetts Institute of Technology, Cambridge

L. Huang, University of Tennessee, Knoxville: Liposomal delivery of proteins and peptides.

H. Okada, Takeda Chemical Industries, Ltd., Osaka, Japan: One-month release injectable microspheres of leuprolide acetate.

R. Langer, Massachusetts Institute of Technology, Cambridge: Controlled polymeric delivery systems for small molecules and polypeptides.

SESSION 7: GLYCOPROTEINS

Chairperson: D.R. Bangham, National Institute for Biological Standards and Control, Herts, England

J.U. Baenziger, Washington University, St. Louis, Missouri: Structure and function of glycoprotein hormone oligosaccharides.

H. Kinoshita, Chugai Pharmaceutical Co., Ltd., Tokyo, Japan: Pharmacokinetics of recombinant erythropoietin in rats.

J.R. Rasmussen, Genzyme Corporation, Boston, Massachusetts: Targeting of glucocerebrosidase to macrophages.

SESSION 8: CELLULAR APPROACHES

Chairperson: D.R. Marshak, Cold Spring Harbor Laboratory, New York

D.B. Glass, Emory University, Atlanta, Georgia: Potent, selective peptide inhibitors of cAMP-dependent protein kinase—Structure-function and biostability studies.

J.A. Thompson, National Heart, Lung and Blood Institute, Bethesda, Maryland: Implantable bioreactors—Modern concepts of gene therapy.

J.R. Murphy, Boston University Medical Center, Massachusetts: Diphtheria-toxin-related growth-factor fusion genes—Model systems for target-cell-receptor-specific toxins.

P.W. Trown, XOMA Corp., Berkeley, California: Immunotoxins—Chemistry, biology, and clinical efficacy.

SESSION 9: REGULATORY ASPECTS II

Chairperson: D.T. Liu, U.S. Food and Drug Administration, Bethesda, Maryland

E. Esber, Center for Biologics Evaluation and Research, U.S. Food and Drug Administration, Bethesda, Maryland: U.S. perspectives on evaluation of therapeutic biological products.

S.L. Jeffcoate, National Institute for Biological Standards and Control, Herts, England: The European approach to the regulation of therapeutic proteins produced by the new biotechnologies.

SESSION 10: CYTOKINES

Chairperson:

G. Tosato, U.S. Food and Drug Administration, Bethesda, Maryland: Interferon-β-2/B-cell-stimulating factor 2, interleukin 6—A novel cytokine that regulates B- and T-cell growth.

D.L. Urdal, Immunex Corporation, Seattle, Washington: Hematopoietic growth factors—From cloning to clinic.

M. Masui, Otsuka Pharmaceutical Co., Ltd., Rockville, Maryland: Heterogeneity of recombinant products. Human interleukin-Iα and -Iβ.

N. Katre, Cetus Corporation, Emeryville, California: Chemical modification of interleukin-2—A potent drug-delivery system.

M.J. Hawkins, National Cancer Institute, Bethesda, Maryland: Ex vivo activation of leukocytes.

D.R. Bangham, National Institute for Biological Standards and Control, Herts, England: Summary and thoughts for the future.

Development and Application of Molecular Markers to Problems in Plant Genetics

November 1-November 4

ARRANGED BY

B. Burr, Brookhaven National Laboratory, Upton, New York
T. Helentjaris, Native Plants, Inc., Salt Lake City, Utah
S. Tanksley, Cornell University, Ithaca, New York

SESSION 1: SUMMARY OF MAIZE EFFORTS

B. Burr, Brookhaven National Laboratory, Upton, New York: Introduction.

M.G. Murray, Agrigenetics Corporation, Madison, Wisconsin: General considerations on building an RFLP linkage map with specific reference to maize.

B. Burr, Brookhaven National Laboratory, Upton, New York: The application of recombinant inbred lines in the analysis of linkage of RFLP loci and their relationship to traits of interest.

D.A. Hoisington, University of Missouri, Columbia: Correlation of RFLP work with existing maps and coordination of multiple group efforts.

G.E. Hart, Texas A&M University, College Station: Use of existing genetic tools in wheat as they might be applied to RFLP analysis.

P.J. Sharp, Institute of Plant Science Research, Cambridge, England: Construction of RFLP maps in wheat and other related species.

R.C. Shoemaker, Iowa State University, Ames: RFLP analysis in soybean and the special problems using self-pollinated species.

SESSION 2

N. Young, Cornell University, Ithaca, New York: The application of RFLPs to studies in plant evolution—The rice and *Solanaceae* synteny stories.

M.K. Slocum, Native Plants, Inc., Salt Lake City, Utah: The genomic structure of related *brassica* species and subspecies studied by RFLP analysis.

E. Meyerowitz, California Institute of Technology, Pasadena: An RFLP map for *Arabidopsis* and its genetic applications.

Open discussion: Summaries of other mapping efforts.

Moderator: T. Helentjaris, Native Plants, Inc., Salt Lake City, Utah

SESSION 3

R.W. Michelmore, University of California, Davis: Use of an RFLP map for lettuce in the analysis of host-parasite interactions.

M.T. Clegg, University of California, Riverside: Studies of genetic variation between plants by sequence and RFLP analysis.

Roundtable: The use of RFLPs in the study of evolution and systematics, with reference to both research and practical applications.

Moderator: J. Doebley, University of Minnesota, St. Paul

J. F. Wendel and M. Lee, Iowa State University, Ames

SESSION 4: RFLPS AND THE ANALYSIS OF QUANTITATIVE TRAIT LOCI (QTL)

J. Romero-Severson, Agrigenetics Corporation, Madison, Wisconsin: Use of RFLPs for analysis of quantitative trait loci in maize—General considerations and potential impact on crop improvement.

C.W. Stuber, North Carolina State University, Raleigh: Comparative studies using both RFLPs and isozymes as molecular markers to analyze multigenic traits in maize.

J. Nienhuis, Native Plants, Inc., Salt Lake City, Utah: The use of RFLPs to analyze multigenic traits in tomato—The simultaneous selection of contrasting traits.

E. Lander, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: Mapping QTLs with RFLPs—Mathematical theory and experimental results.

Roundtable: Theoretical considerations for the application of RFLPs to QTL analysis.

Moderator: N. Cowen, United Agriseeds, Inc., Champaign, Illinois

J.S. Beckman, The Volcani Center, Bet Dagan, Israel; M. Edwards, The Pillsbury Company, LeSueur, Minnesota

SESSION 5

D.S. Robertson, Iowa State University, Ames: Understanding the relationship between qualitative and quantitative genetics.

B. Haughe, Massachusetts General Hospital, Boston: Physical mapping in *Arabidopsis* and possible applications of this approach.

M. Wu, Los Alamos National Laboratory, New Mexico: In situ hybridization to physically map cloned probes in plant chromosomes—Physical-recombination map relationships.

T. Helentjaris, Native Plants, Inc., Salt Lake City, Utah: Future directions for both the technology and its applications.

DNA Technology and Forensic Science

November 28–December 1

ARRANGED BY

J. Ballantyne, Office of the Medical Examiner, Suffolk County, Hauppauge, New York
G.F. Sensabaugh, University of California, Berkeley
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory, New York

SESSION 1: FORENSIC USE OF GENETIC INFORMATION—LEGAL AND SOCIAL ISSUES

Chairperson: A.G. Motulsky, University of Washington School of Medicine, Seattle

G.F. Sensabaugh, University of California, Berkeley: Introduction.

J.L. Peterson, University of Illinois at Chicago: Biological evidence and its impact on judicial decision making.

A.G. Motulsky, University of Washington School of Medicine, Seattle: Genetics and society.

D. Nelkin, New York University, New York: Society's use of data.

A. Westin, Columbia University, New York, New York: General aspects of privacy.

P. Reilly, Shriver Center for Mental Retardation, Waltham, Massachusetts: Regulation of access to genetic data.

SESSION 2: BASIC ISSUES—LEGAL AND SCIENTIFIC

Chairperson: G.F. Sensabaugh, University of California, Berkeley

P. Neufeld, New York, New York: The Frye test and the admissibility of scientific evidence.

R.P. Harmon, Alameda County District Attorney's Office, Oakland, California: The Frye test—Considerations for DNA fingerprinting.

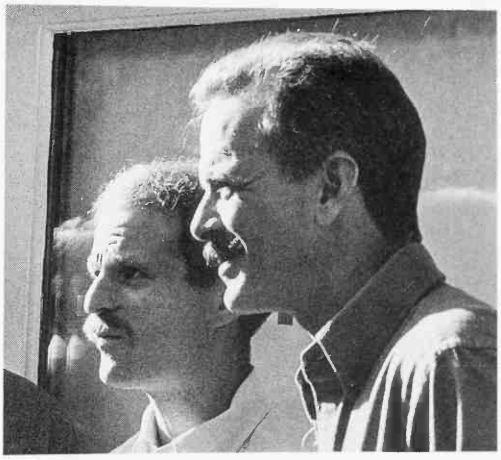
M. Katzer, Office of the District Attorney, County of Albany,

New York: Review of present cases.

C.T. Caskey, Baylor College of Medicine, Houston, Texas: A critical evaluation of the laboratory techniques.

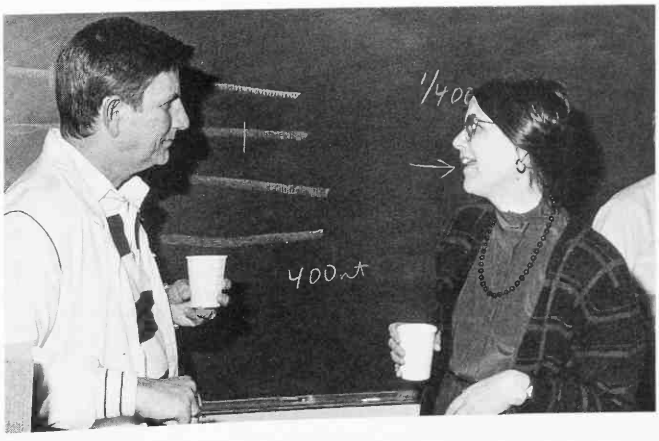
E.S. Lander, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: The requirements for population studies.

E. Lander, R. Roberts, P. Neufeld



B. Budowle, R. Harmon

D. Werrett, A. Jeffreys



T. Caskey, J. Bashinski

SESSION 3: TRANSFER OF DNA TECHNOLOGY TO THE FORENSIC LABORATORY

Chairperson: J.W. Hicks, FBI Laboratory Division, Washington, D.C.

J.S. Bashinski, Oakland Police Department Crime Lab, California: Laboratory accreditation, training, and certification of staff in the forensic context.

M.L. Baird, Lifecodes Corporation, Valhalla, New York: Quality control and quality assurance.

E.T. Blake, Forensic Science Associates, Richmond, California: DNA analysis and its integration into traditional forensic serology.

Discussion: Practical experiences of the transfer of DNA technology to the forensic laboratory.

Discussants:

J.W. Hicks, FBI Laboratory Division, Washington, D.C.

J. Ballantyne, Office of the Medical Examiner, Suffolk County, Hauppauge, New York

H. Lee, Connecticut State Police Forensic Science Laboratory, Meriden

W.C. Stuber, Metro-Dade Police Department Crime Laboratory, Miami, Florida

B.D. Gaudette, Royal Canadian Mounted Police Central Forensic Laboratory, Ottawa, Ontario

D. Werrett, Home Office Research Establishment, Reading, England

SESSION 4: ADVANCED DNA TECHNIQUES WITH APPLICATION IN THE FORENSIC LABORATORY

Chairperson: C.T. Caskey, Baylor College of Medicine, Houston, Texas

S. Odelberg, University of Utah School of Medicine, Salt Lake City: Tandemly repeated DNA and its applications in forensic biology.

D.D. Garner, Cellmark Diagnostics, Germantown, Maryland: Current case experience with single-locus hypervariable probes.

R. Higuchi, Cetus Corporation, Emeryville, California: Applications of the polymerase chain reaction in forensic science.

A.J. Jeffreys, University of Leicester, England: Minisatellite probes and the polymerase chain reaction.

G.L. Trainor, DuPont Company, Wilmington, Delaware: Fluorescence detection nucleic acid analysis.

M. Hunkapiller, Applied Biosystems, Inc., Foster City, California: Detection systems for DNA sequencing and specific nucleotide sequences.

SESSION 5: ESTABLISHMENT, MAINTENANCE, AND REGULATION OF DATABASES

Chairperson: R. Roberts, Cold Spring Harbor Laboratory, New York

S.D. Rose, Collaborative Research, Inc., Bedford, Massachusetts: Standardization of systems—Essential or desirable?

E.A. Rathbun, FBI National Crime Information Center, Washington D.C.: The NCIC experience.

K.K. Kidd, Yale University School of Medicine, New Haven, Connecticut: The human gene-mapping database.

T.G. Marr, Los Alamos National Laboratory, New Mexico: An analysis system and database for gel images.

D. Boggs, U.S. Court of Appeals, Louisville, Kentucky: Summary.

The Polymerase Chain Reaction

December 11–December 14

ARRANGED BY

H.A. Erlich, Cetus Corporation, Emeryville, California
R. Gibbs, Baylor College of Medicine, Houston, Texas
H.H. Kazazian, Jr., Johns Hopkins Hospital, Baltimore, Maryland

SESSION 1: BASIC TOPICS

Chairperson: T.A. Kunkel, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina

T.A. Kunkel, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: DNA polymerase fidelity.

D. Gelfand, Cetus Corporation, Emeryville, California: Enzymes in PCR.

P. Keohavong, Massachusetts Institute of Technology, Cambridge: Fidelity of DNA amplification in vitro.

R.K. Saiki, Cetus Corporation, Emeryville, California: Optimization of PCR.

M.S. Lee, University of Texas, Houston: Detection of chromosomal translocations by sequence amplification.

M. Perucho, California Institute of Biological Research, La Jolla: Application of PCR to the diagnostic detection of mutant *ras* oncogenes in human tumors by the RNase-A mismatch-cleavage method.

D. Goldgaber, State University of New York, Stony Brook: Problems with fidelity of *Taq* polymerase in searching for mutation in the human *PRP* gene.

SESSION 2: HUMAN GENETIC DISEASE MUTATIONS

Chairperson: O. Smithies, University of North Carolina, Chapel Hill

H.H. Kazazian, Jr., Johns Hopkins Hospital, Baltimore, Maryland: Use of PCR in clinical diagnosis of genetic disease.

S.L.C. Woo, Howard Hughes Medical Institute, Baylor College of Medicine, Houston, Texas: Mutations in phenylketonuria.

D. Valle, Johns Hopkins Hospital, Baltimore, Maryland: Mutation detection and structure-function studies at the ornithine aminotransferase locus.

K. Tindall, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Molecular analysis of mutations at *gpt* locus in Chinese hamster ovary cells.

R. Williamson, St. Mary's Hospital Medical School, London, England: Application of PCR to cystic fibrosis – Prenatal diagnosis and carrier testing.

R. Gibbs, Baylor College of Medicine, Houston, Texas: *HPRT* mutations and competitive oligonucleotide priming.

A.A. van Zeeland, State University of Leiden, The Netherlands: Sequence determination of point mutations at the *HPRT* locus in mammalian cells using *HPRT* cDNA prepared from total cellular RNA.

D. Ginsburg, Howard Hughes Medical Institute, University of Michigan Medical School, Ann Arbor: Human von Willebrand's disease – Analysis of platelet mRNA by PCR.

SESSION 3: ANALYSIS OF HIGHLY POLYMORPHIC REGIONS

Chairperson: R. Williamson, St. Mary's Hospital Medical School, London, England

H.A. Erlich, Cetus Corporation, Emeryville, California: HLA class II polymorphisms – Detection and evaluation.

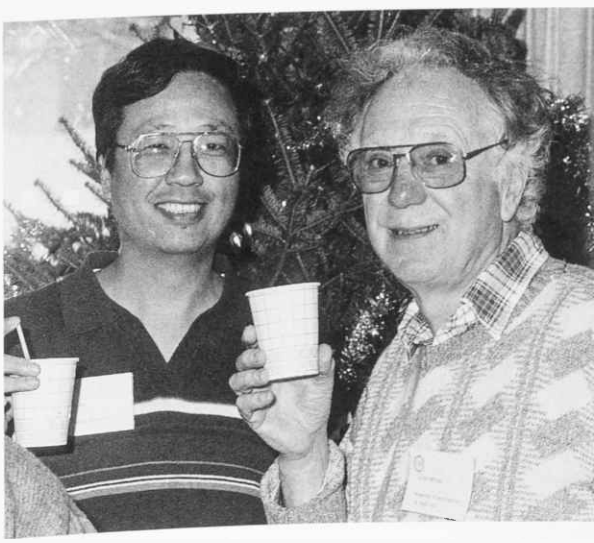
J. Weber, Marshfield Medical Research Foundation, Wisconsin: Length polymorphisms in abundant $(dC\text{-}dA)_n \cdot (dG\text{-}dT)_n$ repeats.

J.A. Todd, John Radcliffe Hospital, Oxford, England: Cloning immune response genes.

G.F. Sensabaugh, University of California, Berkeley: PCR applications in forensic science.

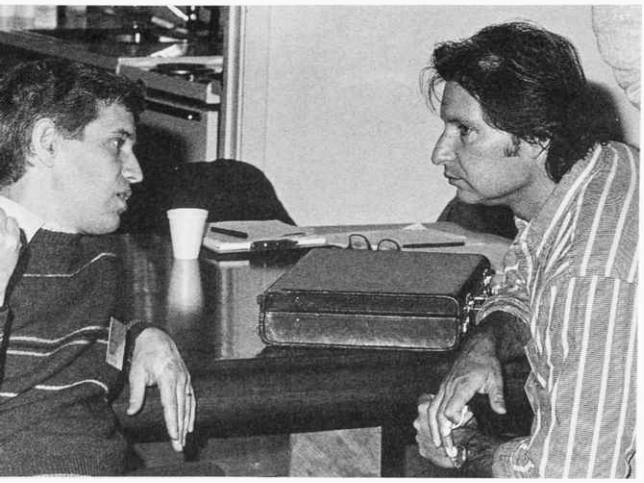
A.F. Markham, ICI Diagnostics, Norwich, Cheshire, England:

N. Arnheim, H. Kazazian



S. Woo, O. Smithies

Specificity and reproducibility of the PCR.
R.M. Myers, University of California, San Francisco: PCR and denaturing gradient gels.



T. Kunkel, H. Erlich

L.S. Lerman, Massachusetts Institute of Technology, Cambridge: Analysis of single-base changes in the human genome.

SESSION 4: DETECTION OF RARE SEQUENCES

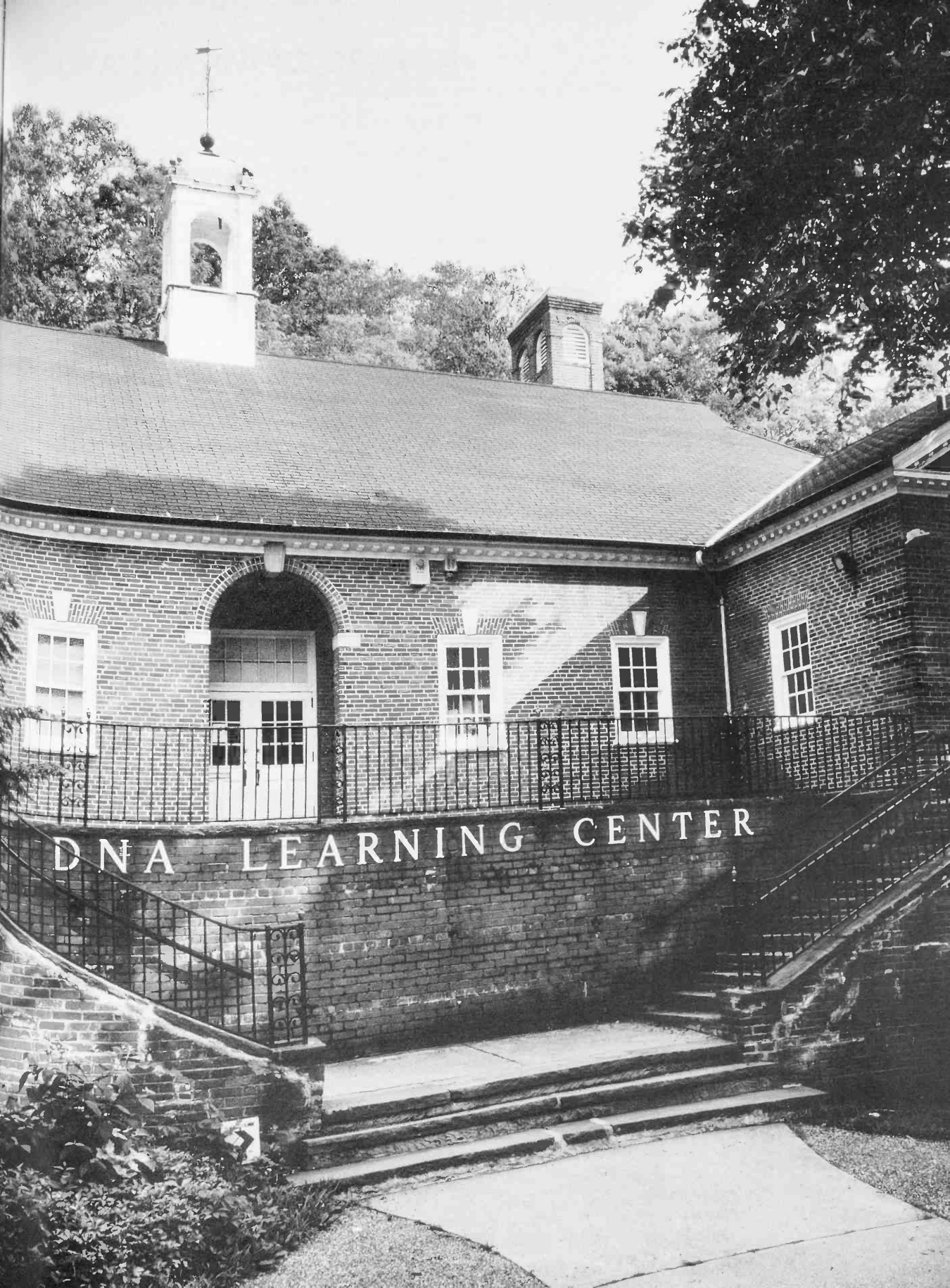
Chairperson: **K.B. Mullis,** XYTRONYX, Inc., San Diego, California

J.J. Sninsky, Cetus Corporation, Emeryville, California: HIV.
B.J. Poiesz, State University of New York Health Science Center, Syracuse: The use of PCR in the detection, quantification, and characterization of human retroviruses.
G. Schochetman, Centers for Disease Control, Atlanta, Georgia: HIV detection.
T.R. Broker, University of Rochester School of Medicine, New York: Synthesis of human papillomavirus cDNAs by PCR amplification.
N. Arnheim, University of Southern California, Los Angeles: Single-cell templates/gene mapping.
O. Smithies, University of North Carolina at Chapel Hill: Use of PCR for detection of targeted gene modifications.
J.S. Chamberlain, Baylor College of Medicine, Houston, Texas: Multiplex PCR for DMD diagnosis.

