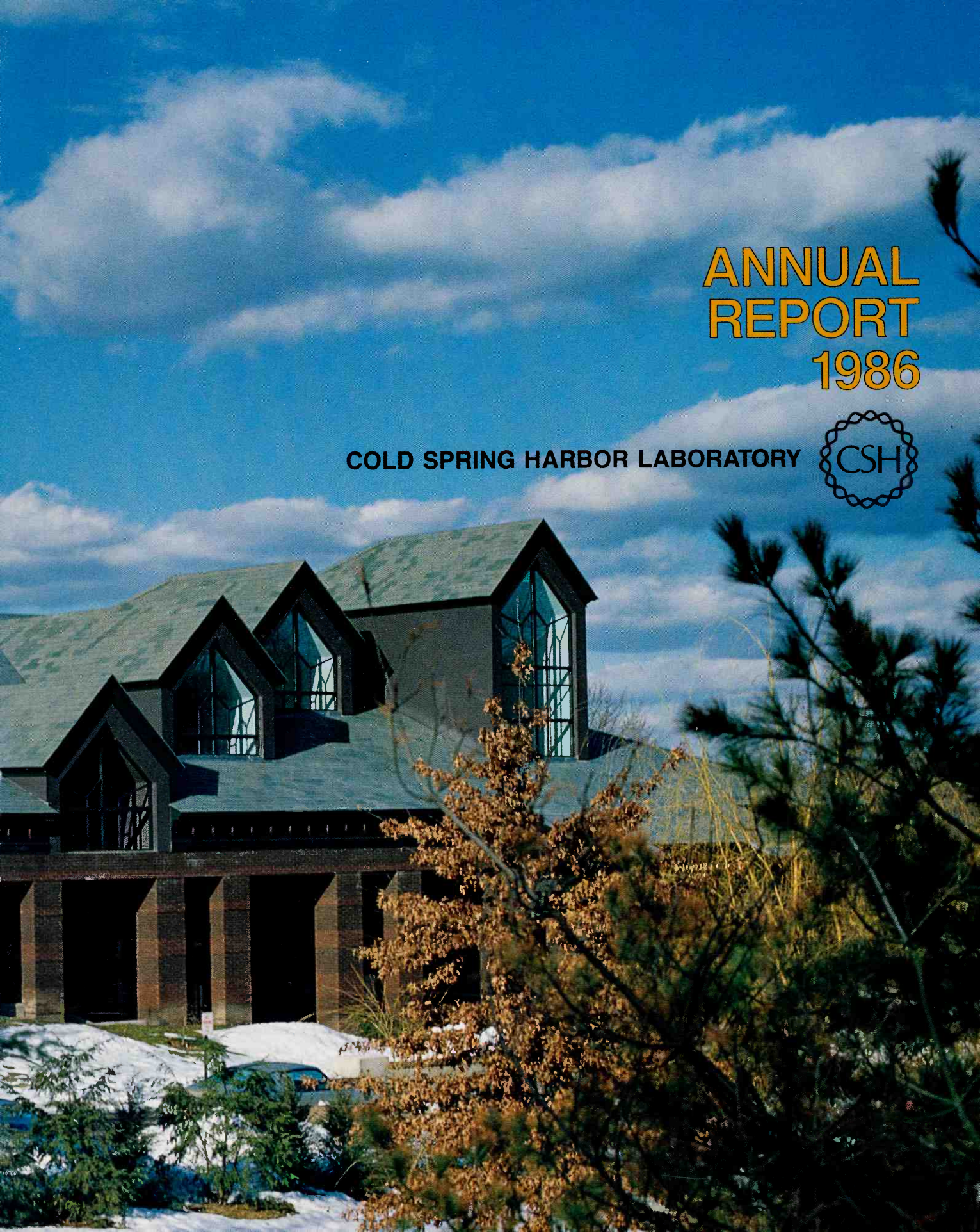

ANNUAL REPORT 1986

COLD SPRING HARBOR LABORATORY

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Cold Spring Harbor Laboratory
Box 100
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Book design Emily Harste
Editors Elizabeth Ritcey, Dorothy Brown, Annette Kirk
Photography Susan Zehl, Herb Parsons, David Micklos
Cover: Oliver and Lorraine Grace Auditorium (photo by S. Zehl)

Contents

(*Back row*) M. Scharff, B. Clarkson, J.D. Watson, W.H. Page, R.L. Cummings, T. Whipple, C. Basilico, H.E. Sampson (*Middle row*) T.J. Knight, R. Landau, Mrs. G.N. Lindsay, J. Klingenstein, Mrs. S. Hatch, F.M. Richards, G.W. Cutting, Jr. (*Front row*) O.R. Grace, W.S. Robertson, T.E. Shenk, W. Everdell

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Representation on the Board of Trustees itself is divided between community representatives and scientists from major research institutions. Ten such institutions are presently represented on the Board of Trustees: Albert Einstein College of Medicine, Columbia University, Harvard University, Massachusetts Institute of Technology, Memorial Sloan-Kettering Cancer Center, New York University, Princeton University, The Rockefeller University, The State University of New York at Stony Brook, and Yale University.

Also represented as participating institutions are the Wawepex Society and the Long Island Biological Association (LIBA). The Wawepex Society was formed in the mid-nineteenth century as a philanthropic arm of the Jones family, who supported the formation of the Laboratory. LIBA's origins began in 1890 when members of the local community became involved as friends and with membership on the Board of Managers of the Biological Station in Cold Spring Harbor, under the aegis of the Brooklyn Academy of Arts and Sciences. When the Brooklyn Academy withdrew its support of the Biological Station, community leaders, in 1924, organized their own association that actually administered the Laboratory until the Laboratory's reorganization as an independent unit in 1962. Today, LIBA remains a nonprofit organization organized under the Department of Social Welfare of the State of New York and represents a growing constituency of "friends of the Laboratory." Its 500 members support the Laboratory through annual contributions and participation in fund drives to raise money for major construction projects.

The Laboratory is chartered as an educational and research institution by the Board of Regents of the Education Department of the State of New York. It is designated as a "public charity" by the Internal Revenue Service.

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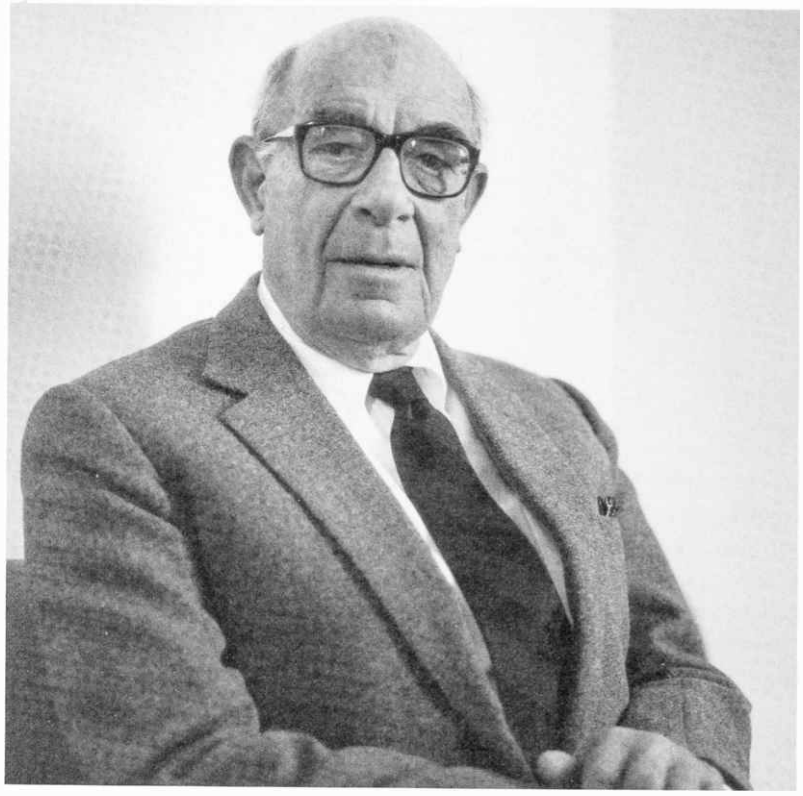


A Tribute to Walter Hines Page

Walter H. Page retired in November as chairman of the Board of Trustees, concluding 25 years of active service to the Laboratory and a legacy of family stewardship stretching unbroken for more than 60 years.

The Page family's commitment to science at Cold Spring Harbor began in 1924 when Walter's father, Arthur W. Page, became a founding director of the Long Island Biological Association (LIBA). Including 13 years as president, Arthur Page served continuously on the LIBA board until 1958, when Walter became president. Jane Nichols Page (Walter's wife) was LIBA vice-president from 1952–1958. In 1962, Walter led negotiations that culminated in the merger of the Biological Laboratory and the neighboring Carnegie Department of Genetics to form what we now know as Cold Spring Harbor Laboratory. In 1972, he helped bring into existence the Robertson Research Fund, the Laboratory's major endowment. He was elected chairman of the Board of Trustees in 1980.

Born in nearby Huntington, Walter attended Milton Academy and graduated from Harvard University in 1937. Choosing a career in banking, he served the Morgan Bank and its successor, the Morgan Guaranty Trust Company, at home and abroad until his retirement in 1979 as chairman of the board. He also served as director of numerous companies, including United States Steel Corporation, the Royal Group, Inc., and the Saudi International Bank.

Over the years, the Laboratory has benefited from the counsel of this truly wise man and from his consummate business acumen. We look forward to his continued advice as an honorary trustee and caring neighbor.

DIRECTOR'S REPORT

Our genetic uniqueness, like that of all other forms of life, is determined by the information present in DNA, the chromosomally located chemical substance out of which our genes are made. Encoded within the sequences of the four nucleotide bases (A, G, C, and T) that are used to construct all DNA molecules are the instructions to make the very large numbers of specific proteins found in all cells. These proteins, many of them enzymes, make possible the orderly growth and multiplication of all cells as well as the developmental progression of fertilized eggs into multicellular organisms.

That DNA plays such a central role in cellular existence only became unambiguously clear in 1953. Until then, most chemically oriented biologists still thought that genetic specificity resided in the protein component of chromosomes. This belief persisted despite the key 1944 announcement that bacteria could be genetically transformed by the addition of pure DNA. At that time, however, the relationship of bacteria to other forms of life was very unclear, leaving open the question of whether DNA also carried the genetic instructions of higher cells. Serious arguments about the chemical identity of genes ended when the double helix was found to possess the essential attribute of a genetic molecule, a structure that allows its accurate copying to yield progeny molecules identical in structure to that of the parental DNA.

In the now over 30 years that have elapsed since the finding of the double helix, all the general principles that underlie the functioning of DNA within cells have been established. The genetic information within DNA is used not only to make progeny DNA molecules, but also to make RNA, a second form of nucleic acid that utilizes essentially the same four-letter alphabet used by DNA. RNA molecules in turn serve as the source of information to order the 20 different amino acids that are used to construct the polypeptide chains of proteins. Once we learned how DNA works, the nature of genes, the genetic determinants first found by Mendel, became clear at the molecular level. A gene is a linearly contiguous group of nucleotide bases within a DNA molecule that specifies the sequence of a specific polypeptide chain. The number of different genes located along a DNA molecule varies greatly from only a few for the smaller viral DNA molecules to many thousands along the much larger chromosomes of higher organisms. As far as we know, only one DNA molecule is found within a given chromosome. The DNA molecules isolated from very big chromosomes are thus much larger than those of small chromosomes.

The simplest cells, those of bacteria, contain only one type of DNA molecule. They each encode from 1 to some 5000 different genes. Many more genes are

needed to code for the much larger number of proteins that underlie the existence of the much more complex higher plants and animals. The genetic information of each human being, for example, is carried by 46 DNA molecules, 23 from the male parent and 23 from the female parent. Except for the X and Y sex chromosomes, each chromosome contributed by the male parent is equivalent in size and function to one contributed by the female parent (homologous chromosomes). There thus exist 24 different types of human chromosomes, 22 autosomes (non-sex chromosomes), 1 X chromosome, and 1 Y chromosome.

The total number of human genes dispersed on the corresponding 24 different human DNA molecules is far from known. Until now, only some 1000 different proteins have been assigned to specific chromosomes. Arguing from the size of known genes, however, we can predict that at least 50,000 different human genes exist, and few human geneticists will be surprised if the total finally established does not exceed 100,000 genes. Until the advent of recombinant DNA procedures, and the powerful new procedures for sequencing DNA, the molecular nature of these genes was not open to analysis. Now, however, given the possession of a relatively small amount of the protein product, procedures exist for the virtual routine cloning and subsequent sequencing of the corresponding gene. Already more than a hundred different human genes have been cloned and over a million base pairs of human DNA sequences are now known. Although this is a large number, it still is only 1/3000 of the total human genome ($3x10^9$). Even with a steady increase in the rate at which new genes are cloned, we would not expect to see the whole human genome established in much less than 50 years from now.

To achieve this goal much faster, say by the turn of this century, will require a highly organized and focused effort of a magnitude that the world of molecular biology has not yet experienced. Conceivably, a sum of several billion dollars and some 30,000 scientist-years would be consumed by the project. So the human genome project strikes many biologists as a form of big science that cannot be organized with effective peer review. They worry that it might run badly out of control, using up sums of money so large that it would necessarily have a negative impact on the rate at which we go about solving fundamental biological problems.

Human geneticists, however, have a very different perspective. To them, the possession of the complete human DNA sequence would be a resource of inestimable value. So they are much less concerned about altering the way their science is done. To have it within our grasp and not go for it strikes them as an act of gross irresponsibility to society. Given the human DNA sequence, the finding and understanding of the many genes underlying currently intractable diseases like the various cancers, schizophrenia, cystic fibrosis, and muscular dystrophy would certainly be accelerated.

This extraordinary potential for understanding so many important diseases at the genetic level has only come about over the past decade. Until very recently, the inherently nonexperimental nature of human genetics has made it lag far behind the genetics of the experimentally much more malleable organisms like *E. coli*, the yeasts, *Drosophila*, and the mouse. It was not until 1968 that the first human gene was mapped to an autosome. Human genetics only began to take off with the development of somatic cell hybridization techniques, as well as the working out of powerful cytogenetic techniques which revealed discrete banding patterns that allowed the characterization of each human chromosome. Now the pace at which new genetic diseases can be identified and then mapped goes even faster because of the application of recombinant DNA procedures. As soon

as a gene is cloned the resulting DNA probes can be used to assign a location on a precise chromosome.

There furthermore exist many human "gene libraries," each made up of a very large number of human DNA fragments (members) individually inserted into autonomously replicating DNA molecules (vectors). The size of the library needed to carry the human genome depends upon the length of the DNA inserted into the DNA vector. Most existing gene libraries were made with approximately 5×10^4-base-pair segments inserted into the so-called cosmid vectors. To effectively encompass the human genome of 3×10^9 base pairs, library sizes of greater than 10^5 members are needed to statistically ensure that virtually all the human genome is on hand. Although the making of such a gene library is virtually routine, the exact ordering of these library members is a manyfold more challenging task. Only over the past several years have techniques begun to be developed to achieve this goal. Particularly crucial will be the new pulsed-field gel electrophoretic procedures for separating megabase-size (10^6) DNA fragments as well as the development of artificial yeast chromosome vectors into which these megabase fragments can be stably cloned. Given the routine employment of such megabase vector systems, the size of the human gene library can be reduced to only several thousand members, thereby greatly reducing the task first of assigning them to separate human chromosomes (regions) and then of determining their exact order along their respective chromosomes.

Equally important, recombinant DNA procedures can now generate a very large number of new genetic markers to be used in family linkage studies. These markers reflect the very large numbers of differences (polymorphisms) in nucleotide sequence that characterize the genomes of all organisms. These variations generate differences in the DNA-cutting patterns made by different DNA-cutting (restriction) enzymes. Already, restriction fragment length polymorphisms (RFLPs, sometimes pronounced "riflips") have been used to allow the first mapping of the mutant genes responsible for Huntington's disease and cystic fibrosis.

The way has thus been opened up not only for prenatal diagnosis, but also for the eventual isolation of the respective disease-causing genes and the working out of their biochemical functioning. Because polymorphisms in DNA sequences are so common, a large number of RFLP probes can be made for any desired chromosomal segment. They now provide a way to map genetically the human genome to a much higher resolution than can ever be provided by genetic markers that reflect protein differences. Through the possession of only several hundred RFLP markers randomly dispersed over the 24 human chromosomes, it should be possible to find the appropriate location of almost any simple genetic disease for which family linkage studies exist. And through the possession of tenfold more RFLPs, these traits can be mapped to within the 1 megabase (10^7 bases) lengths that soon we will be able to clone using newly developed artificial yeast chromosome vectors.

The time will thus soon be at hand when it will be possible to make an overlapping set of cloned DNA fragments that collectively encompass each of the 24 human chromosomes. To do so will require an organized effort reaching over 3 to 6 years and costing some 25-50 million dollars. When this job is done, the appropriate DNA will be available for analysis as soon as a given genetic disease has been assigned a precise map location. This DNA can then be quickly sequenced so as to spot the gene responsible for the respective disease.

Given the ordered set of human DNA clones, the question then will arise as to whether we should sequence them immediately or wait and see if they are

genetically interesting before so proceeding. In part, the way we shall respond will be a function of the cost of DNA sequencing. At today's cost of sequencing of from 1 to 2 dollars per base pair, at least 3 billion dollars would be required. There is every reason to believe, however, that by the time an ordered set of DNA clones exists, the cost of sequencing may be reduced to one-fifth to one-tenth of the present cost, if not more. If so, the mapping and sequencing of the human genome might cost as little as a half billion dollars.

This is still a sum of money far in excess of that spent on any previous tightly focused biological effort. Special efforts will thus have to be made first to obtain this money and then to organize the effort so that we shall do it well, reaching our final goals with a minimum of false starts and with a final precision we have every reason to trust. The many obvious short-term advantages of having the set of overlapping human DNA clones provide strong reasons for accomplishing this mapping job as soon as possible. It will most certainly help us get much faster to the heart of complex genetic disorders like depression, schizophrenia, and alcoholism. We should also waste no time in quickly devoting considerable funds toward the development of the sequencing technologies that will not only greatly lower the final cost, but also reduce the number of highly trained scientists needed for the project. There already is a great shortage of individuals who manipulate DNA well. Even if all the funds were now on hand, it is far from clear that we could ever attract that many top-flight individuals to work on a sequencing project whose nature is highly repetitive.

Setting a completion date of not more than 15 years from now, if not by the year 2000, may well prove necessary if we are to do the job well. Unless the goal can be reached by those who organize the effort, the job may not be well thought out. In any case, the total cost per year of the program is unlikely initially to be much greater than 25–50 million dollars rising toward the end to say 100 million dollars per year. This sum is less than 2% of today's NIH budget and most likely would be closer to 1% of the total NIH budget when the job is done.

The real problem that faces us is thus not the cost of the Human Genome Program, but how to get it going, seeing both that the right people are in charge and that they work under an administrative umbrella that will not tolerate uncritical thinking and so will never promise more than the hard facts warrant. Only a governmental body has reason to assume that it has the means to ensure ultimate success, and within the United States only the NIH, the NSF, and the Department of Energy (DOE) have missions that are commensurate with their taking on a significant role.

Until now, only the DOE has seriously considered the matter and appointed a senior committee to give it advice. Already it has proposed to Congress that it have the mandate to oversee the project and has requested 10 million dollars over the next fiscal year to commence the job. The reason they give for their managing the effort—to be able to evaluate better the damage to our genomes by environmental mutagens—is forced and in fact weakens their case by making them appear at best disingenuous and at worst stupid. A much better reason for thinking them suitable is their past track record of managing well the construction of so many of the large-particle accelerators needed by the physics community. There is also the presumption that additional monies given to them would not be taken from the pockets of NIH research grants. Despite these advantages, DOE sponsorship nonetheless brings chills to many senior members of the DNA world. They worry that the DOE's track record in supporting high-quality extramural research in biology has never matched that of either the NIH or the NSF and that their way of giving out grants in the absence of true peer selection procedures will

inevitably lead to support of too much of the second rate. Equally disquieting to the DNA world is the fact that none of the national labs that the DOE manages is a leader in recombinant DNA procedures. So the DOE cannot now draw on any deep in-house expertise to help administrate even a modestly sized human genome effort.

A quite different set of reasons seem to preclude the NSF from taking the lead. Its budgets for biology have never been large, and so a major Human Genome Program would consume a dangerously large fraction of its total effort in biology. So the success of this program might only be accomplished by harming those other biological disciplines, like evolutionary studies, for which the NSF is the main research support. On the other hand, the NSF's increasing preoccupation in promoting American technological vigor argues that it should take an active role in supporting the technological advances that will reduce the cost of mapping and sequencing and so ensure that the budget for the Human Genome Program never gets out of control. Clearly, the NSF should at least maintain a vigorous junior voice within the final consortium of agencies that handle this project.

The obvious lead agency thus remains the NIH. Not only does it routinely handle very large sums of money, but the nature of the human genome will increasingly be at the heart of the research that it supports over the next several decades. The diseases that it wishes to banish, if not due to infectious agents, will more and more be shown to have their origins in the imperfections of our individual genetic instructions. Over the long term, fighting diseases without thinking in terms of DNA will not be the perceived correct way to proceed. We might have thus expected that the NIH would be campaigning hard to start up a vigorous Human Genome Program. Until now, however, no strong voice within the NIH has emerged to whom this project would naturally be entrusted. The reason for this apparent lack of self-interest may be AIDS. Clearly, the NIH must lead the national challenge to control this fearsome affliction, and in a real sense its future creditability with the American people will depend upon its ability to mount an outstanding AIDS program that will successfully bring together the very best of our national medical research talents. It has to put AIDS at the top of its agenda and ensure that the massive new monies for AIDS research are spent intelligently fast as opposed to just fast. The NIH at this moment thus may feel itself unable to give the Human Genome Project the deep attention that it soon will need. The question of who will manage this nation's effort thus remains unresolved.

No matter where the funds come from, the question will remain as to where they should be used. Clearly, much of the research needed to improve current mapping and sequencing strategies will occur in academic surroundings. Administering these efforts should involve no difficult decisions. On the other hand, the final hard-core mapping and sequencing tasks will best be done in large, especially designed "production centers." Conceivably, these production centers should be located within one or more of our national labs like Brookhaven or Livermore. Alternatively, they could be privately owned, operating under contracts from the appropriate funding agency. The decision between these alternatives will hopefully rest on the inherent motivations and past track records of the key individuals prepared to manage production centers. At present, there are very few individuals qualified to run them, and we may not be able to get rolling as fast as we might wish. So an initial early emphasis of the Human Genome Program should be the setting up of training programs centered on mapping and sequencing procedures.

To help in the initiation and then the monitoring of an effective national program, an ad hoc organization, that could be called the Human Genome

Organization (USA), should be set up. Its members should include the individuals now serving on the several national genome committees functioning in behalf of the National Academy of Sciences, the Department of Energy, and the Office of Technology Assessment of the U.S. Congress. Initially, this organization could sponsor a series of small, expert meetings whose reports might prove invaluable to the several potential funding agencies as they decide how to set up their own respective programs. Optionally, the Human Genome Organization might be funded from a source independent of agencies like DOE, NSF, and NIH and so be free to persue an independent intellectual course that lets its opinions fall where they should.

Also to be solved is how the program within the United States will be coordinated with efforts carried out in other countries. If they wished, either Western Europe or Japan could by themselves take on this project and it must be assumed that they will initiate their own efforts. So a new international body should soon be formed to ensure that collaboration, not competition, marks the relationship between these efforts in various parts of the world. In a real sense, the exact sequence of the human genome will be a resource that should belong to all mankind. So it is a perfect project for us to pool our talents, as opposed to increasing still further the competitive tensions between the major nations of the world.

It is thus clear that in saying now is the time to go for the total human genome sequence, we are taking on an effort far different from those that previously preoccupied us. Countless planning sessions and then years of hard repetitive work lie before us. The final goal when reached should be more than worth the effort. In seeing the exact order of the several billion base pairs that give to us the ultimate basis for our uniqueness, we will have reached a major milestone in human history. The task from then on will be to learn to read these messages. In so doing, we shall remain very intellectually challenged for many centuries still to come.

HIGHLIGHTS OF THE YEAR

Grace Auditorium Dedicated

The dedication of the Oliver and Lorraine Grace Auditorium on June 1 marked the beginning of the second half-century of scientific meetings at Cold Spring Harbor. Fittingly, the ceremony was held on Sunday during the 51st Symposium on Quantitative Biology. A standing room only crowd jammed the 360-seat facility to hear the keynote address, "Scientists and the Future," presented by Daniel E. Koshland, Jr., professor of biochemistry at the University of California, Berkeley, and editor of *Science*.

A plaque inside the front door emphasizes that construction of the facility "was made possible through the generosity of over 400 members of the Long Island Biological Association, including a special gift from Lorraine and Oliver Grace."

Designed by Centerbrook Architects of Essex, Connecticut, the auditorium at once meshes with the historic architecture of the local environment and evokes its cosmopolitan function as a gathering place for scientists from around the world. During its first season of use, the facility won uniform praise from meeting participants who appreciate that the discussion of high-level science is not inhibited by comfortable surroundings.

In addition to its conference function, the auditorium is also "home" to 20 staff members. On the main level is the Public Affairs Office, and on the lower level are the Meetings Office, Samuel Freeman Computer Center, and the Quest Protein Gel Analysis Center.

Walter H. Page at Grace Auditorium dedication

51st Symposium Focuses on Human Genetics

The choice of the sweeping topic for the 51st Symposium, "The Molecular Biology of *Homo sapiens*," signified a turning point in modern biology: a time when the extraordinary techniques of DNA research are yielding up a wealth of detailed knowledge about the human genetic material. Human genetics was the focus of only one of the first 50 Symposia (in 1964), but it will be a topic that will be returned to again and again during the next half-century.

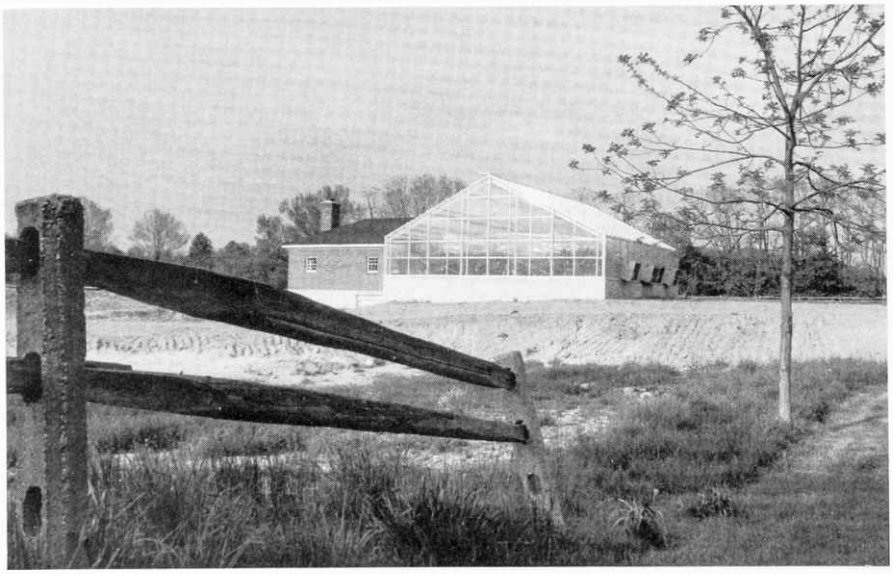
An air of excitement was palpable throughout the meeting; there was a satisfying feeling that DNA science had at last come of age. Contrary to past Symposia, when results were most often of no immediate concern to the "average person on the street," research team after research team presented results with significant implications for human health.

Significant Growth of the Summer Meetings Program

Attendance at Cold Spring Harbor and Banbury meetings rose from 2900 in 1985 to 3600 in 1986—a growth of 23%. Much of this expansion can be attributed to the predictable, annual support now provided by industry. Since inception of the Corporate Sponsor Program in 1984, overall meeting attendance has increased 42% and corporate attendance increased 48%. Moreover, the stable base of support offered by Sponsors has allowed us to increase by one-third the number of meetings offered each summer.

Plant Genetics Program Gathering Critical Mass

In 1986, we reached the turning point in our effort to rebuild a major plant genetics program at Cold Spring Harbor. With the Uplands Farm Experiment



Plant Genetics Lab

Station fully operational, in the spring we broke ground for the Arthur W. and Walter H. Page Laboratory of Plant Genetics. When the 6500-square-foot Page Laboratory is complete in summer 1987, we will have one of the nation's best facilities for the study of plant genetics.

The arrival of Senior Staff investigators Venkatesan Sundaresan (in December) and Stephen Briggs (in January) substantially increased our focus on maize genetics. Dr. Briggs, who has a joint appointment with Pioneer Hi-Bred International, is working to isolate genes that confer resistance to the corn pathogens, such as southern rust. Dr. Sundaresan, who joins us from the University of California, Berkeley, studies the role of transposable genetic elements in corn development. Recently appointed to join us in the fall of 1987 is Tom Peterson who will come to us after a three-year postdoctoral period working on maize in Canberra, Australia in the Laboratory of Jim Peacock.

X-ray Crystallography Comes to CSH

The installation of specialized equipment for X-ray analysis will soon allow us to work out the three-dimensional configuration of DNA and protein molecules. Already arrived to head up the Laboratory's X-ray structure group are John Anderson, from Harvard University, and Jim Pflugrath, from the Max-Planck Institute in Munich, Germany.

J. Anderson and J. Pflugrath

For many years, X-ray crystallography studies on macromolecules were outside the realm of possibility for any but the largest institutions. Construction and maintenance of X-ray generators/detectors required a full-time physicist, and computers with sufficient power to analyze diffraction patterns could fill a room. Now, dependable generators and detectors can be purchased "off the shelf," and super microcomputers the size of a travel trunk provide ample computing speed.

Our move into X-ray crystallography was made possible by grants totaling over $1 million from the Lucille P. Markey Charitable Trust, the Oliver S. and Jennie R. Donaldson Trust, and Mr. and Mrs. Oliver R. Grace. A large portion of the Hershey building has been renovated for the X-ray structure group and includes offices, a seminar room, and a computer suite. A "wet" lab in Demerec Laboratory provides all equipment for protein purification and crystal preparation, with the X-ray generator and diffraction equipment occupying adjacent rooms.

Monoclonal Antibody Agreement with Amersham International

In October, the Laboratory signed an $800,000 joint research agreement with Amersham International plc. The five-year program, which focuses on the production of monoclonal antibodies for research purposes, follows other research agreements with Monsanto Company (1984, $2.06 million) and Pioneer Hi-Bred International, Inc. (1985, $2.5 million). Amersham will have first right to license and distribute any antibodies arising from the 20–25 cell fusions to be carried out each year at Cold Spring Harbor utilizing Amersham funding.