SESSION 5: ALTERNATIVES, AUTOMATION, AND THE FUTURE

Chairperson: **R.M. Myers,** University of California, San Francisco

H. Lehrach, Imperial Cancer Research Fund Laboratories, London, England: Approaches to a large-scale analysis of mammalian genomes.
L.J. McBride, Applied Biosystems, Inc., Foster City, California: Thermal cycling and automated fluorescent DNA sequencing.
R.K. Wilson, California Institute of Technology, Pasadena: Rapid analysis of T-cell–receptor gene structure and expression.
P.J. de Jong, Lawrence Livermore National Laboratory, California: In vitro mutagenesis via PCR.
R.B. Wallace, Beckman Research Institute of the City of Hope, Duarte, California: Alternative to PCR.
K.B. Mullis, XYTRONYX, Inc., San Diego, California: Variations on the polymerase chain reaction.


DNA LEARNING CENTER

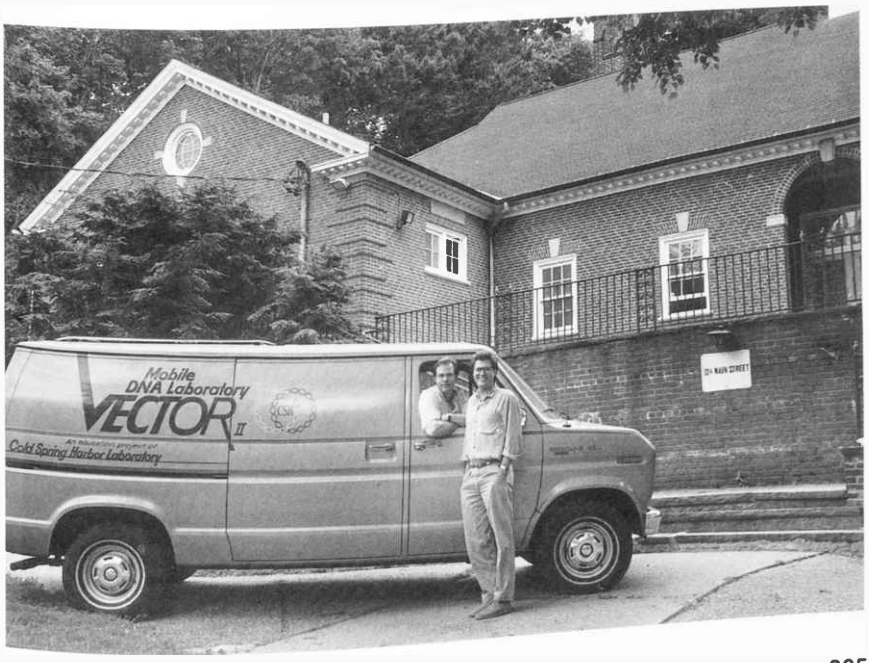
DNA LEARNING CENTER

David A. Micklos, Director
Mark V. Bloom, Assistant Director

This Annual Report marks the emergence of the DNA Learning Center (DNALC) as a new operating unit of Cold Spring Harbor Laboratory. Like its sister Banbury Center, founded in 1976, the DNALC has a separate operating budget and advisory board. The DNALC extends the traditional postgraduate education and research mission of Cold Spring Harbor Laboratory to the college, precollege, and public levels. The DNALC collaborates extensively with the Public Affairs Department and Banbury Center, which have overlapping roles in interpreting science and interacting with segments of the public.

The DNALC is the culmination of the DNA Literacy Program, which was initiated 5 years ago as the nation's first laboratory-based program to retrain precollege teachers in molecular genetics. At that time, many felt that we were a little mad to think that widespread public understanding of one molecule (DNA) could be so important. In fact, our first attempt for federal grant support of this program met with failure.

The dedication of the Learning Center on September 18th, 1988 marked the coming of age of that "crazy" idea. Opening-day tours of the facility were attended by 800 students, teachers, friends, and employees of Cold Spring Harbor Laboratory. A standing-room-only crowd of 400 packed Grace Auditorium for the dedication ceremony, at which Dr. Robert Pollock, Dean of Columbia College, delivered the keynote address, "Reading DNA." Backed by the prestige of Cold Spring Harbor Laboratory, the event sent a clear message that there is now room in the world for at least one institution devoted entirely to biotechnology education.



Cold Spring Harbor Laboratory Director James D. Watson with Robert Pollock, Dean of Columbia College, who delivered the keynote address at the Learning Center dedication on September 18, 1989. Huntington Town Supervisor Toni Rettaliata (above) also spoke.

Scientists have already begun to write the history of discovery that has led us into the biotechnology era. Most of us cannot participate in discovery; however, each of us can help write the history of the first society to dwell in the gene age. Only through widespread education can we ensure a society that shepherds the benevolent use of genetic technology for the good of all its citizens.

Cold Spring Harbor Laboratory has provided for the world a model of what a research institute should be—a quiet place where people can solve the problems of biology. Similarly, we hope that the DNALC will provide a model of how a science education institute can help solve the problems of scientific illiteracy. Now, by our example, we must show the world that there is need for other DNA education units as well. To the extent that we prove that the general public is eager and able to learn about biotechnology, we also provide models of informal science education for other institutions to follow.

The Human Genome Project

Written in the approximately 100,000 human genes is the molecular code-script that, to a large extent, determines the life and health of each individual. This entire complement of genetic instructions encoding human life is called the human genome.

During the last 15 years, biologists have gained the extraordinary ability to dissect precisely the molecules of DNA that comprise the human genome. The molecular dissection of the fundamental units of heredity 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 pinpoint changes in the DNA molecule that predispose one to illnesses such as cancer, muscular dystrophy, Alzheimer's disease, and manic depression.

Until recently, the molecular exploration of the human genome has proceeded in a relatively uncoordinated fashion, with many scientific teams working independently on one or several genes of interest. The information so gathered can be likened to the individual squares of a patchwork quilt, each with its own story to tell. The inauguration of the Human Genome Project in 1988 marked the beginning of a national commitment to knit the patches of genetic research into a cohesive whole. Its goal is to determine the sequence of the estimated 3 billion bits of molecular information—the arrangement of nucleotide rungs of the DNA

The Search for Life exhibit includes a multi-projector slide show and a genetic code game. (Photo by Edward McCain.)

ladder—that constitute the entire code-script of human life. The Human Genome Office, established within the National Institutes of Health, now coordinates the collection, storage, and dissemination of research data collected by thousands of scientists throughout the country.

Possession of an increasingly complete code-script of hereditary information will bring numerous benefits to man- and womankind. Gene-mapping techniques have already made possible accurate DNA diagnosis of a number of debilitating illnesses. By pushing back the threshold of early disease detection, DNA diagnosis should increase therapy options and play a positive role in personal health management. Increased knowledge of 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 acceptance as the most definitive evidence of identity in rape, murder, and paternity cases.

The day is not far off when medical doctors may maintain personal genetic profiles of their patients, and DNA fingerprints may replace thumb- and fingerprints on file with law enforcement agencies. Guidelines concerning access to and use of this information must be rigorously enforced to ensure protection of individual privacy rights.

Barbara McClintock previewed *The Search for Life* exhibit.

Building DNA Literacy

Biology, and with it, our society, has truly stepped into a new era. 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.

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. Within the scientific community, there is concern that shortages of researchers and technicians will retard the growth of biotechnology and limit its full potential.

The nation's schools are the logical place to begin building a DNA literate public. Unfortunately, biology curricula have evolved over the years by simply cramming in more and more facts. Survey data show, for example, that the vast majority of high school biology teachers spend most of class time lecturing from textbooks. Yet even the textbooks they teach from are typically 5 to 10 years out of date! 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 current research or society.

Survey data also tell us that the majority of elementary school students are enthusiastic about science; however, student interest in science decreases dramatically through the junior and senior high school years. This suggests that young people are not turned off to science itself, but to the manner in which it is taught. Children start their lives as natural scientists. Turning over a rock is an expression of a seemingly innate curiosity about the living world. Rather than building upon this natural interest, formal science education effectively squelches young people's interest in science.

Experience-based learning—like the child's rock-turning inquiry—has long been touted as the means to increase student interest and comprehension of science. However, 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.

The excitement of the Human Genome Project offers an important opportunity to reorganize public biology education. Now is the time to sweep clean the granny closet of biology education to make room for the excitement of modern biology—to replace recitation of facts with frequent laboratory investigation. It is time to let the awesome beauty of the DNA molecule integrate biological phenomena for young people as it has for the last two generations of scientists.

Laboratory Field Trips to the *Bio2000* Teaching Laboratory

We live in an age when young people are buffeted by all manner of distractions that keep them from pondering the mystery of life. Students 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 the intellectual pleasure and social relevancy of that wonderful mystery. Thus, as plans were made for

renovation of the Learning Center, priority was given to creating a teaching facility where hands-on laboratory experiences could be offered to the public. Designed to accommodate 24 participants, the *Bio2000* Laboratory was conceived as a model teaching facility of the 21st century. Anticipating that we could never service all the people interested in doing a DNA manipulation lab, we had a glass window-wall installed between the *Bio2000* Laboratory and the adjacent "observation room." We hope soon to add a closed-circuit television system, with monitors in both the laboratory and observation room, that will enable lab participants and observers to have a close-up view of the instructor's demonstrations.

In developing a laboratory field trip program, our aim was to put modern DNA technology within the reach of precollege science students and teachers. Lab experience gives the student a working knowledge of the possibilities and limitations of DNA technology, which is the basis for rational evaluation of the social and personal implications of biotechnology. Practical experience with DNA techniques, which until recently have been the sole province of researchers, reinforces that student experiments have current relevance and are "real science." For the motivated student, a DNA lab experience encourages information-seeking behavior, such as independent reading and research. The laboratory field trip also affords instructors a "micro-teaching" experience, giving them a nonthreatening exposure to DNA lab technology. Observing the Learning Center staff interact with their own students provides convincing proof that it is indeed possible for *real* students to perform DNA experiments.

The laboratory field trip program was initiated in spring of 1988, following completion of the *Bio2000* Laboratory. The program was an immediate success; every lab 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.

The *Bio2000* Laboratory, under renovation below, is visited by 2,800 students each year, including these from Hillcrest High School in Jamaica, Queens. (Photo by Mort Kelman.)

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.

In an era when fewer teachers have the time or equipment to offer meaningful lab experiences, the laboratory field trip program is a model for a cost-effective means to provide pooled laboratory resources to a local region. The *Bio2000* Laboratory has functioned at full capacity since the day it opened, serving 2800 students (160 classes) in its first year of operation. 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 lab 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.

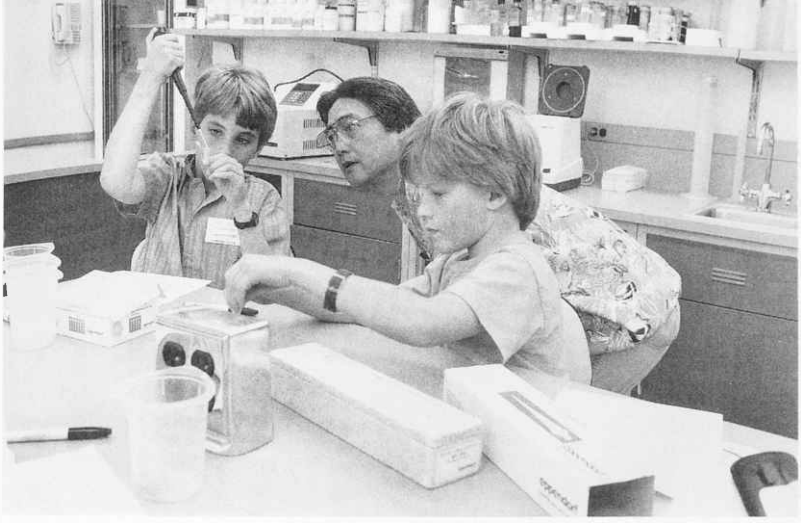
DNA Laboratories Come of Age

Over the years, we have fought the contention of many educators that DNA manipulation labs are too esoteric, too expensive, or too difficult for the high school setting. There is now growing conviction that such laboratories are, in fact, essential to a general biology education. This sentiment has been legitimized by the Educational Testing Service, which will recommend teaching bacterial transformation and DNA restriction analysis labs in the 1989–1990 Advanced Placement (AP) syllabus. These labs will likely become compulsory for AP students in 1993–1994.

As early as 1985, we were training local Long Island teachers to perform these experiments in the high school classroom. So, foresighted Long Island teachers will have been doing these labs as much as a decade before the majority of American biology teachers. Surely the students of these teachers have been similarly ahead of their college-bound peers.

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. Labs 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 comparable or better results, than their academically gifted peers. Success with a 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* for a Saturday laboratory program called "Fun with DNA." During two introductory sessions, the youngsters observed and categorized *Drosophila* 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.



"Fun with DNA" marked our first venture into primary science education. These gifted 5th and 6th graders were the youngest group of students ever to perform a restriction analysis. The National Science Foundation has challenged science educators to focus attention on improving grade school science.

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, and saddening, because we can only wonder in how precious few of these the spark of science will be kept alive through the remainder of their precollege schooling.

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. Our successful *DNA Science Workshop* arose from a collaboration with eight neighboring school districts on Long Island—the Cold Spring Harbor Curriculum Study. These schools were used as a proving ground to develop a laboratory curriculum that illustrates the basic techniques of molecular genetics. Using equipment identical to that found in research laboratories, participants performed nine experiments that culminate in the production and analysis of recombinant-DNA molecules. The laboratory protocols were initially tested in spring 1985, and during that summer, the first training workshop was offered to introduce local high school teachers to the curriculum.

Enthusiasm from participants and interest from numerous educators around the country suggested the desirability of making the workshop available to teachers nationwide. Through a grant from Citibank, N.A., the first *Vector* van was purchased and equipped. In summers 1985 and 1986, a total of 266 educators attended eight workshops.

Receipt in 1987 of major 3-year grants from the National Science Foundation and the Josiah Macy, Jr. Foundation lent legitimacy to the proposition that it is indeed possible to "backpack" a DNA laboratory to essentially anywhere in the nation. These grants provide key support for our teaching staff, as well as stipend and travel expenses for workshop participants. They also allowed us to initiate a program of weekend follow-ups during the fall and winter to keep up the interest of participants and introduce teaching innovations.

Recognizing the educational value of this workshop experience, the State University of New York at Stony Brook agreed to offer a credit option to *Vector* workshop participants nationwide. Teachers who complete both a workshop and follow-up are eligible for three graduate credits from the Continuing Education

Senior Staff Scientist Ed Harlow gave a student lecture on "Recessive Oncogenes," focusing on the childhood cancer, retinoblastoma.

Department. A workshop has been held at the Stony Brook campus each summer since 1987, sponsored by the University's Center for Biotechnology.

Demand for courses in 1987 was great enough to justify the purchase of a second *Vector* van that enables two workshops to be taught simultaneously in different parts of the country. In summer 1987, 307 educators attended 14 workshops around the country. Two additional workshops were conducted for technicians and researchers at the Cleveland Clinic. Nearly 250 teachers were instructed by us at 13 sites in 1988.

The Evaluation Program

Through fall 1989, we have instructed nearly 1100 high school and college instructors in DNA science workshops. The majority of these individuals completed both a pre-survey at the beginning of the workshop and a post-survey at the end of their week-long training experience. In fall 1988, we began a mail survey to follow up on all teachers who had completed the workshop prior to 1988. The response of our "alumni" was overwhelming; 90% returned completed surveys. The good response was in large part due to adhering strictly to a detailed formatting guideline and to a series of follow-up mailings extending over 2 months.

We have just begun the arduous task of entering this mass of data for computer analysis. The amount of information is staggering—each case may have up to 280 bits of data. Furthermore, the amount of data increases each year, as we add new cases and follow up on a new class of "alumni." The total number of cases will increase by nearly one third in winter 1989, when we add some 300 teachers who have independently taught our workshop in collaborative programs in North Carolina, California, and Wisconsin.

By the end of 1989, we will have comparable data on nearly 1600 teachers who have taken the *DNA Science Workshop* over the last 5 years. Taken together, these data represent a substantial sampling of lead biology teachers nationwide and perhaps the most ambitious long-term study of high school biology teachers ever undertaken. From their responses, we hope to determine the characteristics of the "pioneer" teachers who will spearhead biotechnological literacy. We are especially interested in learning how lead teachers seek information and overcome constraints in converting positive attitudes about molecular biology into innovative teaching behavior.

Our data represent a treasure to be used and shared by opinion researchers and educators nationwide. However, we have only begun to scratch the surface of this load of data; layer after layer of insights remain to be revealed. Currently, we

do not have sufficient staff time to do justice to the task. We do, however, have the nucleus of a strong evaluation team to carry on this and other educational research. Dave Micklos combines the perspective of a communication researcher with past experience in opinion research with a major public relations firm. John Kruper, who is using part of the survey data as the basis of his doctoral dissertation at the University of Illinois at Chicago, brings an educational perspective. We now need to locate specific funding to develop a full-time evaluation unit that focuses on the interface of biotechnology and society.

Initiating a Collegiate *Vector* Program

Our experience over the past 4 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. In 1986 and 1987, a total of 28 college faculty teachers participated in our program. Follow-up survey data, collected in 1988, indicate that they were excited about their experience, and most have already begun to implement laboratories from the workshop into their teaching.

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.

Two-day workshops at regional meetings of the American Society of Microbiology (ASM) provide another means for introducing our hands-on approach to college educators nationwide. The success of workshops held in Seattle, Washington and Valley Forge, Pennsylvania in 1988 prompted us to expand our collaboration to five sites in 1989—East Lansing, Michigan; Louisville, Kentucky; Denver, Colorado; Houston, Texas; and Minneapolis, Minnesota.

Educational Collaborations

The Curriculum Study has grown to include 20 Long Island school districts, which receive numerous benefits, including lectures by scientists, reduced admission 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 1988, minority/rural students and teacher chaperones representing each of the Macy-sponsored programs convened for a 2-week workshop at Tuba City, Arizona, within the Navajo Indian Reservation. 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 focus expanded to natural history, geology, and cultural anthropology. In addition to tours of the natural wonders of the Grand Canyon and Monument Valley, and ancient Indian ruins at Wapatki National Monument and Canyon de Chelly, the students also experienced Native American culture first-hand during a 2-day "live-in" with Navajo families. In summer 1989, the format was repeated, this time relying on the cultural offerings of New York, including the Metropolitan Museum, Bronx Zoo, Museum of Natural History, Broadway, and the New York Mets.

Another ongoing 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 39 schools, where BioPrep teachers have instructed 1300 students.

Through our collaboration with the Macy BioPrep Program, we continue to explore creative means to advance biology instruction in the many resource-poor schools in rural America. Beginning in summer 1989, we will provide laboratory instruction over the TI-IN United Star Network. This partnership between public education institutions and private enterprise uses satellite technology to bring live instructional programs to 750 schools in 32 states. The initial three-part broadcast on bacterial transformation, described above, should give AP teachers nationwide the confidence to rapidly introduce this experience into their laboratory program.

In several states, educational consortia have adopted our workshop as a mechanism for introducing teachers to the techniques of DNA manipulation. In many other locations, aspects of our workshop are being implemented one step at a time, as equipment and supplies become available.

The 1986 workshop held at the University of California at Davis prompted the creation of a state-supported instructional program. With funding from the National Science Foundation, 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 northern California each year, and they

In addition to students, DNA analysis labs are offered to special interest groups. Assistant Director Mark Bloom interprets a DNA gel with seniors from Farmingdale University.

have also "cloned" our *Vector* van approach to teaching. A minivan supplied by Genentech, Inc.—dubbed *Helix I*—carries equipment to participating schools, where teachers and some 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. The program also makes available, on a rotating basis, 24 equipment sets to help teachers begin to implement DNA laboratories. In 1988, some 100 schools, representing nearly a third of the schools in the state, 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 become a standard part of the Institute's summer program.

Eye-catching banners crafted by Jan Eisenman of Laurel Hollow.

Materials Development

Our goal has been to modify current research protocols to minimize expense while maximizing safety and reproducibility in the teaching laboratory. However, we strive to maintain the integrity of research methods so that novices need not relearn techniques as they progress to advanced lab work or to a research setting. Experiments are not reduced to the "add A to B" mentality that pervades some laboratory experiences and effectively obscures the process of science. For example, we have learned that having controls performed by every lab team is essential to student interpretation and to sorting out anomalies that invariably arise.

The success of our *DNA Science* protocols lies in their extensive testing and refinement over a long period of time. A deceptively large amount of fine adjustment is required to effectively transfer research techniques into the classroom. Thus, research biologists may encounter difficulties when they attempt to transfer their own protocols and reagents into the teaching laboratory. Molecular biology professors who run training workshops for the North Carolina program have been impressed by the consistent results obtained with the *DNA Science* protocols.

In our quest to make the *DNA Science* course as nearly foolproof as possible, we have gone as far as to develop new plasmids—named simply pAMP and pKAN. Restriction digests of these plasmids yield restriction fragments of markedly different sizes, making gel interpretation straightforward. They are highly amplified in *E. coli*, giving consistent yields in minipreparations. These are, to our knowledge, the only DNA molecules specifically engineered for educational purposes. Many teachers have indicated that time for setup and preparation is now the most serious constraint to teaching DNA laboratories. Their need for "one-stop shopping" and quality-assured reagents led us to collaborate with the Carolina Biological Supply Company, which distributes all reagents and equipment necessary to perform the experiments in our lab/text. A range of product options is offered—from bulk reagents, to multi-use reagent systems, to throwaway kits.

Executives from biotechnology and health care companies, pictured here with Banbury Center Director Jan Witkowski (second from left), performed a lab as part of the Baring Brothers conference on immunology.

We regularly test lab equipment to assess appropriateness for student use, and we are collaborating with suppliers to design and adapt equipment to meet the cost and safety requirements of the education marketplace. For example, we helped to test the first ultraviolet transilluminator designed with student use in mind. Our collaboration with Carolina Biological has resulted in the production of low-cost electrophoresis equipment. A colony transformation kit developed at the DNA Learning Center is proving to be very popular among high school biology teachers and should make it easier for large numbers of AP teachers to perform this recommended experiment.

We plan to develop 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 (PCR). In each case, we will 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 and Perkin-Elmer Cetus to develop PCR for educational purposes. Of great interest is a kit that would allow 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 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.

We also recently joined with the National Air and Space Administration (NASA) on a unique project that combines the latest in space and biotechnology. The SEEDS Project—for Space Exposed Experiment Developed for Students—began in 1984, when 12.5 million tomato seeds were delivered into low Earth orbit by the Space Shuttle. The flight seeds will be retrieved in 1989 and, together with ground-based control seeds, will be distributed to more than 50,000 classrooms nationwide in the spring of 1990. Students in grades 5 through 12 will have the opportunity to design, execute, and interpret their own experiments using these unusual specimens. Our participation will include training a team of NASA education specialists in PCR and gel electrophoresis so that they can train teachers to analyze the DNA from their "space seeds" to look for cosmic-ray-induced mutations.

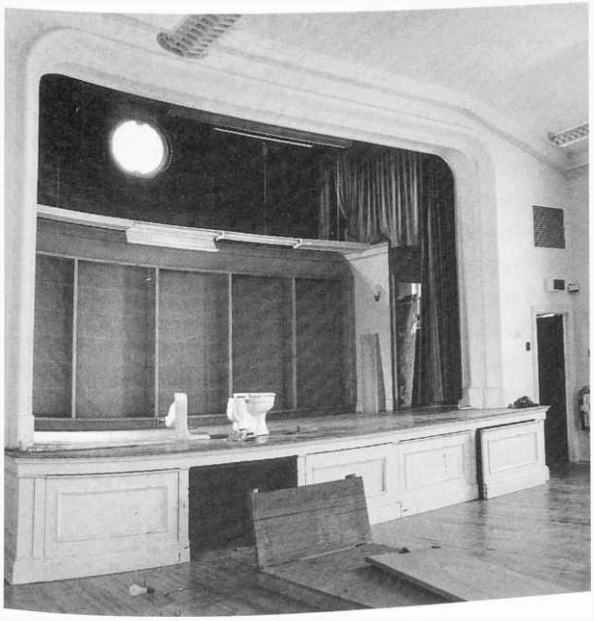
Exhibit Development

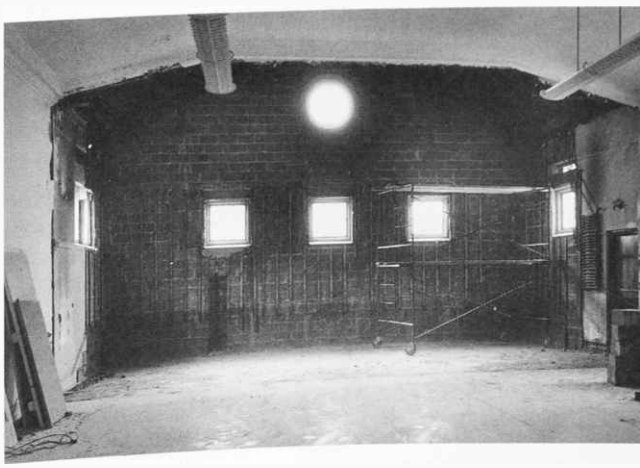
In preparation for our museum/teaching function, more than $400,000 was expended in 1987–1988 to entirely revamp the heating, air conditioning, and electrical systems of our 1925 schoolhouse; to renovate laboratory, exhibit, and office space; and to upgrade parking. A world-class museum program was inaugurated 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. 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 the *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.

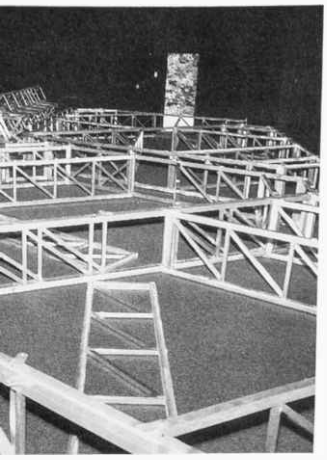
Exploring the Human Genome Exhibit

Our exhibit will approach the genome project from the standpoint of potential gains in understanding the genetic basis of human disease. Although there are more than 3000 known inherited diseases of humans, the causative gene has been identified for only a fraction of these. Mapping disease genes to their exact locations on the chromosomes will facilitate diagnosis, and determining the genetic instructions they encode should lead to improved therapies. The exhibit will focus on several model genetic diseases, including thalassemia, sickle cell anemia, muscular dystrophy, Burkitt's lymphoma, familial colon cancer, retinoblastoma, Alzheimer's disease, manic depression, and Huntington's disease—which illustrate different molecular mechanisms of disease. This case study approach will allow the visitor to learn about both genetic disease and methods of molecular genetic analysis.





Installation of the *The Search for Life* exhibit required major renovation of our building, which was built in 1925 as the grade school of Cold Spring Harbor Village.



DNA Detective/DNA Diagnosis

The natural variability of human life—eye color, hair color, body features, and physical and mental abilities—is determined by genetic instructions encoded in DNA molecules that make up the chromosomes of our cells. Thus, it is not surprising that biologists have identified specific chromosomal regions where the chemical structure of the DNA molecule varies from person to person. Such variable regions are called DNA polymorphisms—for "many forms." Like physical traits, DNA polymorphisms are passed on from parent to child in a Mendelian fashion. The ultimate expression of individual identity, DNA polymorphisms are now revolutionizing forensic medical science, paternity determination, and disease diagnosis.

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. The exhibit, which emphasizes the interaction of science and society, is situated adjacent to the *Bio2000* Laboratory, where hands-on experiments emphasize the methods of science.

The exhibit consists of five Formica-laminated modules—two video modules and three case modules. The video modules, each containing a television monitor, confront visitors as they enter the exhibit area. A short video cycles continuously on each monitor, presenting the scientific basis of DNA polymorphisms and the steps involved in making a DNA fingerprint.

Each case module consists of three back-lit 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 individuals 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."

We intend to develop new cases throughout the year, rotating them into the exhibit on a regular basis. The serialization of cases and the ease of exchanging case materials between one or more modules make it cost effective to create a

rotating "gallery" of DNA fingerprint cases. The Technology Center in Silicon Valley, San Jose, California, plans to install a unit for its opening in 1990, and several other museums have expressed serious interest. The initial cases illustrate various applications of DNA fingerprinting and historical precedents in law, medicine, and society:

Ghana Immigration Case (*1985*). In this case, DNA fingerprints were used to prove the family relationship between an English woman and her child, who wished to emigrate from Ghana. This was the first use of DNA fingerprints in a court of law. 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.

DNA Diagnosis of Muscular Dystrophy (*1988*). This case shows one of the first uses of DNA fingerprints in family genetic medicine. The inheritance of a DNA polymorphism, linked to a causative gene for muscular dystrophy, is traced from "carrier" mother to affected son.

Exploring the Uses of Multimedia

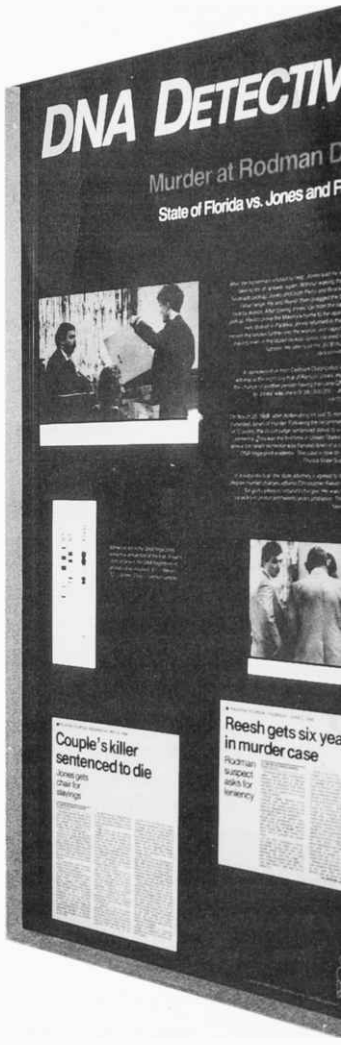
In the last several decades, we have witnessed the virtual perfection of several audiovisual technologies: television, video, computers, and random-access laser discs. Taken alone, none has lived up to its potential as a teaching tool. This is because each learning exercise is only as good as its creator. To the extent that the producer's or programmer's conceptual framework for linking ideas overlaps that of the learner, the presentation will be more or less successful.

Recently, computer researchers have begun to explore methods to link these computo-audiovisual technologies into a flexible system that potentially allows individuals to structure their own learning experience. Multimedia can essentially be thought of as an extension of "windows" technology that allows one to access and display information, from several different sources, simultaneously on a single screen.

The multimedia network consists of an array of stored audiovisual and textual information—an information field—and a set of computer-encoded decision points at intersections of pathways through that field. A command issued at a decision point (by keyboard or mouse) allows one to select a pathway and to rapidly access information stored at addresses along that pathway. Textual information is retrieved from storage in the computer's random-access memory, and audiovisual information is retrieved from an optical laser disc. The information is presented on a high-resolution television monitor.

The open-endedness of the learning experience increases with the number and connectivity of decision points. By choosing their own pathways to explore the information field, individuals may structure the learning experience according to their own preferences of information use. Thus, it is plausible that the pathway individuals take through an information field in some way mirrors the cognitive structure they use to make sense of the world. For example, some people may prefer an analytic pathway, where one begins with general information and progresses to specific information. Others may prefer a synthetic pathway, beginning with specific information and progressing to the more general.

DNA Detective/DNA Diagnosis is the first major exhibit designed by the staff of the DNA Learning Center. (Photos by Mort Kelman.)



The combination of multimedia with parallel distributed processing (PDP) offers even more tantalizing prospects for education. There has been initial success in creating PDP systems that function in a manner analogous to the human nervous system. These simple neural networks can "learn" to identify patterns of input information, for example, words and shapes.

If a person's choices at various decision points in a multimedia information field are input into a neural network, could it then use these choices as feedback to predict the person's best learning path through that field? After extrapolating a Feedback Predicted Learning Path, could the neural network then direct the multimedia computer to structure (edit) the available information into a personalized learning experience that might even be more effective than one selected by the individual? This is possible, considering that without foreknowledge of every bit of stored information and its access points, individuals must wind their way through the information field. In this sense, periodic input to the neural network would produce a Feedback Corrected Learning Path that would, at the least, straighten out some bends and avoid dead ends.

As yet, multimedia systems are not being widely used in educational or museum settings. This is partly due to the fact that every system is essentially custom-made, and development costs are not trivial. Therefore, we hope to set up a facility like the Apple Multimedia Lab in San Francisco to explore the uses of computer/video disc interfacing in science education. The insights we gain and the programs we develop will serve as models for other science educators. In conjunction with a multimedia laboratory, we hope to set up a student laboratory with 10–15 student stations. Here, students would work with computer programs for DNA sequence analysis and molecular modeling to perform simulations of laboratory procedures and to participate in the testing and development of multimedia productions.

Staff

In June 1988, Mark Bloom was promoted to Assistant Director of the DNA Learning Center in recognition of his dedication to the program. Mark remains primarily responsible for the smooth running of our laboratory teaching programs, including the *Vector* workshops and the *Bio2000* Laboratory. Greg Freyer, currently an assistant professor at Columbia University College of Physicians & Surgeons, continues to supply the specially designed DNA molecules used in our workshops and, together with Mark, conducts research to translate the techniques of molecular biology to the teaching environment.

We were fortunate to obtain the services of John Le Guyader as our new education manager. John comes to us from the Woodmere Academy, where he taught advanced placement biology. He has research experience at the State University of New York at Stony Brook and is an adjunct professor at Dowling College. Arriving in November, John quickly assumed the burden of instructing our daily lab class visits. His background and enthusiasm make him an ideal manager of our *Bio2000* teaching laboratory and strengthen our understanding of the problems facing today's biology educators. The teaching load was also 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.

In January 1989, Susan Zehl left the Laboratory's Public Affairs Department to join our permanent staff as designer. Sue came to Cold Spring Harbor Laboratory in 1985 as a photographic intern for Public Affairs and began a full-time position as photographer/artist following her graduation from The Cooper Union in 1986.

DNA Learning Center staff and associates (left to right) John LeGuyader, David Micklos, Greg Freyer, Susan Zehl, John Kruper, and Mark Bloom.

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*.

Interns, ranging from high school juniors to graduate students, provide critical assistance to our teaching staff. Deserving special mention are John Kruper and Jeff Mondschein. John, who is completing his doctorate in science education at the University of Illinois at Chicago, has had primary responsibility for our evaluation program, which tracks the many hundreds of teachers who have participated in *Vector* workshops over the years. Jeff, who is currently in the pre-med program at New York University, was the first DNA gypsy, traveling coast-to-coast with the first *Vector* tour in 1986. He was joined in summer 1988 by Ken LaMontagne, a native of Williston Park, presently a senior at James Madison University in Virginia. Lab aides Steve Malloy and Chris Inzarillo, both juniors at Cold Spring Harbor High School, have been key to the smooth functioning of the *Bio2000* Laboratory.

In summer 1988, we bid farewell to Ellen Gene Skaggs, who departed to Israel with her husband Jesse. Seemingly possessed of two sets of arms, she with calm precision administered the Curriculum Study and *Vector* workshop programs at a time when they were cottage industries of the Public Affairs and Development Department. She, more than anyone else, is responsible for building the "family" feeling that has made our small group so productive. We miss her every day.

Anne Zollo, gamely stepped in to fill Ellen's shoes and has managed to clear up the residual confusion left from having moved our office quarters too many times in a single year. She contributes greatly to the smooth operation of the Learning Center—juggling travel schedules, appointments, and reservations and maintaining daily contact with educators nationwide.

The opening of the DNA Learning Center to the general public also required organizing a group of volunteers to administer our museum program. Besieged by new responsibilities, we gratefully accepted the help of Anne Meier and Sandy Ordway to solicit and coordinate the participation of volunteers. With their help and that of the other volunteers, the DNA Learning Center is growing, striving to reach its potential as an "exploratorium of DNA."

Volunteer directors Sandy Ordway and Anne Meier in the bookstore, which was renovated with funds provided by the Banbury Fund of Lloyd Harbor.

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Curriculum Study Membership 1988–89

Cold Spring Harbor Central School District
Commack Union Free School District
East Williston Union Free School District
Great Neck Public Schools
Half Hollow Hill Central School District
Harborfields Central School District
Herricks Union Free School District
Huntington Union Free School District
Jericho Union Free School District
Lawrence Public Schools
Locust Valley Central School District
Manhasset Public Schools
Northport-East Northport Union Free School District
North Shore Central School District
Oyster Bay-East Norwich Central School District
Plainview-Old Bethpage Central School District
Portledge School
Port Washington Union Free School District
Sachem Central School District at Holbrook
Syosset Central School District

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
KENTUCKY	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
MINNESOTA	University of Minnesota, Minneapolis*	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
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.

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.

Molecular Approaches to Ion Channel Function and Expression

June 3–June 23

INSTRUCTORS

Snutch, Terry, Ph.D., California Institute of Technology, Pasadena
White, Michael, Ph.D., University of Pennsylvania, Philadelphia

ASSISTANT

Stevens, Meg, B.A., Yale University Medical School, New Haven, Connecticut

The technologies of molecular biology and patch-clamping have provided major revisions and novel approaches for the examination of many neurobiological problems. Applications of these methods to the study of ion channels were taught in this intensive laboratory/lecture course. Students concentrated initially on the basic aspects of these approaches and then proceeded to more integrated studies. *Patch Clamping*: cell-attached, excised patch, and whole-cell recording; design and implementation of recording equipment; theory and analysis of ionic

currents. *Ion Channel Expression*: mRNA isolation and handling; *Xenopus* oocytes as an expression system; characterization of newly expressed channels in oocytes using voltage and patch-clamp methods; monitoring changes in channel expression in PC12 and BC3H1 cells after differentiation using electrophysiology and 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

Brink, Deidre, M.S., University of Oregon, Eugene
Brody, David, M.D., Stanford University, California
Cifuentes, Fredy, M.S., Universidad de Chile, Santiago
Huang, Chi-ming, Ph.D., University of Missouri, Kansas City
Korpi, Esa, Ph.D., Alko, Ltd., Helsinki, Finland
MacKinnon, Roderick, M.D., Brandeis University, Waltham, Massachusetts
Raby, Wilfrid, Ph.D., Montreal General Hospital, Quebec, Canada
Russek, Shelley, B.A., Lederle Laboratories, Pearl River, New York
Soravia, Emilia, M.D., National Institutes of Health, Bethesda, Maryland
Stengl, Monika, M.S., University of Arizona, Tempe

SEMINARS

Margiotta, J., University of California, San Diego. Regulation of neuronal nicotinic acetylcholine receptors.
Willard, A., University of North Carolina. Ionic currents in mesenteric neurons.
Beam, K., Colorado State University. Molecular and developmental studies of muscle Ca^{++} channels.
Levinson, S.R., University of Colorado. Role of nonprotein domains in channel biology.
Horn, R., Roche Institute of Molecular Biology. Perforated patch recording.
Claudio, T., Yale University. Stable transfection of acetylcholine receptors in mammalian cell lines.
Sahley, C., Yale University. Behavioral and cellular analysis of learning or What are all those channels for?
Stevens, C.F., Yale University. What's so exciting about glutamate receptors?

Molecular Embryology of the Mouse

June 3–June 23

INSTRUCTORS

Beddington, Rosa, Ph.D., ICRF, Oxford, England
Robertson, Liz, Ph.D., Columbia University, New York, New York

CO-INSTRUCTORS

Lovell-Badge, Robin, Ph.D., National Institute for Medical Research, London, England
McMahon, Andy, Ph.D., Roche Institute of Molecular Biology, Nutley, New Jersey

ASSISTANTS

Cooke, Lesley, University of Cambridge, England
McMahon, Jill, M.A., Roche Institute of Molecular Biology, Nutley, New Jersey

This course was 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 preimplantation and postimplantation embryos; embryo transfer; establishment, culture, and manipulation of embryo-derived stem cell lines; germ layer separation; chimera formation; nuclear

transplantation; microinjection of DNA into eggs; retroviral infection of embryos; in situ hybridization; immunofluorescence and immunoperoxidase techniques.

PARTICIPANTS

Bucan, Maja, Ph.D., Imperial Cancer Research Fund, London, England
Cheah, Kathryn, Ph.D., Hong Kong University
Downs, Karen, Ph.D., University of California, San Francisco
Hardeman, Edna, Ph.D., Childrens' Research Foundation, Camperdown, Australia
Harris, Thomas, M.S., Albert Einstein College, Bronx, New York
Johnson, Gary, Ph.D., University of Massachusetts, Amherst
Lee, Se-Jin, B.A., Johns Hopkins University, Baltimore, Maryland
Lendahl, Urban, Ph.D., Massachusetts Institute of Technology, Cambridge
Norton, Pamela, Ph.D., Massachusetts Institute of Technology, Cambridge
Pey, Roxana, M.S., University of Chile, Santiago
Sandhu, Faheem, B.A., University of Rochester, New York
Stewart, A. Francis, Ph.D., German Cancer Research Center, Heidelberg
Sutton, Paul, B.A., University of Illinois, Urbana
Whitelaw, Emma, Ph.D., University of Oxford, England

SEMINARS

Avner, P., Pasteur Institute. Genetic resources.
Hogan, B., Vanderbilt University. Extraembryonic membranes and extracellular molecules.
Ruddle, F., Yale University. Mouse homeobox genes.
Wassarman, P., Roche Institute of Molecular Biology. Fertilization.
Mintz, B., Fox Chase Center. Development of the pigmentary system.
Jaenisch, R., Whitehead Institute. Retroviruses and development.
Bradley, A., Baylor College. Genetic manipulation of embryonic stem cells.
Papaioannou, G., Tufts University. Developmental mutants.
Williams, D., Children's Hospital, Boston. Hematopoietic stem cells.
Clark, S., Genetics Institute. Hematopoietic growth factors.
Petersen, A., Ludwig Institute. Development of the peripheral nervous system.
Sapienza, C., Ludwig Institute. Imprinting and its implications.
Cepko, C., Harvard Medical School. Retroviral analysis of neural lineages.
Calof, A., Tufts University. Early development of the central nervous system.
Rossant, J., Mt. Sinai Hospital. Chimaeras in development.
Costantini, F., Columbia University. Analysis of globin genes using transgenic mice.
Hanahan, D., Cold Spring Harbor Laboratory. Transgenics and oncogenesis.
Struhl, G., Columbia University. *Drosophila* development.
Chalfie, M., Columbia University. *C. elegans* development.
Jeffreys, B., University of Texas, Austin. Ascidian development.
Klar, A., Cold Spring Harbor Laboratory. Imprinting in yeast.
Solter, D., Wistar Institute. Nuclear transplantation and imprinting.

Advanced Bacterial Genetics

June 3–June 23

INSTRUCTORS

Berget, Peter, Ph.D., Carnegie Mellon University, Pittsburgh, Pennsylvania
Maurer, Russ, Ph.D., Case Western Reserve University, Cleveland, Ohio
Weinstock, George, Ph.D., University of Texas School of Medicine, Houston

ASSISTANTS

Barrett, B. Kyle, B.S., Carnegie Mellon University, Pittsburgh, Pennsylvania
Cardaman, Richard, B.S., Case Western Reserve University, Cleveland, Ohio
Heath, Joe Don, B.S., University of Texas School of Medicine, Houston

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 covered included isolation, complementation, and mapping of mutations; use of transposable genetic elements; construction of gene fusions; cloning and manipulation of DNA; 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

Brun, Yves, M.S., Université Laval, Quebec, Canada
Caces, Maria Luz, M.S., University of Hawaii, Honolulu
Damon, Inger, B.A., University of Connecticut, Farmington
Dicker, Ira, Ph.D., E.I. du Pont de Nemours & Company, Wilmington, Delaware
Hitchcock, Penny, M.S., University of Tennessee, Memphis
Jenal, Urs, B.S., ETH, Zurich, Switzerland
Kaempf, Charlotte, Ph.D., Cornell University, Ithaca, New York
Khosla, Chaitan, B.S., California Institute of Technology, Pasadena
Krishnan, B. Rajendra, Ph.D., Carleton University, Ottawa, Canada
Libby, Stephen, Ph.D., Kansas State University, Manhattan, Kansas
Lobo, Denise, M.S., Institut de Biologie, Paris, France
Muir, Susie, Ph.D., Research Institute of Scripps Clinic, La Jolla, California
Pierce, Margaret, Ph.D., Oklahoma State University, Stillwater
Puziss, John, B.S., University of North Carolina, Chapel Hill
Radstrom, Peter, M.S., Biomedical Center, Uppsala, Sweden
Zeef, Leo, B.S., Leiden University, The Netherlands

SEMINARS

Ausubel, F., Harvard Medical School. Regulation of nitrogen fixation gene expression in *Rhizobium* and its relation to general models of signal transduction in prokaryotes.