With operating headquarters in Buckinghamshire, England, Amersham International is a major producer of research reagents, including radioactively labeled molecules. Amersham scientist Margaret Raybuck is now working with Senior Staff Investigator Ed Harlow, who is in charge of the monoclonal antibody facility in Sambrook Laboratory.

Cairns Lab

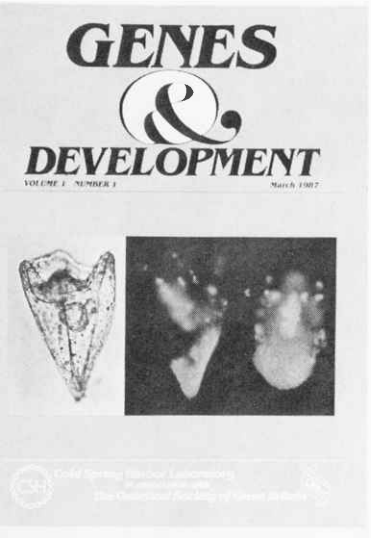
Cairns Electron Microscopy Facility in Operation

The former "mouse house" underwent a spectacular metamorphosis to become an up-to-date electron microscopy suite. The facility is named after John Cairns, who did elegant research here on the nature of viral chromosomes while a visiting fellow in Alfred Hershey's Lab, and who later returned here in 1963 to function as the director as well as to continue research on the mechanism of DNA replication. Upon leaving here in 1973, John became the Director of the Mill Hill Laboratory of the Imperial Cancer Research Fund, moving later to the Harvard School of Public Health as a Professor. Recently, John was honored with a prestigious MacArthur Fellowship in recognition of his extraordinary intellect.

Grants totaling $385,000 from the National Institutes of Health, National Science Foundation, and the Fannie E. Rippel Foundation have made possible the purchase of a Hitachi H-7000 microscope, to be installed in the summer of 1987. A great improvement over the Philips 201 microscope, which has been in use at the Laboratory for over a decade, the Hitachi instrument can produce stereo effects in a "scanning" mode or through backscatter electron imaging.

We have also purchased a computerized image-enhancement system (similar to those used to assemble digital information relayed from space probes) that can make three-dimensional reconstructions, add color, and make intracellular measurements. In addition, the microscope has a "real-time" television monitor, which allows observers to view along with the microscope operator.

The electron microscopy facility is run by Senior Staff Investigator David Spector, who studies the three-dimensional organization of protein and nucleic acid molecules in the cell nucleus. Of particular interest to David is a network of small nuclear ribonucleoprotein particles (snRNPs or "snurps") that play a major role in the RNA splicing events that precede protein synthesis.



We Launch a New Journal

In 1986, a plan was set in motion to publish a new journal in cooperation with the Genetical Society of Great Britain. The title, *Genes & Development*, emphasizes the relationship between gene expression and the phenotype of living things. In casting such a broad net, we wanted a journal sympathetic to the views of both developmental biologists and molecular geneticists. Although the tragedy of Steve Prentis' death just prior to the appearance of the first issue of the journal in March 1987 was a shocking loss, executive editor Grahame Bulfield, editorial board member Mike Mathews, and editor Judy Cuddihy quickly pulled together to keep the publication on schedule. The continuous flow of high-quality papers indicates the scientific community's commitment to keeping Steve's dream alive.

Our Scientific Friends Consider Themselves Alumni

This past summer we wrote to meeting attendees, course instructors, and former staff members asking them to consider how their own careers had benefited from their Cold Spring Harbor experiences and to make a commitment to ensure that the Laboratory's position as a crossroad in science would be maintained.

Our goal is to raise $100,000 for construction of "Alumni Cabin"—one of six eight-bed structures that will replace dilapidated structures currently used to house summer visitors. Response to the appeal was overwhelming; by year's end, nearly 200 scientists (one in four asked!) had contributed almost $34,000 to the Alumni Cabin Campaign. We hope that other alumni will come forward to help us reach our goal by the time construction is complete in 1988.

Creation of the Doubleday Professorship of Cancer Research

In the fall of 1984, upon the dissolution of the Russell and Janet Doubleday Fund, the Laboratory was given a gift of 500 shares of Doubleday & Company. At that time, the value of the shares of the tightly held company was known to be several hundred thousand dollars, but we in no way expected that, upon the sale of the company to the publishing giant Bertelsmann, Inc., we would receive slightly more than 2 million dollars for ourselves. Before receipt of the shares, we indicated to the Doubleday Trust that we would use their funds to support a Doubleday Professorship of Cancer Research. So in February 1987, our Trustees voted to set aside as endowment funds 1.5 million dollars from these proceeds for that purpose. The remaining funds have been used to purchase property virtually surrounded by our land and which contains a house that henceforth will be known as Doubleday Cottage. Now expanded by a previous owner, the house was built in the early 1930s by Hugo Fricke, a scientist then on our staff who purchased the land from the Laboratory. Originally, the Laboratory had a buy-back right, but this restriction was dropped when Dr. Fricke left our staff at the beginning of World War II. Our ability to regain title to this key piece of property and the delightful house located on it is an important event for the Laboratory, and we remain most indebted to the efforts of Edward Pulling, whose intervention with the Doubleday Trust led to our receiving the necessary funds.

Extending Our Role as a DNA Information Center

Cold Spring Harbor is a major international center for the exchange of information on DNA science. In the past, this information-sharing role has been primarily confined to high-level meetings and courses for advanced students and practicing

scientists. However, over the past several years we have moved to extend our educational role to include other audiences that need to be brought up-to-date on advances in DNA science.

Our DNA Literacy Program now offers hands-on training workshops for high school and college teachers across the country. Starting this coming fall, we intend to develop a DNA Learning Center in Cold Spring Harbor Village that will serve as a national center to focus on the problem of biotechnological illiteracy. Our goal is to expose the American public to the potential benefits of molecular biology and to provide an experimental basis for the rational evaluation of biotechnological progress.

Changes in Our Scientific Staff

Senior Staff Scientist Jim Hicks accepted a position to head up PPG Industries' joint research program with the Scripps Clinic and Research Foundation in La Jolla, California. Jim joined the Laboratory as a Staff Investigator in 1978 following a postdoctoral period in Gerald Fink's group at Cornell. He, Jeff Strathern, and Amar Klar helped to firmly establish yeast as a major system for studying development when they showed at the molecular level that the mating-type switches in *Saccharomyces cerevisiae* are achieved through the movement of genetic cassettes.

Three Senior Staff Investigators accepted assistant professorships in academia: Steve Dellaporta in the Department of Biology at Yale University, David Kurtz in the Department of Pharmacology at the University of South Carolina Medical School, and Clive Slaughter in the Department of Biochemistry at the University of Texas Health Science Center.

Steve, who came here to clone maize genes using the *Ac* transposon methodology, was instrumental in developing our current joint research program with Pioneer Hi-Bred International. He came to Cold Spring Harbor as a postdoctoral fellow in Jim Hicks' lab in 1981 from Worcester Polytechnic Institute. David, who studied hormonal control of gene expression, joined the Laboratory as Staff Investigator in 1979 from the Biochemistry Department of the College of Physicians & Surgeons of Columbia University. Clive came to the Laboratory in 1984 from the University of Texas Health Science Center to reestablish a major focus in protein chemistry in the then-new Demerec extension. In returning to the Health Science Center, he also assumed responsibilities as an assistant investigator at the Howard Hughes Medical Institute.

Staff Investigator Paul Thomas, who worked out efficient systems for in vitro translation of proteins, came to the Laboratory as a postdoctoral fellow in 1978 after receiving his Ph.D. from the University of Cambridge. He returned to his native England to assume family responsibilities. Also leaving his Staff Investigator position was Tohru Kataoka, who came to the Laboratory as a visiting scientist in 1983 from Osaka University, and who now has moved to an assistant professorship at Harvard Medical School. Working with Mike Wigler, Tohru cloned genes for key proteins in the *ras*/cAMP pathway in the yeast *Saccharomyces cerevisiae*.

Staff Investigators Steve Hinton, Pablo Scolnik, and Doug Youvan left at the conclusion of our five-year research program on genetics with Exxon Research and Engineering Company. Steve returned to the Exxon Facility in Annandale, New Jersey; Doug went as an assistant professor to the Massachusetts Institute of Technology; and Pablo went to the Experimental Station of du Pont Central Research and Development where he will continue the work he initiated here on plant molecular biology.

New Staff Members

Joining the Laboratory were seven Senior Staff Investigators: John Anderson, Jim Pflugrath, David Frendewey, Michael Gilman, Nouria Hernandez, Dan Marshak, and Venkatesan Sundaresan.

John Anderson and Jim Pflugrath form the nucleus of the new X-ray structure group. John came to the Laboratory following graduate and postdoctoral work at Harvard University where his research involved DNA-binding proteins that selectively bind to specific regions of the DNA molecule. Jim came from the Max-Planck Institute for Biochemistry in Munich, Germany, where he designed major new software and computer graphics programs for obtaining and evaluating X-ray diffraction information.

David Frendewey came from Walter Keller's lab at the German Cancer Research Center in Heidelberg. He is characterizing the biochemical mechanism of pre-mRNA splicing in the fission yeast *Schizosaccharomyces pombe.* Formerly in Robert Weinberg's lab at the Whitehead Institute, Mike Gilman is interested in how extracellular signals of growth factors are communicated to the cell nucleus to ultimately induce transcription of the proto-oncogene c-*fos*.

Nouria Hernandez recently completed a postdoctoral fellowship in Alan Weiner's lab at Yale University. She is studying the assembly of small RNA molecules in the cell nucleus. A protein chemist, Dan Marshak arrived from Michael Brownstein's group at the National Institute of Mental Health, where he had purified nerve extension factor.

Venkatesan Sundaresan was associated with Michael Freeling at the Agricultural Experiment Station of the University of California, Berkeley, before coming to the Laboratory. Sundar studies the transposable element, Robertson's mutator, and its role in generating diversity in maize.

Visiting scientists arriving in 1986 were Arie Admon, Andre-Patrick Arrigo, Loren Field, and Margaret Raybuck. Arie Admon, who completed a postdoctoral fellowship with G.G. Hammes at Cornell University, collaborates with Yakov Gluzman. On sabbatical from the University of Geneva, where he is an assistant professor in the Department of Molecular Biology, Andre-Patrick Arrigo is working with Bill Welch. Now collaborating with Doug Hanahan, Loren Field was a postdoctoral fellow with K.W. Gross at Roswell Memorial Park Institute in Buffalo. Margaret Raybuck, a scientist with Amersham International of England, is working with Ed Harlow.

Visiting scientists who completed their studies at Cold Spring Harbor during the year and returned to their previous or new positions were Diane Esposito to Memorial Sloan-Kettering Cancer Center; Mark Homonoff to Mt. Sinai Hospital; Krystyna-Slaska Kiss to the Institute of Genetics in Szeged, Hungary; and Massimo Romani to Cornell University. Marcello Siniscalco, who co-organized the 51st Symposium on "The Molecular Biology of *Homo sapiens,*" returned to Memorial Sloan-Kettering Cancer Center after sabbatical at Cold Spring Harbor.

Appointment of Douglas Hanahan as a Senior Scientist

In December, the Laboratory's Trustees approved the appointment of Doug Hanahan to the position of Senior Scientist in recognition of his already well-acclaimed studies using transgenic mice. Doug's association with Cold Spring Harbor began in 1978, when he came as a Harvard graduate student to James lab to do recombinant DNA experiments on the collagen gene. At that time, Harvard lacked the so-called safe facilities then thought necessary for work with

recombinant DNA. Later, when he became one of Harvard's prestigious Junior Fellows, he spent even more time here, joining our staff in 1985 as a Staff Investigator and being quickly promoted to Senior Staff Investigator.

Our Senior Scientist position is marked by a rolling five-year appointment, which signifies that salary is now guaranteed by the Laboratory for a five-year period that continuously moves forward with time. This guarantee, however, falls short of the academic tenure offered by many universities. If we are to retain key scientists like Doug for most of their research careers, we must increase greatly our endowment funds specifically earmarked to fund truly tenured positions.

Staff Promotions

Senior Staff Scientists Richard Roberts and Terri Grodzicker accepted newly created leadership positions. As Assistant Director for Research, Rich has primary responsibility for staff recruitment. As Assistant Director for Academic Affairs, Terri coordinates the Laboratory's professional education and meetings programs.

Rich received his Ph.D. in organic chemistry from the University of Sheffield. He came to Cold Spring Harbor as a Staff Investigator in 1972, following a postdoctoral period at Harvard University. Over the years, his research group has isolated more than 25 now commercially available restriction enzymes. He was a key member of the Cold Spring Harbor team that co-discovered the phenomenon of RNA splicing.

Terri also came to Cold Spring Harbor in 1972 for a postdoctoral fellowship with Joe Sambrook after receiving her Ph.D. from Columbia University. Terri was among the first scientists to use restriction enzymes to map genes in tumor viruses. She also demonstrated the feasibility of using virus vectors to correctly express cloned genes in mammalian cells.

David Helfman, who studies the molecular and cellular biology of the cytoskeleton in normal and transformed cells, was promoted to Senior Staff Investigator. He came to Cold Spring Harbor from the Department of Pharmacology of Emory University in Atlanta in 1981 as a postdoctoral fellow in Jim Feramisco's lab and became a Staff Investigator in 1985.

Four postdoctoral fellows trained in Mike Wigler's laboratory accepted positions as Staff Investigators: Scott Powers, Carmen Birchmeier, Daniel Broek, and Takashi Toda. Scott, who came to Cold Spring Harbor after receiving his Ph.D. from Columbia University in 1983, has cloned several genes that affect the *ras*/adenylate cyclase pathway. Carmen received her Ph.D. with M.L. Birnsteil at the University of Zurich and came to the Laboratory in 1984 from the Max-Planck Institute in Tübingen, Germany. She has been exploring new assays for detection of human oncogenes.

Now on a six-month sabbatical at the Medical Research Council in Cambridge, England, Dan Broek studies the biochemical characterization of yeast and mammalian *ras* proteins. He came to Mike Wigler's group in 1984, after receiving his Ph.D. in biochemistry from Rutgers University. Also arriving in 1984, Takashi Toda received his Ph.D. from Kyoto University; he studies the role of *ras* proteins in growth control in yeast.

Others moving up from postdoctoral positions were Andrew Hiatt, Eileen White, and Elizabeth Moran. Andy, who studies genes involved in secondary plant metabolism, received his Ph.D. in biochemistry from Columbia University in 1983. As a Damon Runyon Fellow, he worked with Jim Hicks. Also a Damon Runyon Fellow, Eileen came in 1983 to work in Bruce Stillman's lab, after receiving her Ph.D. from the State University of New York, Stony Brook. She examines the

function of adenovirus E1B tumor antigens in transformation and lytic infections. Elizabeth studies the role of adenovirus E1A protein in regulating transformation and viral transcription. After receiving her Ph.D. in microbiology from New York Medical College in 1983, she came to Cold Spring Harbor as a postdoctoral fellow in Mike Mathew's lab.

Postdoctoral Fellows

Leaving after completion of their postdoctoral terms were William Addison to the University of Alberta (Canada); Jychian Chen to Yale University to work with Steve Dellaporta; Claude Dery to the University of Sherbrooke (Canada); Bob Gerard to the University of Texas Health Science Center; Giovanni Guiliano to the University of Pennsylvania; Janet Hearing to the State University of New York, Stony Brook; Tohru Kamata to the Frederick Cancer Research Facility of the National Cancer Institute; and Yuriko Kataoka to Harvard Medical School.

Also leaving were Antal Kiss to the Institute of Biochemistry of the Hungarian Academy of Science; Richard Kostriken to the Department of Biochemistry at the University of California, San Francisco; John Langstaff to the University of Winnipeg (Canada); Nora Sarvetnick to Genentech Inc. in San Francisco; Marie Wooten to the Hematology Division of the University of Alabama, Birmingham School of Medicine; and Bradley Zerler to Molecular Therapeutics, Inc. in West Haven, Connecticut.

Graduate student Sreenath Sharma received his Ph.D. from the State University of New York, Stony Brook and began a postdoctoral fellowship in Mike Wigler's lab.

Robertson Research Fund

Income from the Robertson Research Fund, the Laboratory's largest endowment fund, continues to be an essential source of salary support for young scientists. In addition, the high cost of living on Long Island makes it imperative that we subsidize rent of many scientists living in the local community.

In 1986, full stipends were provided for visiting scientist Andre-Patrick Arrigo and for postdoctoral fellows Jychian Chen and Guilio Draetta. Supplemental salary/housing support was provided for an additional ten postdoctoral fellows and six graduate students, while Cold Spring Harbor Fellow Adrian Krainer received funding to start an independent research program on RNA splicing.

Two undergraduate researchers were supported for 10-week stays during the summer. Honoraria and travel expenses were provided for speakers attending several Cold Spring Harbor meetings. Robertson funds also allowed the extensive renovations needed to transform the former "mouse house" into the Cairns Electron Microscopy Laboratory, as well as helping with the construction of the new Arthur and Walter Page Laboratory of Plant Genetics.

Undergraduate Research

Our Undergraduate Research Program, which began in 1959, is one of the oldest in the nation. Four-year core support from the Alfred P. Sloan Foundation, as well as grants from the Burroughs Wellcome Fund, American Cyanamid Company, and Pfizer Central Research allowed us to offer a summer of research to 14 exceptional students. The Olney Memorial Fund supported one additional fellow.

Our Banbury Center Became an Even Greater Intellectual Asset

When Charles Robertson gave to us in 1977 his lovely house and beautiful land in Lloyd Harbor, I told him we intended to establish there a site for intense small meetings which today we call the Banbury Center. I imagined then that it would complement well the increasingly large meetings held at the Laboratory itself. But I never anticipated that it would work so well and bring each year such a distinguished group of visitors. Originally, the main emphasis of these meetings was risk-assessment studies that focused on cancer prevention. Since then, through our Corporate Sponsor Program, we have added a series of six meetings on biotechnology-related topics that include such subjects as microbial energy transduction, recombination in yeast, and angiogenesis. Initially, we had to spend much time seeking funds for our risk-assessment series at a time when grants for research had the highest priority. Now through the support of the James D. McDonnell Foundation of St. Louis, we have the freedom to plan for important meetings that may not have obvious funding sources. Beginning in 1988, we shall initiate a collaboration with the Preventive Medicine Institute (Strang Clinic) of New York that will enable us to hold three additional meetings per year on the origins and detection of human cancer. A grant from them will provide half the program support needed over three years and funds to help cover the salary of a cancer-oriented scientist to administer this program, which we hope will result in an important set of timely books.

The tragic death of Steve Prentis this spring has created a gap that will be difficult to fill. A search is now on for the new Banbury Director.

Lab Awarded Bristol-Myers Cancer Grant

At a ceremony held at the Waldorf-Astoria in April, the Laboratory received from the Bristol-Myers Company one of its two annual cancer research grants, which provides $500,000 in unrestricted support over the next five years. The Laboratory became only the 18th institution to receive the prestigious award since 1977. The Laboratory will also be a future site of a Bristol-Myers Symposium on Cancer Research. A primary use of the funds will be the support of postdoctoral fellows working to understand the functioning of oncogenes.

A Major Changeover in Our Board of Trustees

This year brought to conclusion an extraordinary era of leadership in which Walter H. Page, Robert L. Cummings, and Edward Pulling working together presided over the major period of physical and intellectual growth that has made this Laboratory so important to the world of modern biology. An appreciation for Edward Pulling's contributions appeared in last year's Annual Report, and at the beginning of this year's report, we discuss more fully Walter Page's key contributions. I note here that the bylaws of our Board of Trustees limit our major officers to six one-year terms, and thus both Walter Page and Robert Cummings completed their formal service to the Laboratory in November of 1986.

Trustees of the Laboratory are limited to two consecutive three-year terms. Leaving our Board at the conclusion of two such terms are Claudio Basilico, representing the New York University School of Medicine; Mathew Scharff, representing the Albert Einstein School of Medicine; and Robert Webster, representing Duke University. The representative of Princeton University, Thomas Shenk, was succeeded for the balance of his term by Thomas Silhavy. All of these

Robert L. Cummings

Trustees played important roles in seeing that the Laboratory responded to everchanging opportunities provided by the advances of today's molecular biology.

It is with special appreciation that we note the retirement of Robert L. Cummings of Glen Head, who joined the board in 1977 and served as treasurer for the last seven years. During his tenure as treasurer, he tended well a total budget that swelled from $10 million to $25 million. We look forward to his continued close association as an honorary trustee.

Bayard D. Clarkson of Memorial Sloan-Kettering Cancer Center in New York City was elected chairman of the board at the November annual meeting. "Barney," who joined the board in 1968, holds the Enid Haupt Chair for Therapeutic Research at Sloan-Kettering Institute for Cancer Research, where his research involves cancers of blood cells. He is also chief of the Hematology/Lymphoma Service at Memorial Hospital and professor of medicine at Cornell University Medical College.

A partner in the law firm of Davis, Polk & Wardwell, Taggart Whipple was elected to his first term as vice-chairman. David L. Luke, III, chairman of the board and chief executive officer of Westvaco Corporation, was elected treasurer. Tag joined the board in 1983, and Dave became a member last year.

Returning to the board after a two-year absence was Mary Lindsay. William Everdell of the law firm Debevoise & Plimpton became a new individual trustee.

New institutional trustees are Thomas Maniatis, chairman of the Department of Biochemistry and Molecular Biology, Harvard University; David D. Sabatini, chairman of the Department of Cell Biology, New York University School of Medicine; Thomas J. Silhavy, Warner-Lambert/Parke-Davis Professor of Molecular Biology, Princeton University; and Jonathan R. Warner, chairman of the Department of Cell Biology, Albert Einstein College of Medicine.

LIBA Continues to Provide the Strong Community Support We So Depend Upon

Assuming the role of chairman of LIBA in January of 1986, George Cutting has taken on a task performed so uniquely well by his predecessor, Edward Pulling. Happily, I can report that under George's leadership, LIBA continues to function most effectively both in informing our community about our activities and in rallying its most valued financial support. This year, LIBA has played a key role in helping initiate the planning for the centennial of the Laboratory and in setting up the development efforts that will let us look upon our second hundred years with true confidence. Equally important has been the creation of a new joint program of LIBA and the Laboratory, the Cold Spring Harbor Laboratory Associates. Each Associate will donate to the Laboratory an annual gift of at least $1000. Already in this first year, 85 individuals have joined this most important new program.

Creation of the Centennial Fund

The need for new endowment funds to complement those of our Robertson Research Fund has long been apparent. A very important step for ensuring our future thus was initiated last fall through the creation of the Centennial Endowment Fund, which will be one of the major objectives of the fund-raising efforts that will take place in conjunction with the celebration of the Laboratory's 100th birthday in 1990. Initially, this fund will be established through gifts made by our Trustees, past and present, and it is with great appreciation that I can already acknowledge

the major gifts and further pledges made by Bayard Clarkson, Robert L. Cummings, Wendy Hatch, Mary Lindsay, David Luke III, and Taggart Whipple.

Further Plans for the Upper Campus

Upon the site where the Page Motel and the cabins now sit, we have plans for a new, much-improved, short-term housing complex for meetings participants. This complex will consist of a new 30-room lodge and a set of six cabins to be located just above the new parking facilities that we have created to the west (above) of the Page Motel. Linking this parking area with our major parking area along route 25A is a new paved roadway, the Page Parkway, from which a wonderful view can be had over the Grace Auditorium to St. John's Church. The hilltop is now also graced by an octagon-shaped gazebo, whose copper roof originally adorned an early 1920s pump house built on the Snake Hill Road estate of Mrs. Jeanette Taylor. Plans are also being made to site a new research and teaching building to the immediate north of the Lodge. We now envision approximately 24,000 square feet. Besides a full basement, it will have two floors, the lower floor devoted to neurobiology research and the upper floor to year-round expanded teaching programs in neurobiology and structural studies. Optimally, a third accessory building linked to both the Lodge and the new neurobiology-teaching building would provide facilities for a neurobiology library.

Most of the funds needed to build the cabins are either in hand or have been firmly pledged. So, construction of the cabins will begin early in 1988, hopefully in time to allow their completion for use that summer. Funds for the much more expensive lodge-neurobiology complex are now being sought, and already a special gift obtained from the Samuel Freeman Trust will allow us to undertake detailed plans sometime this fall.

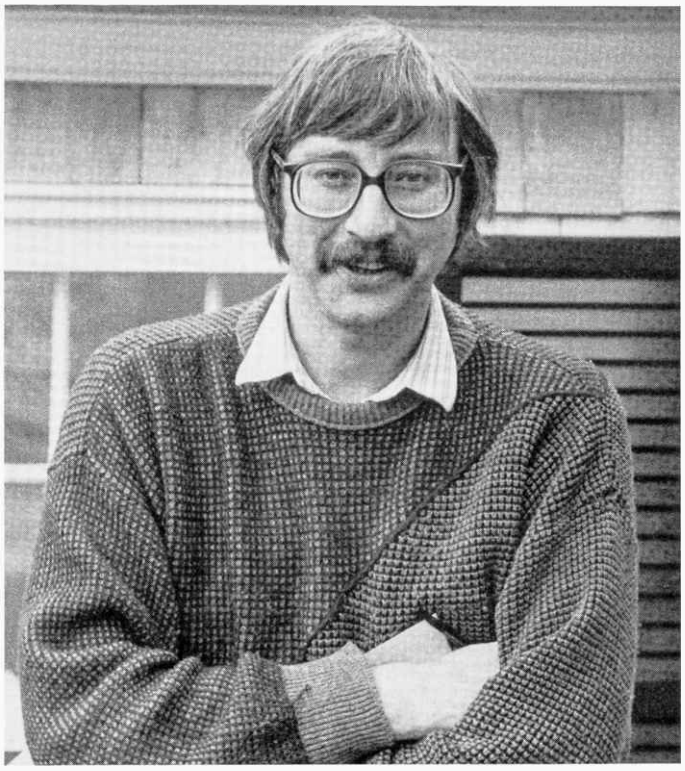
Preparing Ourselves to Function as a Year-round University of DNA

With our centennial only three years distant, it is natural to ask what attributes we shall need to play as important a role in biology worldwide in the 21st century as we have in the 20th century. The answer to this should give us the focus to offer convincing reasons to prospective donors as to why their gifts to our Second Century Campaign will be so important. One attribute most certainly is our ability to house and feed comfortably meetings and course attendees as they begin increasingly to come to us throughout the year as opposed to only during the warmer months. Second, we must have teaching laboratories that will allow our courses to remain at the forefront of the technological development upon which the future of so much of biology will depend. And finally, we must have the endowment funds to guarantee the tenure of the scientists who will maintain the intellectual integrity that major scientists like Barbara McClintock and Alfred Hershey have given to the scientific life at Cold Spring Harbor.

I thus see that in the next few years, the major task for me and my fellow trustees, working in conjunction with supporters in the community and with our by now thousands of concerned alumni, will be to raise funds for the future investments at Cold Spring Harbor that will enable us to match the achievements of the past. We shall not have an easy few years ahead, for we shall have so much to do. But the rewards of success will not let us falter.

June 22, 1987

James D. Watson



Stephen James Prentis
1951–1987

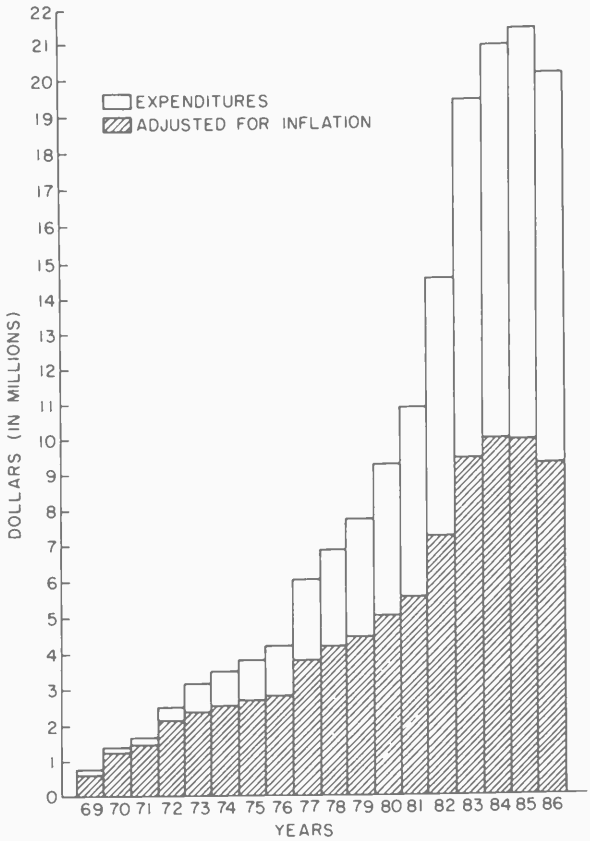
The Laboratory staff gathered at a memorial service in Grace Auditorium on March 6 to mourn the untimely death of Steve Prentis in a car accident on February 28, 1987. During his brief time here, his blithe spirit and enthusiasm had infected all with whom he came in contact.

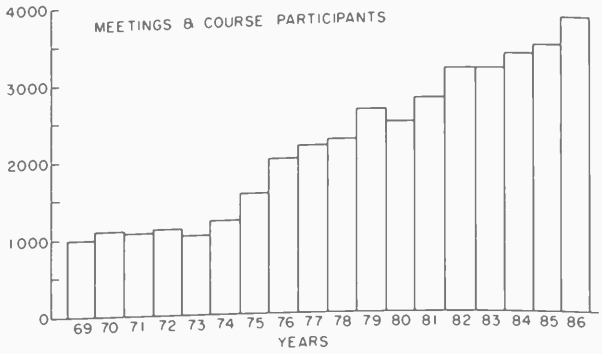
Steve had shouldered dual responsibilities since his arrival in April 1986. As Director of Banbury Center, he administered an extensive meeting program. As Executive Director of Publications, he had labored to make real a long-standing dream for a Cold Spring Harbor journal. Along with colleagues at the Genetical Society of Great Britain, he persuaded prominent scientists in the United States and Europe to join the editorial board and submit papers to the new publication, *Genes & Development*.