Malloy, S., University of Illinois. Genetic analysis of protein structure and function—Mutations that define the active site of proline permease of *Salmonella*.

Shapiro, L., Columbia University College of Physicians & Surgeons. Temporal and spatial regulation of gene expression during *Caulobacter* development.

McClelland, M., University of Chicago. Enzymology for pulse-field mapping of bacterial genomes.

Schuman, H., Columbia University. Genetics of *Legionella* pathogenicity.

Molecular Neurobiology of Human Disease

June 5–June 15

INSTRUCTORS

Black, Ira B., M.D., Cornell University Medical Center, New York, New York

Breakefield, Xandra O., Ph.D., E.K. Shriver Center and Harvard Medical School, Boston, Massachusetts

Gusella, James, F., Ph.D., Massachusetts General Hospital and Harvard Medical School, Boston

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 psychiatirc diseases. Emphasis was also placed on new techniques in neuroscience and molecular genetics that should provide additional insights. Topics included Molecular pathology of neurotransmitter derangement; Developmental plasticity and choice of neurotransmitter phenotype; Synthesis and regulation of neuropeptides; Cellular events in neural regeneration and brain transplantation; Neural pathways involved in pain syndromes; Genetic linkage analysis using DNA polymorphisms; Defects in

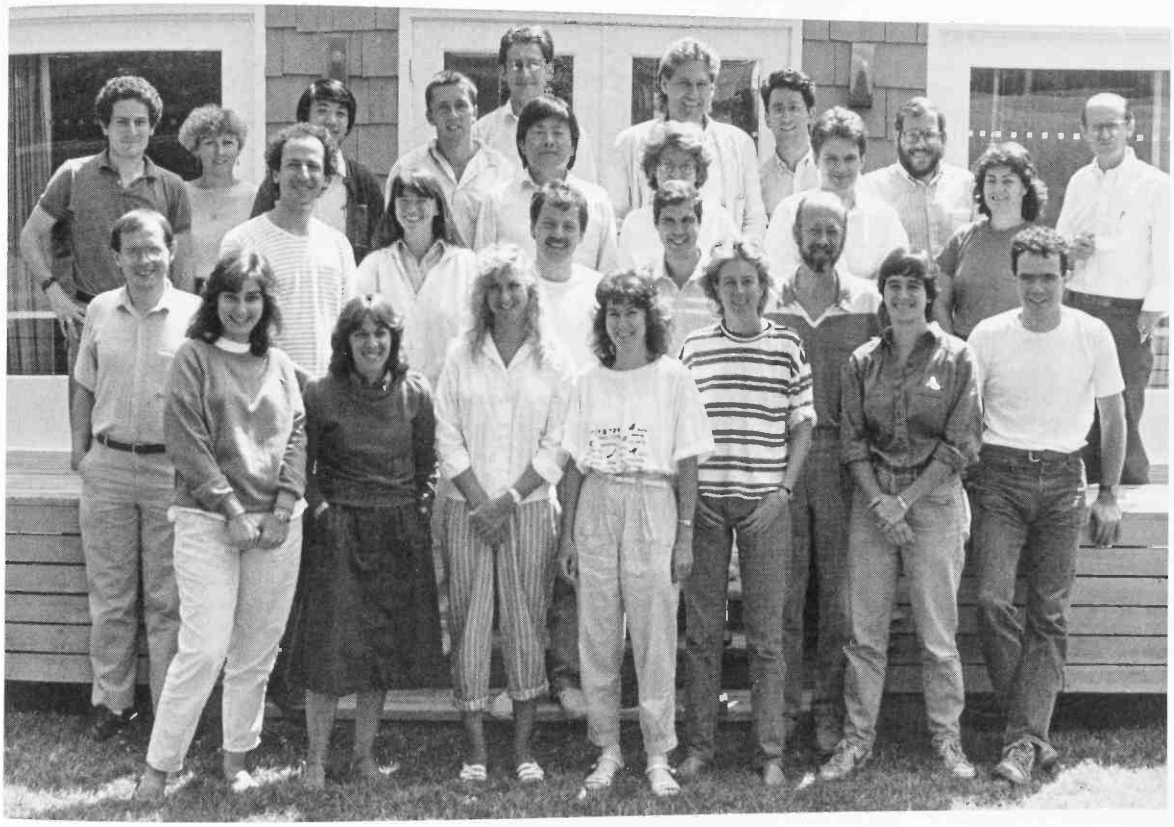
DNA repair; Activation of *onc* genes and genetic homozygosity in neural tumors; Mutations causing the Lesch-Nyhan syndrome and possible means of gene therapy; Biochemistry of the lipidoses; Autoimmune diseases; Brain imaging and metabolism; Epilepsy and seizure disorders; Cell death in degenerative disorders; Viral infections of the nervous system; Experimental models of learning and memory.

PARTICIPANTS

Alsobrook, John, M.S., Yale University, New Haven, Connecticut
Borasio, Gian, M.D., Max-Planck-Institut, Martinsried, Federal Republic of Germany
Chirwa, Sanika, M.S., University of British Columbia, Vancouver
Cohen, Maurice, Ph.D., Abbott Laboratories, Abbott Park, Illinois
Ezzedine, Diala, M.S., E.K. Shriver Center, Boston, Massachusetts
Fischer, Walter, B.S., University of Lund, Sweden
Freimer, Nelson, M.D., Columbia University, New York, New York
Gardella, Joseph, B.S., State University of New York, Stony Brook
Giuffra, Luis, M.D., Yale University, New Haven, Connecticut
Godbout, Martin, Ph.D., Scripps Clinic and Research Foundation, La Jolla, California
Gomez, Maria del Pilar, M.D., Boston University, Massachusetts
Hemperly, John, Ph.D., Becton Dickinson and Co., Research Triangle Park, North Carolina
Hingorani, Vijay, Ph.D., University of Illinois, Chicago
Lock, Christopher, B.S., Imperial Cancer Research Fund, London, England
Melmer, Georg, Ph.D., Hospital for Sick Children, Toronto, Canada
Nagy, Thomas, B.S., University of Calgary, British Columbia
Pollack, Nancy, M.S., Emory University, Atlanta, Georgia
Qiu, Feihua, M.D., Memorial Sloan-Kettering Cancer Center, New York, New York
Raymond, Vincent, Ph.D., Salk Institute, San Diego, California
Rubin, Michael, Ph.D., Columbia University, New York, New York
Saraiva, Maria, Ph.D., Universidade Do Porto, Portugal
Wang, Samuel, B.S., Stanford University, Pacific Grove, California
Watson, Bracie, M.S., Howard University, Washington, D.C.
Welch, Mary, Ph.D., Boehringer Mannheim Corp., Indianapolis, Indiana

SEMINARS

Rossant, J., Mt. Sinai Hospital. Creating animal models using embryonic cells.
Evans, G., Salk Institute. Transgenics—Cell ablation studies.
Sanes, J., Washington University. Retrovirus—Cell lineage studies.
Coleman, D., Columbia University College of Physicians & Surgeons. Biogenesis of myelin membrane.
Milner, R., Salk Institute. Genetic defects in myelin.
Lee, W.H., University of California, San Diego. Retinoblastoma gene and protein.
Mechler, B., Johannes Guttenburg University. *Drosophila* cancer gene.
Anderson, R., Massachusetts Institute of Technology. Visual cortex and modeling.
Roses, A., Duke University Medical Center. Myotonic dystrophy.
Ray, P., Hospital for Sick Children. Duchenne's muscular dystrophy.
Gravel, R., Hospital for Sick Children. Genetic defects in lysosomal enzymes.
Woo, S., Baylor College of Medicine. Molecular basis and population genetics of PKU.
McNamara, J.O., Duke University Medical Center. Epilepsy.
Dingledine, R., University of North Carolina. NMDA receptors.
Uhl, G., National Institute of Drug Abuse. Receptor distribution in brain.
Price, R., Memorial Sloan-Kettering Cancer Center. AIDS in CNS.
Wexler, N., Columbia University. Molecular and human aspects of Huntington's disease.
Schwarcz, R., Maryland Psychiatric Research Center. Cell death in Huntington's disease.
Aguayo, A., Montreal General Hospital. Regeneration in the adult mammalian CNS.
Rakic, P., Yale University School of Medicine. Cell migration and the Hiroshima blast.
Fields, B., Harvard Medical School. Molecular basis of neurotropism.
Stevens, J., University of California, Los Angeles. Herpesvirus pathogenesis of latency.
Davies, P., Albert Einstein College of Medicine. Alzheimer's disease protein.
Gurney, M., University of Chicago. Neuroleukin.
Chao, M., Cornell Medical School. Growth factors.
McGeer, P., University of British Columbia. Neuroimmunology.
Gage, F., University of California School of Medicine, La Jolla. Brain transplantation.
Raichle, M.E., Washington University School of Medicine. PET.
Filipek, P., Massachusetts General Hospital. MRI-based morphometric analyses.



Developmental Neurobiology

June 17–July 1

INSTRUCTORS

Goodman, Corey, Ph.D., University of California, Berkeley
Patterson, Paul, Ph.D., California Institute of Technology, Pasadena

The aim of this lecture course was to review established principles and recent advances in developmental neurobiology. Major topics considered were proliferation, migration, and aggregation of nerve cells; factors influencing the differentiation of neurons; trophic interactions in neural development; patterns, gradients and compartments; genetic programs for development; the guidance of axons to targets; and the formation of synaptic connections. Particular emphasis was given to synapse formation and to mechanisms underlying the specificity of this process. Finally, the operation of developmental principles was examined in the context of the mammalian visual system and in the development of learning and behavior.

PARTICIPANTS

Baron, Margaret, Ph.D., Harvard University, Cambridge, Massachusetts
Basler, Konrad, B.S., University of Zurich, Switzerland
Dudek, Serena, B.S., Brown University, Providence, Rhode Island
Grenningloh, Gabriele, Ph.D., University of Heidelberg, Federal Republic of Germany
Harel, Adrian, M.S., Weizmann Institute, Rehovot, Israel
Hart, Anne, B.S., University of California, Los Angeles
Hill, Caryl, Ph.D., Australian National University, Canberra
Hopkins, Nancy, Ph.D., Massachusetts Institute of Technology, Cambridge
Howard, Kenneth, Ph.D., Columbia University, New York, New York

Hynes, Mary, Ph.D., Columbia University, New York, New York
Kalb, Robert, M.D., Yale University, New Haven, Connecticut
Lugo, Ana, B.S., University of Puerto Rico, Rio Piedras
Moore, Robert, B.S., National Institute for Medical Research, London, England
Mundy, Nicholas, M.A., University College London, England
Nawa, Hiroyuki, Ph.D., California Institute of Technology, Pasadena
Pickford, Lesley, B.S., Stanford University, California
Placzek, Marysia, Ph.D., Columbia University, New York, New York
Rovelli, Giorgio, M.S., Friedrich Miescher Institute, Basel, Switzerland
Sabry, James, M.D., California Institute of Technology, Pasadena
Tonegawa, Susumu, Ph.D., Massachusetts Institute of Technology, Cambridge
Viereck, Christopher, M.S., Friedrich Miescher Institute, Basel, Switzerland
Wilson, Stephen W., Ph.D., University of Michigan, Ann Arbor

SEMINARS

Shatz, C., Stanford Medical School. Neurogenesis, migration, and synaptogenesis in the mammalian visual system.
Cepko, C., Harvard Medical School. Retroviruses and lineage in the CNS.
Hatten, M.E., Columbia University Medical School. Migration in the embryonic CNS.
Raff, M., University College London. Glial lineages in the CNS.
Rubin, G., University of California, Berkeley. Development of the *Drosophila* eye.
Chalfie, M., Columbia University. Neural development in the nematode.
Anderson, D., California Institute of Technology. Neural crest differentiation.
———. Intracellular mechanisms of trophic factors.
Landmesser, L., University of Connecticut. Axon guidance in vertebrates.
Rutishauser, U., Case Western Reserve University. Adhesion molecules in the nervous system.
Jessell, T., Columbia University Medical School. Mechanisms of specificity in the embryonic spinal cord.
Fraser, S., University of California, Irvine. Development of retinotectal specificity.
Bonhoeffer, F., Max-Planck-Institut, Tubingen. Surface molecules and retinotectal connections.
Sanes, J., Washington University Medical School. Development of the neuro-muscular junction.
Purves, D., Washington University Medical School. Synaptic specificity and rearrangement in the PNS.
Kandel, E., Columbia University Medical School. Learning and plasticity of connections in invertebrates.
Nottebohm, F., Rockefeller University. Learning and plasticity in the bird song system.

Neurobiology of *Drosophila*

June 26–July 16

INSTRUCTORS

Bate, Michael, Ph.D., University of Cambridge, England
Campos-Ortega, Jose, M.D., Ph.D., University of Cologne, Federal Republic of Germany
Palka, John, Ph.D., University of Washington, Seattle

ASSISTANTS

Brand, Michael, M.S., University of Cologne, Federal Republic of Germany
Hannaford, Susanah, B.S., 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, taking advantage of the genetic and molecular techniques that are so highly developed for this organism. The course included a crash course on *Drosophila* genetics and other techniques that 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. It also included mutant analysis of complex behaviors, such as courtship, circadian rhythms, learning, and memory. 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

Awad, Timothy A., B.S., University of Washington, Seattle
Baylies, Mary K., B.A., Rockefeller University, New York, New York
Bonini, Nancy, Ph.D., University of Wisconsin, Madison
Delgado, Ricardo, B.S., Universidad de Chile, Santiago
Malsch, Paul L., M.S., Kansas State University, Manhattan
Pflugfelder, Gert, Ph.D., Universitat Würzburg, Federal Republic of Germany
Rutledge, Barbara J., Ph.D., Harvard University, Cambridge, Massachusetts
Steele, Fintan R., M.A., University of Notre Dame, Indiana
Ungar, Anne R., B.A., Northwestern University, Evanston, Illinois
White, Kristin, Ph.D., Massachusetts Institute of Technology, Cambridge

SEMINARS

Aldrich, R., Stanford University. Ion channels in *Drosophila*.
Elkins, T., University of California, Berkeley. Adhesion molecules.
Fischbach, K.-F., University of Freiburg. Brain anatomy and mutants.
Ganetzky, B., University of Wisconsin. *Drosophila* genetics.

Hall, J., Brandeis University. Behavior and its genetic analysis.
Hartenstein, V., University of California, San Diego. Neurogenesis.
Hartley, D., Yale University. Molecular biology of neurogenic mutants.
Hoy, R., Cornell University. Behavior and evolution.
Orr-Weaver, T., Massachusetts Institute of Technology. Germ line transformation.
Ready, D., Purdue University. Eye morphogenesis.
Rubin, G. M., University of California, Berkeley. How to clone interesting genes.
Technau, G., University of Cologne. Cell transplantation.
Timpe, L., University of California, San Francisco. Cellular neurophysiology.
Truman, J.W., University of Washington. Postembryonic neurogenesis.

Molecular and Developmental Biology of Plants

June 26–July 16

INSTRUCTORS

Maliga, Pal, Ph.D., Advanced Genetic Sciences, Oakland, California
Messing, Joachim, Ph.D., Waksman Institute, Rutgers University, Piscataway, New Jersey
Sussex, Ian, Ph.D., Yale University, New Haven, Connecticut

ASSISTANTS

Cruz-Alvarez, Marilyn, Ph.D., Waksman Institute, Rutgers University, Piscataway, New Jersey
Elliston, Keith, B.S., Waksman Institute, Rutgers University, Piscataway, New Jersey
Harper, Elizabeth, B.S., Advanced Genetic Sciences, Oakland, California
McGonigle, Bryan, M.S., Yale University, New Haven, Connecticut
Miller, Ellen, M.S., Yale University, New Haven, Connecticut

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 a 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, as well as informal discussions after their seminars. The laboratory covered established and novel techniques in plant biology, including plant structure and development, nucleic acid manipulations, gene transfer techniques, tissue and cell culture techniques, photosynthesis, and genetics and cytogenetics of maize.

PARTICIPANTS

Benvenuto, Eugenio, Ph.D., ENEA, Rome, Italy
DeVerna, Joseph, Ph.D., Campbell Institute, Davis, California
Gallagher, Jane, Ph.D., City College of New York, New York
Graner, Andreas, Ph.D., Institut fur Resistenzgenetik, Gruenbach, Federal Republic of Germany
Handley, Levis, Ph.D., Westvaco Corp., Summerville, South Carolina
Irish, Vivian, Ph.D., Yale University, New Haven, Connecticut
Johnson, Sheila, B.S., University of California, Davis
Kreiberg, Jette, Ph.D., Aarhus University, Denmark
Mahoney, Deborah, B.A., Princeton University, New Jersey
Moneger, Francoise, M.S., Université Joseph Fourier, Grenoble, France
Paul, Cynthia, Ph.D., Rockefeller University, New York, New York
Pecker, Iris, M.S., Hebrew University, Jerusalem
Sadosky, Alesia, B.S., Pennsylvania State University, University Park
Shiraishi, Hideaki, Ph.D., National Institute, Okazaki, Japan
Vioque, Agustin, Ph.D., Universidad Autonoma, Madrid, Spain
Waibel, Franz, Ph.D., Friedrich Miescher Institute, Basel, Switzerland

SEMINARS

Horsch, R., Monsanto Co. Transformation systems for plants.
Mottinger, J., Rhode Island University. Classical and molecular methods for mapping genes to chromosomes.
Nelson, T., Yale University. C_3 and C_4 photosynthesis gene expression.
Tobin, E., University of California, Los Angeles. Light regulation of photosynthesis genes.
Quatrano, R., E.I. du Pont de Nemours & Company. Polarity induction in *Fucus* zygotes.
Gruissem, W., University of California, Berkeley. Fruit ripening molecular biology.
Levings III, C., North Carolina State University. Mitochondrial genomes and male sterility.
Poethig, S., University of Pennsylvania, Genetic and clonal analysis of corn.
Theologis, S., Plant Gene Expression Center. Hormone-regulated gene expression.
Chumley, F. and B. Sheperd, E.I. du Pont de Nemours & Company. Rice blast disease—Biology and molecular biology.
Meyerowitz, E., California Institute of Technology. *Arabidopsis*—Genes, genome organization, mutants.
Rogers, S., Monsanto Co. Molecular biology of the *gemini* viruses.
Bernatzky, R., University of Massachusetts. Restriction fragment length polymorphism mapping in plants.
Klein, E., Plant Gene Expression Center. Transformation with the particle gun.
Whalen, M., University of California, Berkeley. Interactions of plants with their pathogenic bacteria.
Gillham, N., Duke University. *Chlamydomonas* chloroplast genetics and transformation.
Crouch, M., Indiana University. Developmental biology of pollen.
Meinke, D., Oklahoma State University. Developmental biology and genetic analysis in *Arabidopsis*.
Dellaporta, S., Yale University. Genetic and molecular analysis of the *R* locus in maize.

Molecular Cloning of Eukaryotic Genes

June 26–July 16

INSTRUCTORS

Alt, Fred, Ph.D., Columbia University, New York, New York
Roberts, Thomas, Ph.D., Dana Farber Cancer Institute, Boston, Massachusetts
Yancopoulos, G., M.D., Ph.D., Columbia University, New York, New York

ASSISTANTS

Ma, Avaril, M.D., Columbia University, New York, New York
Morgan, William, B.S., Dana Farber Cancer Institute, Boston, Massachusetts
Oltz, Eugene, Ph.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 and characterization of lymphocyte specific genes were emphasized. Among the topics covered were the construction of cDNA libraries in plasmid or bacteriophage λ vectors, construction of bacteriophage λ and cosmid libraries of high-molecular-weight eukaryotic DNA, screening DNA libraries with gene-specific hybridization probes, purification and characterization of recombinant clones using restriction endonuclease and blot hybridization analyses, and reintroduction and expression of cloned genes in heterologous systems. Strategies for isolating genes that encode rare mRNA sequences were discussed. Guest lecturers discussed the application of molecular cloning procedures to the study of specific eukaryotic gene systems.

PARTICIPANTS

Albino, Tony, Ph.D., Memorial Sloan-Kettering Cancer Center, New York, New York
Biddle, Fred, Ph.D., Alberta Children's Research Center, Canada
Bierer, Barbara, M.D., Dana Farber Cancer Institute, Boston, Massachusetts
Brannon, Patsy, Ph.D., University of Arizona, Tempe
Cheng, Hazel, Ph.D., University of Toronto, Ontario, Canada
Coffey, Robert, M.D., Vanderbilt University, Nashville, Tennessee
Dahl, Karen, M.D., Yale University Medical School, New Haven, Connecticut
Fletcher, Jacqueline, Ph.D., Oklahoma State University, Stillwater
Gahl, William, Ph.D., National Institutes of Health, Bethesda, Maryland
Gilly, William, Ph.D., Stanford University, Pacific Grove, California
Johnson, Thomas, Ph.D., University of California, Irvine
Kornecki, Elizabeth, Ph.D., University of Vermont, Burlington

Larsson, Catharina, M.D., Karolinska Hospital, Stockholm, Sweden
Simpson, Paul, M.D., VA Hospital, San Francisco, California
Stevenson, Mary, Ph.D., Montreal General Hospital, Canada
Stutman, Osias, M.D., Memorial Sloan-Kettering Cancer Center, New York, New York

SEMINARS

Aruffo, S., Massachusetts General Hospital. Techniques for cloning mammalian cell surface proteins.
Ferrier, P. and K. Blackwell, Columbia University. Gene transfer—Techniques to study *Ig* gene recombination.
Harlow, E., Cold Spring Harbor Laboratory. E1A complexes with the retinoblastoma protein.
McKnight, S., Carnegie Institution. Purification and cloning of mammalian transcription factors.
Hood, L., California Institute of Technology. *Ig* gene superfamily—New advances in molecular genetic technologies.
Cantor, C. and C. Smith, Columbia University. Large-scale genome mapping.
Goff, S., Columbia University. Retroviral genetics.
Hanahan, D., Cold Spring Harbor Laboratory. Transgenic systems as models for oncogenesis.
Wigler, M., Cold Spring Harbor Laboratory. Oncogenes/signal transduction.
Kellems, R., Baylor College of Medicine. Molecular genetics of ADA.
Tucker, P., Southwestern Medical Center. Control of *Ig* gene expression.
Sakano, H., University of California, Berkeley. Purification of proteins involved in *Ig* recombination.
Mulligan, R., Whitehead Institute. The Complete Angeler.
Shapiro, L., Columbia University. Control of bacterial gene expression.
Okayama, H., National Institutes of Health. Expression cloning.
Gitsier, J., University of California, San Francisco. PCR and molecular genetics.
Tsukamoto, A., University of California, San Francisco. Int-1 tumorigenesis in transgenic mice.
Weissman, I., Stanford University. Molecular genetics of a reconstructed immune system.
Dixon, R., Merck, Sharp and Dohme. Site-directed mutagenesis—Beta-adrenergic receptor.
Kucherlapati, R., University of Illinois. Reverse genetics.
Piwnica-Worms, H., Dana Farber Cancer Institute. Baculovirus as a tool to study oncogene function.
Robertson, L., Columbia University. Reverse genetics—Part II.

Computational Neuroscience: Motor Control

July 11–July 24

INSTRUCTORS

Atkeson, Chris, Ph.D., Massachusetts Institute of Technology, Cambridge
Bizzi, Emilio, Ph.D., Massachusetts Institute of Technology, Cambridge

ASSISTANT

McIntyre, Joe, B.S., Massachusetts Institute of Technology, Cambridge

This intensive laboratory/lecture course examined computational approaches in motor control, with the theme that understanding of the computational problems, the constraints on solutions to these problems, and the range of possible solutions can help guide research in neuroscience.

The most prominent successes of information-processing approaches have come in areas where strong inputs from neurobiological, behavioral, and computational approaches interact. The goals of this course were to expose students to areas where interdisciplinary approaches have been important and to help students integrate computational approaches into their own research. The course included a computer-based laboratory so that students could actively explore computational issues, as well as interact with prominent research workers in the field.

The course focused on computational approaches to the study of motor control and their interactions with motor control neuroscience. Examples were taken from

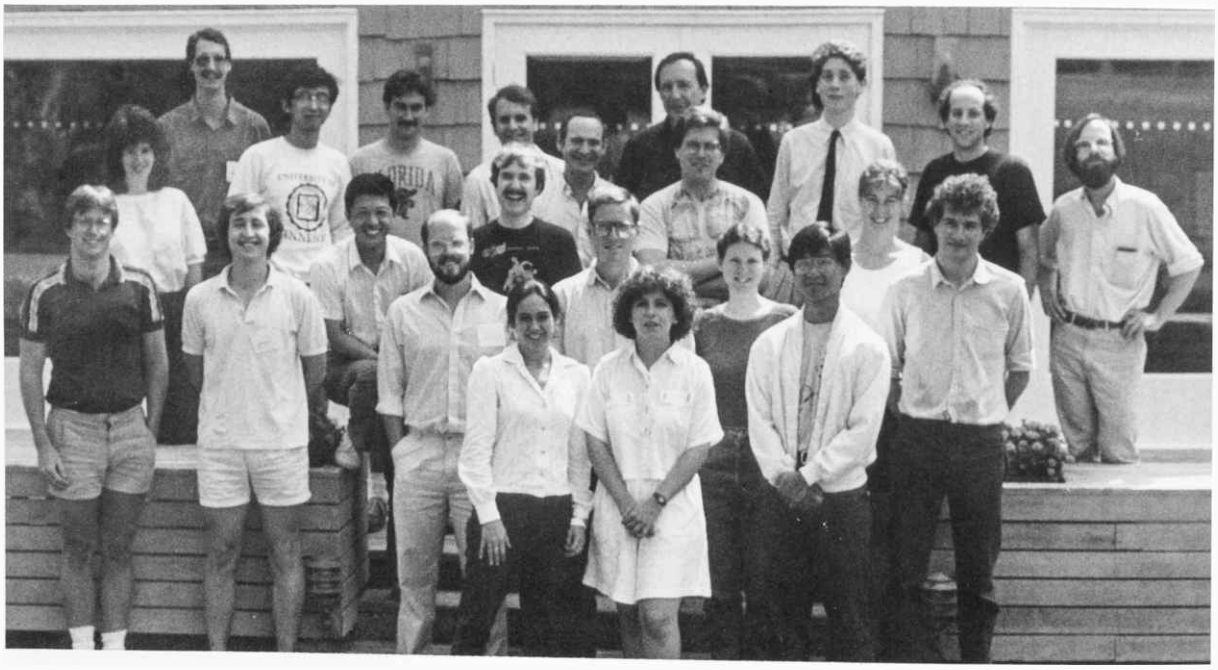
single- and multi-articular arm movements; body posture and locomotion; hand control; oculomotor control; and "simpler" nervous systems. Areas addressed were movement planning, kinematics, dynamics, control, actuation, and sensing.

PARTICIPANTS

Amos, Therese, B.S., University of Minnesota, Minneapolis
Bracewell, Robert, B.A., Massachusetts Institute of Technology, Cambridge
Bronte-Stewart, Helen, M.S., University of Pennsylvania, Philadelphia
Carter, Randy, M.S., Case Western Reserve University, Cleveland, Ohio
Colgate, James, M.S., Massachusetts Institute of Technology, Cambridge
Connor, Nadine, M.A., Speech Motor Control Labs, Madison, Wisconsin
Cooper, Scott, B.A., Columbia University, New York, New York
Gnadt, James, Ph.D., University of Alabama, Birmingham
Gourdon, Antoine, B.S., CNRS, Paris, France
Haggard, Patrick, B.A., Haskins Laboratories, New Haven, Connecticut
He, Jiping, M.S., University of Maryland, College Park
Henis, Ealan, M.S., Weizmann Institute, Rehovot, Israel
Hoff, Bruce, M.S., University of Southern California, Los Angeles
Jacobs, Robert, M.S., University of Massachusetts, Amherst
Kent, Linda, B.S., Drexel University, Philadelphia, Pennsylvania
Shen, Liming, B.S., University of Minneapolis, Minnesota
Thompson, Clay, M.S., Massachusetts Institute of Technology, Cambridge
Vetter, Monica, B.S., University of California, San Francisco
Watanabe, Takashi, B.S., University of Rochester, New York

SEMINARS

Sparks, D., University of Alabama. Role of the superior colliculus and other brainstem areas in the control of saccadic eye movements.
Wurtz, R., National Eye Institute. Physiology of the pursuit system.
Hogan, N., Massachusetts Institute of Technology. Using optimal control theory to model behavior—Minimum jerk movements.
Flash, T., Weizmann Institute of Science. Multijoint minimum jerk movements.
Hogan, N., Massachusetts Institute of Technology. Impedance control.
Mussa-Ivaldi, F., Massachusetts Institute of Technology. Multijoint arm studies.
Flash, T., Weizmann Institute of Science. Multijoint simulations.
Kalaska, J., University of Montreal. Neural representations for motor control.
Humphrey, D., Emory University Medical School. Central control of voluntary arm and hand movements.
Hollerbach, J., Massachusetts Institute of Technology. General tactile sensing issues, robot sensors.
Johnson, K., Johns Hopkins University. Mechanoreceptive transduction in the hand.



Hollerbach, J., Massachusetts Institute of Technology. General hand control issues, robot hands.
Johansson, R., University of Umea. Utilization of tactile input in the control of grip.
Jordan, M., Massachusetts Institute of Technology. Introduction to connectionism.
Raibert, M., Massachusetts Institute of Technology. Dynamics of running in robots.
Loeb, G. Queens University. Models of the cat hindlimb.

Yeast Genetics

July 18–August 7

INSTRUCTORS

Hieter, Phil, Ph.D., Johns Hopkins University, Baltimore, Maryland
Rose, Mark, Ph.D., Princeton University, New Jersey
Winston, Fred, Ph.D., Harvard University, Cambridge, Massachusetts

ASSISTANTS

Gerring, Saundra, B.S., Johns Hopkins University, Baltimore, Maryland
Malone, Elizabeth, B.A., Harvard University, Cambridge, Massachusetts
Vallen, Liz, M.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

Armstrong, John, Ph.D., Imperial Cancer Research Fund, London, England
Cardenas, Maria, Ph.D., Texas College, Fort Worth
Chan, Yuen-Ling, Ph.D., University of Chicago, Illinois
Halula, Madelon, Ph.D., Medical College of Virginia, Richmond
Heitman, Joseph, M.S., Rockefeller University, New York, New York
Hyman, Linda, Ph.D., Tufts University, Boston, Massachusetts
Klis, Frans, Ph.D., University of Amsterdam, The Netherlands
Lahue, Robert, Ph.D., Duke University, Durham, North Carolina
Prat, Annik, Ph.D., CNRS, Gif-sur-Yvette, France
Riggs, Daniel, Ph.D., University of California, San Diego
Ruusala, Tarmo, Ph.D., Yale University, New Haven, Connecticut
Sperry, Ann, Ph.D., University of Texas, Dallas
Springer, Mathias, Ph.D., Institut de Biologie, Paris, France
Tibbetts, Michael, B.S., Wesleyan University, Middletown, Connecticut
Weil, Peter, Ph.D., Vanderbilt University, Nashville, Tennessee
Zimarino, Vincenzo, M.D., National Cancer Institute, Bethesda, Maryland

SEMINARS

Sternglanz, R., State University of New York, Stony Brook. DNA topoisomerases and their roles in DNA replication and transcription.
Kurjan, J., Columbia University. Cell-cell interaction involved in yeast mating.
Klar, A., Cold Spring Harbor Laboratory. Yeast mating-type interconversion.
Carlson, M., Columbia University. Glucose repression in yeast.
Guarente, L., Massachusetts Institute of Technology. DNA binding and transcriptional activation by three yeast activators: HAP1, HAP2, and HAP3.
Wigler, M., Cold Spring Harbor Laboratory. *RAS* control pathways in yeast.
Hinnebusch, A., National Institutes of Health. Translational control of transcriptional activator, GCN4.
Stevens, T., University of Oregon. Genetic approaches to vacuole protein sorting.
Petes, T., University of Chicago. Meiotic recombination in yeast.
Fink, G., Whitehead Institute. Regulation of amino acid biosynthesis in yeast.
Sherman, F., University of Rochester. Posttranslational modification of cytochrome c.
Broach, J., Princeton University. Once more around the 2-micron circle.
Beach, D., Cold Spring Harbor Laboratory. Cell-cycle regulation in yeast.
Fox, T., Cornell University. Translational control of yeast mitochondrial genes.
Douglas, M., University of Texas Health Science Center. Organization of the yeast nuclear pore complex.
Botstein, D., Genentech, Inc. Genetics of the yeast cytoskeleton.

Molecular Probes of the Nervous System

July 19–August 8

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

GUEST INSTRUCTORS

Lagenaur, Carl, Ph.D., University of Pittsburgh, Pennsylvania
Pintar, John, Ph.D., Columbia University, New York, New York

ASSISTANT

Prouty, Steven, M.S., Medical College of Pennsylvania, Philadelphia

This course was designed for cell, molecular, and neurobiologists 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 hybridization, immunoassays (ELISA and 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. Biological assays in tissue culture were designed to demonstrate functional relevance of specific molecules. Computer-aided data analysis of protein and nucleotide sequences also was introduced.

PARTICIPANTS

Abosch, Aviva, B.A., University of Pittsburgh, Pennsylvania
Allendoerfer, Karen, B.A., Stanford University, California
Brady, Linda, Ph.D., National Institutes of Mental Health, Bethesda, Maryland
Carpenter, Melissa, B.S., University of California, Irvine
Carrithers, Michael, B.S., University of Illinois, Urbana
Goldman, Steven, Ph.D., Cornell University Medical College, New York, New York
Granger, Ellen, Ph.D., Florida State University, Tallahassee
Keller, Flavio, M.D., University of Zurich, Switzerland
Mallat, Michel, Ph.D., INSERM, Paris, France
Mandell, James, B.A., Cornell University Medical College, New York, New York
Soghomonian, Jean, Ph.D., University of Montreal, Quebec, Canada
Trainer, Vera, M.S., University of Miami, Florida

SEMINARS

Fleischman, J., Washington University School of Medicine. Immunoglobulins and the Ig super family.
Roulet, D., Massachusetts Institute of Technology. T-cell development and function.
Kearney, J., University of Alabama. B-cell development, function, and the network theory.
Lagenaur, C., University of Pittsburgh School of Medicine. Cell-surface interactions in neural development.
Alt, F., Columbia University. Immunoglobulin gene rearrangements.
Sharff, M., Albert Einstein College of Medicine. Somatic mutations and the antibody repertoire.
Cohen, S., Hahnemann University School of Medicine. Interleukins and growth factors of the immune system.
Schwartz, M., Weizmann Institute. Molecular aspects of nerve regeneration.

Hockfield, S., Yale University School of Medicine. Molecular aspects of synapse formation and brain organization.
Kaufman, D., University of California, Los Angeles. Molecular cloning of neural genes.
Lindstrom, J., Salk Institute. Molecular organization of the acetylcholine receptor.
Pintar, J., Columbia University College of Physicians & Surgeons. Molecular aspects of pituitary development.

Advanced Molecular Cloning and Expression of Eukaryotic Genes

July 19–August 8

INSTRUCTORS

Botchan, Michael, Ph.D., University of California, Berkeley
Myers, Richard, Ph.D., University of California, San Francisco
Rio, Don, Ph.D., Whitehead Institute, Cambridge, Massachusetts

ASSISTANTS

Brodsky, Michael, B.A., University of California, San Francisco
Kaufman, Paul, B.A., Whitehead Institute, Cambridge, Massachusetts
Robbins, Alan, B.A., E.I. du Pont de Nemours & Co., Wilmington, Delaware

The focus of this course was on how to manipulate cloned eukaryote genes to probe questions on expression, structure, and function. Students created and screened both genomic and cDNA libraries from various organisms with established, as well as experimental, protocols. A variety of transfection techniques were used to introduce cloned DNA molecules that had been manipulated in vitro into *Drosophila* and vertebrate cells in culture. As a model system for this approach, we examined *cis*- and *trans*-acting components involved in the regulation of eukaryote gene expression. Mutants were generated by a variety of methods, including oligo-directed and random mutagenesis procedures. New

methods for generating and physically isolating mutant DNAs, as well as standard DNA sequencing techniques, were employed. Physical methods for screening mutant genes in cellular genomic DNA were also discussed and demonstrated. Guest lecturers included speakers who discussed present problems in eukaryote molecular biology, as well as technical approaches to their solutions.

PARTICIPANTS

Agrwal, Neera, B.S., Michigan State University. East Lansing
Aladjem, Mirit, B.S., Tel Aviv University, Israel
Boris, Kathleen, M.S., George Washington University, Washington, D.C.
Giallongo, Agata, Ph.D., Istituto di Biologia dello Sviluppo, Palermo, Italy
Griswold-Prenner, Irene, B.A., University of California Medical Center, Los Angeles
Kagnoff, Martin, M.D., University of California, San Diego
Lasmoles, Françoise, Ph.D., INSERM, Paris, France
Leask, Andrew, B.S., University of Chicago, Illinois
Lee, Brendan, B.S., State University of New York, Brooklyn
Lo, Donald, B.S., Yale University, New Haven, Connecticut
Powers, Maureen, B.S., University of California, Davis
Ratanavongsiri, Jitra, M.S., University of Alberta, Edmonton, Canada
Sorrentino, Rosa, Ph.D., Universita di Roma, Italy
Teitz, Tal, M.S., Tel Aviv University, Ramat Aviv, Israel
Wefald, Franklin, M.D., Duke University, Durham, North Carolina
Williams, Lewis, Ph.D., University of California, San Francisco

SEMINARS

Sharp, P., Massachusetts Institute of Technology. Structure of the transcriptional complex.
Guarante, L., Massachusetts Institute of Technology. Transcriptional regulatory elements in yeast.
Mulligan, R., Massachusetts Institute of Technology. Gene transfer systems.
Spradling, A., Carnegie Institution of Washington. Genetic methods for studying meiosis in *Drosophila*.
Tjian, R., University of California, Berkeley. Structure of transcriptional factors in metazoans.
Chan, M., Cornell Medical College. Receptors in eukaryotic cells.
Maniatis, T., Harvard University. Organization and control of gene expression at the interferon locus.
Evans, R., Salk Institute. Steroid receptors.
Ptashne, M., Harvard University. Theoretical notions regarding transcriptional regulation in eukaryon.

Molecular Biology of the Nervous System

July 26–August 8

INSTRUCTORS

Evans, Ron, Ph.D., Salk Institute, La Jolla, California
McKay, Ron, Ph.D., Massachusetts Institute of Technology, Cambridge
Reichardt, Louis, Ph.D., University of California, San Francisco
Zipursky, Larry, Ph.D., University of California, Los Angeles

This lecture course was designed for neuroscientists who wish to understand the concepts and methods of molecular biology and their application to problems in neuroscience. The participants were drawn from a wide range of backgrounds. The methods of recombinant DNA technology were introduced in a series of lectures. These were followed by lectures from visiting faculty. The lectures covered a variety of topics to give an overview of the molecular mechanisms underlying the development and function of the nervous system.

PARTICIPANTS

Baron, Miron, M.D., New York State Psychiatric Institute, New York
Blackshear, Ann, Ph.D., Tennessee State University, Nashville
Bulleit, Robert, Ph.D., California Institute of Technology, Pasadena
Buonamici, Matilde, Ph.D., Farmitalia Carlo Erba, Nerviano, Italy
Farkash, Yigal, M.S., Hebrew University, Jerusalem, Israel
Friedman, Beth, Ph.D., Yale University, New Haven, Connecticut

Gavras, Haralambos, M.D., Boston University, Massachusetts
Gladstein, Laura, B.A., University of Pittsburgh, Pennsylvania
Gullapalli, Sharada, Ph.D., Indian Institute of Science, Bangalore, India
Hamilton, Bryan, M.S., University of Wisconsin, Madison
Hayes, Tim, M.S., Massachusetts Institute of Technology, Cambridge
Liu, Shumo, M.S., Massachusetts Institute of Technology, Cambridge
Marcom, Kelly, B.S., Baylor College of Medicine, Houston, Texas
Matteoni, Rafaele, Ph.D., Center of Molecular Biology, Rome, Italy
Smith, Martin, Ph.D., Upjohn Co., Kalamazoo, Michigan
Taplitz, Susan, Ph.D., California Institute of Technology, Pasadena
Wang, Yonping, M.S., University of Rhode Island, Kingston

SEMINARS

Green, M., Harvard University. Transcription and RNA processing in eukaryotes.
Myers, R., University of California, San Francisco. Long-range structure of the genome.
Smith, J., National Institute of Medical Research, London. Early steps in amphibian development.
Kater, S., Colorado State University. Regulating growth cone function.
Reese, T., National Institutes of Health. How axons work.
Mitchison, T., University of California, San Francisco. Cell biology of neurite extension.
Chikaraishi, D., Tufts University. Molecular biology of catecholamine biosynthesis.
Kenyon, C., University of California, San Francisco. Molecular genetics of nematode development.
Staunton, D., Center for Blood Research, Boston. Cell-surface receptors in immune function.
Jessel, T., Columbia University. Axon guidance in the mammalian spinal cord.
Bastiani, M., University of Utah. Axon guidance in the insect nervous system.
Curran, T., Roche Institute. Transcriptional regulation in the nervous system by the *fos/jun* complex.
Sweatt, D., Columbia University. Mechanisms regulating synaptic strength.
Schwarz, T., University of California, San Francisco. Molecular biology of voltage-regulated channels.
Reed, R., Johns Hopkins University. Molecular biology of olfaction in mammals.
Patrick, J., Salk Institute. Molecular biology of the acetylcholine receptor.
Zucker, C., University of California, San Diego. Visual transduction in the insect eye.
Kennedy, M., California Institute of Technology. Biochemistry of phosphokinases and synaptic function in the mammal.
Madison, D., Yale University. Physiology of long-term potentiation in the mammalian hippocampus.
Andersen, R., Massachusetts Institute of Technology. Network analysis of mammalian cortical function.

Molecular Cloning of Neural Genes

August 10–August 30

INSTRUCTORS

Eberwine, Jim, Ph.D., Stanford University, California
Evinger, Marian, Ph.D., Cornell University School of Medicine, New York, New York
Schachter, Beth, Ph.D., Mt. Sinai Medical Center, New York, New York

ASSISTANTS

Fox, Susan, Ph.D., New York University Medical School, New York
Inman, Irene, M.S., Stanford University, California

This intensive laboratory/lecture course was intended to provide scientists with a basic knowledge of the use of the techniques of molecular biology in examining questions of neurobiological significance. Particular emphasis was placed on using and discussing methods for circumventing the special problems posed in the study of the nervous system; for example, examination of low abundance mRNAs in extremely heterogeneous cell populations.

The laboratory work included mRNA quantitation methods (S1 assay, etc.), preparation of hybridization probes, library construction (plasmid, λgt11 and IST procedure), plaque and colony screening techniques (probe hybridization, antibody interaction, and activity assays), DNA sequencing, eukaryotic cell transfection, in situ hybridization, and in situ transcription. The lecture series, by invited speakers, focused on how molecular biology techniques can be used to supplement more standard neurobiological tools in examining the nervous system.