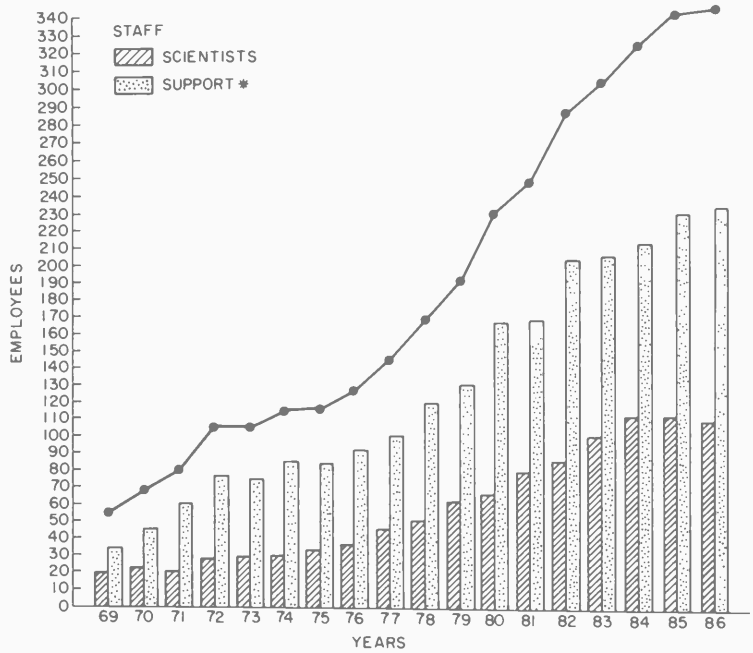
Steve built his reputation as a brilliant scientific editor at Elsevier Publications in Cambridge, England, where he was Managing Editor of three popular scientific journals: *Trends in Biochemical Sciences*, *Trends in Biotechnology*, and *Trends in Genetics*. He was the author of numerous scientific articles, and his widely praised book *Biotechnology: A New Industrial Revolution* has been translated into several languages.

Steve's death came only several days before copies of the first issue of *Genes & Development* arrived, bearing a preface that, in part, read: "Taking a decision to launch a scientific journal is never simple, not least because its real value can only be properly measured after it comes into existence." Tragically, the real value of Steve's contribution to science can now only be measured in his absence.

DEPARTMENTAL REPORTS







*Consists of Technical Support, Core Services, Publications, Library, Buildings and Grounds, Information Services, Administrative Personnel, Banbury Center

ADMINISTRATION

Last year, there was serious concern that deep cuts in federal funding for research would aggravate the deficit projected for the Laboratory's operating budget. However, such cuts only partially materialized, and with strong support from industry, foundations, and private individuals, we were able to approximately balance revenues and expenditures after allowing for depreciation. The better-than-expected financial results were also due to close attention to controlling administrative costs.

In 1987, we will be faced with replacing the substantial revenues received in recent years from the just-completed five-year research support agreement with Exxon Corporation. At the same time, we will need to find start-up money for the extraordinary group of young scientists recruited during the past year. Funding for the plant research program must also be completed, particularly including construction of the new Arthur and Walter Page Laboratory of Plant Genetics. Finally, we will need money to build and operate the expensive new facilities that are required for the Laboratory's dream of becoming a *year-round* teaching institution of DNA science and a center of neuroscience research. Nongovernment funding sources will be essential to this effort. In this regard, we must keep in mind the basic academic character of science at Cold Spring Harbor Laboratory and the importance of freedom to do interesting research without regard for commercial application.

Growth of the Laboratory will aggravate the already expensive and intrusive problems of maintenance, security, sewage treatment, and waste disposal. These problems must be resolved without altering the essential character of the Laboratory and the "village of science" atmosphere that has made this place unique. We must be especially sensitive to the feelings of our neighbors in the villages of Laurel Hollow and Lloyd Harbor. Their feelings of pride in having a world-class institution in their backyard must be coupled with confidence that the Laboratory will not be a financial burden and that we share their concern for the natural beauty of the area.

Crucial to achieving the Laboratory's future goals are the managers who head our various nonscientific departments: commercial relations, publications, public affairs, library, buildings and grounds, personnel, grants management, accounting, and purchasing. Although the contributions of these exceptionally qualified and motivated managers are great, too often they receive most notice when we lose one of them. The tragic death of Steve Prentis in an automobile accident stunned and saddened us all. Art Brings, Director of Safety, and Mike Balamuth, Director of Data Processing, departed to establish their own consulting firms. Art has made the Laboratory a pacesetter in the areas of biohazard and radiation safety. We are delighted that he will be returning to the Laboratory after about a six-month absence. Mike was invaluable in making all areas of the Laboratory computer-literate and was the architect of our shortly to arrive state-of-the-art system of networked PC-based microcomputers. His replacement, Fred Stellabotte, has had extensive experience with such systems at TWA and Brookhaven National Laboratory.

The fall of 1986 was marked by the retirement of Walter H. Page as Chairman of the Board and Robert L. Cummings as Treasurer. Much is written elsewhere in this report about their contributions to this institution. They are good friends to all of us who have relied on their leadership and good sense.

The charts preceding this report portray the upward path of operations. The challenge will be to manage future growth in a way consistent with the excellence that has been the hallmark of Cold Spring Harbor Laboratory.

G. Morgan Browne

BUILDINGS AND GROUNDS

As predicted in last year's Annual Report, 1986 brought with it much work for the Buildings and Grounds Department. The Laboratory's agreement to conduct research with Pioneer Hi-Bred International prompted a major renovation and construction of several buildings at CSHL and its off-grounds properties. This year, the Genetics Field Experimental Station at Uplands Farm was essentially completed with the construction of a potting shed and a 2200-square-foot greenhouse. The height of this greenhouse will accommodate a winter corn crop so that researchers will be able to grow corn all year long. Pioneer funding also made possible the addition to Delbrück Laboratory. We completed a new foundation for the Firehouse, which was relocated 90 feet north to make room for the Delbrück addition. As of this writing, the Delbrück addition is up and the roofing is being installed.

The existing structure of the Mouse House was completely rebuilt and two rooms were added. Now named the John Cairns Laboratory, the building will house an electron microscopy facility. In Hershey, we are converting the apartment into offices and the repair shop area into a computer graphics analysis facility to accommodate the newly arrived crystallographers. We also began work on their laboratory as well as on Mike Wigler's laboratory at Demerec. The kitchen area was reworked and a coldroom was installed.

Early in the year, we rebuilt Dave Helfman's laboratory in McClintock. The laboratory had been destroyed by a serious fire caused by a short circuit in a gel box. Although smoke leaking through the vents caused some damage to the kitchen and hall, the rest of the building was spared because the laboratory door had been closed.

Along with new construction and renovations, equally important is the upkeep of all our other buildings. This year, the exterior of Olney and the trim on Demerec Lab were painted, and over at Banbury, the trim on Robertson house was repainted.

1986 saw the completion of many of our ongoing projects. The work on James Lab was essentially finished with the completion of the lower floor. Furnishings for the offices and computer rooms in Grace Auditorium were installed and personnel were moved in before the summer meetings and courses began. The landscaping around Grace, the building of the Gazebo, and the grading and surfacing of the Motel parking lot were all completed. A copper roof for the gazebo was donated to CSHL by the Taylor Estate in Lloyd Harbor. It was removed from the pump house and stored behind Page Motel until our carpenters completed the structure and foundation of the gazebo. After the landscaping of the hill behind Grace was completed, the roof was hoisted into position and the structure was painted.

The Gazebo

In the spring, our Electrical Department began working on the site lighting and the never-ending job of burying all electrical, TV, and telephone lines so that we could remove all the utility poles along Bungtown Road. By November, half of the poles were removed, and hopefully, the rest will be gone by late spring of 1987.

In looking back at our accomplishments for the year, I find that we completed more work. Our own Buildings and Grounds building was reorganized to make room for the Safety office, the B&G Purchasing Department, and Peter Stahl's office. (Pete was promoted to Maintenance Manager from his position at Banbury.) In our free time, we shoveled and plowed snow in the winter, planted bulbs and flowers in the spring, cut grass in the summer, raked leaves in the fall, and so on. All in all, we had a very productive year.

Jack Richards

PUBLIC AFFAIRS AND DEVELOPMENT

Public Affairs

It has been very satisfying over the past several years to see the maturation of the Laboratory's public affairs function. Our organization has clearly reached a stage in its evolution where it is large enough—and successful enough—to share its unique expertise with the many nonscientific audiences with need for accurate, up-to-the-minute information about DNA science. The hallmark of this transition has been a heightened sense of social responsibility and an organizational commitment to public education that extends from the Board of Trustees to the scientific staff. Just as innovation has been crucial to our scientific progress, so, too, have we been imaginative in our response to the problem of public understanding of DNA science.

The DNA Literacy Program, explained in greater detail in the education section of this report, has become the nation's most ambitious program to prepare individuals to understand recombinant DNA and the social issues it generates. Last summer, we introduced the *Vector* Mobile DNA Laboratory, which brought hands-on training in DNA science to 210 high school and college instructors from 12 states and Great Britain.

The Genetic Knowledge of Man, a briefing workshop cosponsored with Shearson Lehman Brothers Inc., brought together scientific and business leaders to discuss the extraordinary applications of DNA research to health care in the United States. Attending the October conference were scientists from leading research institutions and corporate executives from health care and investment companies: Tom Maniatis, Harvard; David Botstein, Massachusetts Institute of Technology; Charles Cantor, Columbia; Harold Varmus, University of California, San Francisco; Leroy Hood, California Institute of Technology; Michael Brown, University of Texas; Thomas Caskey, Baylor College of Medicine; Allan Wilson, University of California, Berkeley; Curt Engelhorn, Boehringer Mannheim; Henry Wendt, SmithKline Beckman; Erik Danielsson, Pharmacia; Leo Jack Thomas, Eastman Kodak; Abramo Virgilio, Bristol-Myers; Sigi Ziering, Diagnostic Products; Richard Williams, Erbamont; John Wilkerson, The Wilkerson Group; Kenneth Merrill, Tambrands; and Harvey Sadow, Boehringer-Ingelheim.

The Laboratory entered the video era with the release in January of the documentary *The Biological Revolution: 100 Years of Science at Cold Spring Harbor.* This 30-minute video has done much to extend the reach of our public affairs program; it is now possible (and cost-effective) to send the excitement and beauty of the Laboratory to any part of the country. The documentary aired in the spring on Cablevision systems throughout the Metropolitan New York area and has sold briskly to high school science teachers across the nation. We were especially pleased that the production was awarded the first prize for video documentary in the 1986 competition of the Suffolk County Motion Picture and Television Commission.

Development

In anticipation of the Laboratory's centennial, major steps were taken in formulating an articulated development program to ensure a second century of scientific excellence. A comprehensive plan, setting forth institutional goals and development objectives, was approved by the Board of Trustees. John F. Harper & Company conducted a survey of the directors of both the Laboratory and the Long Island Biological Association (LIBA) and provided many important insights about how our organization is perceived and considered within the Long Island community.

As our program unfolds, we will want to draw participation from an ever-widening circle of friends and patrons. The key to success will be a number of individuals who believe enough in our work to push the Laboratory to the very top of their priority lists. There is so much to be done over the next three years.

Central to our overall development effort is an increased emphasis on annual giving. We now have in place three programs aimed at building constituencies that support the Laboratory with predictable, annual gifts (detailed in the grants and contributions section).

Established in 1984, the Corporate Sponsor Program has encouraged the biotechnology industry to support our summer meetings program. Proceeds from the program have encouraged increased interaction between industry and academic scientists and have allowed a 40% growth in our meetings program.

In 1986, the Alumni Cabin Campaign became our first attempt to reach meeting attendees, course instructors, and departed staff members whose careers have benefited from the Cold Spring Harbor experience. Response to an appeal to support construction of "Alumni Cabin" (one of six four-bedroom cabins planned to replace existing, dilapidated housing) exceeded all expectations. By the end of 1986, nearly 200 scientists (one in four asked) have contributed almost $34,000. We hope that after reaching the goal of $100,000 by 1988, a good habit will have been created, and we will continue to receive annual gifts from our alumni.

In cooperation with LIBA, we have instituted for calendar year 1987 a third annual giving program that is aimed at our constituency of local friends. Cold Spring Harbor Laboratory Associates, who contribute $1,000 or more annually, receive a special education program designed to bring them face-to-face with the world of biotechnology. In the first year, proceeds from the program will be used to support four postdoctoral researchers to be called LIBA Fellows.

Staff Expansion

Joining the staff after serving extensive internship periods were Photographer/Artist Susan Zehl and DNA Workshop Coordinator Christine Bartels. A recent graduate

of The Cooper Union in Manhattan, Susan is in charge of nontechnical photography for the Annual Report and does design and graphics for public affairs publications. A native of West Germany, Christine administers the expanding *Vector* DNA Workshop Program and other educational efforts. With graduate degrees in both biochemistry and science journalism, she also assumes writing chores. Jericho High School junior Jeff Mondschein performed admirably as summer lab aide with the *Vector* program. Holding everything together is Departmental Assistant Ellen Skaggs, who has special responsibility for liaison with members of the Corporate Sponsor Program. Joan Pesek, whose husband Dan Marshak is the Laboratory's protein chemist, joined the administrative staff in Nichols as development liaison for LIBA.

David Micklos

LIBRARY SERVICES

Library Responds to Changing Needs of the Laboratory's Science

In 1986, a variety of changes in our scientific direction challenged the library to examine its collection and to provide new materials. The arrival of the crystallographers required the purchase of three major serial titles and their backsets. We also saw an increased interest in both plant and yeast molecular biology and neurobiology as the laboratory prepared to expand its work in those disciplines. Our protein chemistry section was also expanded.

On December 1, we began a three-month experiment, sending each day to our senior scientists photocopies of the tables of contents of 44 journals selected by them. Each scientist created his or her own profile using the ten most frequently cited journals and five titles specific to the individual specialty. The project will be evaluated in March 1987; its success to date indicates that it will most likely continue.

Reference Services and Archives

Our reference department has expanded its online access to include databases available from the National Library of Medicine. The following have been added: Registry of Toxic Effects of Chemical Substances, and Cancerline, which provides access to cancer research projects and clinical trials/protocols. The addition of these databases and the increase in new area research resulted in a 37% increase in online searches this year.

To accommodate this increased demand for services without increasing our professional staff, we reorganized the reference complex on the second floor. The terminal used for searching has been moved to the gallery at the top of the stairs, which provides an atmosphere conducive to the reference interview required to establish an accurate search.

During the summer, we received the remaining contents of the Jane Davenport Harris DeTomasi estate. Jane Harris was the daughter of Charles B. Davenport, the

Laboratory's Director from 1898 to 1934. She was also the wife of Reginald D. Harris, the Laboratory Director responsible for establishing the Cold Spring Harbor Symposium on Quantitative Biology. An exciting collection, this truckload of material included Laboratory, Harris, and Davenport memorabilia that will eventually, when sorted and cataloged, greatly enrich our knowledge of the history of the Laboratory.

Annual Growth of Permanent Collection

In 1986, the book collection grew by a net total of 1403 bound volumes, bringing the total number of volumes to 30,501. The serial collection numbers 503 unique titles, of which 358 are periodicals and 145 are cataloged series. This year 15 titles were added and 6 were withdrawn.

This year, we have begun the tedious, yet important, process of weeding out our scientific book collection. This involves determining where new editions must replace old ones and buying new titles to enhance subject areas that are at the cutting edge of scientific research.

Storage Facility Access Easier Than Expected

It has been more than a year since a library storage facility was established at the East Side School in Cold Spring Harbor to house the older materials from our overflowing journal collection. There was some concern that service to our scientists for those volumes would be impeded; however, the retrieval system that we have designed is working smoothly for both the library staff and the scientists. In 1986, 287 volumes were retrieved and returned to storage. It is clear that the items sent to storage in 1985 were chosen wisely, since the 287 volumes represent only 3.7% of the collection housed at the school. Library staff members stop at the school to and from work or following their lunch hour. Although our published turnaround time is 24 hours, we will always retrieve a volume immediately in an emergency.

A storage facility can only provide for little-used materials. The main library is at capacity; realistically, more space is needed. Plans exist for renovation of the second floor and the attic, which would ensure comfortable library service for 5 years. We hope to revitalize these plans late in 1987.

Susan Cooper

PUBLICATIONS

Several innovations and developments were introduced into our publications program in 1986. These have been designed to extend the range of scientific information we provide and to increase the efficiency with which this can be achieved.

Integration of Banbury Publications into the Main Publishing Program

From an organizational point of view, one of the most important developments has been the incorporation of books emanating from the Banbury Center into CSHL's main publications program. Although the editorial tasks related to the *Current Communications in Molecular Biology* series (based on meetings held at the Banbury Center) have always been handled by Nancy Ford and her staff at Urey Cottage (on the grounds of CSHL), similar editorial and production tasks related to books in the Banbury Report series had been undertaken by an independent editor at Banbury Center. In an attempt to increase the speed and efficiency with which the Banbury Reports are published, they will now be handled within the framework of our main publications operation. There will still be a resident editor at Banbury Center, but it is hoped that this organizational change will eliminate some duplication of effort. In this context, it is noteworthy that, as mentioned in the Banbury Director's report, a new series of books on the *Origins and Detection of Human Cancer* will be launched as a result of the agreement reached last year between the Banbury Center and the Preventive Medicine Institute/Strang Clinic.

New Technology Aids Production Efficiency

Nancy Ford and her colleagues have had yet another very active year, during which we produced 13 titles, 10 reprints, abstracts for all of our meetings, plus, of course, this Annual Report. As noted in previous reports, we have been "keyboarding" manuscripts on site for some time now. This method of reducing typesetting costs, while maintaining and even improving quality, has worked very successfully. In 1986, virtually all publications were handled in this manner, enabling us to take over some of the traditional tasks of the typesetter. A further advance has been the installation of equipment that can read floppy disks provided by authors. This is already reducing costs and, equally important, reducing errors and increasing speed. We expect that the percentage of articles we receive on floppy disks will increase rapidly in the next few years.

Three New Themes in Editorial Development: A Journal, Videos, and a High School Manual

Three novel aspects of our publishing program have begun to emerge over the past year: scientific journals, video tapes, and materials for high school students.

CSHL's first journal will be entitled *Genes & Development*, and we aim to establish it as one of the first-rank journals for molecular biologists. It will contain research articles that elucidate the manner in which genes control every stage of development, from an egg to an adult organism. *Genes & Development* is being published in association with the Genetical Society of Great Britain, and the response from the scientific community in terms of papers submitted to the journal has already been immensely favorable. The first issue will appear in March 1987. After more than fifty years of successful book publishing, this venture into journal publishing marks a significant step in our publication efforts. We are currently examining a small number of other possible journal projects for the future.

The Laboratory's video *The Biological Revolution: 100 Years of Science at Cold Spring Harbor* was prepared in 1985 in anticipation of the Centennial in 1990. It traces the major developments in biology by focusing on the many significant events and achievements that took place at CSHL. Since this video tape would clearly be of great interest to teachers and high school students, it was

decided to make it widely available to them, and it is now generating a great deal of attention.

In 1986, we concluded an agreement with Carolina Biological Supply Co. to produce and sell a high school/junior college laboratory manual entitled *Recombinant DNA for Beginners*. This company is the major supplier of biological materials and equipment to schools, and they will be assembling the laboratory kit that will enable students to carry out the experiments described in the manual.

It is quite clear that video tapes are becoming increasingly important in teaching at all levels, from school students to advanced researchers. Our very successful series of laboratory manuals, which was extended this year with the publication of *Manipulating the Mouse Embryo*, might very usefully be augmented by instructional videos that would demonstrate some of the techniques involved. We are currently investigating the possibility of producing one or more such video tapes.

Symposia and Other Well-established Book Series

The reception given throughout last year to Symposium 50 was even better than we have come to expect for all the Symposium volumes. It is interesting to note that its topic, *Molecular Biology of Development*, matches that of the new *Genes & Development* journal rather closely. Volume 51 is based on the very timely 1986 Symposium concerning the *Molecular Biology of* Homo sapiens. This area of research has seen countless fundamental advances in the past few years, and, with the current discussions concerning the feasibility and desirability of sequencing the entire human genome, this volume is sure to be greatly valued for many years. Other publications in our regular series included the fourth volume of *Cancer Cells*: *DNA Tumor Viruses*; *Vaccines 86*; and five books in the *Current Communications in Molecular Biology* series: *Mechanisms in Yeast Recombination*, *Microbial Energy Transduction*, *Translational Control*, *DNA Probes*, and *Computer Graphics and Molecular Modeling*. This last title is particularly interesting in view of the Laboratory's newly established research activities in this area.

Laboratory Manuals

As mentioned previously, our series of laboratory manuals for researchers has been very successful. *Manipulating the Mouse Embryo*, added to the list in 1986, presents a lucid and detailed description of studying mammalian development and producing transgenic mice, a technique that is being used to great effect by researchers at CSHL and is yielding very important information.

Several new manuals are currently being developed for publication, plus, of course, the second edition of *Molecular Cloning*: *A Laboratory Manual* by Tom Maniatis, Ed Fritsch, and Joe Sambrook. The first edition of this manual has now sold over 50,000 copies.

Marketing Department Extends Its Services

Without an effective method of letting potential readers know about our publications, our editorial and production efforts would be largely in vain. 1986 brought several innovations in the marketing department, headed by Susan Cooper, which have built upon several developments in recent years.

CSHL Notebook, our thrice yearly newsletter, is distributed to 45,000 people. It has proved to be very popular, allowing us to provide advance information on new

publications, meetings, and courses. In addition, the *Notebook* has carried various questionnaires that have helped us define the needs of scientists for publications and other services.

In 1986, we also used, for the first time, a professionally designed booth on which our publications can be displayed at scientific meetings. Six such meetings were attended last year, and our publications were displayed at eight additional meetings. We made our first appearance at the Frankfurt Book Fair, which provided useful information on overseas distribution and the views of the many booksellers who carry our titles. The marketing department has, of course, been actively engaged in publicizing our new titles, especially *Genes & Development*, and has now begun to become involved in selling advertising space to manufacturers who wish to advertise in the journal. All of these activities have been in addition to the normal and vital tasks of publishing our catalogs, designing advertisements, and maintaining one of the best and most-up-to-date mailing lists available.

Order Fulfillment and the Warehouse Complete Another Year of Smooth Operations

No news is usually good news when it comes to fulfilling orders for books and dispatching them. The very low level of inquiries about missing orders or late deliveries has reassured us that the Fulfillment and Warehouse Department, led by Charlaine Apsel, continues to provide a very rapid turnaround of orders. The advent of our new journal has required the implementation of new systems for handling monthly subscriptions, in addition to our regular one of book sales.

Two Valued Employees Leave the Publications Department in December

In December, Doug Owen left CSHL after seven years as an editor. His work during this period brought him into contact with literally hundreds of scientists from the Laboratory and elsewhere, all of whom appreciated his cheerful and professional approach. All of his colleagues in the Publications Department wish him well in his new career with Genetics Institute in Boston.

In the fall, Barbara Skelly left after working in our warehouse for three years. She was present during a time of many changes in this essential part of our operations, and her quiet efficiency was greatly appreciated.

Steve Prentis

* * *

Mere hours after completing the above report, Steve tragically lost his life in a car accident. His zest for the new and innovative, so clearly reflected in the enthusiasm with which he approached the expansion of the publications program into new areas, is a legacy we will cherish and work to fulfill in his memory.


RESEARCH

TUMOR VIRUSES

The laboratories comprising this group employ the DNA tumor viruses, adenovirus and SV40, to probe fundamental cellular mechanisms of gene regulation and growth control at the molecular level. Drawn together by this research theme as well as by common research facilities, the group also shares a joint source of funding in the form of a large Program Project grant.

1986 was something of a vintage year for the Tumor Virus group. As recorded in the pages that follow, it was a good year at the lab bench. Moreover, on the strength of past achievements and of future promise, our application to the National Cancer Institute for continued support was well received. With renewal for a fourth 5-year term, this Program Project grant remains the longest-running as well as the largest at the Laboratory. As recipients of this grant, we count ourselves doubly fortunate: Under its aegis have flourished both individual research projects and collaborative ventures among the constituent groups, and within the framework of the grant, we have been able to bring in new blood and new technologies. This year, in April, we recruited Dan Marshak as head of the Protein Chemistry Laboratory, replacing Clive Slaughter who moved to Dallas at the end of 1985. Dan's expertise in protein analysis and peptide synthesis has already played a part in more than one project and will doubtless contribute to many others, while he simultaneously develops his own interests in both tumor and neurobiology.

The complement of the Tumor Virus group now stands at eight laboratories, divided equally between the James/Sambrook complex and the Demerec building. Reflecting the independent status of the four James/Sambrook research groups, their reports appear separately for the first time under the headings of Adenovirus Genetics, Molecular Biology of SV40, Protein Immunochemistry, and Transcription Control, instead of collectively as the Molecular Biology of Tumor Viruses. These reports are accompanied by those of the Demerec laboratories: DNA Synthesis, Nucleic Acid Chemistry, Protein Chemistry, and Protein Synthesis.

ADENOVIRUS GENETICS

T. Grodzicker R. Cone, X.-C. Fu, E. Lamas, M.P. Quinlan, D. Chao, R. Chisum, M. Goodwin, P. Hinton, M. Jaramillo

Work in this section has concentrated on the properties of the adenovirus oncogene early region 1A (E1A). This gene possesses diverse functions in transcription and transformation, expressed through a multiplicity of polypeptide products. The complexity results from alternative splicing, which yields different primary translation products, and from extensive posttranslational modifications. Our analysis involves studies using an array of E1A gene mutants and expression vectors, with the aim of understanding the functions of the several gene products and of increasing the range of primary cell types available for investigation.

Analysis of Epithelial Cell Proliferation and Immortalization by the E1A 12S Gene Product

M.P. Quinlan, D. Chao, P. Hinton, T. Grodzicker

We have been studying the ability of the 12S gene product of the E1A region of adenovirus to immor-

talize primary epithelial cells, using an adenovirus 5 (Ad5) 12S virus in which the E1A region is replaced by the cDNA of the 12S mRNA. The 12S protein induces quiescent primary baby rat kidney (BRK) epithelial cells to synthesize cellular DNA and to proliferate in the presence and absence of serum, ultimately resulting in their immortalization, but not transformation. Furthermore, cell lines established from primary BRK cells infected with Ad5 12S retain many of their original epithelial cell characteristics, such as morphology, poor plating efficiency, and the expression of cytokeratins.

Infection of primary BRK cells with the Ad5 12S virus results in the production of a growth factor(s) that can induce uninfected primary epithelial cells to proliferate. 12S immortalized epithelial cells do not constitutively produce detectable levels of growth factor. However, superinfection of these cells with the 12S virus results in the transient production of growth factor(s). This growth factor is associated with high-molecular-weight complexes from which it can be released by high-salt treatment. The growth factor(s) is stable for about 6 weeks at 4°C. Unlike EGF, TGF, or PDGF, it is sensitive to acid and heat. In collaboration with D. Marshak (Protein Chemistry Section), we have determined that the growth factor binds to DEAE-cellulose and that its activity is potentiated by heparin. Preliminary observations, in collaboration with E. Lamas and R. Cone (this section), suggest that the growth factor induced by 12S also affects liver epithelial cells and hepatocytes.

A genetic analysis was undertaken to identify regions of the 12S gene product that are necessary for induction of cell proliferation and growth-factor production. A series of deletion mutants that produce amino-terminal nonsense fragments ranging in size from 106 to 150 amino acids was produced by BAL-31 digestion (P. Whyte and E. Harlow [Protein Immunochemistry Section] and E. Ruley [MIT]). Thus, none of these mutants produce proteins containing amino acids encoded by the carboxy-terminal exon of the 12S gene. Another class of mutants consisted of amino-terminal fragments that were in-frame upon ligation to the *Xba*I site in the second exon, and thus encode the carboxy-terminal 68 amino acids. These mutants are internal deletion mutants. The mutant DNAs were reconstructed into virus and used to infect BRK cells.

Immunoprecipitates from infected BRK cells demonstrate that polypeptides of the expected size are synthesized. None of the mutants exhibit the *trans*-activation function of E1A. Thus, they fail to express the E2 72K protein and are host range with respect to their ability to grow on HeLa and 293 cells. All of the mutants lacking the carboxy-terminal amino acids were localized to both cytoplasm and the nucleus, whereas wild-type 12S and the mutants containing the carboxy-terminal 68 amino acids were localized to the nucleus. Mutants producing a polypeptide encoded by at least the first 402 nucleotides of the E1A region (134 amino acids) are able to initiate cellular DNA synthesis and proliferation, but they are unable to maintain the epithelial cells in a proliferative state and thus are not immortalized. No growth factor was detectable from cells infected with these mutants. They are, however, able to cooperate with Ha-*ras* in transformation (P. Whyte, Protein Immunochemistry Section). The presence of the carboxy-terminal 68 amino acids from the second exon confers upon the first exon of the 12S gene the ability to maintain epithelial cell DNA synthesis and proliferation, to produce the growth factor, and to immortalize epithelial cells. We are presently mapping and analyzing more precisely the carboxy-terminal domain required for epithelial cell survival and immortalization.

Construction of Retrovirus Vectors for the Establishment of Primary Cells

R. Cone, T. Grodzicker, M. Jaramillo

Retroviruses are extremely useful tools for the efficient introduction of genes into animals and primary cells in culture. Over the past year, we have constructed or obtained from other laboratories recombinant retroviruses expressing many of the known nuclear oncogenes, including the adenovirus E1A 12S and 13S proteins, SV40 T antigen, polyoma middle T antigen, polyoma large T antigen, murine c-*myc*, murine v-*fos*, and murine p53. We are currently in the process of constructing viruses that express adenovirus E1B proteins and E1A plus E1B. Our interest in these viruses is twofold. First, we would like to develop methods for the routine establishment of certain differentiated cell types via infection of the primary cells with recombinant oncogene-containing retroviruses. Second, we would like to learn more about the mechanism(s) by which these diverse gene products induce cell proliferation in quiescent primary cells.

Our initial work has focused on viruses that we constructed which express either the 12S or 13S adenovirus E1A proteins. These two viruses express equivalent levels of 12S and 13S E1A proteins in infected NIH-3T3 cells, comparable to protein levels seen in 293 cells. The 12S virus is capable of immortalizing primary cells from nearly every rat organ tested, including kidney, heart, liver, pancreas, thyroid, and brain. Most of these cells have an epithelioid morphology, and many are true epithelial cells, as judged by positive staining with anticytokeratin antibodies. Surprisingly, the 13S protein had much less immortalization potential than the 12S, even though immunofluorescence studies showed that the protein was stably expressed in the nuclei of primary cells. This observation provides support for the model of Spindler et al. (*J. Virol. 53:* 742 [1985]) that the 12S protein is required for the full activation of the DNA replication machinery of quiescent epithelial cells.