PARTICIPANTS

Carbonetto, Salvatore, Ph.D., Montreal General Hospital, Canada
Chang, Alice, Ph.D., Indiana University, Indianapolis
Dame, Margaret, Ph.D., Abbott Laboratories, Abbott Park, Illinois
Denburg, Jeffrey, Ph.D., University of Iowa, Iowa City
El-Fakahany, Esam, Ph.D., University of Maryland, Baltimore
Henderson, Christopher, Ph.D., INSERM, Montpellier, France
Jen, Joanna, B.S., Yale University, New Haven, Connecticut
Maycox, Peter, Ph.D., Max-Planck-Institute, Martinsried, Federal Republic of Germany
McNamara, James, M.D., VA Hospital, Durham, North Carolina
Nelson, James, Ph.D., McGill University, Montreal, Quebec, Canada
Philipson, Kenneth, Ph.D., University of California School of Medicine, Los Angeles
Sobel, Andre, Ph.D., INSERM, Paris, France
Stretton, Antony, Ph.D., University of Wisconsin, Madison
Suen, Chen-Shian, B.S., Mt. Sinai Medical Center, New York, New York
Turetsky, Dorothy, B.S., University of California, San Francisco
Yool, Andrea, Ph.D., Research Institute of Scripps Clinic, La Jolla, California

SEMINARS

Sudhof, T., Southwestern Medical College, Dallas. Molecular structure of synaptic vesicle proteins.
Hahn, B., University of Colorado Medical Center. Gene expression in the mammalian brain—Developmental and evolutionary perspectives.
Curran T. and D. Cohen, Roche Institute of Molecular Biology. Fos on the brain.
Goodman, R., Tufts University. Tissue-specific and -regulated expression of somatostatin.
Claudio, T., Yale University. Gene transfer techniques.
Roberts, J., Mt. Sinai School of Medicine. Regulation of neuroendocrine gene expression.
Chao, M., Cornell University Medical College. Functional expression of the NGF receptor.
Breakefield, X., E. K. Shriver Center, Boston. Molecular biological approaches to understanding human neurologic diseases.
Ramachandran, J., Neurex Corp. Structural basis and functional diversity of muscarinic acetylcholine receptor subtypes.
Neve, R., Children's Hospital, Boston. Human genes for GAP-43 and the Alzheimer amyloid precursor protein—Genetic and neurobiological studies.

Macromolecular Crystallography

October 6–October 19

INSTRUCTORS

Deisenhofer, Johann, Ph.D.*, University of Texas Southwestern Medical School, Dallas
Jones, Alwyn T., Ph.D., University of Uppsala, Sweden
McPherson, Alexander, Ph.D., University of California, Riverside
Pflugrath, James, Ph.D., Cold Spring Harbor Laboratory, New York
Remington, S.J., Ph.D., University of Oregon, Eugene

* 1988 Nobel Laureate in Chemistry.

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

Chandrasegaran, Srinivasan, Ph.D., Johns Hopkins University, Baltimore, Maryland
Chen, Ying, B.S., Cornell University, Ithaca, New York
Clarke, Frank, Ph.D., Ciba-Geigy Corporation, Summit, New Jersey
Coghlan, Vincent, B.S., University of California, Irvine
Eggleston, Drake, Ph.D., Smith Kline & French Laboratories, King of Prussia, Pennsylvania
Forest, Katrina, B.S., Princeton University, New Jersey
Gajhede, Michael, M.D., Copenhagen University, Denmark
Knighton, Daniel, B.S., University of California, San Diego
Lyon, Mary, Ph.D., Scripps Clinic and Research Foundation, La Jolla, California
Nollet, Kenneth, B.A., Mayo Foundation, Rochester, Minnesota
Palenik, Gus, Ph.D., University of Florida, Gainesville
Ramakrishnan, Venki, Ph.D., Brookhaven National Laboratory, Upton, New York
Schuller, David, B.A., Washington University Medical School, St. Louis, Missouri
Shen, Betty, Ph.D., Argonne National Laboratory, Argonne, Illinois
Swepston, Paul, Ph.D., Molecular Structure Corporation, College Station, Texas
Takeda, Yoshinori, Ph.D., NCI-Frederick Cancer Research Facility, Maryland
Twigg, Pamela, M.S., University of Alabama, Huntsville
Wardell, Mark, Ph.D., University of California, San Francisco

SEMINARS

Reeke, G., Rockefeller University. Data scaling and merging/ROCKS.
Sweet, R., Brookhaven National Laboratory. X-ray sources and optics.
Bricogne, G., L.U.R.E., Orsay. The Fourier transform.
———. The phase problem.
Hendrickson, W., Columbia University. Phasing with anomalous dispersion.
Saper, M., Harvard University. Molecular averaging.
Fitzgerald, P., Merck Sharp & Dohme Research Laboratories. Molecular replacement.
Hendrickson, W., Columbia University. Crystallographic refinement.
Brunger, A., Yale University. Molecular dynamics.

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.

1988

January

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.

W.M. Marzluff, University of Florida, Gainesville: Multiple regulatory steps in histone mRNA metabolism.

Meinrad Busslinger, Genentech Inc., South San Francisco, California: CCAAT displacement protein and the regulation of human globin gene expression.

Lindsay Williams, EMBL, Heidelberg, Federal Republic of Germany: Effects of polyomavirus middle T oncogene on embryonic stem cells and chimeric mice.

Patricia Smith Churchland, University of California, San Diego: The mind as a neurobiological machine.

Gilbert Jay, NCI, National Institutes of Health, Bethesda, Maryland: Viral oncogenes in transgenic mice—Model for study of human cancers.

February

Sara Lavi, University of Tel Aviv, Israel: Carcinogen-induced over-replication of SV40 in Chinese hamster cells in vivo and in vitro.

Martyn Goulding, University of Auckland, New Zealand: Cyclic AMP induces stable expression of c-*fos* in a mast cell line.

Nicole Le Douarin, Institute d'Embryologie College de France, Nogent-sur-marne, France: Differentiation of the peripheral nervous system from the neural crest—Cell determination and influence of environmental factors.

Masataka Nakamura, Institute for Virus Research, Kyoto University, Japan: Regulation of the promoter activity of human T-cell leukemia virus type I.

Bill Reznikoff, University of Wisconsin, Madison: The regulation of Tn*5* transposition.

Amalendra Kumar, New York University Medical Center, New York: Domain structure and RNA binding properties of some hnRNA proteins.

Gottfried Schatz, Biocenter, Basel, Switzerland: Protein unfolding and protein translocation across membranes.

Ann Wrinkler, University of Missouri, Columbia: Selective recognition of higher-order snRNP complexes by monoclonal antibodies.

Peter Peterson, Iowa State University, Ames: Structure and function of the *En* transposable element system in maize.

Sung-Hou Kim, University of California, Berkeley: Three-dimensional structure of human *ras* oncoprotein and its biochemical implications.

March

Helen Brady, St. Louis University Medical Center, Missouri: Alternative pre-mRNA processing in region E3 of adenovirus—Evidence for competition between splicing and polyadenylation reactions.

Judy Fox, Rockefeller University, New York, New York: GPI-PLCs—Possible role in signal transduction.

David W. Marshak, University of Texas Medical School, Houston: Function of neuropeptides in the vertebrate retina.

Shamshad Cockcroft, University College, London, England: G proteins, inositol lipid signaling pathway, and exocytosis.

Arpad Parducz, Hungarian Academy of Science, Szeged, Hungary: Fine structural changes in synapses during the transmission of a single nerve impulse.

Tom Moss, Laval University, Quebec, Canada: Multiple mechanisms of ribosomal transcription enhancement.

Tony Pryor, CSIRO, Canberra, Australia: Genetic approaches to cloning and fine structure of a gene for rust resistance in maize.

Michele Sawadogo, Rockefeller University, New York, New York: Interaction of transcription factors and RNA polymerase II with the adenovirus major late promoter.

M. Yanagida, Kyoto University, Japan: New nuclear proteins implicated in chromosome organization and separation in fission yeast.

Graham Tebb, EMBL, Heidelberg, Federal Republic of Germany: Transcription of *Xenopus* UsnRNA genes.

Roger Brent, Harvard Medical School, Cambridge, Massachusetts: Studying higher eukaryotic regulatory proteins with transcription regulation in yeast.

April

Peter Fantes, University of Edinburgh, Scotland: Chromosome function in yeast and mammalian cells.

Ed Chang, Public Health Research Institute, New York, New York: Expression of heterologous genes in *Bacillus*.

Grant McFadden, University of Alberta, Edmonton, Canada: Biology and replication of Shope fibroma virus.

Ingrid Grummt, University of Würzburg, Federal Republic of Germany: *cis*-Acting elements and *trans*-acting factors involved in initiation and termination of mouse rDNA transcription.

Kevin Brady, Indiana University, Bloomington: Chemical, environmental, and phytopathogen stress-induced genes in tobacco.

Nat Heintz, Rockefeller University, New York, New York: Factors regulating histone gene expression during a cell cycle.

Gary Ruvkun, Harvard University, Cambridge, Massachusetts: Temporal control of the C. *elegans* cell lineage by *lin-14*.

May

O. Prem Das, Waksman Institute. Piscataway, New Jersey: A novel recombinational event at a storage protein locus in maize.

Jeffrey A. Kazzaz, Case Western Reserve University, Cleveland. Ohio: Tissue specificity of myosin heavy chain transcripts and isoforms in *Drosophila melanogaster*.

Tom Dutchman, University of Wisconsin, Madison: Targeted gene modifications in embryonic stem cells.

Robert Martienssen, University of California, Berkeley: A nuclear gene in maize involved in photosynthetic membrane organization and its regulation by Robertson's mutator.

Francis Barany, Cornell University Medical College, New York, New York: How *Taq*I restriction endonuclease recognizes its cognate sequence.

Christolph Mueller, Stanford University, California: Molecular mechanism of cell-mediated cytotoxicity in vivo.

Peter Hornbeck, National Institutes of Health, Bethesda. Maryland: Signal transduction in lymphocytes.

June

Eric Richards. Harvard Medical School. Boston, Massachusetts: Isolation of a telomere from *Arabidopsis*.

Jeffrey W. Kelly, Rockefeller University, New York. New York: Design and chemical synthesis of the lambda Cro repressor.

M. Gerry Neuffer. University of Missouri. Columbia: Orange pericarp in maize; filial expression in a maternal tissue.

July

Brian Hauge, Massachusetts General Hospital. Boston: Progress toward a physical map of the *Arabidopsis* genome.

Brian Gavin, Yale University School of Medicine, New Haven. Connecticut: A GC-box-binding protein mediates transcriptional control of the minute virus of mice P4 promoter in human and murine nuclear extracts.

Hans-Georg Simon, Max-Planck-Institute for Immunobiology. Freiburg, Federal Republic of Germany: T-cell-specific serine proteinase—cDNA cloning and expression of the gene in T lymphocytes.

Edgar Serfling, University of Würzburg, Federal Republic of Germany: Functional analysis of the mouse interleukin enhancer.

August

Robin Allshire, MRC, London, England: From fission yeast chromosomes to human telomeres.

Ueli Grossniklaus, Biocenter, Basel, Switzerland: Enhancer traps in *Drosophila*.

Aneel Aggarwall, Harvard University, Cambridge, Massachusetts: Structure of the 434 repressor-operator complex at 2.5-angstrom resolution.

David A. Christopher, University of Arizona, Tucson: Organization and expression of ribosomal protein genes on the *Euglena* chloroplast genome—The presence of novel chloroplast introns.

Walter Keller, Biozentrum, Basel, Switzerland: Processing (splicing and 3′ end formation) of nuclear mRNA precursors in vitro.

John Leavitt, Institute for Medical Research, San Jose, California: Use of protein profiling for molecular diagnosis of disease.

Rodrigo Bravo, EMBL, Heidelberg, Federal Republic of Germany: Identification of genes induced by growth factors in mouse fibroblasts.

September

William Phares, University of California, Berkeley: Transduction of proto-*src* sequences in tissue culture by transformation-defective RSV with an internal *src* deletion.

Stan Brown, Fred Hutchinson Cancer Research Center, Seattle, Washington: Suppressor analysis of 4.5S RNA of *E. coli*.

October

Nava Segev, Genentech, Inc., South San Francisco, California: The small yeast GTP-binding protein, YPT1, is associated with secretion.

Ann Tsukamoto, University of California, San Francisco: The *int-1* proto-oncogene in transgenic mice induces mammary gland abnormalities and tumorigenesis.

Per Nielsen, Odense University, Denmark: Optimization of plasma desorption mass spectrometry—Application to protein structure.

Bengt Westermark, University of Uppsala, Sweden: Differences in transforming activities and biological functions of the various isoforms of PDGF.

Jeffrey Way, Columbia University, New York, New York: Mec-3—A homeobox-containing gene that controls neuronal development in *C. elegans*.

November

Rick Thompson, National Institutes of Health, Bethesda, Maryland: Application of an in vitro basement membrane invasion assay to the study of human breast cancer and Kaposi's sarcoma.

Dirk Bohmann, University of California, Berkeley: AP-1—A family of *jun*-related transcription factors.

Alexei Ryazanov, Institute of Protein Research, Pushchino, USSR: Phosphorylation of elongation factor 2 and its role in the regulation of protein synthesis.

Sam Benchimol, Ontario Cancer Institute, Toronto, Canada: Inactivation of the cellular p53 gene is associated with Friend-virus-induced erythroleukemia.

James Lillie, Harvard University, Cambridge, Massachusetts: Transcription activation by adenovirus E1A protein.

Henry Sadowski, University of Rochester Medical Center, New York: Growth-factor-induced phosphorylation in Swiss 3T3 cells.

December

Lucio Miele, National Institutes of Health, Bethesda, Maryland: Phospholipase A_2 inhibitory peptides from the region of highest similarity between uteroglobin and lipocortin.

John Cairns, Harvard Medical School, Boston, Massachusetts: Spontaneous mutation.

Michael Finney, Massachusetts General Hospital, Boston: *unc-86*, a *C. elegans* neural developmental gene, is homologous to mammalian transcription factors.

Uli Schibler, University of Geneva, Switzerland: A biochemical approach to tissue-specific gene expression.

Richard Gibbs and Jeff Chamberlain, Baylor College of Medicine, Houston, Texas: The polymerase chain reaction—Recent developments and application.

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, 301 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 well over 100 applicants, took part in the program, which was supported by the Alfred P. Sloan Foundation, Burroughs Wellcome Fund, and Cold Spring Harbor Laboratory Funds.

Jennifer Brown, Yale University
Research Advisor: **Kim Arndt**
Cloning a transcription factor of the yeast HIS4 gene by expression library screening.

Franco Carlotti, University of Cambridge
Research Advisor: **Andrew Rice**
Human immunodeficiency virus *tat* protein function.

Emily Chan, Harvard University/Radcliff College
Research Advisor: **Richard Roberts**
mRNA splicing extracts from mammalian tissues.

Emad Gharavi, City College of New York
Research Advisor: **Michael Mathews**
Cloning of PCNA by screening rat and human genomic libraries in bacteriophage λ and isolation of genomic DNA fragments.

Lisa Gloss, Michigan State University
Research Advisor: **Robert Franza**
Expression of three eukaryotic nuclear proteins.

Daniel Grief, Stanford University
Research Advisor: **John Anderson**
Purification of the *myc* oncoprotein.

Ulrich Grossniklaus, University of Basel
Research Advisor: **Nouria Hernandez**
trans-Activation of the human U2 small nuclear RNA promoter.

Beth Hance, Moravian College
Research Advisor: **Elizabeth Moran**
Activation of cellular gene expression by the adenovirus E1A gene products.

Junjiro Horiuchi, Stanford University
Research Advisors: **Bruce Stillman** and **Toshiki Tsurimoto**
Isolation of putative human chromosomal origins of DNA replication.

Seth Karp, Harvard University
Research Advisor: **David Beach**
Cell-cycle regulation.

Chris Leptak, Yale University
Research Advisor: **David Frendewey**
S. pombe mRNA splicing in vitro.

Brandon Lloyd, Grinnell College
Research Advisor: **Venkatesan Sundaresan**
Cell-cycle control in *S. cerevisiae*.

Melissa Macias, University of Texas
Research Advisor: **James Pflugrath**
Purification of p13suc1.

Sharon Perez, Wellesley College
Research Advisor: **Michael Gilman**
Transcriptional activation of the c-*fos* gene.

Mia Schmiedeskamp, University of Michigan
Research Advisor: **Daniel Marshak**
Determination of S100B levels in chick embryo cerebral cortex at successive stages in development.

Ann Schroeder, University of California, Davis
Research Advisor: **David Spector**
DHFR mRNA localization in mammalian nuclei.

Tanya Whitfield, Cambridge University
Research Advisor: **Winship Herr**
HIV-1 *tat/tar* interaction.

Albert Yan, Princeton University
Research Advisor: **David Helfman**
Rat tropomyosin gene control.

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 student's 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.

In addition to the four-week courses, a series of one-day marine biology workshops was offered to students. Studies on the marine ecology of L.I. Sound were conducted aboard Little Jenny, a restored 19th century vessel based in Huntington. Students were able to study the Sound chemically, physically, and biologically using the ship's instrumentation. The three-day Adventure Education class took students on an 18 mile bike hike to Caumsett State Park, a 12-mile canoe trip on the Nissequogue River, and a day of sailing on Little Jenny.

PROGRAM DIRECTOR

William M. Payoski, M.A., Adjunct Professor,
Nassau Community College

INSTRUCTORS

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
Noah Newman, B.A., science teacher candidate
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
Marine Biology Workshops

FINANCIAL STATEMENT

FINANCIAL STATEMENT

BALANCE SHEET
year ended December 31, 1988
with comparative figures for year ended December 31, 1987

ASSETS

	Operating Funds		Endowment & Similar Funds	Land, Building, & Equipment Funds	Total All Funds	
	Unrestricted	Restricted			1988	1987
Cash and cash equivalents	$4,499,218	$372,408	$382,094	$9,406,587	$14,660,307	$12,797,099
Marketable securities	—	—	2,676,487	152,875	2,829,362	1,948,138
Accounts receivable:						
Publications (less allowance for doubtful accounts of $24,000 in 1988 and $21,000 in 1987)	230,056	—	—	—	230,056	218,831
Other	158,657	—	—	—	158,657	84,563
Grants receivable	—	853,813	—	—	853,813	1,559,866
Accrued interest receivable	153,443	—	—	79,083	232,526	268,592
Publications inventory	602,807	—	—	—	602,807	425,832
Other assets, principally prepaid expenses	603,653	—	—	—	603,653	349,536
Contract deposit	—	—	—	161,143	161,143	161,143
Investment in employee residences	—	—	—	747,156	747,156	938,221
Land, buildings and equipment:						
Land and improvements	—	—	—	2,579,440	2,579,440	2,233,486
Buildings	—	—	—	21,614,551	21,614,551	20,518,291
Furniture, fixtures and equipment	—	—	—	1,736,200	1,736,200	1,417,131
Laboratory equipment	—	—	—	4,550,493	4,550,493	4,318,510
Library books and periodicals	—	—	—	365,630	365,630	365,630
	—	—	—	30,846,314	30,846,314	28,853,048
Less accumulated depreciation and amortization	—	—	—	9,774,795	9,774,795	8,490,284
Land, buildings and equipment, net	—	—	—	21,071,519	21,071,519	20,362,764
Construction in progress	—	—	—	2,774,090	2,774,090	348,688
Total assets	$6,247,834	$1,226,221	$3,058,581	$34,392,453	$44,925,089	$39,463,273

LIABILITIES AND FUND BALANCES

	Operating Funds		Endowment & Similar Funds	Land, Building, & Equipment Funds	Total All Funds	
	Unrestricted	Restricted			1988	1987
Liabilities:						
Accounts payable and accrued expenses	$1,343,374	$17,478	—	$481,296	$1,842,148	$1,863,227
Note payable to Robertson Maintenance Fund	—	—	—	670,000	670,000	—
Loan payable	—	—	—	4,000,000	4,000,000	6,500,000
Deferred revenue	548,806	1,208,743	—	—	1,757,549	1,977,048
Total liabilities	1,892,180	1,226,221	—	5,151,296	8,269,697	10,340,275
Fund balances:						
Unrestricted	4,355,654	—	—	—	4,355,654	4,300,665
Endowment and similar funds	—	—	3,058,581	—	3,058,581	1,937,311
Land, building and equipment:						
Expended	—	—	—	20,434,689	20,434,689	18,111,309
Unexpended—Donor restricted	—	—	—	7,958,695	7,958,695	3,787,934
Unexpended—Board authorized	—	—	—	847,773	847,773	985,779
Total fund balances	4,355,654	—	3,058,581	29,241,157	36,655,392	29,122,998
Total liabilities and fund balances	$6,247,834	$1,226,221	$3,058,581	$34,392,453	$44,925,089	$39,463,273

STATEMENT OF SUPPORT, REVENUE, AND EXPENSES AND CHANGES IN FUND BALANCES
year ended December 31, 1988
with comparative figures for year ended December 31, 1987

	Operating Funds		Endowment & Similar Funds	Land, Building, & Equipment Funds	Total All Funds	
	Unrestricted	Restricted			1988	1987
Support and Revenue:						
Public support—contributions and grants	$638,659	$3,270,742	$1,010,449	$6,001,669	$10,921,519	$8,677,267
Government grant awards	—	8,102,876	—	—	8,102,876	6,861,271
Indirect cost allowances	5,643,433	—	—	—	5,643,433	4,544,846
	6,282,092	11,373,618	1,010,449	6,001,669	24,667,828	20,083,384
Other revenue:						
Program fees	956,795	—	—	—	956,795	793,476
Rental income	89,658	—	—	—	89,658	86,501
Publications	1,640,491	—	—	—	1,640,491	1,646,913
Dining services	1,118,037	—	—	—	1,118,037	990,389
Rooms and apartments	544,753	—	—	—	544,753	420,387
Distribution from Robertson Maintenance and Research Funds	130,000	800,000	—	—	930,000	925,000
Investment income	212,056	—	178,650	730,777	1,121,483	2,027,134
Miscellaneous	258,248	—	—	138,205	396,453	70,844
Total other revenue	4,950,038	800,000	178,650	868,982	6,797,670	6,960,644
Total support and revenue	11,232,130	12,173,618	1,189,099	6,870,651	31,465,498	27,044,028
Expenses:						
Program services:						
Research	—	9,138,957	—	—	9,138,957	8,008,814
Summer programs	549,632	1,872,226	—	—	2,421,858	2,278,121
Publications	1,719,187	—	—	—	1,719,187	1,449,178
Banbury Center conferences	5,574	515,529	—	—	521,103	386,922
DNA Learning Center programs	4,901	358,438	—	—	363,339	211,738
Total program services	2,279,294	11,885,150	—	—	14,164,444	12,334,773