We have also investigated the growth properties of these 12S E1A immortalized cells. Epithelial cells obtained from 12S retrovirus infection of heart and liver were maintained in culture for 10 months without any sign of crisis. At 3 months, these cells were unable to grow in soft agar or cause tumors in syngeneic rats, although the addition of an activated *ras* gene caused rapid focus formation and complete transformation of the cells, as judged by their ability to form tumors with a latency of 6–12 weeks.

To determine if E1A immortalized cells can retain differentiated functions, we have turned to thyroid tissue as a model. Preliminary results (discussed in detail below) suggest that both rat and human thyroid cells infected with the 12S retrovirus remain highly differentiated, as assayed by their ability to respond to thyroid-stimulating hormone, and to express thyroglobulin. Because of the many bioassays for thyroid function, thyroid cells should provide an excellent system for determining the efficacy of different oncogenes for immortalization with retention of the differentiated phenotype.

Characterization of Differentiated Human Thyroid Cell Clones

R. Cone [in collaboration with T.F. Davies, Mount Sinai Hospital]

The establishment of differentiated cell lines from normal, primary cultures is a difficult procedure, particularly with human tissues. For example, there are very few human cell lines that retain endocrine function. Although primary human thyroid cells can be cultured for several weeks, no proliferation of these cells can be obtained and no functional cell lines have been isolated. For long-term studies of human thyroid function, and for studies of thyroid cells as immune targets in thyroid autoimmune disease, it is crucial to develop human thyroid cell lines. We have attempted to do this using the 12S E1A retrovirus vector described above, transferred into the ψAM cell line for production of virus capable of infecting human cells, as well as using an adenovirus vector in which the E1 region was replaced by SV40 T antigen (Van Doren and Gluzman, *Mol. Cell. Biol. 4:* 1653 [1984]).

A histologically confirmed, 22-week-old, human fetal thyroid was minced, digested with a dispase/collagenase solution, and plated as a monolayer in medium 199 with 10% FCS. After 2 days, the cells were infected with the T-antigen adenovirus or the 12S E1A retrovirus. The T-antigen–adenovirus-infected cells were split at low density, and rapidly growing epithelial colonies were isolated and expanded. The E1A-retrovirus-infected cells, which initially grew slowly, were split at low density and cloned on the basis of a G418 resistance marker encoded by the retrovirus. Mock-infected cells were unable to proliferate under these conditions.

After 3 months of growth in culture, approximately 30 clones continued to proliferate. One T-antigen clone (TAD2) and three E1A clones (12S2, 12S3, and 12S4) were selected for further analysis. One sensitive assay of thyroid cell function is the ability to respond to thyroid-stimulating hormone (TSH). Thyroid cells respond to this pituitary hormone with an activation of adenyl cyclase and an increase in intracellular cAMP. This second messenger greatly increases the metabolic activity of these cells, inducing thyroglobulin expression and enhancing thyroid hormone production. 12S2, 12S3, and 12S4 were sensitive to less than 10 μU/ml of bovine TSH (bTSH), whereas TAD2 required greater than 100 μU/ml bTSH when assayed for cAMP accumulation. Maximal cAMP concentration attained was 1200–1400 and 500 fmoles per 10^5 cells for the E1A and T-antigen clones, respectively, representing a four- to sevenfold increase in intracellular cAMP concentration. Since the basal TSH level in the blood is around 10 μU/ml, these clones respond to TSH at biologically relevant levels. Immediately following G418 selection, 12S E1A

clones were found to be positive for thyroglobulin expression by immunofluorescence using a monoclonal antibody, A3. We are currently quantitating levels of thyroglobulin secretion in these clones by radioimmunoassay.

Although it is too early to tell if our cell clones will become permanent cell lines, these studies indicate the potential of viral vectors for the establishment of proliferating clones of differentiated human endocrine cells. Future studies will concentrate on the response of these clones to known regulators of thyroid function in vivo, notably TSH, thyroid hormone, and iodide, with the eventual goal being the study of the regulation of thyroid-specific gene expression in a cell-culture system.

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DNA SYNTHESIS

B. Stillman E. White, J. Diffley, M. Fairman, W. Heiger, G. Prelich, S. Smith, A. Denton, B. Faha, P. Newman, S. Penzi

The DNA Synthesis laboratory continues to investigate two aspects of eukaryotic molecular biology, namely, (1) the mechanism and control of DNA replication in eukaryotic cells and (2) the functions of the adenovirus early region 1B (E1B)-encoded tumor antigens. A significant shift in focus on the first subject has occurred over the past year by increasing our effort on SV40 DNA replication, which has already yielded valuable insight into cellular replication proteins and their regulation. At the same time, studies on adenovirus DNA replication, replication of cellular chromosomes in yeast, and adenovirus E1B tumor antigens continue with substantial progress.

Highlights of the past year include the purification and characterization of a cellular protein that is required for SV40 DNA replication. This replication protein is a cell-cycle-regulated protein called proliferating cell nuclear antigen (PCNA) or cyclin, and we have demonstrated that it is a processivity factor for a novel DNA polymerase called polymerase δ. This finding suggests that multiple DNA polymerases are involved in eukaryotic DNA replication; previously, it was thought that a single polymerase (α) was involved. Furthermore, since PCNA is cell-cycle-regulated, it may play a regulatory role in DNA replication.

Another interesting observation came from genetic studies on the function of the adenovirus-encoded E1B 19,000-molecular-weight (19K) tumor antigen, which has been studied in this laboratory for a number of years. We have clearly demonstrated that the 19K tumor antigen acts as a negative regulator of adenovirus gene expression during the productive infection of human cells. This phenomenon has been observed in a number of hu-

man cell lines, but the magnitude of regulation varies between different cells, suggesting the involvement of cellular proteins in this process. The negative regulation of gene expression by the E1B 19K protein is mediated by its effect on the adenovirus E1A proteins, which normally function as positive regulators of early viral gene expression. Thus, it appears that we have uncovered a complex regulatory pathway that controls adenovirus gene expression, although much effort is needed to completely understand how this regulation works.

SV40 DNA Replication

M. Fairman, W. Heiger, G. Prelich, B. Faha, B. Stillman

The small DNA tumor virus, SV40, has long been studied at Cold Spring Harbor Laboratory, providing a valuable tool for examining macromolecular processes in the mammalian cell. We have continued this tradition and have used the SV40 genome as a model for studying mammalian DNA replication. This work primarily involves studying SV40 DNA replication in a cell-free system consisting of an extract derived from human 293 cells that is supplemented with purified SV40 T antigen, the virus-encoded replication protein. The main goals are to (1) identify the cellular proteins that are involved in SV40 DNA replication, (2) study the mechanism of DNA synthesis, and (3) study the function of SV40 T antigen in this process. In last year's Annual Report, we described the initial characterization of this cell-free system, the DNA sequence requirements for SV40 origin function and assembly of chromatin during DNA replication.

CELLULAR REPLICATION PROTEINS

To identify cellular proteins that are required for DNA replication, a cell-free extract capable of supporting replication from the SV40 origin region has been fractionated into multiple components. Initially, three cellular protein fractions were derived, each of which was required for complete replication of the genome. These fractions, designated II, A, and B, were the starting point for further purification of individual replication proteins, and we have concentrated on fraction B. Using conventional column chromatography, we have purified a single protein from fraction B that is required, in the presence of T antigen and fractions II and A, for complete DNA replication. This protein has a molecular weight of 36,000 (36K) and is required for the complete elongation of DNA synthesis in vitro, but not for the initiation of replication. Therefore, fractions II and A, in the presence of SV40 T antigen, are capable of forming the presynthesis complex at the origin of replication (described in last year's Annual Report), as well as a limited amount of DNA synthesis.

During the purification of the 36K replication protein, we noted that its physical properties were similar to the properties displayed by a previously identified cellular protein, alternatively called the proliferating cell nuclear antigen (PCNA) or cyclin. This protein was first detected by immunofluorescence in proliferating cells with sera from patients with the autoimmune disease, systemic lupus erythematosus (SLE), and was subsequently shown by the Protein Synthesis group to be identical to a cell-cycle-regulated protein called cyclin. Cyclin was identified as a protein that is preferentially synthesized in the S phase of the cell cycle and was suspected to be involved in cellular growth control. We therefore collaborated with M. Kostura and M. Mathews (Protein Synthesis Section) and D. Marshak (Protein Chemistry Section) to investigate the relationship between PCNA and our replication protein. It was shown that PCNA and the replication factor copurified over several chromatographic columns, that they were both recognized by the human autoantibodies, and that both have an identical amino acid sequence at the amino terminus of the protein. Furthermore, we demonstrated that the purified replication protein was able to block the nuclear immunofluorescence pattern normally seen when cells are stained with sera from SLE patients (see Fig. 1). This result demonstrated that the variable, S-phase-specific nuclear immunofluorescence pattern observed with the SLE autoantibodies was due to PCNA. Since other investigators had noted that the PCNA immunofluorescence pattern appeared to coincide with sites of cellular DNA synthesis, we concluded that PCNA is involved in cellular DNA replication as well as SV40 DNA replication.

Shortly after the identification of the 36K replication protein as PCNA or cyclin, we noticed that this protein had physical properties similar to those of yet another cellular protein that was recently purified and shown to stimulate the activity of DNA polymerase δ, a novel DNA polymerase found in mammalian cells. In collaboration with K. Downey

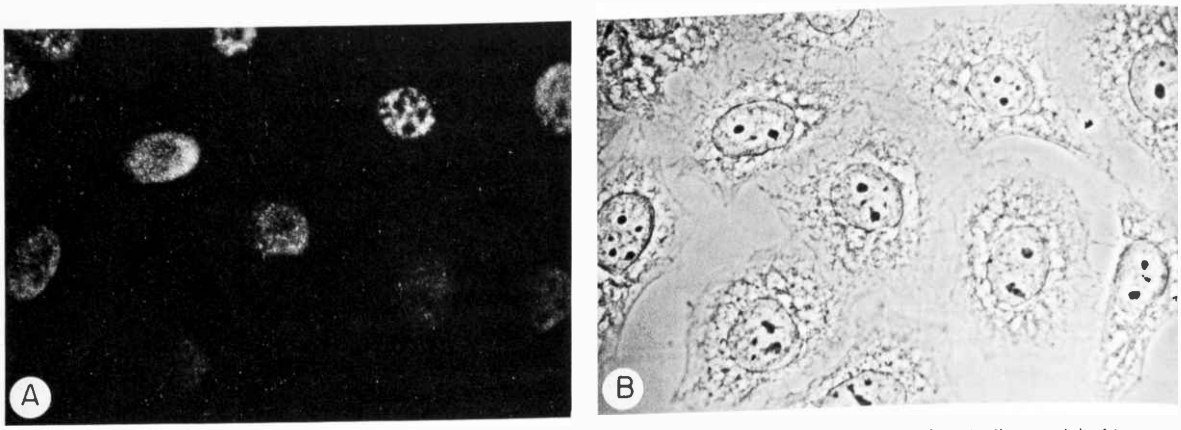

FIGURE 1 Immunofluorescent localization of PCNA, a protein required for SV40 DNA replication in vitro, to the nuclei of human HeLa cells using human autoantibodies. HeLa cells were fixed onto a glass coverslip and incubated with human autoantibody (obtained from M. Mathews, Protein Synthesis Section). After eluting excess antibody, a second fluorescein-labeled goat anti-human antibody was used as a reporter antibody. (*A*) Fluorescence micrograph; (*B*) phase-contrast micrograph. Note the variable staining pattern in these asynchronously growing cells.

and A. So (University of Miami Medical School), we demonstrated that the PCNA was identical to the polymerase-δ auxiliary protein. Both proteins stimulate the activity of the polymerase on primed, single-stranded DNA by increasing the processivity of the polymerization reaction. These results raise the interesting possibility that DNA polymerase δ, in addition to DNA polymerase α, is involved in SV40 and cellular DNA replication. Previously, it was thought that only DNA polymerase α was required for replicative DNA synthesis. We are currently seeking to identify directly the polymerases involved, with the aid of monoclonal antibodies. Similarly, studies are continuing to determine the role of PCNA in replicative DNA synthesis, as well as characterization of other cellular proteins. To this end, a cellular protein derived from fraction A that is required for formation of the presynthesis complex at the replication origin has been partially purified and its function in replication has been determined.

SV40 T ANTIGEN

SV40 T antigen is a multifunctional protein that is required for viral DNA replication and gene expression throughout the virus life cycle. It causes malignant transformation of many cells. In collaboration with Y. Gluzman's laboratory (Molecular Biology of SV40 Section), we have previously studied the multiple activities of T antigen using purified proteins expressed from various mutants. Two functions that reside within the T-antigen polypeptide, the site-specific origin DNA-binding activity and an ATPase activity, are both required for DNA replication in vitro. Recently, T antigen was shown to contain a DNA helicase activity (Stahl et al., *EMBO J. 5:* 1939 [1986]) that can unwind duplex DNA, probably at the replication fork. The ATPase activity of the protein is associated with this helicase activity. These multiple functions probably reflect the multiple roles that T antigen plays in various stages of SV40 DNA replication; it facilitates the formation of a multiprotein complex prior to initiation of replication and most likely unwinds the parental duplex DNA during the elongation stage of replication.

In collaboration with I. Mohr and Y. Gluzman (Molecular Biology of SV40 Section), a regulatory role for T antigen in origin recognition has been studied. It was demonstrated that dephosphorylation of SV40 T antigen with alkaline phosphatase, which removed most, but not all, phosphate groups from the molecule, stimulated the origin-binding activity of T antigen, but not its ATPase activity. Concomitant with the increased origin-binding potential of the partially dephosphorylated protein was an increased ability to support DNA replication in vitro. This suggests that phosphorylation of SV40 T antigen during infection may contribute to the temporal regulation of DNA replication. In the future, it will be of value to examine the interaction

of phosphorylated and dephosphorylated forms of SV40 T antigen with the cellular proteins that are required for formation of the presynthesis complex at the origin of replication.

DNA-Protein Interactions at Viral and Cellular Origins of DNA Replication

J. Diffley, S. Smith, B. Stillman

Replication origins from widely diverse organisms commonly contain sites for high-affinity, sequence-specific, DNA-binding proteins adjacent to highly conserved, immutable A+T-rich domains. In SV40, the virus-encoded large T antigen binds at sites adjacent to a 17-bp A+T-rich domain. Deletion of even 2 bp from this domain, or deprivation of T antigen from the system, inactivates the origin both in vivo and in vitro. In most serotypes of adenovirus, the cellular protein nuclear factor I (NFI) binds with high affinity to a sequence abutting an A+T-rich domain highly conserved among all serotypes of adenovirus. NFI was first detected and purified from HeLa cell nuclear extracts, using as an assay the stimulation of in vitro adenovirus DNA replication in the presence of purified viral replication proteins. Subsequently, we described a rapid and sensitive assay for NFI, exploiting its high affinity for DNA fragments containing the origin of adenovirus replication. Using this assay to follow NFI activity, we extensively purified NFI in the presence of protease inhibitors, using a combination of conventional and affinity chromatography. The final fraction of NFI was highly enriched for a polypeptide of 160 kD. Since this report, we have raised polyclonal antibodies against the 160-kD polypeptide and have shown that this protein is present in cell extracts from diverse cell/tissue types, including chick embryo brain, mouse L cells, and rat liver. Significantly, strongly antigenic polypeptides between 52 and 67 kD were detected in extracts from rat kidney and liver. Other groups have purified forms of NFI with polypeptides in exactly this range, and we are currently attempting to determine if they are derived from a 160-kD precursor or if there are multiple proteins that can bind to a similar site in the adenovirus origin. Furthermore, we are continuing to study the role of the 160-kD NFI in adenovirus replication.

Since the initiation of both adenovirus and SV40 replication requires virus-encoded proteins, in addition to cellular factors, it is difficult at present to assess the degree to which understanding these initiation systems will be helpful in understanding the initiation of cellular DNA replication. To address this problem directly, we have undertaken a functional analysis of a potential cellular origin of DNA replication from the yeast *Saccharomyces cerevisiae.*

Using the filter-binding assay described for NFI, we have identified and purified two very different proteins that interact specifically with the yeast chromosomal replicator ARS1. The first protein, ARS-binding factor I (ABFI), was identified in crude extracts and recently purified to near homogeneity. ABFI is a high-molecular-weight (300,000) native protein consisting of polypeptides of approximately 140,000. This protein binds specifically within a domain previously identified as being important for ARS function and which flanks the essential A+T-rich ARS consensus sequence. In this respect, ARS1 seems to be similar in overall anatomy to the origins of both SV40 and adenovirus replication. Although ABFI does not bind to all ARS elements, it does bind to an ARS within the silent mating-type cassette, the HMR E region.

The second protein, ARS-binding factor II (ABFII), was also identified in crude extracts and purified to homogeneity as a 17-kD polypeptide. Unlike ABFI, ABFII is not a sequence-specific binding protein, exhibiting only a modest preference for binding ARS1 sequences. Interestingly, however, it was shown by retardation of DNA-protein complexes in polyacrylamide gels that ABFII bends ARS1 DNA while not affecting control fragments from pBR322. Furthermore, in the presence of topoisomerase I, ABFII can introduce superhelical turns in any relaxed, covalently closed, circular plasmid DNA. In many respects, this protein resembles the *Escherichia coli* HU protein, a small, high-mobility-group-like protein that also exhibits the supercoiling phenomenon. The HU protein is required for *E. coli oriC* DNA replication in vitro to help specify initiation of replication at the correct sequence. Since ABFII resembles the HU protein and exhibits ARS1-dependent DNA-bending activity, it is possible that ABFII is involved in yeast DNA replication. By combining these biochemical studies with a genetic approach to understanding the function of these two ARS-binding proteins, we

expect to gain further insight into the process of initiation of chromosomal DNA replication in yeast.

Functions of the Adenovirus E1B Tumor Antigens

E. White, A. Denton, B. Stillman

Expression of adenovirus E1A and E1B gene products is required for the activation and regulation of early gene expression and cellular transformation. Several biological functions have been attributed to the E1A gene products: (1) *trans*-activation of transcription of adenovirus early genes and some cellular genes, (2) repression of enhancer-dependent promoters, (3) stimulation of cellular DNA synthesis and proliferation in quiescent cells, and (4) immortalization of primary rodent cells in culture. Considerably less is known about the function of the major E1B gene products, the 19K and 55K tumor antigens. Although the E1B 55K protein has been assigned a role in the transport and stability of mRNA and the shutoff of host-cell protein synthesis, the E1B 19K protein function is less understood.

Our research over the last few years has demonstrated that the E1B 19K tumor antigen is required to protect host-cell chromosomal DNA from degradation during productive infection. It also appears that the presence of the E1B 19K protein in the nuclear envelope is required to preserve the structural integrity of cellular DNA. Furthermore, the host range of adenovirus, as well as the morphology of the infected cell, is grossly affected by the E1B 19K gene product. Until recently, it has been difficult to correlate the roles of the E1B 19K protein in DNA protection, limiting host range, and modification of cell morphology with any biochemical function. Therefore, the function of this E1B-encoded polypeptide in either infection or transformation has remained unclear.

To assess the role of the E1B 19K tumor antigen during infection and transformation, we have relied on the characterization of adenovirus mutants that carry defined lesions in the E1B 19K gene. Experiments carried out over the last year have elucidated a number of important findings regarding E1B 19K protein function. Foremost is the identification of a functional interaction between the E1A and E1B 19K gene products. The E1A 13S and 12S gene products are responsible for inducing the degradation of host-cell chromosomal DNA in the absence of the 19K protein, and, in turn, the E1B 19K protein functions in repressing E1A-dependent *trans*-activation of adenovirus early gene transcription.

E1A GENE PRODUCTS INDUCE DNA DEGRADATION

We had determined previously that infection of human HeLa cells with adenovirus mutants that fail to express the E1B 19K polypeptide results in the degradation of host-cell chromosomal DNA (*deg* phenotype) and enhanced cytopathic effect (*cyt* phenotype). These phenotypes are the result of early gene functions and occur prior to viral DNA replication. We have sought to identify the early gene product(s) that cause these phenotypes by constructing double-mutant viruses. A series of viruses were constructed that are unable to express functional E1B 19K protein and, in addition, varied in their ability to express each of the other early gene products. Double-mutant viruses were then assayed for the *cyt* and *deg* phenotypes, with the expectation that eliminating expression of both the E1B 19K protein and the early gene product that causes these phenotypes would result in reversion to the wild-type phenotype (cyt^+ and deg^+). Analysis of E1A-E1B double-mutant viruses yielded just such a result.

Mutant viruses were constructed such that the individual 13S, 12S, or 9S E1A cDNA gene was expressed in place of the intact E1A gene, in conjunction with an E1B 19K gene mutation (Fig. 2). The viruses were then assayed for *cyt* and *deg* in infected HeLa cells. Expression of either 13S or 12S E1A gene products was sufficient to induce DNA degradation and enhanced cytopathic effect. Expression of only the 9S E1A cDNA gene, however, resulted in reversion to the wild-type cyt^+ and deg^+ phenotypes, despite the absence of functional E1B 19K protein. Therefore, expression of either the E1A 13S or 12S gene products, which possess the *trans*-activation and transforming activities of E1A, is either directly or indirectly responsible for inducing DNA degradation and enhanced cytopathic effect during infection with E1B 19K gene mutant viruses. Normally, during an infection with the wild-type virus, the presence of functional E1B 19K protein prevents this from occurring. Current efforts center around mapping the functional domain within the E1A proteins that causes the *deg* and *cyt* phenotypes in the absence of the E1B 19K protein, with

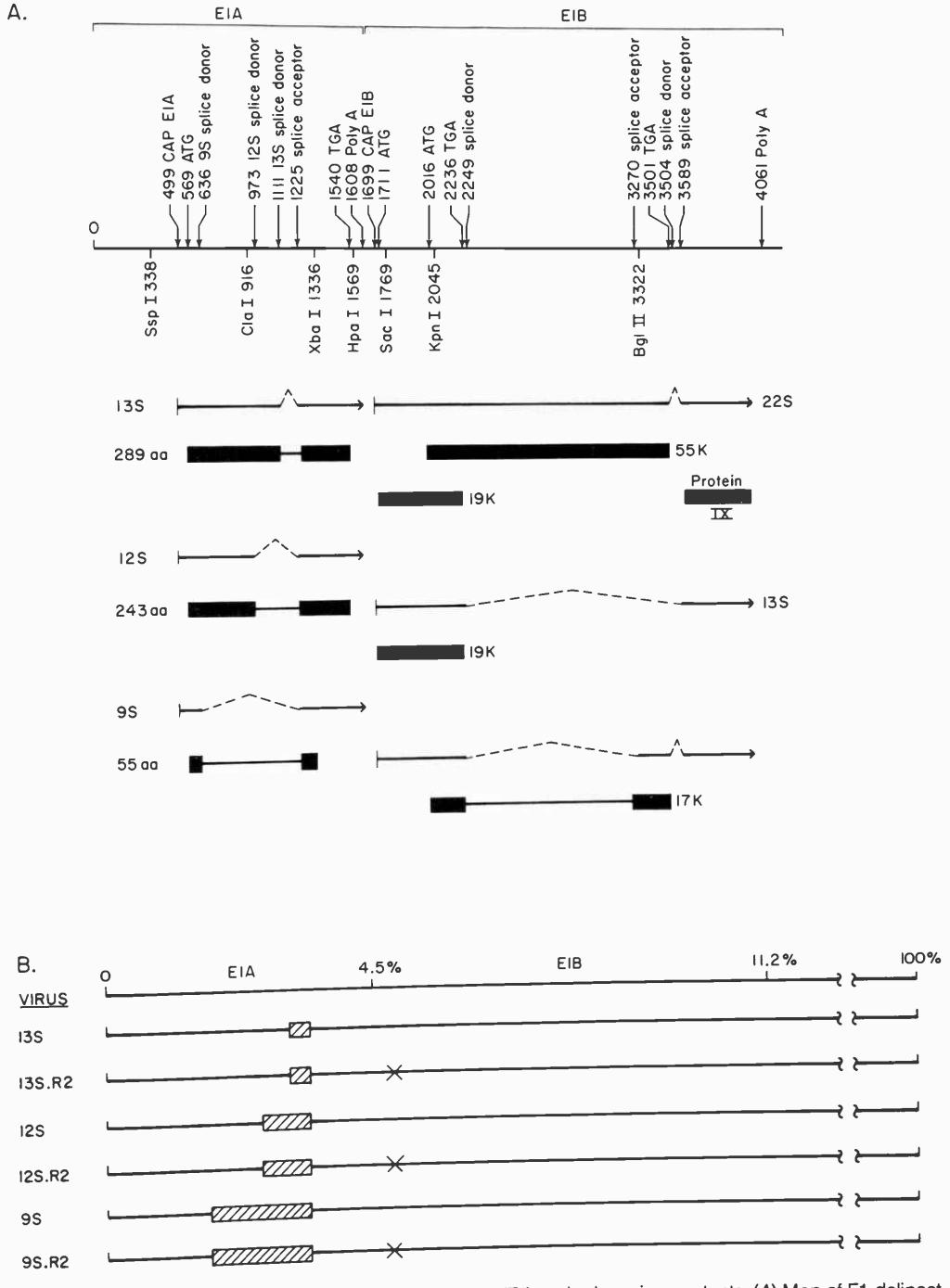

FIGURE 2 Schematic representation of early region 1 (E1) and adenovirus mutants. (*A*) Map of E1 delineating positions of differentially spliced mRNAs (thin arrows with carets indicate introns removed by splicing), corresponding translation products (closed boxes), and relevant restriction sites. Numbers indicate base pairs beginning from the left end of the genome. (*B*) Map of mutant virus genomes, with numbers indicating map units (in % genome length) from the left end of the genome. Hatched boxes indicate deletions of intron sequences due to the insertion of cDNA genes. × indicates position of point mutation and amino acid substitution in the E1B 19K gene and protein, respectively.

the ultimate intention of elucidating the biochemical relationship between E1A and E1B 19K gene products.

E1B 19K TUMOR ANTIGEN REPRESSES E1A-DEPENDENT *TRANS*-ACTIVATION

Another important finding that resulted from the characterization of adenovirus E1B 19K gene mutant viruses is that infection with these viruses results in the overexpression of the E1A proteins. The production of elevated levels of E1A proteins leads to overstimulation of early gene transcription and to an accelerated viral infection. These effects of the E1B 19K gene product on E1A protein levels and subsequent transcriptional *trans*-activation were dependent on the expression of either the E1A 13S or 12S gene products. Eliminating expression of E1A 13S and 12S genes by infection with an E1A 9S virus abolished any effect of the E1B 19K tumor antigen on early gene expression.

We have concluded from these and other experiments that the function of the E1B 19K tumor antigen is to down-regulate the levels of E1A protein during adenovirus infection. The absence of this important regulatory phenomenon disturbs the temporal sequence of transcription during productive infection and impairs the ability of adenovirus to transform rodent cells.

The work on bacteriophage λ set a precedent for the importance of repressor mechanisms in the control of gene expression. Given the pivotal role of λ repressor, similar mechanisms might be expected to exist in eukaryotic systems. Further experiments will determine whether adenovirus E1B 19K gene mutant viruses represent an analogous system to the repressor mutants of λ. Nevertheless, defining the precise mechanism by which the E1B 19K protein represses E1A-dependent *trans*-activation of transcription should yield insight into the regulatory mechanisms of eukaryotic gene expression.

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MOLECULAR BIOLOGY OF SV40

Y. Gluzman A. Admon, P. Argani, R. Gerard, D. McVey, I. Mohr, W. Ryan, R. Chisum, J. Cossetti, R. Frank, M. Strauss

The SV40 T antigen is a large, multifunctional protein generated from the early region of the viral genome. It plays a role in the replication of the viral DNA, in its transcription, and in SV40-mediated cellular transformation. Our current studies focus on the relationship between the structure of this protein and its different functions. In particular, we have been investigating the importance of post-translational modifications, most notably phosphorylation, and defining the protein's origin-binding domain. At the same time, we have continued our functional analysis of DNA sequences in the origin itself.

Posttranslational Modifications of SV40 T Antigen and Their Effect on T-antigen Functions

Y. Gluzman, I. Mohr, A. Admon, W. Ryan

To examine the effect of phosphorylation on SV40 large T antigen, we purified ^{32}P-labeled T antigen, enzymatically dephosphorylated it with calf intestinal alkaline phosphatase (CIAP), and assessed its ability to act in several biochemical assays. The phosphatase treatment successfully removed 80% of the ^{32}P label from large T antigen. Although CIAP treatment did not affect the ability of the protein to hydrolyze ATP, this partially dephosphorylated material displayed an increase in the ability to direct SV40 origin-specific DNA synthesis in vitro. Furthermore, this increase was most profound at early time points in reactions programmed with suboptimal amounts of T antigen, where a 20-fold activation was observed.

To check the interaction of normal and partially dephosphorylated T antigens with the elements of the SV40 origin of DNA replication, we used the T-antigen–DNA complex immunoprecipitation technique (McKay, *J. Mol. Biol. 145:* 471 [1981]), in which equimolar ratios of three SV40 fragments were used. These fragments contain either a complete wild-type origin (sites I and II), site I, or site II element (see Fig. 1). The precipitation assay, which was used in all of the experiments described

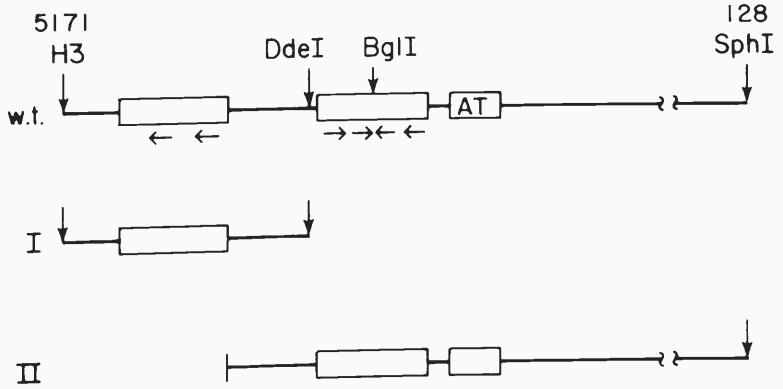

FIGURE 1 Three fragments of SV40 DNA containing the wild-type origin (w.t.), site I (I), or site II (II) were cloned into the pAT153 plasmid. The wild-type plasmid (bp no. 5171-128) and site II (bp no. 5209-128) were cloned between *Hin*dIII and *Sph*I sites of the plasmid (Stillman et al., *EMBO J. 4*: 2933 [1985]); site I (bp no. 5171-5232) was cloned between *Hin*dIII and *Bam*HI (no. 485) of pAT. For the assay, all of these plasmids were mixed in equimolar ratio and digested with *Taq*I, which released four common plasmid fragments and three unique fragments: 295 bp (w.t.), 257 bp (II), and 233 bp (I). The mixture of fragments was labeled by filling in the ends and used in the assay.

below, reflects the stability of T-antigen–DNA complexes and does not address the question of initial recognition of the DNA sites. The ability of the partially dephosphorylated T antigen to bind specifically to DNA sequences contained within the SV40 origin of replication was significantly altered, when compared to that of untreated T antigen. CIAP-treated material demonstrated at least a fourfold increase in binding to site II, a core element necessary for SV40 DNA replication, and a twofold increase in binding to site I. The nature of these quantitative changes is currently being investigated in depth utilizing DNA footprinting techniques. Preliminary data demonstrated a ninefold increase in the ability of the T antigen to protect specifically site II sequences following treatment with CIAP.

T antigen contains phosphorylated amino acids that are localized at an amino-terminal cluster and a carboxy-terminal cluster. Each region contains several phosphoserine residues and a single phosphothreonine residue. Since treatment of purified T antigen with CIAP only removes 80% of the phosphate from the polypeptide, it is of interest to us to determine those sites that are sensitive to enzymatic dephosphorylation and to mediate the observed changes in DNA replication/binding. To this end, we developed a method for quantitative analysis of the phosphoamino acids of the T antigen. This method allows us to quantitate the amount of phosphoamino acid simultaneously with all other amino acids. We have started peptide mapping of the T-antigen protein by trypsin proteolysis and reversed-phase chromatography. We are currently in the process of identifying the modified peptides as well as quantitating the phosphoamino acids on each peptide.

Expression of SV40 T Antigen in *Escherichia coli*

Y. Gluzman, R. Gerard, M. Strauss, I. Mohr

It is intriguing to envision that the numerous posttranslational modifications present on large T antigen directly modulate the multiple activities of this protein, influence its interaction with other cellular components, or even target the protein to specific intracellular locations. To produce T antigen lacking all of the mammalian modifications, we have made use of an *Escherichia coli*-based expression system. The coding sequences for SV40 large T antigen or truncated T antigens were cloned into an expression plasmid between the phage T7 promoter and transcriptional terminator. This plasmid was used to transform bacteria that express T7 RNA polymerase under the control of the *lac* UV5 promoter. Addition of IPTG to the culture induces the synthesis of T7 RNA polymerase, and this in turn activates the transcription of the gene(s) cloned downstream from the T7 promoter (Studier and Moffatt, *J. Mol. Biol. 189*: 133 [1986]). Full-length T antigen (708 amino acids) produced in this manner and purified by immunochemical techniques possesses ATPase activity, and replicates SV40 origin containing DNA in vitro; however, this occurs at substantially reduced levels when compared to T antigen purified from a mammalian source. The full-length protein produced in *E. coli* fails to bind to a fragment containing only site II, a core element of SV40 DNA replication, whereas its mammalian counterpart binds to fragments containing either site (see below). These data strongly implicate the importance of posttranslational modifications of T antigen on its biological activities.

Analysis of the Origin-binding Domain of SV40 T Antigen

Y. Gluzman, M. Strauss, D. McVey, P. Argani, R. Frank

To determine the minimum protein domain that is responsible for T-antigen binding to the origin of SV40 DNA replication, we have isolated a series of SV40 T-antigen mutants that code for amino-terminal fragments of different lengths. Mutant DNAs were used to produce truncated T-antigen proteins in *E. coli* using the host vector system described above. To screen the origin-binding properties of truncated T antigens in soluble *E. coli* extracts, we used the T-antigen–DNA complex immunoprecipitation technique, as described above. Full-length T antigen (708 amino acids), produced in *E. coli*, precipitates separate fragments containing wild-type or site I, but not site II (see above). Truncated T-antigen proteins that are 272 and 266 amino acids long precipitate only the wild-type fragment at very low efficiency. However, a trun-

cated T antigen containing 259 amino acids regains origin-binding activity to the wild-type fragment at levels comparable to that of full-length T antigen; binding to site I or site II is not detectable. Simultaneous analysis of the equivalent 259-amino-acid-long T-antigen fragment produced in HeLa cells demonstrated increased binding activity to the wild-type origin; furthermore, binding to site II, but not site I, was easily detected. These data further support the importance of posttranslational modifications in affecting the biological properties of T antigen.

At present, we are analyzing a larger set of amino-terminal T-antigen fragments produced in *E. coli.* They form three classes with respect to their origin-binding activities. One class behaves like full-length T antigen. A second class binds to the wild-type and site I fragments; in addition, it also binds to site II, although at a reduced level. A unique mutant binds to site-I-containing fragments better than to wild-type or site II fragments. The endpoints of these mutations are being determined by sequence analysis.

Functional Analysis of the Role of the A+T-rich Region and Upstream Flanking Sequences in SV40 DNA Replication

Y. Gluzman, R. Gerard

The origin of SV40 DNA replication consists of two major units: the core origin of DNA replication, which includes T-antigen-binding site II, and the A+T-rich region adjacent to the core. The late boundary of the minimal origin of replication of SV40 DNA lies upstream of the A+T-rich region. As we had previously reported, deletion of only a few bases into the adenine-thymine (AT) stretch results in a DNA template defective for replication both in vivo and in vitro (Stillman et al., *EMBO J. 4:* 2933 [1985]). For a more detailed analysis of the AT region and its role in origin function, such deletion mutations have been reconstructed into an SV40 genome containing an intact early promoter-enhancer region. The resulting mutants synthesized wild-type levels of T antigen but were defective for replication and would not form plaques on CV-1 monkey cells.

Replication-competent phenotypic revertants were selected after transfection of large quantities of the replication-defective viral DNAs into CV-1 cells. DNA sequence analysis showed that most of these revertants contained insertions or point mutations that partially regenerate the length of the AT stretch. These genotypic alterations were shown to be responsible for the revertant phenotype by replication analysis in vivo of subcloned revertant origin fragments. In general, our results emphasize the importance of the AT region to SV40 origin function. However, one revertant retained the altered AT region but deleted six nucleotides upstream. Experiments using this mutant indicate that the 21-bp repeats identified as part of the early transcriptional promoter may compensate for defects in SV40 DNA replication in vivo caused by mutations in the A+T-rich region when positioned at an appropriate distance from the core origin.

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NUCLEIC ACID CHEMISTRY

R.J. Roberts A.S. Bhagwat, L. Dalessandro, J. Hasapes, F.B. Johnson, C. Keller, A. Kiss, D. Kozak, A. Krainer, C. Marcincuk, B. Mollet, K. Otto, L. Schoenherr, A. Sohail, N. Sullivan, M. Wallace, K. Weule, B. Zerler

Repair of DNA Damage Caused by 5-Azacytidine in *Escherichia coli*

A.S. Bhagwat

5-Azacytidine (5-azaC), an analog of cytidine in which the C—H group at position 5 is replaced by a nitrogen, has a multitude of biological effects in a wide variety of organisms. These effects range from mutagenesis and inhibition of cell growth for bacteria to an increase in sister chromatid exchange in hamster cells, induction of differentiation in mouse cells, and induction of synthesis of proteins, such as hypoxanthine-guanine phosphoribosyl transferase and fetal globin, in human cells. At the biochemical level, 5-azaC has been shown to cause hypomethylation of DNA, to produce defective ribosomal RNAs and tRNAs, and to inhibit protein synthesis. The ability of 5-azaC to cause hypomethylation of DNA is believed to be the cause of many of the phenotypic effects of the drug.

In vitro studies have shown that 5-azaC acts as a mechanism-based inhibitor of cytosine methylases. Most cytosine methylases link covalently to position 6 of cytosine as a normal intermediate in their action. Following the transfer of a methyl group from *S*-adenosyl methionine to position 5 of cytosine, the enzyme unlinks itself. 5-azaC, when incorporated into DNA or RNA at the site of methylation, sabotages this reaction by making the transfer of the methyl group to position 5 impossible. Hence, cytosine methylases form stable covalent complexes with DNA (or RNA) containing 5-azaC.

The presence of such complexes in vivo has not yet been demonstrated. If these complexes can exist stably in vivo, they would be expected to interfere with essential cellular functions such as DNA replication and hence cause cell death. A gene designated *dcm* codes for the only known DNA cytosine methylase in the K-12 strain of *Escherichia coli.* But contrary to expectation, wild-type *E. coli* is fairly resistant to 5-azaC. We have taken this as evidence for the existence of a repair pathway(s) for the DNA damage caused by the drug and have investigated the genes involved in such repair.

Several interesting points have emerged from this study. First, a *recA-lexA*-dependent pathway for the repair of damage caused by 5-azaC exists. *recA* and *lexA* control the inducible DNA repair ("SOS" repair) in *E. coli,* and mutants that are defective in inducible repair are very sensitive to the drug. The requirement for the induction of SOS-repair genes can be largely eliminated by overproducing the RecA protein in the cell. Thus, RecA must play a direct role in the repair. In contrast, *uvr*-dependent excision repair does not appear to play a role in the ability of cells to survive the damage. Together, these data suggest that the repair of DNA damage caused by 5-azaC in *E. coli* may be repaired by the "postreplication repair" pathway, which uses the ability of RecA to mediate homologous recombination.

Second, several lines of evidence suggest that phosphorylation of 5-azaC and not of its decomposition or degradation products, such as 5-azauracil (5-azaU), is responsible for the lethal damage. In contrast, we have found that the bacteriostatic effects of 5-azaC are caused largely by the conversion of the drug to 5-azaU and can be partially overcome by the addition of an excess of normal uracil in the growth medium. Since the uracil does not inhibit the conversion of 5-azaC to 5-aza-dCTP, the potentially lethal effects arising from DNA damage are unchanged. This is of great practical value since it permits the use of 5-azaC for the selection of mutant methylases. Normally, selection is complicated by the bacteriostatic effects caused by the drug that are unrelated to DNA methylation. However, by including uracil in the growth medium, the bacteriostatic effects of 5-azaC are eliminated, and hence *recA*$^-$ cells carrying a defective methylase gene are able to grow in the presence of 5-azaC.

Finally, we have also found that *dcm*⁻ *recA*⁻ and Δ*dcm recA*⁻ mutants of *E. coli* are significantly more sensitive to 5-azaC than their *recA*⁺ parents. This suggests either that *E. coli* carries an unknown DNA cytosine methylase(s) or that 5-azaC causes lethal damage in additional ways not presently understood.

Structure of *Eco*RII Endonuclease and Methylase Genes and Their Relationship to Other Restriction Endonuclease and Methylase Genes

A.S. Bhagwat, F.B. Johnson, K. Weule [in collaboration with S. Som and S. Friedman, SUNY-Health Science Center, Brooklyn, New York]

The *Eco*RII restriction-modification (R-M) enzymes from *E. coli* are the prototype for one of the largest set of isoschizomers, i.e., enzymes that recognize the same DNA sequence. Over 40 restriction endonucleases, including *Eco*RII, recognize the sequence 5′-CC(A/T)GG-3′. Like *Eco*RII, each of these enzymes is expected to be accompanied by a modification methylase that recognizes the same DNA sequence. Although all of these enzymes recognize the same DNA sequence, they do so in different ways. For example, if this sequence is not methylated, *Eco*RII endonuclease cleaves the DNA before the first cytosine. In contrast, *Bst*NI endonuclease (from *Bacillus stearothermophilus*) cleaves the sequence after the second cytosine, regardless of whether either cytosine is methylated at position 5. Several other interesting differences between these isoschizomers are also known. Hence, *Eco*RII and its isoschizomers present a fascinating system for the study of sequence-specific interactions between proteins and DNA.

We have cloned, mapped, and determined the nucleotide sequences of the *Eco*RII genes. We have also cloned a gene from the *E. coli* chromosome, *dcm*, that codes for a methylase gene with specificity identical to that of the *Eco*RII methylase. The *dcm* gene has been mapped to a 1.5-kb fragment. Parts of the *Eco*RII genes were cloned in M13 phage vectors, and the DNA sequence was determined by the dideoxy chain-termination method. The genes are convergently transcribed, and the corresponding termination codons are separated by 33 nucleotides. The direction of transcription of the endonuclease gene determined from the nucleotide sequence is consistent with the direction predicted from the random protein fusions constructed between the endonuclease and the β-galactosidase gene. The predicted size of the methylase, 54,564 daltons, agrees well with the observed size of the purified protein (55,000 daltons). At present, uncertainty in the precise start of the endonuclease gene prevents us from predicting the size of the endonuclease monomer.

We have used the nucleotide sequences of the *Eco*RII genes and their predicted protein sequences as starting points for a comprehensive search for sequence homologies among R-M systems. The database includes sequences of 11 endonucleases and 17 methylases. This study has resulted in several interesting observations: (1) All cytosine methylases share several common protein domains. These conserved domains are four to ten amino acids long and are shared by methylases that recognize similar DNA sequences, as well as by methylases that recognize very different DNA sequences. Thus, *Eco*RII, *Msp*I (from *Moraxella species*; recognition sequence, CCGG), and *Hha*I (from *Haemophilus haemolyticus*; recognition sequence, GCGC) share at least six regions of protein sequence identity. *Msp*I and *Hha*I enzymes also share a great deal of DNA sequence homology. (2) Adenine methylases share little homology with the cytosine methylases or with each other. The only adenine methylases to share protein sequence homology are *Dpn*II (from *Streptococcus pneumoniae*) *dam*, and *Eco*RV; the latter two are from *E. coli.* They recognize identical or related sequences (GATC or GATATC). (3) None of the endonuclease genes or proteins share significant homology with any methylase or with each other. Thus, even in the cases where the two methylase proteins show significant homology with each other, the corresponding endonucleases are nonhomologous. There is also significant rearrangement around homologous methylase genes. The *Eco*RII endonuclease and methylase genes are adjacent to each other in a 5′→3′/3′←5′ fashion, whereas the *Msp*I genes are arranged in a 3′←5′/5′→3′ fashion.

Overexpression of the *Msp*I Methylase

B. Mollet

The *Msp*I restriction-modification system, which recognizes the DNA sequence 5′-CCGG-3′, has

been previously isolated and sequenced (P.-M. Lin and R.J. Roberts, unpubl.). A 3-kb-long DNA fragment, containing the *Msp*I endonuclease and methylase gene, has now been subcloned into a pBR322 derivative plasmid. The endonuclease gene has been deleted by further subcloning, and the new clones have been tested for maintenance of *Msp*I methylase activity. The inducible promoter pTac has been introduced 85 bp in front of the first ATG start codon of the 418-amino-acid-long open reading frame encoding the *Msp*I methylase. When *E. coli* cells carrying the overproducing plasmid are induced with IPTG, a protein band of about 50 kD appears on SDS protein gels of a crude extract. The size of the protein is in good agreement with the calculated molecular weight of 47,664 for the *Msp*I methylase. After induction, this accounts for about 1% of the total cell mass of *E. coli*.

Cloning of the *Bsp*RI Restriction Endonuclease Gene

A. Kiss

Analysis of the protein sequences deduced from the DNA sequences of cloned modification methylases has revealed structural similarities between several enzymes that recognize identical or related DNA sequences. This opens the way for a more detailed analysis of the mechanism by which these enzymes recognize their target sequence. Much less information is available for restriction endonucleases, mainly because few genes have been cloned and even fewer genes have been sequenced. Furthermore, those endonucleases whose sequences have been determined display different specificities. To date, no isoschizomers have been compared at the sequence level.

We previously cloned and sequenced the gene encoding the *Bsu*RI endonuclease from *Bacillus subtilis* (recognition sequence, GGCC). To find out whether restriction enzymes with the same specificity share sequence homology, we began cloning the *Bacillus sphaericus* enzyme *Bsp*RI, which is an isoschizomer of *Bsu*RI. Initially, we tried to use hybridization as a means of identifying suitable clones. Hybridization at low stringency (56°C in 6× SSC) allows the *Bsp*RI and *Bsu*RI methylase genes to hybridize. We tested whether this also holds for the endonuclease genes. We have been unable to find, in Southern experiments, conditions under which the *Bsu*RI endonuclease gene would specifically hybridize to *B. sphaericus* DNA. This effectively prevented the use of this method as a selection to clone the *Bsp*RI endonuclease gene.

As an alternative approach, we cloned several large fragments of chromosomal DNA by selecting for the methylase. If the endonuclease gene is located close to the methylase gene, as has been found in many systems, then this strategy would allow both genes to be cloned. We obtained several clones carrying the methylase gene on overlapping fragments, but none of them showed *Bsp*RI endonuclease activity.

Another method to clone the *Bsp*RI endonuclease gene was tried. A host, already carrying the methylase, was used, and selection for endonuclease function was carried out by infecting the clones with nonmodified phage. This also failed. These failures were not entirely surprising because cloning of many restriction genes has not been successful in other laboratories. This can basically happen for two reasons: Either the gene is not expressed in the cloning host or it is deleterious to the cell.

One way to circumvent this problem is to try to clone the endonuclease gene, or part of it, by using an oligonucleotide hybridization probe that was synthesized on basis of the protein sequence. To do this, we purified the *Bsp*RI endonuclease to homogeneity, and a partial amino-terminal amino acid sequence was determined in an automatic gas-phase sequencer. The sequence was Ala Gln ? Lys Tyr Gly Ala Leu Glu Gln Lys Val Ala Asn Ile Phe Ile Asn. Using this information, we devised a mixed probe of 17-mer oligonucleotides that correspond to amino acid residues 10–14 and to the first two nucleotides of the triplet coding for Ile-15. The oligonucleotides were synthesized in two pools, each containing 64 different sequences. These oligonucleotide probes were used in Southern experiments to hybridize to *B. sphaericus* chromosomal DNA. In 6× SSC at 37°C, both probes hybridized to several fragments. No hybridization was detected under more stringent conditions. We also tested whether these probes hybridized to any of those plasmids mentioned earlier, which in addition to the methylase gene carry large pieces of *B. sphaericus* DNA. Pool I was found to hybridize to one of these plasmids. This plasmid carried the largest piece of *B. sphaericus* DNA downstream from the methylase gene. The analysis of this plasmid is now in progress.

TABLE 1 Simple Hexanucleotide Palindromes

1st	2nd A	C	G	T	3rd
A	–	–	*Bgl*II	–	A
	–	–	*Eco*47III	*Cla*I	C
	*Hin*dIII	*Mlu*I	*Stu*I	*Ava*III	G
	*Ssp*I	*Spe*I	*Sca*I	–	T
C	–	*Nco*I	*Pvu*I	–	A
	*Pma*CI	*Sma*I	–	*Xho*I	C
	*Pvu*II	*Sac*II	*Xma*III	*Pst*I	G
	*Nde*I	*Avr*II	*Sph*I	*Afl*III	T
G	*Eco*RI	*Sph*I	*Bam*HI	*Sna*I	A
	*Aat*II	*Nae*I	*Nar*I	*Sal*I	C
	*Sac*I	*Bse*PI	*Apa*I	*Apa*LI	G
	*Eco*RV	*Nhe*I	*Kpn*I	*Hpa*I	T
T	–	*Bsp*HI	*Bcl*I	–	A
	*Sna*BI	*Bsp*MII	*Mst*I	*Asu*II	C
	–	*Nru*I	*Bal*I	–	G
	–	*Xba*I	–	*Aha*III	T

Restriction Endonucleases

R.J. Roberts, K. Weule

The collection of restriction endonucleases continues to grow, and more than 750 such enzymes are now known; 140 different specificities have been characterized, including 123 type II enzymes. During the last year, 25 new enzymes have been isolated and characterized as part of a collaborative program with I. Schildkraut and D. Comb (New England BioLabs). Among these, only one, *Bsp*HI (TCATGA), displays a new sequence specificity. The remaining enzymes are isoschizomers of known endonucleases. Several other enzymes, that appear to display new sequence specificities are still being examined. As shown in Table 1, 49 of the possible 64 enzymes that could recognize hexanucleotide palindromes have now been discovered.

Sequence of the *Hpa*II Methylase Gene

A. Kiss, J. Hasapes, K. Weule

Previously, we determined the complete sequence of the genes of the *Msp*I restriction-modification system. The *Msp*I endonuclease recognizes the sequence CCGG and cleaves between the two C residues. The methylase recognizes the same sequence, and methylates the outer cytosine. Recently, the *Hpa*II methylase has been cloned by G. Wilson at New England BioLabs. This methylase also recognizes the sequence CCGG, but differs from the *Msp*I methylase in that it methylates the inner cytosine residue. To allow a comparison of these two enzymes, we analyzed the sequence of the *Hpa*II methylase gene. The gene was obtained on a 2-kb insert in pBR322. The insert was transferred to M13mp19 in both orientations and was used to prepare a series of phased deletions suitable for sequence analysis. It should be noted that whereas the original pBR322 plasmid carrying the gene was completely resistant to *Hpa*II digestion, both M13 clones showed only partial resistance when their Rf DNA was isolated from JM107. Sequence analysis of the gene is almost complete.

Purification of Factors Involved in Nuclear Pre-mRNA Splicing

A.R. Krainer

My long term goal is to identify, purify, and characterize factors necessary for nuclear pre-mRNA splicing in mammalian cells. Previous work with HeLa and *Saccharomyces cerevisiae* in vitro systems showed that pre-mRNA splicing occurs via a two-step pathway, each step consisting of a cleavage and a ligation reaction. At present, it appears that, in addition to several unknown protein factors, the small nuclear ribonucleoproteins (snRNPs) U1, U2, U4/U6, and U5 are involved in the first step of the reaction. The U1 snRNP is involved in the selection of 5′ splice sites by RNA-RNA base pairing. U2 and U5 appear to bind at the branch point and 3′ splice sites, respectively. However, the mechanism of action of these snRNP particles is not known. They may act in a stoichiometric manner to bind to specific sites and fold the pre-mRNA into a conformation that is optimal for the cleavage-ligation reactions. Alternatively, the snRNAs may be ribozymes, with intrinsic catalytic activities, or the snRNP polypeptides may have enzymatic activities that are brought to their sites of action by their associated snRNAs. Precedents for each of these types of mechanisms are, for example, self-splicing RNAs, RNase P, and ribosomes. Elucidation of the detailed mechanisms of action of snRNPs and of other splicing components will require their purification in an active form.

To purify snRNPs that are active, at least with respect to their involvement in the splicing reaction,

I have chosen to try an immunoaffinity scheme. The basic methodology was developed by R. Luhrmann and co-workers, who showed that snRNPs could be bound specifically via their snRNA moieties by antibodies directed against modified nucleosides. Gentle elution from the antibody columns could be accomplished by competition with the relevant free nucleoside. My goal is to apply this methodology to snRNPs present in splicing extracts and to test the activity of the purified snRNPs by biochemical complementation assays involving the restoration of splicing activity to extracts depleted of snRNPs.

With the help of C. Bautista and E. Harlow (Protein Immunochemistry Section), I have recently obtained two mouse monoclonal antibodies specific for 2,2,7-trimethylguanosine (m_3G). This modified nucleoside is present in the characteristic cap structures of all known snRNPs, except U6. These antibodies can be used to immunoprecipitate specifically and efficiently the snRNPs U1, U2, U4/U6, and U5 from a crude splicing extract, with retention of all of the previously characterized snRNP polypeptides. The snRNPs bound to a m_3G monoclonal antibody column can be eluted by competition with free m_3G at moderate ionic strength. I am presently scaling-up this purification step to obtain concentrated snRNPs that can be assayed for splicing and splicing complex formation. I am also employing these antibodies to analyze the polypeptide composition of snRNPs under different conditions.

Ongoing *myc*-associated Projects

N. Sullivan [in collaboration with R.A. Watt, Smith Kline and Beckman]

To approach the function of the human c-*myc* protein, the coding sequence has been cloned and inserted into bacteria, and the protein has been overexpressed. The subsequently purified protein forms a basis for the biochemical and structural studies currently in progress.

Several monoclonal antibodies directed against the bacterially produced human c-*myc* protein have been isolated and mapped by immunoblotting against truncated derivatives of the c-*myc* protein. Two of these antibodies, B_3 and E_{10}, which do not compete in enzyme-linked immunosorbent assays, map to the amino terminus of c-*myc*, whereas another antibody, B_7, recognizes an epitope in the carboxyl terminus of this protein. These antibodies are being used in attempts to block the action of c-*myc* in vivo by microinjection and to make affinity columns for the isolation of *myc*-associated complexes.

The c-*myc* protein is capable of binding to DNA, although no specificity has yet been demonstrated. Other experiments, namely, nuclease digestion of fixed cells, indicate that the c-*myc* protein may interact with nuclear RNA but not DNA. Thus, nucleic-acid-binding experiments are now in progress (1) to determine the kinetics of the *myc*-RNA association and (2) to identify cellular sequences with which c-*myc* may interact.

In collaboration with J. Anderson (Demerec Laboratory), we have initiated studies to crystallize both the c-*myc* protein and two truncated derivatives that correspond to the amino- and carboxy-terminal proteins of the protein. These are currently purified from broken *E. coli* lysates by detergent extraction, followed by anion-exchange and gel-filtration chromatography. This protein, which is currently 95% pure, is being purified further for the purposes of crystallization. Together with a functional domain analysis, we hope that the X-ray crystal structure of the c-*myc* protein will lead us to a better understanding of how this protein interacts with nucleic acid.

That c-*myc* colocalizes with snRNPs leads us to hypothesize a functional role for *myc* in RNA processing. This is being investigated by examining the formation of splicing complexes in nuclear extracts prepared from a c-*myc*-expressing cell line. Does the *myc* protein associate with larger complexes as they form from smaller precursors and exogenous RNA? In addition, immunoprecipitation experiments are being performed in an attempt to identify potential direct associations between c-*myc* and proteins of the snRNP particles.

Adenovirus-2 Early Region 1A Gene Products

B. Zerler, L. Schoenherr

In collaboration with E. Moran and M. Mathews (Protein Synthesis Section), we are continuing to study the relationship between the structure of the adenovirus early region 1A (E1A) gene and the biological roles of this gene in host-cell immortalization and the regulation of gene expression. Com-

parison of the amino acid sequences of the E1A regions of several adenovirus serotypes shows that the E1A regions are highly conserved within three distinct domains. Domains 1 and 2 are common to both the 12S and 13S products and encompass amino acids 22–45 and 120–139, respectively. The third domain consists essentially of the 13S unique region and encompasses amino acids 140–185. Initially, plasmids and viruses containing the separate E1A 12S and 13S cDNA products were isolated. These constructs identified domain 3 (the 13S unique region) as being necessary for efficient activation of viral gene expression, but not for the induction of host-cell immortalization. Subsequent analysis by construction of in-frame and site-specific point mutations (Moran et al., *Mol. Cell. Biol. 262:* 3470 [1986]) identified domain 2 as an active site for the stimulation of proliferation of primary baby rat kidney (BRK) cells and for the ability to cooperate with the *ras* oncogene to transform primary BRK cells fully; in addition, a nonconserved region (amino acids 86–120) was identified between domains 1 and 2 that is dispensable for the functions associated with domains 2 and 3.

During this past year, we used these mutants to analyze the changes in host-cell gene expression that occur during the early stages of adenovirus-induced proliferation of quiescent primary BRK cells. We have identified different host-cell products, associated with DNA replication and transformation, whose activation appears to be dependent on different domains of the E1A products.

Nuclear runoff and cytoplasmic RNA dot blots showed that expression of the DNA replicative enzyme, thymidylate synthase (TS), occurred 8 hours after infection of quiescent primary BRK cells with either wild-type or the E1A 13S cDNA virus. The kinetics of expression of TS were essentially the same as that of the adenovirus DNA-binding protein (DBP). Expression of TS and DBP was not detected by 8 hours or even by 16 hours after infection of BRK cells with the 12S cDNA virus or the E1A host-range mutant virus *hr*3, which contains a single amino acid change within domain 3. TS and DBP expression eventually occurs during 12S viral infection, but not until some time between 16 and 24 hours postinfection. Expression of TS and DBP was not impaired during infection with the E1A mutant transformation-defective virus E1A 928 that contains a single amino acid change within domain 2.

The expression of a different cellular product associated with DNA replication and transformation, proliferating cell nuclear antigen (PCNA), appears to be independent of the E1A activity required for the efficient activation of DBP or TS. PCNA expression was detectable by immunoprecipitation by 8 hours postinfection of primary BRK cells with either the 12S or 13S cDNA viruses. PCNA expression was not impaired in BRK cells infected with the E1A 928 or E1A *hr*3 mutant viruses, and it was not impaired in cells infected with viruses containing in-frame deletions of domain 2, domain 3, or the nonconserved region between domains 1 and 2. We also found that all the mutant viruses were able to stimulate DNA synthesis, measured by [^{3}H]thymidine incorporation into acid-precipitable material, in quiescent primary BRK cells to levels at least 50% of wild type.

These experiments identified two cellular products, TS and PCNA, that are activated during adenovirus infection of primary BRK cells. Early activation of TS correlates with the function of E1A required for the efficient activation of early viral genes (domain 3) and may be associated with usurpation of the host DNA replication machinery for viral replication. Early activation of PCNA and stimulation of DNA synthesis occurred in primary BRK cells infected with mutant viruses that in combination excluded all but the amino-terminal 85 amino acids of the E1A products. Activation of PCNA and stimulation of DNA synthesis appear to be independent of the early region gene-activation functions associated with domain 3, as well as the transformation-related functions associated with domain 2. These activities are also independent of the nonconserved amino acids between domains 1 and 2. On the basis of these results, we felt that a function of conserved domain 1 (amino acids 22–45) involved the activation of PCNA and the stimulation of DNA synthesis.

To test this idea more directly, we made a single amino acid change (Leu-49–Phe-49) in domain 1 and an in-frame deletion of amino acids 20–85 that removed the entire domain. 12S cDNA viruses containing the Leu–Phe mutation did not exhibit altered growth characteristics compared with wild-type virus during infection of HeLa cells, and the mutant was able to stimulate DNA synthesis to wild-type levels during infection of primary BRK cells. However, the amino acid change resulted in a 2–3-kD decrease in apparent molecular mass of both the 12S and 13S E1A proteins, whereas a different Leu–Phe change at position 173 (the *hr*4

mutation) did not result in any apparent change in the mobility of the E1A proteins. Virus containing the in-frame deletion that excludes domain 1 was unable to activate PCNA, stimulate DNA synthesis, or cooperate with the *ras* oncogene to transform primary BRK cells fully. Plasmids containing this deletion were still able to activate the adenovirus E3 promoter in a transient expression assay, indicating that domain 1 is not involved in the *trans*-activation of early region genes.