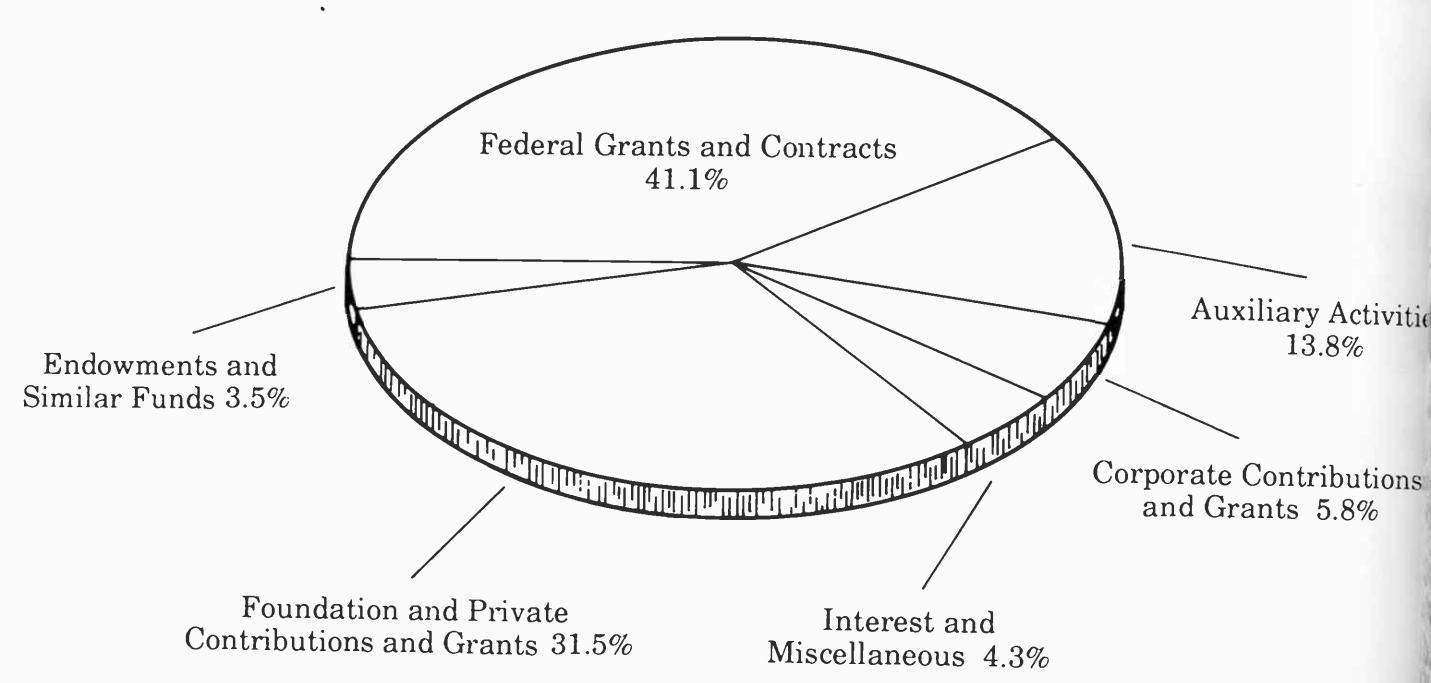
Supporting services:						
Direct research support	485,915	—	—	—	485,915	504,153
Library	339,915	—	—	—	339,915	287,905
Operation and maintenance of plant	3,259,354	—	—	—	3,259,354	2,945,532
General and administrative	2,890,857	—	—	—	2,890,857	2,017,976
Dining services	1,167,405	—	—	—	1,167,405	1,034,927
Interest	—	—	—	339,055	339,055	423,154
Total supporting services	8,143,446	—	—	339,055	8,482,501	7,213,647
Depreciation	—	—	—	1,286,159	1,286,159	1,126,349
Total expenses	10,422,740	11,885,150	—	1,625,214	23,933,104	20,674,769
Excess of support and revenue over expenses	809,390	288,468	1,189,099	5,245,437	7,532,394	6,369,259
Other changes in fund balances:						
Transfer to unexpended plant funds	(754,401)	(95,000)	—	849,401	—	—
Capital expenditures	—	(261,297)	—	261,297	—	—
Transfer to restricted funds	—	67,829	(67,829)	—	—	—
Net increase in fund balance	54,989	—	1,121,270	6,356,135	7,532,394	6,369,259
Fund balance at beginning of year	4,300,665	—	1,937,311	22,885,022	29,122,998	22,753,739
Fund balance at end of year	$4,355,654	—	$3,058,581	$29,241,157	$36,655,392	$29,122,998

NOTE: Copies of our complete, audited financial statements, certified by the independent auditing firm of Peat, Marwick, Main & Co., are available upon request from the Comptroller, Cold Spring Harbor Laboratory.

COLD SPRING HARBOR LABORATORY

SOURCES OF REVENUE

YEAR ENDED DECEMBER 31, 1988



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 won 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. However, the continued 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 upon 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, 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, 1988–December 31, 1988

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. Watson	7/87–6/90	5,346,907
	HIV Program Project, Dr. Watson	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	9/86–8/89	578,929
	Dr. Beach	7/88–6/93	1,064,173*
	Dr. L. Field	4/87–3/92	568,521
	Dr. Feramisco	4/85–3/88	535,534
	Dr. Franza	9/85–11/88	522,439
	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. Hanahan	8/87–7/88	312,634
	Dr. Helfman	9/85–12/88	616,510
	Dr. Hernandez	7/87–6/92	1,293,754
	Dr. Klar/Futcher	7/81–6/90	2,411,676
	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	9/86–8/88	216,446
	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. Watson	4/87–3/88	117,413
	Dr. Watson	4/88–3/89	121,971*
	Dr. Welch	4/84–11/88	676,359
	Dr. Wigler	7/85–6/92	8,426,929
	Dr. Zoller	9/85–8/88	545,752
Equipment Support	Dr. Marshak	2/88–2/89	189,000*
	Dr. Roberts	6/87–5/88	38,991*
	Dr. Roberts	6/88–5/89	27,331*
	Dr. Spector	2/87–2/88	165,000*

* New Grants Awarded in 1988

Grantor	Program/Principal Investigator	Duration of Grant	Total Award
Fellowships	Dr. Cheley	10/86–3/88	37,494
	Dr. J. Field	10/86–9/89	63,996
	Dr. Kessler	8/88–7/91	93,996*
	Dr. McLeod	2/85–1/88	63,996
	Dr. Meyertons	5/88–4/91	63,996*
	Dr. Morris	11/86–10/89	82,008
	Dr. Munroe	10/87–4/88	28,000
	Dr. Potashkin	10/85–9/88	64,886
	Dr. Stern	11/88–11/91	63,996*
Training Support	Institutional, Dr. Grodzicker	7/78–8/89	1,291,646
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
	Neruobiology Short Term Training, Dr. Hockfield	5/82–4/90	723,939
	Neurobiology Short Term Training, Dr. Hockfield	6/79–3/89	922,887
Meeting Support	*C. elegans*	1987 & 1989	61,874
	RNA Processing	1988–1991	9,500
	Symposium	1988	27,774*
	Cancer Cell Meeting	1988	5,000*
	Conference on Mouse Molecular Genetics	1988	16,000*
	Ribosome Synthesis	1988	3,000*
NATIONAL SCIENCE FOUNDATION			
Research Support	Dr. Herr	6/88–5/91	240,000*
	Dr. Klar	8/86–7/88	220,000
	Dr. Marshak	7/87–12/90	190,000
	Dr. Roberts	1/83–5/90	640,000
	Dr. Roberts	2/87–1/89	359,262
	Dr. Roberts	8/87–1/90	43,106
	Dr. Sundaresan	5/87–10/90	270,000
	Dr. Zoller	8/88–1/92	285,000*
Equipment Support	Dr. Spector	3/87–2/89	95,000*
Training Support	Undergraduate Research Program, Dr. Herr	6/87–5/88	32,000
Course Support	Plant Molecular Biology, Dr. Grodzicker	8/86–1/90	137,490
Meeting Support	*C. elegans*	1987–1989	22,819
	Ribosome Synthesis Conference	1988	8,000*
	Symposium	1988	5,000*
	RNA Processing Conference	4/88–3/91	30,000*
	Chlamydomonas Conference	1988	5,000*
	Conference on Mouse Molecular Genetics	1988	13,875*
	Molecular Neurobiology of Aplysia Conference	1988	13,000*
	Intermidiates in Genetic Recombination Conference	1988	4,000*

* New Grants Awarded in 1988

Grantor	Program/Principal Investigator	Duration of Grant	Total Award
DEPARTMENT OF ENERGY			
Meeting Support	*Chlamydomonas* Conference	1988	3,000*
	Symposium	1988	9,000*
OFFICE OF NAVAL RESEARCH			
Course Support	Computational Eye Movement Workshop	7/88–6/91	62,125*
NONFEDERAL GRANTS			
Research Support			
Aaron Diamond Foundation	Dr. Anderson	12/88–11/89	200,000*
American Boremene, Inc. (Subcontract)	Drs. Feramisco, Bar-Sagi	9/87–3/88	16,498
American Cancer Society	Dr. Hanahan	7/87–6/89	160,000
	Dr. Moran	1/88–12/90	90,500*
	Dr. Spector	7/87–6/89	162,000
	Drs. Stillman, Gluzman, Welch-Institutional Award	7/82–6/88	220,000
	Dr. Gilman-Institutional Award	7/88–6/90	80,000*
	Dr. Wigler	4/87–3/89	20,000
	Dr. Wigler, Professorship	1986–2012	1,333,333
Amersham International	Dr. Harlow	11/86–10/91	799,635
AmFAR	Dr. Franza	12/87–11/88	78,000
Howard Hughes Medical Institute	Neurobiology Support	1987–1990	1,000,000
J.N. Pew Jr. Charitable Trust	Dr. Watson	4/87–4/90	260,000
Juvenile Diabetes Foundation	Dr. Hanahan	9/86–9/88	66,000
LIBA	Dr. Hernandez	7/87–6/88	20,000
	Dr. Spector	4/87–3/88	27,000
	Dr. Frendewey	4/88–3/89	25,000*
	Dr. Peterson	4/88–3/89	25,000*
Muscular Dystrophy Association	Dr. Feramisco	1/87–1/88	50,764
	Dr. Helfman	7/86–6/89	94,500
	Dr. Mathews	1/87–6/90	100,718
Mellam Family Foundation	Dr. Franza	12/88–11/89	50,000*
Monsanto Company	Cooperative Research	10/84–9/90	2,089,200
New England Biolabs	Dr. Roberts	1988	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
Scripps Clinic	Dr. Hanahan (subcontract)	1/86–9/88	356,806
Equipment Support	Fannie E. Rippel Foundation	3/87–12/88	125,000
Fellowships			
American Cancer Society	Dr. Conway	10/88–9/91	63,000*
	Dr. Ryan	7/88–6/90	90,000*
Bristol-Myers Company	Fellowship Support	6/86–5/91	500,000
Bioseeds International	Plant Fellowship Support	7/88–6/89	25,000*
Cancer Research Institute	Dr. Young	8/85–7/88	73,500
	Dr. Sturm	9/86–8/89	79,500
	Dr. Efrat	9/86–1/89	62,313

* New Grants Awarded in 1988

Grantor	Program/Principal Investigator	Duration of Grant	Total Award
Damon Runyon-Walter Winchell Cancer Fund	Dr. Michaeli	3/87–2/90	69,000
	Dr. D. Roberts	9/88–3/90	36,250*
Esther and Joseph A. Klingenstein Fund, Inc.	Dr. Marshak	7/87–6/90	100,000
Juvenile Diabetes Foundation International	Dr. Grant	7/87–6/89	51,000
LIBA	Eight Fellowships	1987–1989	200,000
Life Science Research Foundation	Dr. Colicelli	9/86–8/89	105,000
Medical Research Council of Canada	Dr. Mizzen	6/87–5/88	38,052
Merck Sharp & Dohme Research Laboratories	Graduate Student Support	12/88–11/90	30,000
Muscular Dystrophy Association	Dr. Lees-Miller	7/88–6/89	23,000
NYS Health Research Council	Dr. Bautch	9/86–8/88	39,000
Oncogene Science Fellowship Fund	Dr. Watson	1987–1989	25,000
Weizmann Institute of Science	Dr. Gerst	9/88–8/89	28,500*
Training Support			
Alfred P. Sloan Foundation	Undergraduate Research Program	1985–1988	74,000
Burroughs Wellcome Foundation	Undergraduate Research Program	1987–1988	16,560
Grass Foundation	Neurobiology Scholarship Support	1980–1988	141,670
Lucille P. Markey Charitable Trust	Scholarship Support	1985–1989	250,000
Robert H.P. Olney Memorial Fund	Undergraduate Research	1988	2,443*
Course Support			
Alfred P. Sloan Fund	Computational Neuroscience Course	1986–1989	140,000
Amersham International plc	Advances in Molecular Cloning and Expression of Eukaryotic Gene Courses	1988	2,500*
Howard Hughes Medical Institute	Neurobiology Courses	1987–1990	1,000,000
Ester and Joseph A. Klingenstein Foundation	Neurobiology Support	1982–1988	300,000
Meeting Support			
Accurate Chemical and Scientific Support	Ribosome Conference	1988	175*
Amoco Corporation	*Chlamydomonas* Conference	1988	1,000*
Beckman Instruments	Ribosome Conference	1988	175*
	Liver Gene Expression Conference	1989	500
Bio-Rad Lab 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
E.I. DuPont de Nemours, & Company	Ribosome Conference	1988	300*
	Liver Gene Expression Conference	1989	300
Foundation for Microbiology	*Chlamydomonas* Conference	1988	2,500*
International Society for Plant Molecular Biology	*Chlamydomonas* Conference	1988	1,500*
Lucille P. Markey Charitable Trust	53rd Symposium: Molecular Biology of Signal Transduction	1988	15,000*
Merck & Co., Inc.	Ribosome Conference	1988	500*
Mettler Instrument Corp.	Ribosome Conference	1988	200*
Milligen	Ribosome Conference	1988	200*
Nestle, S.A.	Liver Gene Expression Conference	1989	1,000
U.S. Biochemical Corp.	Liver Gene Expression Conference	1989	175

* New Grants Awarded in 1988

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*Total Award*
Monsanto Company	*Chlamydomonas* Conference	1988	3,000*
Nippon Zeon Co., Ltd.	*Chlamydomonas* Conference	1988	1,000*
Promega Biotech	Ribosome Conference	1988	200*
Rockefeller Foundation	Vaccines Conference	1986–1990	45,000
United States Biochemical Corporation	Ribosome Conference	1988	175*
Worthington Biochemical Corporation	Ribosome Conference	1988	250*
VWR Scientific-Rochester	Ribosome Conference	1988	175*
Zoecon Research Inst./Sandoz Crop Protection Corp.	Chamydomonas Conference	1988	1,000*

* New Grants Awarded in 1988

DNA LEARNING CENTER

Grantor	*Program*	*Duration of Grant*	*Total Award*
FEDERAL GRANTS			
NATIONAL SCIENCE FOUNDATION			
	Teacher Enhancement Program	6/87–11/90	415,928
STATE GRANTS			
New York State Legislature	The Search for Life	1988	74,595*
NONFEDERAL GRANTS			
Boehringer Mannheim Biochemicals	Core Support	1988	$ 2,000*
Brinkmann Instruments, Inc.	Core Support	1988	10,000*
Harweb Foundation	Core Support	1988	1,500*
J.M. Foundation	Core Support	1988	25,000*
Richard Lounsbery Foundation	Core Support	1988	30,000*
Josiah Macy, Jr. Foundation	Core Support	7/87–6/90	490,850
The Banbury Fund	Renovation	1988–1989	56,200*
Center for Biotechnology, SUNY Stony Brook	Vector Workshop	1988	10,550*
Fotodyne Inc.	Vector Workshop	1988	4,000*
Samual Freeman Charitable Trust	Vector Workshop	1988	10,000*
Cold Spring Harbor	Curriculum Study	1988	500*
Commack School District	Curriculum Study	1988	500*
East Williston School District	Curriculum Study	1988	500*
Great Neck School District	Curriculum Study	1988	500*
Harborfields School District	Curriculum Study	1988	3,000*
Herricks School District	Curriculum Study	1988	500*
Huntington School District	Curriculum Study	1988	500*
Jericho School District	Curriculum Study	1988	500*
Lawrence School District	Curriculum Study	1988	500*
Locust Valley School District	Curriculum Study	1988	1,500*
Manhasset School District	Curriculum Study	1988	500*
Northport-East Northport School District	Curriculum Study	1988	500*
North Shore School District	Curriculum Study	1988	500*
Oyster Bay-East Norwich School District	Curriculum Study	1988	500*
Plainview-Old Bethpage School District	Curriculum Study	1988	500*
Portledge School	Curriculum Study	1988	500*
Port Washington School District	Curriculum Study	1988	500*
Sachem School District	Curriculum Study	1988	500*
Syosset School District	Curriculum Study	1988	500*
Aboff's Inc.	The Search for Life Exhibit	1988	50*
Agway Petroleum Corporation	The Search for Life Exhibit	1988	200*
Amity Westchester	The Search for Life Exhibit	1988	100*
Andrew Goetzs Sons, Inc.	The Search for Life Exhibit	1988	100*
Apple Bank	The Search for Life Exhibit	1988	150*
Automatic Data Processing	The Search for Life Exhibit	1988	1,000*
Badge Agency, Inc.	The Search for Life Exhibit	1988	1,000*
Barek-Karpel Industries, Inc.	The Search for Life Exhibit	1988	100*

* New Grants Awarded in 1988

Grantor	Program	Duration of Grant	Total Award
Bethesda Research Laboratories Life Technologies, Inc.	The Search for Life Exhibit	1988	10,000*
Blackman General Offices	The Search for Life Exhibit	1988	200*
Brinkmann Instruments, Inc.	The Search for Life Exhibit	1988	1,000*
BRL Life Technologies	The Search for Life Exhibit	1988	10,000*
Brunswick Appraisal Corp.	The Search for Life Exhibit	1988	150*
Budget Instant Print	The Search for Life Exhibit	1988	200*
Dale Carnegie & Associates, Inc.	The Search for Life Exhibit	1988	100*
C.N.D. Supply Corp.	The Search for Life Exhibit	1988	1,000*
Damson Oil Corporation	The Search for Life Exhibit	1988	1,000*
Dunkin Donuts, Inc.	The Search for Life Exhibit	1988	1,000*
Fairchild Weston System, Inc.	The Search for Life Exhibit	1988	1,000*
Grumman Corporation	The Search for Life Exhibit	1988	10,000*
Harris Trust	The Search for Life Exhibit	1988	90*
Hickey's Carting, Inc.	The Search for Life Exhibit	1988	1,000*
Huntington Business Products	The Search for Life Exhibit	1988	100*
Huntington Market, Inc.	The Search for Life Exhibit	1988	1,000*
I. Janvey & Sons, Inc.	The Search for Life Exhibit	1988	250*
Jeff's Seafood	The Search for Life Exhibit	1988	300*
Kollmorgen Corporation	The Search for Life Exhibit	1988	1,000*
Limco Manufacturing Corp.	The Search for Life Exhibit	1988	1,000*
Meenan Oil Company, Inc.	The Search for Life Exhibit	1988	1,500*
Meyer, Suozzi, English & Klein	The Search for Life Exhibit	1988	1,000*
Miller, Anderson & Sherrerd	The Search for Life Exhibit	1988	5,000*
New York Telephone	The Search for Life Exhibit	1988	1,000*
Northville Industries Corp.	The Search for Life Exhibit	1988	1,000*
Official Offset Corp.	The Search for Life Exhibit	1988	2,500*
Pall Corporation	The Search for Life Exhibit	1988	5,000*
Peat Marwick	The Search for Life Exhibit	1988	1,000*
The Roslyn Savings Bank	The Search for Life Exhibit	1988	1,000*
Servo Corporation of America	The Search for Life Exhibit	1988	1,000*
Spinnaker Travel, Ltd.	The Search for Life Exhibit	1988	1,000*
The Stebbins Fund	The Search for Life Exhibit	1988	500*
James F. Straub, Inc.	The Search for Life Exhibit	1988	500*
Tambrands, Inc.	The Search for Life Exhibit	1988	5,000*
Thermo Electron	The Search for Life Exhibit	1988	1,000*
The Tiles Investment Company	The Search for Life Exhibit	1988	1,000*
Vecco Instruments	The Search for Life Exhibit	1988	1,000*
W.A. Baum Company	The Search for Life Exhibit	1988	150*
Wentworth Films, Inc.	The Search for Life Exhibit	1988	100*
Winston Foundation	The Search for Life Exhibit	1988	5,000*
Individual Contributors	The Search for Life Exhibit	1988	8,105*
Mr. and Mrs. G. Morgan Browne			
Bernadette Castro			
Mr. Peter O. Crisp			
Katya Davey			
Eleanor Greenan			
Mrs. Sinclair Hatch			
Phyllis Satz			
Cynthia R. Stebbins			
Mr. Byam K. Stevens, Jr.			
Joseph A. Suozzi, Esq.			
Mr. and Mrs. Walter C. Teagle, III			
Mr. and Mrs. Richard Wesley			

* New Grants Awarded in 1988

BANBURY CENTER

Grantor	Program/Principal Investigator	Duration of Grant	Total Award
FEDERAL GRANTS			
Meeting Support			
U.S. Department of Agriculture	RFLPs 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*
NONFEDERAL SUPPORT			
Meeting Support			
Agrigenetics Corp.	Molecular Biology of Plants Conference	1988	1,000*
Alfred P. Sloan Foundation	Journalists and Congressional Workshops	1985–1989	162,000
Bionetics Research	Control of HIV Expression	1988	3,000*
Biotech Research Laboratories, Inc.	Control of HIV Expression	1988	3,000*
Calgene	RFLPs and the Molecular Biology of Plants Conference	1988	1,000*
California Biotechnology	Therapeutics Peptides and Proteins Conference	1988	3,000*
Chugai Pharmaceutical Co., Ltd.	Therapeutics Peptides and Proteins Conference	1988	5,000*
Collaborative Research, Inc.	DNA Technology and Forensic Science Conference	1988	5,000*
Hoffmann-La Roche, Inc.	Control of HIV Expression	1988	2,000*
ICI Americas Inc.	DNA Technology and Forensic Science Conference	1988	5,000*
ICI Seeds	RFLPs and the Molecular Biology of Plants Conference	1988	2,000*
Kabivitrum	Therapeutic Peptides and Proteins Conference	1988	3,000*
Lifecodes Corporation	DNA Technology and Forensic Science Conference	1988	5,000*
Merck Sharp & Dohme Research Laboratories	Control of HIV Expression Conference	1988	7,500*
Molecular Device Corp.	Therapeutic Peptides and Proteins Conference	1988	500*
Otsuka Pharmaceutical Research Center	Therapeutic Peptides and Protein Conference	1988	1,500*
The Plant Cell Research Institute, Inc.	RFLPs and the Molecular Biology of Plants Conference	1988	5,000*
Perkin Cetus Elmer	Polymerase Chain Reaction	1988	35,000*
Repligen Corporation	Control of HIV Expression Conference	1988	3,000*
Sumito Pharmaceutical Research Center	Therapeutic Peptides and Proteins Conference	1988	5,000*
Sungene Technologies Corporation	RFLPs and the Molecular Biology of Plants Conference	1988	1,000*
Takeda Chemical Industries Ltd.	Therapeutic Peptides and Proteins Conference	1988	5,000*
Toyobo New York Inc.	Therapeutic Peptides and Proteins Conference	1988	5,000*
Yamanouchi Pharmaceutical Co., Ltd.	Therapeutic Peptides and Proteins Conference	1988	5,000*

* New Grants Awarded in 1988

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

Mr. and Mrs. Cliff Bates
Ms. Helen Bilbrey
Mr. David C. Clark
Mr. Laurence M. Clum
Mrs. John L. Davenport
Mr. and Mrs. Raymond C. Doop
Mr. and Mrs. James J. Feeney
Mrs. Pearl S. Fisher
Ms. Helena Gaviola
Dr. and Mrs. Richard L. Golden
Mr. and Mrs. Gerald Griffin
J.W. Hirschfeld Agency, Inc.
Mr. and Mrs. Dennis R. Holland
Mr. and Mrs. Andrew Hulko
Interboro Institute
Mr. and Mrs. Marc W. Jensen
Mrs. Mary Kelly
Mr. and Mrs. Richard E. Leckerling
Ms. Mary T. Maher
Mr. and Mrs. William E. Mahoney
Mr. and Mrs. Harold T. McGrath
Mr. and Mrs. Glenn W. Mullen
Mr. and Mrs. John E. Nelson
Mr. and Mrs. John A. Pieper
Mr. and Mrs. Bernard Silverwater
Mr. and Mrs. Henry E. Spire
Mr. and Mrs. Henry H. Spire
Dr. and Mrs. James D. Watson
Mr. and Mrs. George Weber

In memory of . . .