We also observed that the domain-1 deletion mutant virus and viruses containing mutations in domain 2 were unable to stimulate proliferation of primary BRK cells, as visualized by vital staining 6 days after infection. This result was not unexpected for the domain-1 mutant, since it does not activate PCNA or stimulate DNA synthesis. Domain-2 mutants, however, do not affect the ability of the E1A products to activate PCNA or stimulate DNA synthesis in quiescent primary BRK cells. To examine more closely the point in the cell cycle that domain-2 mutants fail to pass, we determined the ability of wild-type and mutant viruses to stimulate infected BRK cells to enter mitosis. We used the drug nocodazole to block cells in metaphase and found that domain-2 mutants were extremely impaired in their ability to induce mitosis. Therefore, it appears that a function of domain 1 is to stimulate quiescent primary BRK cells to enter G_1 of the cell cycle, whereas a function of domain 2 enables the BRK cells to progress from G_1 to mitosis.

We have shown that conserved domain 3, unique to the 13S gene product, is required for the accelerated activation of adenovirus early region genes and the cellular TS gene but is dispensable for primary BRK cell proliferation. Domain 2 is required for the induction of mitosis and the stimulation of proliferation of primary BRK cells but is dispensable for the stimulation of DNA synthesis and the early activation of viral genes. Amino acids 20 through 85, which includes conserved domain 1, appear to be sufficient for activation of PCNA and the stimulation of DNA synthesis; however, these activities of domain 1 are not sufficient to induce cell proliferation.

We are extending our analysis of the functions of domain 1 by making single-site alterations in this region, and we are continuing to use the domain-1 and domain-2 mutants to explore the regulation of host-cell proliferation. In addition, we are using TS cDNA clones that contain mutations in the 5′ regulatory region to determine sequences responsive to the *trans*-activation functions of the E1A products.

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PROTEIN CHEMISTRY

D.R. Marshak A. Admon D. Carroll
G. Binns N. Santoro

The Protein Chemistry Section is involved in the purification, structural analysis, and chemical synthesis of proteins and peptides. In the laboratory, we employ state-of-the-art instrumentation and methodologies for protein chemistry. The chemical analyses of proteins are essential to our group's efforts to understand the basic mechanisms of cell proliferation and cancer. Proteins are the molecular phenotypes of genes, and the cellular responses to changes in gene expression during oncogenesis are due to the functions of the protein products of those genes. Our investigations of protein structure and function have shed light on the molecules that transform normal cells into cancers.

We have focused our research in four major projects: (1) analyses of posttranslational modifications on products of the oncogenes, adenovirus early region 1A (E1A) and SV40 T antigen; (2) purification and characterization of a new mitogenic factor that is secreted by epithelial cells infected by adenovirus; (3) structural analysis of the proliferating cell nuclear antigen (PCNA) that has been identified as a DNA replication factor; and (4) development of new methods of protein sequence analysis and peptide synthesis. We have completed several structures using mass spectrometry, an emerging technique in protein chemistry. Our improved methods of peptide synthesis allow us to build proteins of relatively large size (50–100 amino acids). Thus, advanced technologies combined with our multidisciplinary approach to important biological questions create an exciting atmosphere for protein chemical analysis.

Modification of Oncogene Products

D. Carroll, A. Admon, N. Santoro, D. Marshak
[in collaboration with E. Harlow, C. Stephens,
and Y. Gluzman, Cold Spring Harbor Laboratory]

The protein products of the adenovirus E1A gene and the SV40 T antigen gene are heterogeneous in size and charge. Some of the heterogeneity can be attributed to alternative splicing mechanisms that give rise to different mRNAs; however, even the products of specific mRNAs produce heterogeneous products. To uncover the basis of this heterogeneity, we have investigated the posttranslational modification of the E1A proteins and T antigen.

The E1A proteins can be immunoprecipitated from in vitro translation mixtures using antibodies developed by E. Harlow (Protein Immunochemistry Section). Treatment of the E1A proteins with phosphatases significantly decreases the heterogeneity of the E1A proteins on two-dimensional gel electrophoresis, suggesting that many of the modifications are phosphorylations. Two missense mutants in E1A-containing point mutations at serine residues 132 and 219 were analyzed by immunoprecipitation and electrophoresis. The results indicated that phosphorylation of these two serine residues are the first two modification steps for E1A. The mutant virus at Ser-132 has a reduced ability to transform cells. Thus, the phosphorylation at this residue might be a crucial step in the activation of E1A during transformation.

To pursue this phenomenon further, we are attempting to identify and characterize the kinase that is responsible for the phosphorylation of Ser-132. We constructed a synthetic peptide consisting of 14 residues surrounding the phosphorylation site at residue 132. This peptide blocks the phosphorylation of E1A in vitro, and it appears that the peptide itself is phosphorylated. We are currently using this peptide as substrate to measure and purify the E1A kinase. In addition, we have constructed a series of other peptides that are designed to be specific inhibitors of the enzyme.

Our studies on T-antigen phosphorylation take advantage of the availability of large amounts of the protein from overproduction systems in HeLa cells or from *Escherichia coli.* We fragment the protein into pieces using enzymatic digestions and then isolate the peptide fragments by high-performance liquid chromatography (HPLC). A. Admon (this section) has developed a new method for analysis of the phosphorylated amino acids in the peptides: The peptides are first subjected to complete enzymatic digestion using immobilized amino peptidase and carboxyl peptidase. The resulting amino acids are labeled with phenylisothiocyanate, and

the derivatives are separated by HPLC. This separation permits the quantitation of all of the unmodified amino acids as well as the phosphorylated amino acids. We anticipate that this new procedure will allow us to identify rapidly the multiple phosphorylation sites on T antigen.

Further studies of the kinases that catalyze the phosphorylation of T antigen are now in progress using synthetic peptide substrates. Because T antigen and E1A share regions of structural homology, the research on T antigen and E1A phosphorylation are intertwined. We hope to identify enzymes that are fundamental to the activation of nuclear oncogenes and to cellular growth.

Mitogenic Factor Induced by Adenovirus

D. Marshak, N. Santoro [in collaboration with M. Quinlan and T. Grodzicker, Cold Spring Harbor Laboratory]

When adenovirus infects primary cultures of baby rat kidney cells, the epithelial cells rapidly proliferate. Using serum-free defined media, we found that the fibroblasts were overgrown by the immortalized epithelial cells. The adenovirus responsible for the establishment of these cells has had the E1A region removed and replaced by a cDNA for the E1A 12S gene product. This virus construction does not allow complete transformation or lytic growth of the virus, but the virus does cause quiescent cells to proliferate. The conditioned media harvested from the infected epithelial cells cause other uninfected cells to proliferate. The mitogenic factor in the media is heat-labile and sensitive to trypsin digestion, indicating that the factor is likely to be a protein.

We have begun to characterize this mitogenic factor biochemically. From the media, the factor is retained by ultrafiltration membranes with a 100,000-molecular-weight cutoff and is found in the pellet upon centrifugation at 100,000*g*. After treatment with 4 M sodium chloride, however, the mitogen is freely soluble at neutral pH, and it does not sediment upon subsequent ultracentrifugation. Thus, the protein appears to be associated with a high-molecular-weight component, perhaps a cell matrix protein such as fibronectin or laminin. Further evidence that supports this hypothesis resides in the fact that heparin potentiates the mitogenic activity, and heparin is representative of glycosaminoglycans that naturally bind to the cell matrix and to a family of mitogenic proteins. Preliminary studies indicate that the factor is acidic, with a molecular weight of greater than 12,000.

Our future studies on this factor will focus on the purification and complete structural analysis of the protein. We will also initiate studies to identify a receptor for the mitogenic factor. Eventually, we hope to understand the mechanism of action of the factor and the way in which the adenovirus 12S E1A gene product stimulates its expression. Most of the time, when wild-type adenovirus infects a tissue, the virus finds adult, quiescent cells, not proliferating cells. As part of the earliest response after infection, it is advantageous for the virus to stimulate the host cell to secrete mitogenic factor, so that the surrounding cells proliferate. Such a paracrine response would allow the progeny viruses to encounter a proliferating tissue, thus boosting the infectivity of the viral particles and accelerating oncogenesis. We believe that this mitogenic factor may be an important part of the mechanism of viral oncogenesis.

Proliferating Cell Nuclear Antigen

D. Marshak [in collaboration with G. Prelich, M. Kostura, M. Mathews, and B. Stillman, Cold Spring Harbor Laboratory]

PCNA is a protein that was initially identified by an autoantiserum from patients with the autoimmune disease, systemic lupus erythematosus. PCNA is localized to the nucleus of proliferating normal cells and tumor cells, but it is not detected in quiescent cells. The expression of PCNA is under cell-cycle control such that it is preferentially synthesized during the S phase. During the course of their studies on PCNA, M. Kostura and M. Mathews (see Protein Synthesis Section) were able to purify the protein from human cells and rabbit tissues by following immunoreactivity to the autoantiserum. We succeeded in identifying the first 26 amino acid residues of the protein. G. Prelich and B. Stillman (DNA Synthesis Section) had isolated a protein that was active as a DNA replication factor, and they noticed that its chemical and physical properties were similar to those of PCNA. We showed by sequence analysis of the DNA replication factor that the amino-terminal 26 residues were identical to those of PCNA. Taken together with a variety of biochemical, immunological, and func-

tional data, our structural analyses have helped to prove that PCNA is a DNA replication factor.

Structural Analysis of Peptides

D. Marshak, N. Santoro, G. Binns [in collaboration with B. Fraser, Food and Drug Administration, Bethesda, Maryland]

We have completed the structure of several novel peptides using a multidisciplinary approach. Our approach involves three stages: (1) purification, (2) characterization, and (3) application. Purification relies on an accurate biological or chemical assay to measure the yield of active protein at each step. Characterization should include several independent and complementary methods of structural analysis, e.g., amino acid analysis, chemical sequence analysis, and physical determination of mass, usually by mass spectrometry. The application of structural information is the most important part of our strategy. Deciphering the complete structure of the protein is not the end of our work; rather, structural information permits us to study the relationships between the detailed structure and the function of the molecule. Often, these studies of structure and function involve site-directed mutagenesis, site-specific antibodies, and synthetic peptide analogs of the protein.

The structures of several polypeptides have been elucidated during the past year using our multidisciplinary strategy. The structure of a new gonadotropin-releasing hormone (GnRH) was elucidated by a combination of mass spectrometry and chemical analysis. Mass measurements by physical methods are quite accurate; in this case, the measured mass of the GnRH molecule was 1226.54 and the actual mass was 1226.59. Proof of the structure was further demonstrated by synthesis of the molecule by chemical methods and using the synthetic peptide to stimulate ovulation in test animals.

The amino-terminal peptide structure of endothelial cell growth factor β (ECGF-β) was determined by mass spectrometry. This molecule has an acetylated alanyl residue at the amino terminus, and subsequent proteolytic cleavages of the blocked protein produces the daughter molecules, ECGF-α, and acidic fibroblast growth factor. Our studies have helped to demonstrate that the ECGF-β is a precursor for this family of mitogens.

We have improved our methods of solid-phase peptide synthesis using an automated instrument, the Applied Biosystems 430A. Peptides are prepared by condensations of t-Boc amino acids as symmetric anhydrides on solid-phase systems according to the method of Merrifield. This procedure is automated using the synthesizer, and we sample the resin at each step for quantitative analysis of the coupling efficiency. Recent procedures include double-coupling cycles, with capping of the unprotected chains with acetic anhydride. This allows the synthesis of longer (>40 residues) peptides and simplifies the purification procedure. The crude products of such syntheses generally contain the major desired product and a series of smaller termination fragments of the peptide, but very few deletion products. This is an advantage because the multiple deletion products are difficult to separate from the desired product. The peptides are removed from the resin by treatment with hydrogen fluoride or trifluoromethane sulfonic acid, precipitated twice with ether, and filtered on a gel permeation column for desalting. The resulting crude peptide products are further purified by preparative HPLC on reversed-phase columns, 1–2 inches in diameter. The products are routinely characterized by amino acid analysis, sequence analysis, and analytical rechromatography on HPLC.

During the past 3 years, we have been able to analyze synthetic peptide products by fast atom bombardment mass spectrometry through a collaboration with B. A. Fraser (Food and Drug Administration, Bethesda, Maryland). Our general procedure is to analyze fraction samples from the HPLC purification of the peptide. If the peptide mass is within approximately 0.1 amu of the expected mass, then we accept the material for use in biological experiments. In addition, we have been able to identify several interesting by-products of the chemical synthesis, such as dehydration, fluoridation, and alkylation. Treatment of peptides with aspartyl-(*O*-benzyl ester)-Gly sequences with strong acid under dehydrating conditions (as in cleavage from the resin) leads to cyclization of the side chain to yield an aspartimide. The use of the *O*-cyclohexyl ester of aspartic acid greatly reduces this cyclization. The dehydrated products can be separated by isocratic HPLC and display a nominal mass 18 amu lower than the desired product. In another case, treatment of a peptide with hydrogen fluoride resulted in three products with additions of 19 amu. Preliminary analysis of these products suggests that under certain conditions, free radical of fluorine ($F^{\cdot}$) can

add to a stable free radical at a tertiary carbon atom to form an alkyl fluoride derivative. This occurs on residues of isoleucine and valine predominantly. Finally, alkylation and oxidation of indole rings in tryptophan residues have been routinely identified in peptide products. This most frequently occurs during the cleavage procedures under strong anhydrous acid, presumably by a free radical mechanism.

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PROTEIN IMMUNOCHEMISTRY

E. Harlow K. Buchkovich, A. Lele, C. Stephens, P. Whyte, B. Ahrens, C. Bautista, R. McGuirk, V. Meshan, C. Schley, J. Wiggens, N. Williamson

The Protein Immunochemistry Laboratory consists of two units, one is a research group that is studying the functions of nuclear oncoproteins and the other serves as a central facility for the production of monoclonal antibodies. The Monoclonal Antibody Facility is run by Carmelita Bautista. In collaboration with scientists at Cold Spring Harbor Laboratory, the facility handles all stages of the production of monoclonal antibodies. During the last year, they have produced 12 different panels of hybridomas secreting antibodies specific for a wide range of antigens. Descriptions of many of these projects can be found in the individual research projects discussed in this Annual Report.

Research in the Protein Immunochemistry Laboratory focuses on the function of nuclear oncoproteins such as the adenovirus early region 1A (E1A) and cellular p53 proteins. Much of our work over the last year has concerned the analysis of the E1A proteins. Several years ago, we prepared a panel of monoclonal antibodies specific for the E1A polypeptides, and these reagents have allowed us to investigate a number of unusual properties of these proteins. During the last year, we have completed a major segment of our studies on the physical characterization of the E1A proteins. These studies have shown that the E1A region codes for five mRNAs, that the primary translation products of these five mRNAs are highly modified during posttranslational processing, and that the E1A proteins form a series of stable complexes with host-cell polypeptides. Our goal now is to use this new information to help understand the molecular basis for the functions of the E1A proteins in both viral growth and transformation.

Heterogeneity of the Adenovirus Early Region 1A Proteins

C. Stephens, E. Harlow

When the adenovirus E1A proteins are separated by two-dimensional, isoelectric-focusing polyacrylamide gel electrophoresis, the E1A-specific polypeptides can be resolved into approximately 80 different species. This polypeptide heterogeneity arises from at least two sources: (1) The primary

transcript of the E1A region is differentially processed to yield five mRNAs, and (2) these mRNAs direct the synthesis of a polypeptide that undergoes extensive posttranslational modification. The combination of these two mechanisms results in a large number of protein species arising from a single genetic locus. We have begun to study the role that these mechanisms play in contributing to the different functions attributed to the E1A proteins.

The two best-characterized E1A transcripts are the 13S and 12S mRNAs. These two mRNAs can be first detected shortly after infection, and they are synthesized throughout the lytic cycle. During the late phase of infection, three other mRNAs are produced from the E1A region. Until recently, only one of these mRNAs had been identified and characterized. The previously characterized mRNA is known as the 9S mRNA, and it has been cloned and analyzed in several laboratories. The structures of the 9S, 12S, and 13S mRNAs are shown in Figure 1. The first evidence for other mRNAs came from the analysis of E1A proteins immunoprecipitated with monoclonal antibodies raised against a bacterially produced E1A antigen. In addition to the 12S and 13S products, polypeptides with relative molecular weights of 30,000 and 35,000 could be detected during the late phase of infection. Judging from the localization of the epitopes for the monoclonal antibodies that specifically bound to these proteins, it was determined that the previously described 13S, 12S, or 9S mRNAs could not encode E1A proteins with the immunochemical properties of the 30K and 35K polypeptides. Previously, three groups had described a 30K E1A polypeptide that now appears to be one of the species discussed here.

To characterize the origin of the 30K and 35K polypeptides, poly(A)$^+$ mRNA was prepared from different stages of infection and used to program in vitro translations. A 30K protein that was indistinguishable in size and in immunochemical properties from the in-vivo-synthesized protein was detected in these translations. A cDNA library was constructed from RNA prepared from cells at late times after infection, and a clone was isolated that coded for the 30K protein. In keeping with the nomenclature for the E1A mRNAs, the 30K mRNA is referred to as the 10S mRNA. The structure of this RNA is shown in Figure 1. The 10S mRNA contains a new splice between nucleotides 637 and 853 as well as the splice found in the 12S mRNA. In the analysis of this clone, it was noticed that if the late

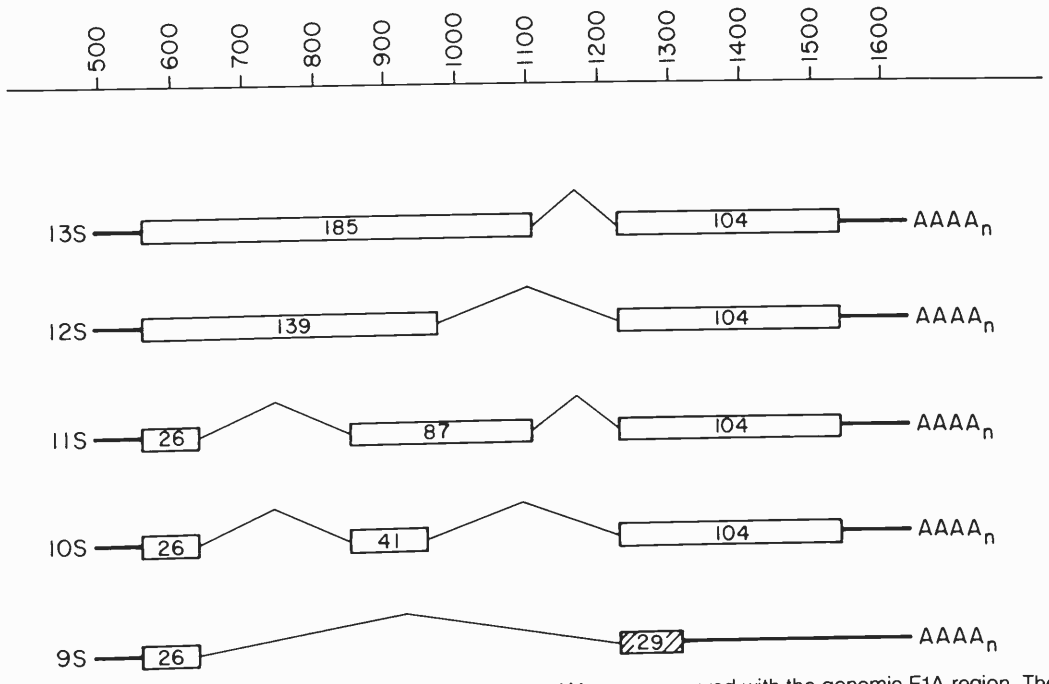

Figure 1 Adenovirus type-5 E1A mRNAs. The five E1A mRNAs are compared with the genomic E1A region. The solid lines show the structures of the mRNAs, and the open boxes represent the coding region for the E1A polypeptides.

splicing event that is found in the 10S mRNA was linked to the 13S splice, a protein with the properties of the 35K protein could be synthesized. A cDNA construct was prepared by combining the appropriate segments of the 10S and 13S cDNAs and was designated the 11S cDNA. Both the 10S and 11S cDNA clones directed the synthesis of proteins that were identical to the 30K and 35K proteins found during viral infections. The sequence of these clones indicated that at late times in adenovirus infection, a novel series of splice sites are used to produce E1A-specific RNAs. These RNAs are referred to as the 10S and 11S mRNAs, and their structures are shown in Figure 1. Both the 10S and 11S mRNAs encode proteins that lack 72 amino acids in the amino-terminal region of the 12S and 13S E1A proteins. The amino acid sequence that is removed during splicing of the 10S and 11S mRNAs contains a region that is highly conserved between adenovirus serotypes. Together, the appearance of these mRNAs at late times in infection and the sequence conservation between divergent adenovirus serotypes suggest that this region may be important for an E1A function that is not required during the late phase of infection. This suggestion is supported by data collected with adenovirus mutants that carry a 10S or 11S cDNA in place of the wild-type E1A sequences.

These viruses will not replicate on cells that readily support the growth of wild-type viruses. We are currently constructing mutant viruses that are unable to use the late splice donor and acceptor sites, and these mutants should provide a system to evaluate the role of these late proteins in the viral lytic cycle. Interestingly, plasmids that direct the synthesis of the 30K and 35K polypeptides fail to cooperate with the human *ras* oncogene to yield transformed foci when transfected into baby rat kidney cells. These data suggest that the 72 amino acids removed by this late splicing event may be important for one or more of the E1A functions essential for adenovirus transformation.

In addition to the different E1A mRNAs, the heterogeneity of the E1A proteins is also generated by posttranslational modification. We are interested in what role these modifications play in the functions of the E1A proteins. Using pulse-chase experiments, we have identified the primary translation products of both the 12S and 13S mRNAs. These experiments have also shown that the addition of modifying groups moves the primary translation products to more acidic isoelectric-focusing positions and that some of the additions also decrease the relative mobility of the proteins on polyacrylamide gels. Both of these changes are consistent with the addition of acidic modifying groups such as phosphate. Previous work has shown that the E1A proteins are phosphorylated on serine residues. Treatment of the immunoprecipitated E1A proteins with either bacterial alkaline phosphatase or calf intestinal alkaline phosphatase simplifies the patterns seen on two-dimensional gels, and most of the E1A polypeptides then run at a position identical to that of primary translation product. This result suggests that phosphorylation accounts for much, if not all, of the E1A protein modification. By two-dimensional gel analysis of E1A missense mutants, we have identified residue Ser-132 (13S numbering) as a modified residue. Similar analyses have been used to confirm the results of A. Tsukomoto and A. Berk that the serine residue at position 219 is also modified. The Ser-132 site is of particular interest because mutants that change this serine disrupt two biological activities associated with E1A. The first is a decrease in the efficiency with which E1A can transform baby rat kidney cells in cooperation with an activated *ras* protein, and the second is that the ability of E1A to repress the transcription of genes found adjacent to some enhancers is lost. In collaboration with D. Carrol and D. Marshak (see Protein Chemistry Section), we have also shown that in rabbit reticulocyte lysates, the modification of Ser-132 can be blocked by the addition of a 15-amino-acid synthetic peptide with the identical sequence to the region around Ser-132. Pulse-chase experiments have shown that the serine modifications at positions 132 and 219 are the first modifications that are added to the E1A proteins. We are presently trying to determine what other sites on the E1A proteins are modified and whether these modifications are necessary for E1A functions.

Mapping the Regions of the Adenovirus E1A Proteins That Are Needed for Transformation

P. Whyte, E. Harlow [in collaboration with E. Ruley, Massachusetts Institute of Technology]

As with the transforming proteins of other DNA tumor viruses, the adenovirus E1A proteins have evolved to perform a number of different functions during viral infection. To help determine which

regions of the E1A proteins are important in viral transformation, a series of deletion mutants has been constructed to locate the boundaries of regions essential for these functions. Reports by M.P. Quinlan, D. Chao, P. Hinton, and T. Grodzicker (Adenovirus Genetics Section), and this section (P. Whyte, K. Buchkovich, A. Lele, C. Schley, N. Williamson, and E. Harlow; see below) discuss the use of these mutants in studies of viral replication and association with host-cell polypeptides, respectively. This study presents the results of experiments that help to localize the minimal region of the E1A proteins necessary for transformation. Two types of assays have been used. First, plasmids that contain the mutant E1A-coding regions cloned behind either the E1A promoter or mouse metallothionein promoter are transfected into baby rat kidney cells in conjunction with a plasmid that contains an activated *ras* oncogene. In these cotransfection assays, transformed foci are scored approximately 3 weeks after the introduction of the E1A mutant and *ras* plasmids into recipient cells. Second, the same plasmids are transfected into baby rat kidney cells alone, and mass cultures of transfected cells are scored for their ability to continue to divide under standard tissue-culture conditions.

Only the sequences common to the first exons of the 12S and 13S mRNAs are necessary for cooperation with the *ras* oncogene to yield transformed foci. When mutant E1A proteins shorter than the amino-terminal 139 amino acids are tested for cooperation, a gradient of the number of transformed foci is found. Although the 139-amino-acid fragment will produce near wild-type levels of foci, transfection of a plasmid that makes a 127-amino-acid fragment fails to yield any transformed foci. Between these two extremes, proteins that have intermediate lengths produce an intermediate number of foci per plate. Similarly, the amino acids from 1 to 139 are sufficient to immortalize rat fibroblast cells in culture, and there appears to be a similar decrease in the number of established cell lines as the length of the protein is shortened.

Similar types of deletion mutants have been prepared beginning at the amino terminus of the E1A proteins. All mutants in this region that have been tested are deficient in cotransfection assays. These include a point mutation that changes the second amino acid from an arginine to a glycine residue. Previous work from other laboratories has shown that mutants in the amino-terminal 14-amino-acid residues are not necessary for transformation by viral infection. We are currently testing the ability of the viruses carrying the amino-terminal mutations to transform cells in an effort to resolve this apparent discrepancy.

Association of the Adenovirus E1A Proteins with Host Polypeptides

P. Whyte, K. Buchkovich, A. Lele, C. Schley,
N. Williamson, E. Harlow

In addition to the adenovirus E1A proteins, monoclonal antibodies specific for the E1A polypeptides precipitate a series of proteins with relative molecular weights of approximately 28,000, 40,000, 50,000, 60,000, 80,000, 90,000, 105,000, 107,000, 130,000, and 300,000 from adenovirus-infected and -transformed cells. The most prominent of these are the 105K, 107K, and 300K polypeptides. Three lines of evidence suggest that these proteins form stable protein complexes with the E1A proteins: (1) the 105K, 107K, and 300K proteins are not recognized directly by the any of the anti-E1A monoclonal antibodies, (2) these polypeptides copurify with a subset of the E1A proteins, and (3) complexes of these proteins and E1A proteins can be formed in vitro. Because the in vitro association experiments use labeled extracts from cells that do not contain any adenovirus DNA, the complexed proteins must be encoded by host DNA.

To identify the domains of the E1A proteins that interact with the 105K, 107K, and 300K cellular polypeptides, deletion mutants that remove regions of the E1A-coding sequences have been constructed. Immunoprecipitations of the E1A–host polypeptide complexes from cells infected with the E1A deletion mutants have shown that the binding sites for the 300K, 107K, and 105K polypeptides are located in the portion of E1A encoded by the first exon of the 12S and 13S mRNAs. All of our studies to date suggest that the host-cell polypeptides other than the 105K, 107K, or 300K proteins are not bound directly to E1A and are apparently precipitated because they are associated with one of these three proteins. The binding site for the 300K protein is found between amino acid 1 and 85 (13S numbering). The 105K and 107K proteins appear to have identical or overlapping binding sites located between amino acids 121 and 139. Using monoclonal antibodies raised against the 107K or 105K proteins, we have shown that although the 105K and

300K proteins can bind to the same E1A molecule, the 105K protein is not found in the same E1A complex as the 107K protein. In addition, gradient and high-performance liquid chromatography (HPLC) size fractionation, followed by immunoprecipitation with anti-E1A antibodies, results in separate peaks for the E1A-107K and the E1A-300K-105K complexes. Together, our results have suggested that there are at least four forms of E1A in infected or transformed cells. These are (1) E1A associated with 107K, (2) E1A associated with 300K, (3) E1A associated with both 300K and 105K, and (4) free E1A. This provides an attractive model to explain the multifunctional capacity of the E1A proteins.

To help assign functions to the various E1A–host protein complexes, we have begun to characterize the biological activities of E1A mutants that have lost the ability to bind to the cellular proteins. Perhaps the most interesting observation in these studies is the correlation between the localization of the binding sites for the host proteins and the regions of E1A that are necessary for transformation. All of the E1A mutants that have lost a binding site for one of the host proteins have also lost the ability to cooperate with an activated *ras* oncogene in the transformation of primary rat cells. However, the converse is not true. Several point mutations in E1A (e.g., the 952 mutant of E. Moran and M. Mathews, see Protein Synthesis Section) that do not cooperate with an activated *ras* oncogene still bind with wild-type affinity to all of the host proteins. Although further work will be necessary to confirm these findings, they suggest that binding to the host proteins may be necessary, but not sufficient, for transformation. We are currently preparing a large series of point mutations within the binding regions for the cellular polypeptides to help in these studies.

Using monoclonal antibodies raised against immunoaffinity-purified E1A–host protein complexes, we have begun analysis of the host proteins in cells that have not been exposed to adenovirus and in cells that have been infected with other adenovirus serotypes. Both the 107K and 105K proteins are normal constituents of many of the mammalian cells we have tested to date. In addition to the 105K and 107K proteins, the antibodies raised against these proteins precipitate novel polypeptides from uninfected cells. The anti-105K antibodies also immunoprecipitate an 85K polypeptide, whereas the anti-107K antibodies bring down 68K and 65K proteins. We are currently investigating whether these proteins are recognized directly by the monoclonal antibodies or whether they are precipitated via a protein-protein complex with the 105K or 107K polypeptides. If the latter is true, it is interesting to speculate that these proteins may be cellular homologs for the adenovirus E1A proteins.

Expression of Adenovirus E1A Proteins and Oncogenic *ras* Proteins in the Rat Cell Line REF52 Leads to Amplification of the Endogenous p53 Oncogene

E. Harlow, R. Franza, C. Schley

The rat cell line REF52 was isolated from a primary cell culture of rat embryonic fibroblasts as a rare descendant that had overcome senescence and survived several cycles of passage under standard mammalian tissue-culture conditions. This cell line is resistant to transformation by an activated *ras* oncogene but will produce fully transformed foci when the activated *ras* gene is cotransfected with the adenovirus E1A gene. During the analysis of transformed cells prepared from cotransfection of the E1A and *ras* genes, we noticed that the levels of the cellular oncoprotein p53 were significantly higher in these cells than in the parental REF52 cells. Comparsion of the levels of p53 proteins in cells that contained either the E1A or *ras* gene alone showed that the p53 levels were similar to those of the parental line and well below the levels found in cotransfected lines. Further study revealed that the mRNA levels for p53 were correspondingly higher in the E1A plus *ras*-transformed cells. Southern hybridization showed that the endogenous p53 gene was amplified at least 50-fold in these cells, compared with that of parental, E1A-alone, or *ras*-alone lines. Similar hybridization studies showed that other rat endogenous genes were not amplified, nor were the transfected E1A or *ras* genes. Together, these data suggest that higher levels of p53 protein may convey some selective advantage to cells in tissue culture when both the E1A and *ras* proteins are expressed. We are currently testing other cell lines for similar amplifications. One interesting possibility is that the p53 protein serves as an intermediate in the signaling pathways stimulated by the *ras* and E1A polypeptides and that higher levels of p53 protein may allow or be necessary for rapid growth in tissue culture.

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PROTEIN SYNTHESIS

M.B. Mathews A.P. Rice, C.V. Déry, M. Kostura, J.M. Langstaff, A. Maran, E. Moran, G.F. Morris, C.C. Bunn, C. Herrmann, K. Mellits, R.P. O'Malley, M. Freedman, A. Kamenick, L. Manche, R. Packer, P.A. Wendel

Work in the Protein Synthesis Section continues to pursue the themes of transcriptional and translational control, with special emphasis on the adenovirus early region 1 oncogenes and virus-associated (VA) RNA products as probes of these processes. Our interest in autoantibodies in human immune disease has also borne fruit this year, with the identification of an antibody-reactive epitope and of the protein PCNA (proliferating cell nuclear antigen) as a DNA replication factor. The latter finding, made in conjunction with members of the DNA Synthesis and Protein Chemistry Sections of the Tumor Virus group, is particularly gratifying as it forges a link between the themes of autoimmunity, replication, and transformation. One project that has now drawn to a close in this section (though it continues at Cold Spring Harbor in Bill Welch's laboratory) is our work on heat shock, a topic that for some time had been indissolubly linked to Paul Thomas, who returned to England and will be sorely missed by many at the Lab.

VA RNA and Translational Control

A.P. Rice, M. Kostura, A. Maran, K. Mellits, R.P. O'Malley, M.B. Mathews

Adenovirus encodes two small RNAs, the VA RNAs, transcribed by RNA polymerase III. These RNAs are approximately 160 nucleotides long and accumulate to high concentrations in the cytoplasm of adenovirus-infected cells. The role of the major VA RNA species has been elucidated through study of HeLa cells infected with a mutant, *dl*331, deficient in the production of VA RNA_I. As discussed in last year's Annual Report, in *dl*331-infected HeLa cells, protein synthesis fails during the late phase of infection because of defective polypeptide chain initiation. The defect results from phosphorylation of the eukaryotic initiation factor 2 (eIF-2) on its α-subunit and the consequent trapping of a second initiation factor known as GEF (guanine nucleoside exchange factor or eIF-2B). The protein kinase responsible is DAI, the double-stranded RNA-activated inhibitor of protein synthesis. This enzyme, found in uninfected cells in an inactive form, is activated during the late phase of infection with *dl*331, a process antagonized by VA RNA_I during infection with wild-type virus.

Previous studies had suggested that the activator present in *dl*331-infected cells is double-stranded RNA (dsRNA): Deproteinized cytoplasmic extracts of these cells were able to activate the quiescent DAI enzyme from uninfected, interferon-treated cells. (Interferon induces elevated synthesis of the enzyme, but dsRNA is needed for activity.) Furthermore, the activation exhibited the unusual bell-shaped concentration curve that is characteristic of

activation by dsRNA. We have now confirmed that the component behaves like dsRNA in several additional ways. First, when fractionated on the basis of secondary structure using column chromatography, the activator was recovered in the fraction that contains dsRNA. Second, the activator was found to be sensitive to heating at 100°C, a treatment expected to denature dsRNA, and to be restored by annealing under conditions allowing renaturation of the duplex. Finally, the activity was destroyed by incubation with RNase III, a dsRNA-specific enzyme. From these experiments, we conclude that *dl*331-infected cells contain dsRNA that is capable of accounting for the observed DAI activity. As anticipated, when purified away from VA RNA, dsRNA was also detected in extracts of wild-type adenovirus-infected cells. More surprisingly, however, when the cytoplasmic RNA from uninfected cells was similarly fractionated and tested, the dsRNA-rich fractions also activated DAI in like fashion, despite the fact that DAI activity is low or undetectable in uninfected cell extracts. This finding casts some doubt on the notion that dsRNA is the DAI activator in *dl*331-infected cells, although it is not yet clear whether this dsRNA behaves like an activator of DAI in uninfected cells or whether there exist cellular factors that, like VA RNA_I analogs, antagonize its action. Investigations along these lines are currently under way.

DAI is induced by interferon treatment of animal cells and is believed to participate in cellular defenses that block viral replication. It is activated in interferon-treated cells after infection with some RNA viruses (reovirus, encephalomyocarditis virus) and correlates with inhibition of growth of these viruses. In contrast, many of the more complex DNA viruses appear to encode functions capable of blocking DAI action. Adenoviruses encode VA RNA_I; Epstein-Barr virus also encodes two similar RNA polymerase III genes that are able, in part, to substitute for VA RNA_I^-; and vaccinia virus also inhibits DAI activity, although the mechanism is unknown. Its importance to the antivirus response and the regulation of protein synthesis notwithstanding, little is known about the mode of action of DAI. What is known hints at rather subtle and interesting regulatory mechanisms—inhibition rather than activation by high concentrations of dsRNA and by short or imperfect duplex RNA molecules, and the association of autophosphorylation with the activation reaction are two such features. To elucidate the interaction of DAI with its activators and antagonists, we have purified the enzyme and prepared a number of defined RNA ligands. The latter include a set of mutant VA RNA_I genes carrying linker-scanning or deletion mutations in the 3′ half of the RNA coding sequence, permitting study of the sequence and structural motifs relevant to its function. These mutants have been tested for their ability to rescue translation in a transient expression assay. Plasmids containing wild-type or mutant VA RNA genes are transfected into human 293 cells, which are infected with *dl*331 virus 24 hours later and labeled with [^{35}S]methionine at 48 hours. The results show that the RNA is unexpectedly sensitive to structural changes. The linker-scanning mutants and most of the deletion mutants allow production of less than 10% as much hexon (a viral late protein) as the wild-type VA RNA_I gene, although they make comparable levels of steady-state RNA. Two deletion mutants were, however, able to rescue late protein synthesis efficiently. We are now in the process of analyzing the secondary structure of the mutant RNAs to ascertain the role of duplex regions in VA RNA function.

Viewed as a protein kinase, DAI phosphorylates a highly restricted set of substrates. Only four such substrates have been recognized to date: a 68-kD polypeptide and the α-subunit of eIF-2, both in the cytoplasm of many (if not all) mammalian cells; histones, basic DNA-associated nuclear proteins; and a 90-kD polypeptide that we have recently identified in reticulocyte lysates. The 68-kD polypeptide substrate copurifies with DAI activity and, as mentioned above, is thought to be a kinase itself; its autophosphorylation is believed to be an integral aspect of the enzyme's activation. When activated, DAI will phosphorylate eIF-2 on its α-subunit, and it is this phosphorylation that impairs the ability of eIF-2 to recycle, resulting in the inhibition of initiation of protein synthesis.

We first observed phosphorylation of the 90-kD polypeptide while assaying kinase activity in crude and partially purified DAI preparations from rabbit reticulocyte lysates. The reaction requires dsRNA and follows the characteristic biphasic dsRNA concentration curve, suggesting that the 90-kD polypeptide is a substrate of DAI. To study the 90-kD polypeptide further, we purified the protein to apparent homogeneity and found that it is separable from DAI activity and has no detectable intrinsic kinase activity, being phosphorylated only in reactions where dsRNA and DAI activities are

included. These experiments demonstrate that the 90-kD polypeptide is indeed a newly identified substrate of DAI.

Less expectedly, we found that the 90-kD polypeptide can be cross-linked to VA RNA_I by irradiation with UV light, indicating that the two moieties bind each other tightly and suggesting that the 90-kD polypeptide may regulate DAI activity. To look at this interaction more closely, we have used a nitrocellulose-filter-binding assay with RNAs of various kinds. The 90-kD polypeptide has a high affinity for VA RNA_I and 5S RNA, but not for tRNA. We also generated a set of dsRNA molecules of precisely known sizes by in vitro transcription of a DNA fragment from bacteriophage λ. The DNA was introduced into a bacterial plasmid between SP6 and T7 RNA polymerase promoters, and complementary transcripts were made in separate reactions. By exploiting appropriate restriction sites within the λ insert, a full-length (2.4 kb) transcript was synthesized as well as complementary transcripts of varying lengths. The transcripts were annealed and then digested with RNase A to remove single-stranded RNA (ssRNA) tails, creating dsRNA structures of defined length. Using the nitrocellulose-filter-binding assay, we found that the 90-kD polypeptide has affinity for dsRNA molecules, but not for ssRNA. It therefore seems that the protein may be recognizing the duplex regions in the VA and 5S RNAs, but not those in tRNA, either because they are too short or because they are buried by tertiary folding.

As a means to approach the function of the 90-kD protein, we have raised a number of monoclonal antibodies against it. In immunoprecipitation reactions with these antibodies, we have been unable to detect the 90-kD polypeptide in HeLa or 293 cells, but it is not yet clear whether this reflects the absence of a homologous protein in these cells or antigenic differences between the rabbit and human proteins. The antibodies are currently being used to immunoprecipitate the 90-kD polypeptide from reticulocyte lysates to see if there is any nucleic acid bound to the polypeptide in vivo. We also plan to use the antibodies to deplete the 90-kD polypeptide from the reticulocyte lysate, to determine whether the ability of the lysate to synthesize proteins is affected.

Functions of the Adenovirus E1A Gene

E. Moran, M.B. Mathews

The early region 1A (E1A) gene plays an important role in adenovirus-mediated oncogenesis, and on its own, it is able to immortalize primary rodent cells. During the past year, we have continued our analysis, in collaboration with B. Zerler and R. Roberts (see Nucleic Acid Chemistry Section), of the relationship between the structure of the adenovirus 2 E1A gene and the biological effects induced by E1A products. We have now constructed and analyzed mutants covering almost the entire E1A coding sequence, from amino acid 22 to the carboxyl terminus at position 289, and have made substantial progress in correlating various functions of the E1A gene with specific regions of the E1A proteins.

As illustrated in Figure 1, the E1A regions of adenoviruses of different serotypes are composed of alternating regions of high and low amino acid sequence identity. There are three highly conserved regions, two of which (domains 1 and 2) are common to the products of the 12S and 13S E1A mRNAs and one of which (domain 3) consists essentially of the 13S unique region. Our earlier results, together with those from several indepen-

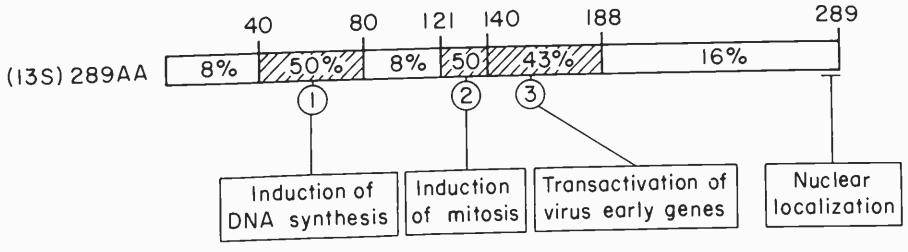

FIGURE 1 Functional domains of E1A. The bar representing the 289-amino-acid-long product of the E1A 13S mRNA is shaded to emphasize regions that are highly conserved among the different adenovirus serotypes. Beneath each conserved domain is shown its function (in some cases still only tentatively assigned).

dent laboratories, showed that the 13S unique region plays an important role in facilitating *trans*-activation of other adenovirus early genes but is completely dispensable for the E1A-dependent induction of proliferation of quiescent primary baby rat kidney (BRK) cells. We then went on to study the role of the 12S/13S common sequences in the control of host-cell proliferation. Since it is likely that viral oncogenes include counterparts of normal cell genes involved in regulating cell growth, the study of E1A should offer insights into the nature of cellular proliferation controls and their mechanisms of action.

By last year, our analysis of the role of the 12S/13S common sequences had shown that the nonconserved amino acid regions from position 86 to 120 and after position 140 were largely dispensable for the E1A activities involved in the induction of primary cell proliferation. In addition, we had shown that the second conserved domain, between positions 120 and 139, plays an essential role in this activity. Deletion of this region, or single missense mutations at position 124, 130, 132, or 135, severely impairs the ability of the 12S E1A product to induce primary cell proliferation and to express other transformation-related activities. None of the domain-2 point mutations interferes with the viral early gene *trans*-activation function, strongly supporting the hypothesis that the induction of cell proliferation and the *trans*-activation of viral early genes are two independent functions. This does not, however, exclude the possibility that gene *trans*-activation is involved in immortalization: Cellular genes activated during immortalization may represent a different class of targets from the viral early genes, and activation of this gene class may require different E1A functions.

During the past year, we asked whether the domain-2 activity required for the induction of cell proliferation is involved in the activation of cell-cycle-regulated host-cell products. Because uninfected primary BRK cells are quiescent with regard to DNA synthesis and proliferation, we studied the role of domain 2 in the activation of cellular products required for the onset of DNA synthesis in quiescent cells. A rapid increase in the level of DNA synthesis begins at 8–10 hours after infection with a 13S or genomic E1A virus and at 12–16 hours after infection with a 12S E1A virus. Transcription of the DNA synthesis enzyme, thymidylate synthase, was accelerated by the presence of a functional 13S product, in a manner similar to that of the viral early genes. In contrast, the appearance of the DNA replication factor PCNA (see below) was induced coordinately with expression of either the 12S product or the 13S product, and thus appears to be independent of the 13S unique region. PCNA may be the first product known to be activated by E1A in response to an activity other than that of domain 3, although it remains to be shown that PCNA is regulated at the RNA level.

Somewhat surprisingly, the induction of DNA synthesis and the E1A-dependent activation of thymidylate synthase and PCNA were unaffected by the presence in the genomic E1A virus of the domain-2 mutation at position 124, although this mutation severely impairs the ability of the E1A products to induce mitosis or proliferation of primary cells. The onset of DNA replication and the activation of PCNA synthesis are about 8 hours delayed during infection with viruses carrying simultaneous mutations in both domains 2 and 3 of the E1A region, but then come close to wild-type levels. The pattern of DNA synthesis observed during infection with these mutants is comparable to that occurring during infection with viruses carrying deletions of nonconserved E1A regions, which remain competent for the induction of rapid cell proliferation.

Each of a series of E1A region deletion mutants, removing in combination all but the amino-terminal 85 amino acids of the E1A region, remains competent to induce DNA synthesis. This result implies, at least indirectly, that more than a single E1A activity is involved in the control of the host-cell cycle and that these activities exert their effects at different points in the cell cycle. There seems to be an activity in the amino-terminal 85 amino acids that is sufficient for the induction of DNA synthesis in quiescent cells, as well as a second activity, localized in domain 2, that is required for progression through mitosis. If any physical interaction between these domains is required for their function, it appears to be independent of the spacing between them, since deletion of 35 intervening amino acids does not interfere with E1A-dependent induction of rapid proliferation in BRK cells.

Separate points of cell-cycle control, operating at the G_1/S-phase and the G_2/mitosis boundaries, have been demonstrated in yeast, and the possibility that the mammalian cell cycle is controlled at two separate points is suggested by the existence of

mutants that arrest in either G_1 or G_2. The identification and study of mammalian cell-cycle-control genes have been difficult, however, possibly because mammalian genes in situ are under complex regulation involving extracellular and cytoplasmic signals. Since the E1A gene seems to exert its effects independently of these signals, it may be an excellent tool for probing the biochemical mechanisms of cell-cycle control.

The amino-terminal 85 amino acids encode only the first of the three conserved domains of the E1A gene. To study the functions of this domain, we have begun a mutational analysis of this region. A deletion removing amino acids 22 to 107 abolishes the ability of adenovirus to induce both cell proliferation and DNA synthesis in infected quiescent primary BRK cells. The mutant retains some ability to activate viral early genes in proliferating cells, however: Using an E3-*cat* transient expression assay in HeLa cells, the viral E3 promoter was activated equally well by a plasmid containing this deletion and by a wild-type E1A plasmid. Nevertheless, viral infection assays at low multiplicity of infection reveal a defect in the ability of this mutant to support normal viral gene activation during productive viral infection. The production of infectious viral progeny or of late viral proteins is faster during infection with the domain-1 deletion mutant virus than during 12S cDNA viral infection, but slower than during wild-type viral infection.

The domain-1 deletion mutant plasmid, like the domain-2 deletion mutant plasmid described last year, has no detectable ability to cooperate with the *ras* oncogene in a cotransfection focus formation assay in BRK cells. Interestingly, foci are formed when both mutant plasmids are transfected simultaneously, together with the *ras* gene, suggesting that the activities of conserved domains 1 and 2 are independent enough to function even when present on different polypeptides. To explore an alternative explanation—that recombination between the two deleted E1A plasmids might regenerate the wild-type gene—single foci produced in the triple plasmid transfection experiment were grown out as stable cell lines. Consistent with the idea of independent domain function, immunoprecipitation revealed the expected set of E1A proteins and no proteins of wild-type appearance. Nevertheless, the possibility remains that recombination takes place at a level below the limit of sensitivity of this technique and that the establishment of the permanent cell lines results from integration of a reconstituted gene producing a single polypeptide containing both domains. This issue will be addressed by further experimental tests.

The independent nature of the conserved domains is supported by their correlation with splicing exons. Domain 3 consists essentially of the 13S unique region, whereas domain 1 is contained within the intron removed in forming two newly identified (10S and 11S) E1A splice products that accumulate late in infection (see Protein Immunochemistry Section). These products resemble the 12S or 13S products except for the removal of sequences coding for amino acids 27–98. Thus, naturally occurring E1A products are now known that contain either all three conserved domains (13S), domains 1 and 2 (12S), domains 2 and 3 (11S), or domain 2 only (10S).

We are continuing to explore the function of domain 1 in more detail, by generating additional deletion and missense mutations in this region and by probing the effects of this domain, alone and in combination with domain 2, on the regulation of the host-cell cycle. Since E1A can bring about both activation and repression of target genes, as well as the control of host-cell proliferation, it will be interesting to see whether the functional domains of E1A can serve as models in identifying active sites in cellular regulatory proteins with homologous functions.

Gene Activation by E1B

C. Herrmann, C. Déry, M.B. Mathews

The second gene required for full transformation of primary cells by adenovirus is the E1B gene, which lies adjacent to E1A at the left end of the viral genome. In last year's Annual Report, we discussed the response of adenovirus promoters to products of the E1A gene. Constructs in which each of the nine adenovirus promoters was fused to the chloramphenicol acetyltransferase (*cat*) gene were transfected into HeLa cells, and the activity of each promoter was assayed by measuring the level of *cat* enzyme produced. We found that all of the early promoters (E1A, E1B, E2e, E3, and E4) are active in HeLa cells and, with the exception of the E1A promoter itself, are stimulated by cotransfection with the E1A gene. The major late promoter (MLP)

and other late promoters (IX, IVa_2, and E2L) are virtually inactive, irrespective of the presence of E1A products. In 293 cells, human cells that constitutively express both E1A and E1B, all of the early promoters and the MLP are active, but the other late promoters (IX, IVa_2, and E2L) are not.

Continuing our study on the regulation of the adenovirus gene expression in HeLa cells using the promoter-*cat* constructs, we discovered that the early promoters, but not the late promoters, are also activated by a product of the other early region 1 gene, E1B. Consistent with a transcriptional effect, the stimulation of E1A-*cat* activity brought about by the presence of the E1B gene correlates with increased levels of *cat* mRNA in the cytoplasm of transfected cells. In contrast to the effects of E1A, however, the E1B gene products are capable of activating expression from the E1A-*cat* construct. Thus, the E1A region can be positively regulated by E1B polypeptides, but not directly by its own gene products.

To examine the mechanism of this effect, we created a series of deletion mutations in the upstream transcriptional control region of the E1A gene. Analysis of these mutants indicates that the stimulation of E1A expression by E1B is sequence-independent. For example, although the expression of E1A-*cat* activity is reduced by deletion of the E1A enhancer elements, the stimulation by E1B is nevertheless retained. We are presently conducting experiments to discover whether the increased mRNA accumulation is due to a transcriptional event (increased synthesis) or to a posttranscriptional event, such as more efficient processing or transport or increased stability.

The E1B region encodes two major proteins, with apparent molecular weights of 19,000 (19K) and 55,000 (55K), as well as several minor proteins. Since investigators in other laboratories have recently shown that the 55K protein facilitates mRNA transport from the nucleus to the cytoplasm of adenovirus-infected cells, it was important to determine which of the individual E1B polypeptides is responsible for the stimulation of E1A expression. We have tested a number of plasmids containing deletions of the E1B region, with the following result. Constructs synthesizing a normal 19K protein but a truncated 55K protein increase E1A expression to levels similar to those of wild-type E1B products, whereas constructs synthesizing a mutant 19K protein are unable to stimulate E1A expression above basal level. These data suggest that the elevated expression of E1A is due to an activity of the E1B 19K protein. Rather different experiments done by E. White (DNA Synthesis Section) suggest that the E1B 19K protein can act as a negative regulator of E1A activity. Work is in progress to resolve the seeming contradiction between these two sets of observations.

The Proliferating Cell Nuclear Antigen

M. Kostura, G.F. Morris, M.B. Mathews

The proliferating cell nuclear antigen, abbreviated PCNA and also known as cyclin, initially attracted our attention because it is recognized by antibodies from the sera of a subset (about 3%) of patients with the autoimmune disease, systemic lupus erythematosus. Synthesis of the protein is regulated during the cell cycle (hence the name cyclin) and is greatly augmented in rapidly dividing and transformed cells (hence PCNA). The protein is made predominantly late in the G_1 phase and early in the S phase of the cell cycle, and, correspondingly, it becomes detectable by immunofluorescence with autoantibody in the cell nucleus during the S phase. These observations suggested circumstantially that PCNA might be involved in cellular DNA synthesis. To explore its function, and explicitly to examine this possibility, we undertook the purification of PCNA by conventional chromatographic methods. In the course of this work, it became increasingly clear that the protein we were purifying on the basis of its immunoreactivity and size (apparent molecular weight of 36,000) resembled in its physical and chromatographic properties a DNA replication factor that was under investigation by G. Prelich and B. Stillman in the DNA Synthesis Laboratory. This factor is required for efficient origin-dependent replication of SV40 DNA in vitro, in a fractionated cell-free system derived from human 293 cells.

Collaborative work subsequently showed that PCNA and the replication factor are indeed one and the same. First, the replication factor comigrates with PCNA (defined antigenically) through several chromatographic steps and in a glycerol gradient. Second, they share the same isoelectric point and mobility in SDS-gel electrophoresis. Third, the replication factor (defined biochemically) blocks both nuclear immunofluorescence and immunoprecipitation reactions with anti-PCNA autoanti-

body. Fourth, analyses conducted with D. Marshak (Protein Chemistry Section) show that the two proteins are identical in sequence for at least the first 26 amino acids from their amino termini. Finally, adsorption by an anti-PCNA antibody column effectively removes PCNA and the replication factor activity.

These data prove beyond all reasonable doubt that PCNA is involved in SV40 DNA replication in vitro. We presume that it plays a similar role in cellular replication in vivo, although there is little direct evidence to offer as yet. The temporal and regulatory circumstances of its synthesis, and its subcellular localization, are consistent with this idea. In addition, the recent demonstration of its identity with an accessory factor required for efficient chain elongation by the cellular DNA polymerase δ (detailed in the report of the DNA Synthesis Section) lends further support.

Studies by M. Lambert (Two-dimensional Gel Laboratory) suggest an involvement with DNA repair mechanisms. Current efforts in our laboratory are directed toward an understanding of the regulation of PCNA synthesis and the cellular consequences of its expression.

Ribonucleoprotein Particles and Autoimmunity

C.C. Bunn, M.B. Mathews

Autoantibodies against cellular components such as PCNA are made in a number of diseases. Last year, we described a trio of autoantibody specificities characteristic of the muscle-wasting diseases polymyositis and dermatomyositis, especially in conjunction with severe pulmonary complications. These antibodies differ from those common in connective tissue diseases like systemic lupus erythematosus in recognizing cytoplasmic constituents, rather than nuclear constituents. All three specificities react with charging enzymes, aminoacyl-tRNA synthetases, and one of them (known as PL-12) additionally reacts with the corresponding tRNA.

It is widely believed that clues to the origin of the autoimmune response and to the etiology of the disease may arise from an understanding of the nature of the antibodies and their reaction with antigen. To this end, we have embarked on a detailed analysis of the PL-12 specificity. Antibodies of this class recognize both alanyl-tRNA synthetase and $tRNA^{Ala}$. So far, we have determined the complete nucleotide sequence of two of the $tRNA^{Ala}$ species immunoprecipitated by PL-12 antibody and the partial sequence of a third species. All three tRNAs contain the anticodon IGC embedded in a common anticodon loop, but there are differences elsewhere in the molecules, including the anticodon stem. Recent data indicate that the antibody actually reacts with the anticodon region. When the immune complex, containing the antibody and tRNA, is exposed to ribonucleases, the anticodon region is found to be protected against digestion. Furthermore, submolecular $tRNA^{Ala}$ fragments containing the anticodon loop retain the ability to interact with antibody, whereas fragments containing other regions of the molecule do not. Half-molecules cut in the anticodon itself fail to bind, emphasizing the importance of the anticodon for antibody recognition. Knowing this fact and that the PL-12 system contains antibodies reactive with both members of an RNA-enzyme complex, it seems possible that the antibodies react with the RNA and enzyme at the sites of their interaction with one another. We are now beginning to address this question.

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TRANSCRIPTIONAL CONTROL

W. Herr R. Aurora, T. Baumruker, S. Burgess, J. Clarke, J. Harper, B. Ondek, A. Shepard, R. Sturm, M. Tanaka

The focus of our research is regulation of transcription and regulation of posttranscriptional mRNA processing. We use SV40 to probe transcriptional control in mammalian cells. Our strategy has been to use viral genetics to identify and characterize functional elements within the enhancer of the SV40 early promoter. Enhancers are promoter elements that can activate transcription from a distant position either upstream or downstream from the transcriptional initiation site. Over the past few years, we have described a series of SV40 enhancer mutants in which three sets of closely spaced double point mutations were engineered into three distinct regions of the SV40 enhancer. These mutations impair both enhancer activity and SV40 growth and have allowed the isolation of numerous viral revertants. These revertants carry characteristic tandem duplications within the mutated enhancer region that are responsible for restored activity. Comparison of the duplication patterns has allowed identification of three separate SV40 enhancer elements called A, B, and C, each between 15 and 22 bp in length. We have now centered our attention on the characterization of these three elements by assaying their intrinsic enhancer activities, the cell specificity of their function, and the nature of the *trans*-acting factors that interact with these elements.

Our results lead to a picture of the SV40 enhancer as a composite of individual enhancer elements, each responding differently to the cellular environment.

To study regulation of posttranscriptional RNA processing, we have focused on the regulation of alternative splicing of the adenovirus early region 1A (E1A) gene. Our main interest is to identify factors that influence alternative splicing of mRNAs, and to this end, we are making use of an in vitro system to study splicing of E1A RNA precursors. Using wild-type and mutant E1A precursors, we have been able to establish a hierarchy of splice site selection in vitro. We are now establishing how alterations in the in vitro splicing reaction can alter the ratios of splicing products.

Functional Elements within the SV40 and Heterologous Enhancers

J. Clarke, M. Tanaka, R. Aurora, W. Herr

During the past year, we have continued our analysis of SV40 revertants of the different SV40 double point mutations (the *dpm* series). Figure 1 shows, with respect to the SV40 early promoter, the location of the A, B, and C SV40 enhancer elements that we have identified by revertant analysis. Also shown is the sequence of each element, along with the base changes involved in each of the *dpm* mutations. The mutations are called *dpm*1, *dpm*2, and *dpm*6 in A, B, and C elements, respectively, and combinations of these mutations are called *dpm*12 for the A + B element mutant, etc. Analysis of revertants of all the pairwise combinations of *dpm* mutations has shown that, by duplication, each of the A, B, and C elements can compensate for loss of enhancer function in the remaining two elements.

During the course of these experiments, we also found that the revertant duplications can arise by illegitimate recombination between separate viral genomes because a chimeric *dpm*2/*dpm*16 recombinant was obtained in a mixed infection with the *dpm*2 and *dpm*16 viruses. To show conclusively that it is duplication of the wild-type elements per se that is responsible for restored enhancer activity of the revertant viruses, we have taken a *dpm*12 revertant, which contains mutated A and B elements and a duplicated C element, and mutated each C element separately. Whichever of the two C elements is mutated, there is loss of virus viability, showing clearly that both elements are important for restored virus viability.

The experiments described above have led to the identification of three separate SV40 enhancer elements. We are taking two related approaches to analyze further elements within the SV40 and heterologous enhancers: The SV40 enhancer has been cloned into polyomavirus, and heterologous viral and cellular enhancers have been cloned into SV40.