Nelson Appet
Irene Bal
Marguerite Bhend
Mrs. C. Buckley, Jr.
John L. Davenport
Thomas Donahue
Pat Famighetti
Joseph Ferrante
Richard Hathorn
Helen Leckerling
Jean Leckerling
Evelyn M. Levin
Barbara A. Mahoney
Jim Maxfield
Michael O'Grady
Stephanie Pall
Mrs. Jerald Rose
Robert Simkins
Alfred Wheeler
Posy White
Frank Zizza

Participating Institutions

Albert Einstein College of Medicine
Columbia University
Massachusetts Institute of Technology
Memorial Sloan-Kettering Cancer Center
New York University Medical Center
Princeton University
Rockefeller University
State University of New York, Stony Brook

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. Access to current research presented at these high-level professional meetings is an important resource for the biotechnology industry.

The annual $15,000 membership commitment from each Corporate Sponsor has enabled the Laboratory to significantly expand this role as a clearinghouse for biotechnical information. In 1988, more than 4000 scientists from around the world attended conferences and advanced training courses at Cold Spring Harbor Laboratory and Banbury Center.

The Corporate Sponsor Program supports a series of Special Banbury Conferences that focuses on basic research as well as emerging areas of research germane to industrial biotechnology. These high-level meetings are the basis for the Laboratory's popular book series *Current Communications in Molecular Biology*. Topics of 1988 conferences were "Ubiquitin," "Viral Vectors," "Cell-Cycle Control," "Cytoskeletal Proteins in Tumor Diagnosis," and "Molecular Markers in Problems of Plant Genetics."

Benefits to Sponsor companies include waiver of all fees for six representatives at Cold Spring Harbor meetings and special Banbury conferences; gratis Cold Spring Harbor and Banbury publications, including the journal *Genes & Development*; and recognition in meeting abstracts and publications.

Since 1984, more than 30 companies have participated in the Corporate Sponsor Program. The membership renewal rate is 90%. The 1988 members of the Corporate Sponsor Program—world leaders in the application of biotechnology to health care, agriculture, and manufacturing—were:

Abbott Laboratories
American Cyanamid Company
Amersham International plc
AMGen Inc.
Applied Biosystems, Inc.
Becton Dickinson and Company
Boehringer Mannheim
Bristol-Myers Company
Cetus Corporation
Ciba-Geigy Corporation
Diagnostic Products Corporation
E.I. du Pont de Nemours & Company
Eastman Kodak Company
Genentech, Inc.
Genetics Institute
Hoffman-La Roche Inc.
Johnson & Johnson
Life Technologies, Inc.
Eli Lilly and Company
Millipore Corporation
Monsanto Company
Oncogene Science, Inc.
Pall Corporation
Pfizer Inc.
Pharmacia Inc.
Schering-Plough Corporation
Smith Kline & French Laboratories
Tambrands Inc.
The Upjohn Company
The Wellcome Research Laboratories, Burroughs Wellcome Co.
Wyeth Laboratories

Summary of Annual Contributions

Unrestricted Annual Contributions

CSHL (LIBA) Associates (1/1/88–12/31/88)	$189,541	
LIBA Members (1/1/88–12/31/88)	40,322	
General	1,445	
Memorials	8,122	
Corporations	15,000	
Foundations	5,000	
Participating Institutions	8,000	
		$267,430

Restricted Annual Contributions

Corporate Sponsor Program	$465,000	
DNA Learning Center	159,615	
Airslie Fund	1,606	
Dorcas Cummings Fund	2,525	
Olney Fund	2,646	
Prentis Memorial	821	
Robertson House	5,000	
		637,213

Total Annual Contributions

$904,643

Second Century Campaign

January 1, 1988–December 31, 1988

Unrestricted Contributions — Total $1,013,000

Anonymous
Mrs. Donald Arthur, Jr.
Mr. and Mrs. Allen L. Boorstein
Mr. G. Morgan Browne
Mr. and Mrs. Samuel R. Callaway
Dr. Bayard Clarkson
Mr. and Mrs. Miner D. Crary, Jr.
Mr. Robert L. Cummings
Mr. and Mrs. Roderick H. Cushman
Mr. George W. Cutting, Jr.
Mr. and Mrs. Norris Darrell, Jr.
Mr. William Everdell
Mr. and Mrs. Henry U. Harris, Jr.
Mr. and Mrs. Sinclair Hatch
Mr. and Mrs. Townsend J. Knight
Mr. and Mrs. George N. Lindsay
Mr. David L. Luke, III
Miller Richard, Inc.
Mr. and Mrs. F. Warren Moore
Dr. David B. Pall
Mrs. H. Irving Pratt
William and Maude Pritchard Charitable Trust
Mr. and Mrs. John R. Reese
Mr. and Mrs. Harvey E. Sampson
Mr. George F. Sprague, Jr.
Mr. Jonathan R. Warner
Mr. Taggart Whipple

Restricted Contributions

ENDOWMENT — $4,820,000

Oliver and Lorraine Grace Director's Chair
Doubleday Professorship for Advanced Cancer Research
Anonymous Professorship in Molecular Neuroscience

PROGRAM — 2,000,000

Howard Hughes Medical Institute

FACILITIES DEVELOPMENT — 19,580,000

Cancer Biology
Neuroscience Center/Lodge
- Arnold and Mabel Beckman Foundation
- Dolan Family Foundation
- Samuel Freeman Charitable Trust
- Lita Annenberg Hazen Trust
- Howard Hughes Medical Institute
- Esther A. and Joseph Klingenstein Fund, Inc.
- Litchfield Charitable Trust
- W.M. Keck Foundation
- James S. McDonnell Foundation
- Individual Contributors

Structural Biology
- Lucille P. Markey Charitable Trust

Page Laboratory of Plant Genetics
- Charles E. Culpepper Foundation
- Ira W. DeCamp Foundation
- Morgan Guaranty Trust Company of New York
- National Science Foundation
- William and Maude Pritchard Charitable Trust
- Individual Contributors

GUEST FACILITIES	1,041,000
10 Guest Houses	
Alumni Cabin Contributors	
Dr. Herbert Boyer	
Mrs. George G. Montgomery, Jr.	
New England Biological Laboratories	
Dr. Mark Ptashne	
Visiting Scientist Guest House	
Russell and Janet Doubleday Fund	
DNA LEARNING CENTER	151,000
Banbury Fund #1	
Banbury Fund #2	
Brinkmann Foundation	
Mr. and Mrs. Henry U. Harris, Jr.	
New York State	
Total Second Century Campaign Contributions	**$28,605,000**

Alumni Cabin Contributors

Since the first Symposium on Quantitative Biology in 1933, Cold Spring Harbor Laboratory has been a major site for discussion of serious science. Each year, visiting scientists from all over the world remember a time when the entire molecular biology community could be comfortably seated in Vannevar Bush Hall. The spectacular growth of molecular biology research worldwide is reflected in attendance at Cold Spring Harbor meetings. Over the last six years, the number of meeting participants has increased 57% to a record 4000 scientists in 1988.

The caliber of science discussed at Cold Spring Harbor has remained consistently excellent; however, facilities once considered rustic have become inadequate. As part of a program to update our guest facilities prior to the Laboratory's Centennial in 1990, meetings attendees, course instructors, and former staff personnel were asked to recall how their careers had benefitted from the *Cold Spring Harbor experience* and to contribute to the **Alumni Cabin** Campaign. **Alumni Cabin** is one of ten eight-person cabins being constructed in a lovely wooded area overlooking Laboratory grounds. In late 1987, ground was broken for six of the new cabins which will be completed in 1989 in time for the April meetings. In generous response to three annual **Alumni Cabin** appeals, 325 biologists from around the world had contributed over $55,000 by the end of 1988. Cold Spring Harbor Laboratory Alumni continue to make donations, and we hope that the goal of $100,000 can be achieved in 1989.

The following individuals contributed to **Alumni Cabin** in 1988:

Dr. Fred Ausubel
Dr. Richard Axel
Dr. Ronald Bauerle
Dr. Gunter Blobel
Dr. Robert M. Blumenthal
Dr. Thomas Blumenthal
Dr. Walter F. Bodmer
Dr. Jeremy Bruen
Dr. Keith Burridge
Dr. Charles R. Cantor
Dr. Mario Capecchi
Dr. Germaine Chipault
Dr. James Crow
Dr. and Mrs. James Darnell
Dr. Julian Davies
Dr. Hajo Deulis
Dr. Kurt Drickamer
Dr. Sarah C.R. Elgin
Dr. Sharyn A. Endow
Dr. Lynn W. Enquist
Dr. Ronald M. Evans
Dr. Georg Fey
Dr. John Fiddes
Dr. Richard A. Firtel
Dr. Beth Friedman
Dr. C.R. Fuerst
Dr. Walter Gehring
Dr. William M. Gelbart
Dr. Martin Gellert
Dr. Nicholas W. Gillham
Dr. Thomas R. Gingeras
Dr. Howard Green
Dr. Maurice Green
Dr. Francois Gros
Dr. Christine Guthrie
Dr. H.T. Hanafusa
Dr. Lawrence E. Hightower
Dr. Bernhard Hirt
Dr. Susan Hockfield
Dr. David Hogness
Dr. Nancy Hopkins
Dr. Tony Hunter
Dr. Jerard Hurwitz
Dr. Richard Hynes
Dr. Yuriko Kataoka
Dr. Tohru Kataoka
Dr. Daniel Koshland
Dr. Samuel Latt
Dr. Michael J. Lenardo
Dr. John Lewis
Dr. Jim Jung-Ching Lin
Dr. Janet I. Macinnes
Dr. Fumio Matsumura
Dr. I. George Miller, Jr.
Dr. Jeffrey Miller
Dr. Gisela Mosig
Dr. Frederick C. Neidhardt
New England Bio-Labs
Dr. Masayasu Nomura
Dr. Richard P. Novick
Dr. Stephen Oroszlan
Dr. Paul H. Patterson
Dr. Manuel Perucho
Dr. Peter Philippssen
Dr. Anthony R. Poteete
Dr. Dale Purves
Dr. Charles M. Radding
Dr. Martin Raff
Dr. Charles C. Richardson
Dr. Rex G. Risser
Dr. Gordon Sato
Dr. Miyo Sato
Dr. Walter Schaffner
Dr. Milton Schlesinger
Dr. Sondra Schlesinger
Dr. Walter Scott
Dr. Charles J. Sherr
Dr. Maxime Singer
Dr. Cassandra L. Smith
Dr. Davor Solter
Dr. A.R. Srinivasan
Dr. David Stacy
Dr. Charles D. Stiles
Dr. Howard M. Temin
Dr. Charles Thomas
Dr. Jeremy Thorner
Dr. G.P. Tocchini Valentine
Dr. James C. Wang
Dr. David Ward
Dr. Peter C. Wensink
Dr. Heiner Westphal
Dr. Masao Yamada
Dr. Keith R. Yamamoto


LONG ISLAND
BIOLOGICAL
ASSOCIATION

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, 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

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.

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.

Officers

Mr. George W. Cutting, Jr., Chairman
Mrs. George N. Lindsay, Vice-Chairman
Mr. James A. Eisenman, Treasurer
Mrs. John P. Campbell, Secretary
Mr. G. Morgan Browne, Asst. Secretary/Treasurer

Directors

Mrs. Donald Arthur
Mrs. Allen L. Boorstein
Mr. Lionel Chaikin
Mr. Arthur M. Crocker
Mr. Lawrence L. Davis
Mr. Joseph P. Downer
Dr. Lester Dubnick
Mrs. Duncan Elder
Mrs. Henry U. Harris, Jr.
Mrs. Sinclair Hatch
Mr. Gordon E. Lamb
Mrs. Walter C. Meier
Mr. Arthur C. Merrill
Dr. Larry Nathanson
Mr. William F. Payson
Mr. Edward Pulling
Mr. John R. Reese
Mr. Douglas E. Rogers
Mr. Byam K. Stevens, Jr.
Mrs. Stanley S. Trotman, Jr.
Mrs. Philip R. von Stade
Dr. James D. Watson
Mrs. Bradford Weekes, III

CHAIRMAN'S REPORT

The interrelationship of the Long Island Biological Association with the Cold Spring Harbor Laboratory is developing into a unique partnership wherein members of LIBA are becoming the sponsors of the Annual Giving Program at Cold Spring Harbor, and at the same time, the members are receiving more and more opportunities to expand their understanding of molecular biology.

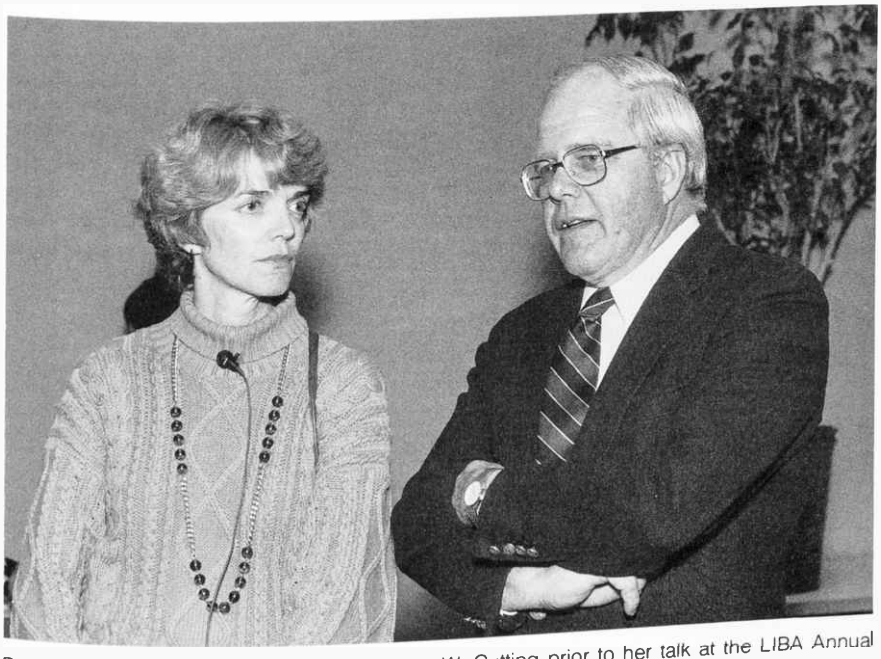
LIBA's Role

The Annual Giving effort results this year were most encouraging. Total contributions were $261,525 versus $182,195 last year, a 44% gain. The Cold Spring Harbor Associate Program, established for those who contribute $1,000 or more, was a major factor in this success.

This success has enabled the Laboratory to fund LIBA Fellowships for Drs. Joseph Colasanti, Bernard Ducommun, Ann Sutton, and Peter Yaciuk. In addition, the New Investigator Start-up Fund awarded grants to Drs. Dave Frendewey and Tom Peterson. And finally, the Laboratory was given the resources to fund unexpected requirements of their scientific programs.

Programs for LIBA Members

During the year, LIBA continued its efforts to increase the understanding of molecular biology throughout its membership. Besides Dr. Churchland's talk titled "The Brain: as a Neurological Machine" at the Annual Meeting, Dr. Eric Kandel, a trustee of the Laboratory and Chief of Psychology at Columbia Presbyterian Hospital, addressed the Dorcas Cummings Memorial Lecture in May on "The



Dr. Patricia Churchland chatting with George W. Cutting prior to her talk at the LIBA Annual Meeting.

Long and Short of Long-term Memory." Dr. Kandel, who attended the Cold Spring Harbor Symposium on Quantitative Biology which was conducted at that time on "The Molecular Biology of Signal Transduction", put forth some insights on functions of the brain. The programs of Dr. Kandel and Dr. Churchland gave the LIBA membrship its first glimpse of the world of neurobiology. This will become an increasingly important field of study at Cold Spring Harbor as the development of a Neuroscience Center emerges.

Additional learning opportunities were arranged for Cold Spring Harbor Laboratory Associates, including the first "hands-on" laboratory workshop for members at the DNA Learning Center. In addition, there was a series of five lectures from January to May by Laboratory scientists Drs. Dan Marshak, Rich Roberts, Mike Gilman, Jim Pflugrath, and Barbara McClintock. Their talks covered a broad spectrum of fields in molecular biology and were enthusiastically received by the participants. These scientists deserve our very special thanks, as the preparation of a talk for the "lay" community to understand is no easy task in the highly technical field of molecular biology.

Following the Dorcas Cummings Lecture, nearly 180 Symposium speakers and Laboratory scientists were entertained at 21 dinner parties in the surrounding community. The parties were a great success and this year's hosts and hostesses have our special thanks. They were:

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Changes in LIBA Directors

At the Annual Meeting special recognition was given to: Jeanne Ball, Mary Crary, Joan Hutchins, Sam Callaway, Rod Cushman, Charles Dolan, George Hossfeld, Jr., and Harvey Sampson, all of whom retired as directors after loyal and effective service.

Elected to replace them were: Jane Boorstein, Susie Trotman, Marjorie von Stade, Joe Downer, Les Dubnick, Gordon Lamb, Larry Nathanson, and Doug Rogers.

Laboratory Highlights

In the fifteen years since recombinant DNA techniques were developed, the number of scientists working in the field has expanded from a few hundred to almost 100,000. The pace of discovery has been expanding rapidly and whole new methods of investigation have been uncovered. Under the leadership of Dr. James D. Watson, the Cold Spring Harbor Laboratory is renowned as a world center for the exchange of ideas, through its meetings programs and through the basic research of its staff of more than 110 scientists. No LIBA report could be complete without a brief summary of some of the highlights of the year on the Laboratory campus.

Banbury Center Programs

Beginning to the east of the harbor, the Banbury Center, under the able leadership of Dr. Jan Witkowski, has conducted several outstanding programs, including an important meeting on gene expression in the AIDS virus, journalist and congressional workshops, and a seminar on the human brain for corporate executives. The interaction between LIBA and the Laboratory was evident as Dr. Patricia Churchland, the closing speaker at the seminar on the brain, returned to Cold Spring Harbor to deliver a talk to LIBA membership at the Annual Meeting in January.

DNA Learning Center

1988 saw the establishment of the DNA Learning Center in the former administration building of the Cold Spring Harbor School District. In March, DNA Workshops were held for the first time in the refurbished building. Here, high school and college instructors can learn the latest developments in molecular biology, enabling them to bring this technology to their students. Workshops are now run daily for students from the surrounding metropolitan area.

Of further note, the DNA Learning Center received a one-year loan of an exhibit from the Smithsonian Institution in Washington titled "The Search for Life: Genetic Technology in the Twentieth Century." This exhibit calls on LIBA members for another supporting activity—staffing for the exhibit.

Page Laboratory Opened

In October, the Laboratory saw the completion of a three-year capital program in plant biology with the dedication of the Arthur W. and Walter H. Page Laboratory. This 6,500-square-foot facility provides the Uplands Farm Field Station with much needed laboratory space and meeting rooms.

Construction was begun on six guest houses, each with four double rooms, to replace the rustic cabins which have served the Laboratory for nearly 50 years. These will be ready for occupancy when the meetings season begins in the Spring.

Dr. Ed Harlow and a participant in the Undergraduate Research Program examine test results.

Research Highlights

The science undertaken at the Laboratory continues on the highest level. The five-year DNA Tumor Virus Project grant from the National Cancer Institute was renewed for the fourth time since its inception in 1972 under Dr. Mike Mathews' supervision. Equally important was the NCI award of a large core grant for the Lab's activities as a specialized cancer center under the coordinating efforts of Dr. Rich Roberts. These two grants total $6 million of support for the cancer research effort.

Highlighting the research successes at Cold Spring Harbor this past year was the disclosure by Dr. Ed Harlow and his staff that a cancer-causing gene (an oncogene) found in a virus apparently acts by blocking the action of a second gene that seems to protect against cancer. This discovery sheds important new light on a mechanism that makes an ordinary cell suddenly erupt into uncontrolled growth, forming a cancer, and will have great impact on the cancer research field.

The Future

The success of LIBA's sponsorship of the Annual Giving Program at the Laboratory is dependent on the members' continuing efforts to introduce their friends to the excitement at the Laboratory, as their contributions will play an important part in the support of science at Cold Spring Harbor. They too will enjoy being a part of the Laboratory team and LIBA will continue to keep its membership apprised of discoveries in the fast-growing field of molecular biology.

George W. Cutting, Jr., Chairman

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