To date, the analysis of SV40 revertants has been carried out in the African green monkey kidney (AGMK) cell line CV-1. Because of a block in DNA replication, the host range of SV40 is largely restricted to AGMK cell lines. The SV40 enhancer may contain more than the three elements described so far, but these putative additional elements may have gone undetected because they can-

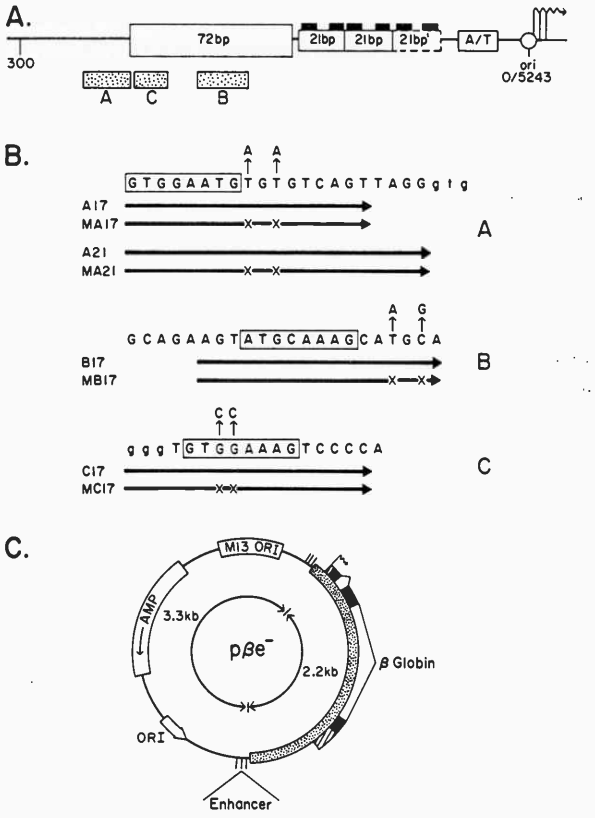

FIGURE 1 SV40 early promoter region showing the location and sequence of the A, B, and C elements, the structure of the synthetic oligonucleotides, and the pβe⁻ expression plasmid. (*A*) Diagram of the SV40 early promoter showing, from right to left, the early transcriptional start sites, origin of replication, AT-rich TATA-like element, one imperfect (dashed box) and two perfect 21-bp repeats complete with Sp1 binding sites (black boxes), and a single copy of the 72-bp element. The A, B, and C enhancer elements (stippled boxes) are shown below their corresponding position within this region. (*B*) The complete nucleotide sequence of each of the three elements A, B, and C is shown in uppercase letters, with flanking sequences shown as lowercase letters. The 8-bp consensus sequences, the SV40 core in A and C and the octamer in B, are boxed. The A, B, and C enhancer-debilitating double point mutations, *dpm*1, *dpm*2, and *dpm*6, respectively, are shown above the wild-type sequences. The sequences included in the oligonucleotides used to create synthetic enhancers are shown below the wild-type sequence by a black arrow; the name of each oligonucleotide is shown to the left. The broken arrows with the Xs indicate the corresponding *dpm*1, *dpm*2, or *dpm*6 mutant oligonucleotides. (*C*) The experimental plasmid pβe⁻ is depicted with the pUC119 sequences as a solid line interrupted with open boxes representing the M13 replication origin, β-lactamase gene, and plasmid origin of replication. The polylinker (hatch marks) is split by insertion of the human β-globin gene sequences (stippled box). The β-globin start site is identified by a wavy arrow, with introns shown as bent lines linked to exons shown as hatched (untranslated) and solid (translated) boxes. The downstream enhancer cloning site (*Sph*I) is labeled enhancer, and its distance from the β-globin start site is shown on the inner circle.

not function effectively in CV-1 cells. To test this possibility, we are using polyomavirus as a vector to isolate SV40 enhancer rearrangements. As with SV40, rearrangements such as duplications within the polyomavirus enhancer can activate enhancer function. Furthermore, growth of polyomavirus in different cell lines is frequently limited by the activity of the enhancer region. Therefore, selection for host-range mutants generally gives rise to new enhancers. These experiments necessitate the construction of a viable polyomavirus-SV40 enhancer recombinant in which the polyomavirus enhancer sequences have been deleted. We have succeeded in constructing such a chimeric virus that can grow in the mouse embryo fibroblast cell line NIH-3T3. We now plan to determine the effect of the *dpm* series of mutations on growth of this chimeric virus and to isolate revertant viruses in those cases where the mutants do not grow.

To study heterologous viral and cellular enhancers, we have performed the complementary experiment by replacing the SV40 enhancer with either one or the other of two separate regions of the polyomavirus enhancer, or with either the immunoglobulin heavy-chain or insulin enhancers. The polyomavirus enhancer region can be divided into two separate restriction fragments, called A and B, that can each act as an enhancer. To test the feasibility of these SV40 enhancer replacement experiments, we have chosen to use these two polyomavirus enhancer fragments because Weber et al. (*Cell 36*: 983 [1984]) have shown that tandem copies of the polyomavirus-"A" enhancer can functionally replace the SV40 enhancer. The SV40 enhancer region was replaced by a single copy of the A or B enhancer, and chimeric viral DNA was transfected into CV-1 cells to assay for virus growth.

We were unable to obtain plaques with either construct, but revertants of the polyomavirus-A enhancer replacement did arise after passage of viral lysates in CV-1 cells. These revertants contained duplications that all spanned a single region 28 bp in length. This 28-bp polyomavirus-A enhancer element contains a consensus sequence that is shared with the adenovirus E1A enhancer and overlaps a 26-bp region that has been shown to confer enhancer activity when present in multiple tandem copies (Veldman et al., *Mol. Cell. Biol. 5*: 649 [1985]). These results suggest that SV40 can be used to identify elements within heterologous enhancers. To date, however, we have not obtained revertants of the polyomavirus-B enhancer replacement, nor of chimeric SV40 carrying the immunoglobulin heavy-chain or insulin gene enhancers.

SV40 A, B, and C Elements Represent Autonomous Enhancer Elements

B. Ondek, W. Herr

SV40 revertant analyses identified the A, B, and C enhancer elements but did not address whether these elements themselves are sufficient for enhancer function. By using short, synthetic DNA oligonucleotides, we have shown that each of these elements can act autonomously as an enhancer when present as multiple tandem copies. Figure 1B shows the sequences of the 17-bp-long double-stranded DNA fragments, called A17, B17, or C17, that we synthesized to assay the activity of individual elements. The fragments were ligated as multiple tandem copies into a plasmid vector containing the human β-globin gene. This vector, called pβe$^-$ (see Fig. 1C), lacks SV40-derived sequences, and the synthetic enhancers are positioned 2.2 kb downstream or 3.3 kb upstream of the initiation site for β-globin transcription. Assay of these constructs, by transient expression in CV-1 cells, showed that the 17-bp fragments representing the B and C elements could function as enhancers. The A-element construction, however, was defective for enhancer function. This led us to test a 21-bp synthetic fragment containing the entire A-element sequence (Fig. 1); multiple tandem copies of this fragment effectively enhance transcription. These results show that each of the three elements that were identified by SV40 revertant analysis possesses intrinsic enhancer function.

To test the authenticity of the activities of these synthetic enhancers, we constructed parallel oligomerized elements containing the series of *dpm* mutations that debilitate these elements within the context of the SV40 enhancer. Each of these mutant oligonucleotide constructs lacks any detectable enhancer activity. In contrast to this drastic inactivity, the effect of the *dpm* mutations is variable when in the context of the SV40 enhancer region. In CV-1 cells, the *dpm6* mutation in the C element has the largest effect on enhancer function, whereas the *dpm1* mutation in the A element has the least effect. Because we now know that the mutations completely inactivate these elements, these results suggest that the relative contribution of

these three elements to SV40 enhancer function in CV-1 cells is A<B<C. We do not know, however, whether these relative activities are due to the intrinsic strength of these elements, their neighboring SV40 enhancer sequences, or their relative position with respect to other promoter elements.

We have used the synthetic enhancers to study the bidirectional activity and copy number requirement of individual enhancer elements. To study directionality, B-element and C-element DNA fragments containing six copies of either element were cloned into pβe⁻ in both orientations and assayed for activity. The results show that these individual elements can each function in either orientation. This suggests that the bidirectional activity of the SV40 enhancer region results from the accumulation of a number of individual bidirectional enhancer elements and is not due to the grouping of a number of unidirectional elements in opposite directions. Analysis of a progressive series of B-element oligomers shows that a single copy of the B element is ineffective in enhancing transcription; with two or more elements, however, enhancer activity increases linearly with each added (up to 14) B-element oligomer.

SV40 Enhancer Elements Display Different Cell-specific Enhancer Activities

A. Shepard, B. Ondek, W. Herr

We have employed the synthetic enhancers described above to study the relative activity of these SV40 enhancer elements in different cell lines. Using the B- and C-element constructs containing six tandem copies of either element (6×B17 and 6×C17), we tested the relative levels of β-globin expression in four different cell lines, in addition to CV-1 cells: NIH-3T3, a mouse embryo fibroblast cell line; NS-1, a nonsecreting mouse myeloma cell line; MDCK, a canine kidney epithelium-like cell line; and HeLa, a human epithelioid cervical carcinoma cell line. Except for one case, these constructs were active in all of these cell lines; the B-element construct was not active in NIH-3T3 cells. Most striking, however, was that the individual elements displayed different activities relative to each other in the various cell types. The 6×B17 enhancer was more active than 6×C17 in CV-1 and MDCK cells, whereas in NIH-3T3, NS-1, and HeLa cells, the 6×C17 enhancer was more active. These results indicate that the SV40 enhancer B and C elements have different cell-specific enhancer activities. In all cases, the closely spaced *dpm* mutations within the B and C elements abolished enhancer function in all cell lines tested. Taken together, the activity of the wild-type oligomers and the inactivity of the mutant oligomers indicate that the B and C elements represent single units of enhancer function.

The differential cell-specific activity of the B and C elements and the ability of these elements to enhance transcription autonomously when duplicated have led us to suggest that the duplication of these elements by the wild-type SV40 72-bp repeat serves to broaden the conditions under which the SV40 enhancer can activate transcription. Figure 2 shows schematically how a duplicated enhancer (2×72) may activate transcription under conditions that the nonduplicated form (1×72) is inactive. At the top of panels A and B is shown the 1×72 (A) and 2×72 (B) enhancers interacting with putative A-, B-, and C-element-specific *trans*-acting factors. Under these conditions, in the presence of all three factors, both enhancers should be active because they are each capable of enhancing transcription. In the presence of just a single B- or C-element-specific factor, however, we predict that the 1×72 enhancer will be relatively inactive, whereas the 2×72 enhancer will still effectively enhance transcription. This is because the activity of 1×72 in the presence of just B- or C-element factors will be similar to the weak activity of the *dpm*16 (A + C mutated) and *dpm*12 (A + B mutated) enhancers, whereas the 2×72 enhancer will activate transcription effectively as do the *dpm*16 and *dpm*12 revertants, which have a single wild-type element duplicated.

These interpretations suggest that by carrying single copies of two different elements that recognize different *trans*-acting factors, the function of an enhancer is restricted to the presence of both *trans*-acting factors. By tandem duplication of these same elements, however, expression is expanded to the presence of either factor. Thus, appropriate manipulation of enhancer elements can either restrict or expand the conditions under which a promoter can operate. Consistent with these interpretations, we find that the activities of the revertant enhancers, which contain duplications of a single functional element, show different host-cell preferences.

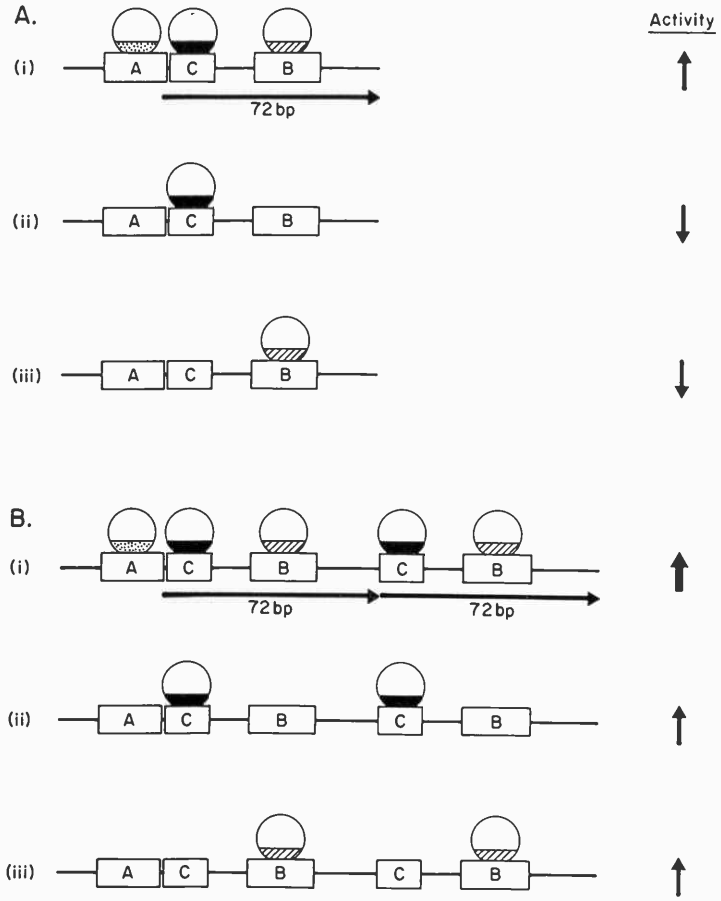

FIGURE 2 Putative interactions and relative activities of the 1×72 and 2×72 SV40 enhancers in the presence of different sets of *trans*-acting factors. The position of the A, B, and C elements is shown by the labeled rectangular boxes, and the regions located within the 72-bp element are shown by the black arrows. Putative element-specific *trans*-acting factors are shown as circles, with the bottom portion shaded differently to emphasize the postulated different DNA-binding specificities of these factors. *A* shows the 1×72 enhancer and *B* the 2×72 enhancer in three states: in the presence of all three A-, B-, and C-element-specific factors (i), the C-element-specific factor alone (ii), and the B-element-specific factor alone (iii). The expected relative activities of each enhancer in these three states are shown as very high activity (heavy up-arrow), high activity (thin up-arrow), and low activity (down arrow).

Late Transcription in Revertants of SV40 Enhancer Mutants

S. Burgess, W. Herr

The late promoter of SV40 overlaps the early promoter in the opposite orientation and is activated by the early gene product large T antigen. Unlike the early promoter, which contains a closely spaced set of transcriptional start sites, the late transcripts are initiated at a large number of sites spread over the entire enhancer region. Although the majority of late RNAs correspond to initiation at sites surrounding nucleotide 325, nearly half of the late RNAs are initiated elsewhere. It is not understood how these initiation sites are selected or how their levels are regulated. We have asked whether point mutations that reduce enhancer function have an effect on late transcriptional initiation site selection. To accomplish this, we isolated RNAs late after infection of CV-1 cells with the viral revertants of the *dpm* mutants.

To measure the abundancies and structure of the late transcripts, CV-1 cells were infected with SV40 and late RNAs were collected when present at maximal levels, as determined by Northern blot analyses. The RNAs were hybridized to one of two ^{32}P-labeled oligonucleotides downstream from the transcriptional initiation sites, and a cDNA was synthesized by reverse transcriptase in the 5′ direction of the transcript. These cDNA fragments were separated by electrophoresis through denaturing polyacrylamide gels alongside sequencing reactions primed with the same oligonucleotide. The relative abundancies of the late transcriptional start sites could thus be determined. The experiments indicate that the *dpm*1 mutations that lie within a cluster of late transcriptional start sites inhibit the use of these initiation sites, but that the different revertant duplications seem merely to duplicate the het-

erogeneous initiation sites. These results are consistent with the hypothesis that late transcriptional initiation sites are influenced by local nucleotide sequence but not "distant" elements such as a TATA box.

Interaction between SV40 Enhancer Elements and Nuclear Factors Isolated from HeLa Cells

R. Sturm, T. Baumruker, W. Herr

To examine the nature of *trans*-acting factors that may interact with the SV40 A, B, and C elements, we have performed "gel retardation" assays using nuclear extracts from HeLa cells. In the gel retardation assay, a radioactive DNA fragment is mixed with a cellular extract, and, after an incubation period to allow for complex formation, it is subjected to electrophoresis through a polyacrylamide gel. DNA fragments, which form complexes that are stable during such an electrophoretic separation, are retarded in mobility with respect to the free fragment. When an SV40 enhancer fragment encompassing the A, B, and C elements (but not the 21-bp repeats) is mixed with a partially fractionated HeLa cell nuclear extract, we observe two major complexes. These complexes can be inhibited by competition with restriction fragments containing six copies of the B17 oligomer (refer to Fig. 1) but not a 6×C17 fragment. This result suggests that the complexes are formed by interaction with the B element.

The B17 oligomers, when placed in tandem, recreate 18 bp of wild-type SV40 sequence. This 18-bp sequence contains two popular sequence motifs: the octamer sequence ATGCAAAG and two near-perfect 9-bp repeats, $AAG^{T}/_{C}ATGCA$. The octamer sequence is shown boxed in Figure 1 and is closely related (7/8 match) to a sequence found in a number of promoters, including histone, snRNA, and immunoglobulin gene promoters. The *dpm*2 mutations, which completely inactivate the B element, lie outside of this octamer sequence. Complex formation is inhibited not only by competition with the wild-type 6×B17 enhancer, but also by the mutant *dpm*2 B17 construct 8×MB17. This has led us to analyze the significance of the octamer sequence.

We have created a further B-element double point mutation called *dpm*7 that maps in the middle of the octamer domain (AT*TT*AAAG; the *dpm*7 mutations are italicized) and also maps in the upstream 9-bp repeat. The effect of the *dpm*7 mutant on virus viability is significantly less than the effect of the *dpm*2 mutations. Preliminary experiments indicate that a synthetic enhancer composed of a *dpm*7-containing 17-bp B oligomer can function to enhance transcription of the human β-globin gene as well as the wild-type 17-bp oligomer. In gel retardation experiments, the complexes formed with the SV40 enhancer are only slightly affected by either the *dpm*2 or *dpm*7 mutations alone but are considerably reduced in quantity when the two sets of point mutations are combined. These results are consistent with interactions with the two 9-bp repeats, rather than with the octamer sequence. This B-domain DNA-protein interaction is being further investigated using dimethylsulfate interference studies and DNase I footprint analysis. We plan to purify this activity further and to use in vitro transcription assays to determine the role, if any, that this DNA-protein interaction plays in SV40 enhancer function.

To date, enhancer elements have not been shown to activate transcription in vitro over large distances; therefore, as a preliminary step to establishment of an in vitro transcription assay, we have placed the synthetic oligomerized A, B, and C elements just upstream of the human β-globin promoter. When assayed in vivo in either HeLa or CV-1 cells, these constructs effectively activate transcription. We now plan to use these constructs to assay the activity of the factor(s) that interacts with the B element.

In Vitro Splicing of Adenovirus E1A Pre-mRNA

J. Harper, W. Herr [in collaboration with R. Roberts, Cold Spring Harbor Laboratory]

We are continuing our experiments on in vitro splicing of adenovirus E1A pre-mRNA. Our primary interest is to evaluate factors that may influence alternative splicing of mRNAs. In general, cellular genes produce a single mRNA by utilization of a single splicing pathway; however, a number of genes produce multiple mRNAs by alternative RNA splicing. In many instances, these mRNAs are differentially expressed in a tissue-specific or temporally regulated manner, suggesting that regula-

tion of RNA splicing may play a role in regulating gene expression during development. We have focused on the adenovirus E1A gene as a model system because of its small size and relatively simple alternative splicing pattern. E1A encodes multiple mRNAs that differ only in the internal sequences spliced out. Three of these, the 13S, 12S, and 9S RNAs, are produced by splicing from different 5′ splice sites to a common 3′ splice site. Expression of these RNAs is differentially regulated during the course of adenovirus infection: 13S and 12S RNAs are expressed throughout the lytic cycle, whereas 9S RNA does not accumulate until late times after infection. Additional E1A mRNAs produced by splicing from the 9S 5′ splice site to an adjacent 3′ splice site also appear to be expressed specifically at late times (see C. Stephens and E. Harlow, Protein Immunochemistry Section). This strengthens the suggestion that the 9S 5′ splice site is active specifically at late times.

Our first goal was to determine the relative strengths of the 13S, 12S, and 9S 5′ splice sites under standard in vitro splicing conditions. Comparison of the splicing products of wild-type and mutant E1A pre-mRNAs in HeLa cell nuclear extracts indicates that *cis*-competition between 5′ splice sites determines the ratio of RNAs produced. Splicing the wild-type E1A substrate yields predominantly 13S products. 9S splicing occurs at about 5% of the level of 13S splicing, and 12S splicing is less than 2% of 13S levels. Splicing a mutant substrate in which the 13S 5′ splice site is inactivated yields predominantly 12S splicing products and 9S splicing products at somewhat higher levels than with the wild-type substrate. Using a double-mutant substrate in which both the 13S and 12S 5′ splice sites are inactivated yields predominantly 9S splicing products. Other E1A splicing products produced at low levels in these reactions have not yet been fully characterized. These results indicate that the 13S 5′ splice site is extremely strong relative to the 12S and 9S 5′ splice sites in these reactions and that competition by the 13S 5′ splice site affects 12S splicing more strongly than it does 9S splicing.

The goal of our subsequent experiments has been to determine whether or not alterations of the splicing extracts can alter the ratios of splicing products. In particular, we have been interested in identifying factors that might modulate the level of the late specific 9S RNA relative to the early 13S and 12S RNAs. We examined the effect of moderate heat treatment of the extracts on their ability to carry out splicing of E1A pre-mRNA. We find that 9S splicing activity in the extract is inhibited more severely by the heat treatment than 12S or 13S splicing activity. This may reflect different affinities of the three 5′ splice sites for a commom heat-sensitive splicing factor or heat sensitivity of a specific factor required for 9S splicing.

We have surveyed a series of different extracts and crude fractions of extracts for activities that might alter the ratio of 9S splicing relative to 12S or 13S splicing during in vitro reactions. Because some of the extracts and fractions assayed are not capable of carrying out in vitro splicing reactions, all activities were assayed in extract-mixing experiments. In these experiments, the splicing reaction is supported by a receptor extract and the extract or fraction to be tested constitutes one sixth of the total extract volume. We have found two activities that have opposite effects on the ratio of 9S to 12S splicing. The first activity, present in nuclear extracts prepared from adenovirus-infected cells at late times after infection, stimulates 9S splicing relative to either 12S or 13S splicing when assayed in an uninfected HeLa cell receptor extract. Extracts prepared from uninfected or early adenovirus-infected cells lack this activity. We find, however, that the particular uninfected cell extract used as a receptor is critical for detection of this activity, and we are currently characterizing this heterogeneity among receptor extracts. The 9S stimulating activity fractionates with high-molecular-weight material on a sizing column and precipitates at an ammonium sulfate concentration between 40% and 70% saturation. The second activity stimulates 12S splicing and inhibits 9S splicing when assayed in a partially heat-inactivated extract. It is found in the fraction of a whole-cell extract that precipitates at an ammonium sulfate concentration between 10% and 30% saturation but is absent from either a mixture of the 10–30% and a 30–60% fraction or a 10–60% fraction, suggesting that some component of the 30–60% fraction inhibits this activity. These activities may represent *trans*-acting factors that regulate the splicing pattern of the E1A pre-mRNA. We are working to purify the factors responsible for these activities.

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In Press, Submitted, and In Preparation

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MOLECULAR GENETICS OF EUKARYOTIC CELLS

This section includes three laboratories using molecular approaches to understanding aspects of the cellular and organismal biology of eukaryotes. The control of the expression of the α_{2u} globulins (see Hormonal and Developmental Control of Gene Expression) is the focus of the laboratory of D. Kurtz. The α_{2u} globulins are products of a multigene family in rats; they are of unknown function. Their expression is under the control of multiple hormones that act at multiple levels as well as under complex developmental control. The control of cell growth (see Genetics of Cell Proliferation) is the focus of the laboratory of M. Wigler. Cellular oncogenes that can positively alter the proliferative capacity of cells are being characterized. A human oncogene (*mas*) that encodes a product similar in structure to the visual rhodopsins has recently been discovered. The oncogene *ros* has been shown to be expressed in glioblastomas and may be a marker for that neoplasia. Particular attention is given to the function of the *ras* oncogenes that are involved in a wide variety of human neoplasia. The *ras* oncogenes have structural and functional homologs in the yeast *Saccharomyces cerevisiae*, and the powerful genetics of yeast is being applied to understanding *ras* function. The laboratory of D. Hanahan (Molecular Biology of Mouse Development) is using the extremely powerful method of germ-line genetic manipulation to study several aspects of gene expression and tumor formation in mice. Introduction into the germ line of the SV40 T-antigen gene under the control of the insulin gene promoter/enhancer leads to the heritable development of pancreatic β-cell tumors. Studies of such mice have led to the discovery of a model for autoimmune diabetes. Other tissues have been targeted for oncogenesis using tissue-specific promoters. Introduction of a papilloma genome leads to the development of dermal fibrosarcomas. This type of approach should lead to deep insights into the neoplastic process and the development of animal models for human disease.

GENETICS OF CELL PROLIFERATION

M. Wigler C. Birchmeier, D. Broek, T. Kataoka, S. Powers, T. Toda, G. Bolger, J. Colicelli, K. Ferguson, J. Field, T. Michaeli, J. Nikawa, P. Sass, S. Sharma, D. Young, S. Cameron, K. O'Neil, M. Riggs, L. Rodgers, G. Waitches

Our laboratory has continued work on growth control in eukaryotes. Two experimental systems are employed: the yeast *Saccharomyces cerevisiae* and cultured mammalian cells.

The yeast studies have grown from a study of the function of mammalian *RAS* oncogenes. Close structural and functional homologs exist in yeast, and they control adenylate cyclase. Two other human oncogenes are under intensive scrutiny: the *ros* gene, which appears to be expressed in glioblastomas and encodes a transmembrane tyrosine kinase, and the *mas* gene, which encodes a protein that resembles the visual rhodopsins in structure.

Growth Control in *S. cerevisiae*

M. Wigler, T. Kataoka, S. Powers, T. Toda, D. Broek,
P. Sass, J. Nikawa, J. Field, K. Ferguson,
J. Colicelli, T. Michaeli, S. Cameron

The effort of this section is directed to define the components of the *RAS/CYR* (adenylate cyclase) pathway in yeast and to study their interactions. Work from previous years (see last year's Annual Report) resulted in cloning the two *RAS* genes *RAS1* and *RAS2*; *SUPH,* a gene required for maturation of *a* factor and *RAS* proteins (Powers et al., *Cell 47:* 413 [1986]); *CDC25,* a gene required for progression through G_1 (Broek et al., *Cell* [1987] in press); *CYR,* which encodes adenylate cyclase; *TPK1, TPK2,* and *TPK3,* which encode the cAMP-dependent protein kinase catalytic subunits (T. Toda et al., in prep.); *BCY1,* which encodes the cAMP-dependent protein kinase regulatory subunit (Toda et al., *Mol. Cell. Biol.* [1987a] in press); *PDE2,* which encodes the high-affinity cAMP phosphodiesterase (Sass et al., *Proc. Natl. Acad. Sci. 83:* 9303 [1986]); and a variety of auxiliary genes of unassigned function (*JUN1, JUN2, SUPC, SCH1, SCH2,* and *SCH9*). There have been several new developments in this area. *CDC25* appears to control *RAS* function, and we have found dominant lethal *RAS* mutations. Biochemical studies now clearly indicate the complete GTP dependence of *RAS*. Progress has been made in purifying yeast adenylate cyclase. Study of the *TPK* genes has led to a better understanding of their role in regulating physiologic responses. We have cloned and sequenced both yeast cAMP phosphodiesterase (*PDE1* and *PDE2*) genes. Disruption of the *PDE*s or mutations in the cAMP-dependent protein kinases (*TPK* genes) have revealed an extraordinarily powerful feedback mechanism for cAMP homeostasis. Progress has been made in characterizing additional genes related to the *RAS/CYR* pathway (*SCH1, SCH2, SCH9, JUN1, JUN2*, and *SUPC*). Our progress is described in greater detail below.

SUPH was originally isolated as an allele capable of suppressing the phenotypic effects of the mutant of $RAS2^{val19}$. The *supH* alleles were found to be *a*-specific sterile. In collaboration with members of I. Herskowitz's laboratory at the University of California (San Francisco), we showed that *supH* strains fail to secrete *a*-factor, fail to palmitylate yeast *RAS* proteins, and fail to palmitylate many other yeast proteins that are normally palmitylated. We derive two conclusions from these findings: (1) The *SUPH* gene product is required for the normal maturation of proteins containing the Cys-*A*-*A*-*X* (where *A* is any aliphatic amino acid and *X* is the terminal amino acid) consensus sequence for palmitylation, and (2) this processing event is required for the maturation and/or secretion of *a*-factor (Powers et al., *Cell 47:* 413 [1986]).

CDC25 was first described by Hartwell et al. (*Genetics 74:* 267 [1973]) as the wild-type allele of a temperature-sensitive mutation causing G_1 arrest. This gene was cloned, sequenced, and characterized (Broek et al., *Cell* [1987] in press). It encodes a 1589-amino-acid protein with no obvious structural similarities to known proteins. Disruption of *CDC25* causes a lethal phenotype that can be suppressed by $RAS2^{val19}$ but not by wild-type *RAS2.* Most recent biochemical experiments indicate that the adenylate cyclase of *S. cerevisiae* lacking *CDC25* has a very low level of activity in the absence of guanine nucleotides. From biochemical and genetic data, we have concluded that the *CDC25* product controls *RAS* protein function (Broek et al., *Cell* [1987] in press). Indeed, we have identified a mutant *RAS* protein that behaves as though it complexes irreversibly with *CDC25* (see below).

RAS1 and *RAS2* genes have been previously characterized (Kataoka et al., *Cell 37:* 437 [1984]; *Cell 40:* 19 [1985]; Powers et al., *Cell 36:* 607 [1984]; Toda et al., *Cell 40:* 27 [1985]). Recent biochemical experiments with *RAS* proteins purified from *Escherichia coli* expression systems indicate that they fail to stimulate adenylate cyclase when bound to a guanine dinucleotide (Field et al., *Mol. Cell. Biol.* [1987] in press). These results strongly confirm the model of *RAS* activation by point mutations that destroy intrinsic GTPase activity. In addition, we have determined that, in vitro, *RAS* bound to guanine diphosphates does *not* compete with *RAS* bound to guanine triphosphates for stimulation of adenylate cyclase (Field et al., *Mol. Cell. Biol.* [1987] in press).

In an unrelated series of experiments, a dominant temperature-sensitive lethal *RAS2* mutation was found during a search for genes capable of suppressing the phenotypic effects of $RAS2^{val19}$. This temperature-sensitive defect cannot be suppressed by high-copy *RAS2* or by high-copy *CDC25* alone, but it can be suppressed by high-copy *CDC25* when at least one copy of wild-type *RAS* is present. These

experiments indicate the existence of a novel class of *RAS* mutants capable of blocking the *RAS* pathway in a dominant fashion. The simplest explanation of the particular allele we have discovered is that it encodes a protein that forms an ineffective complex with *CDC25*. The existence of dominant interfering mutations in regulatory components has important implications for strategies to understand *RAS* function as well as the functions of other oncogenes.

We are still testing the hypothesis that *RAS* has an alternate function in addition to stimulating adenylate cyclase. This hypothesis appears to be needed to explain two curious observations. First, disruption of the *CYR1* gene is not always lethal, whereas disruption of both *RAS* genes invariably is (Toda et al., in *Oncogenes and Cancer,* Japan Scientific Societies Press, Tokyo, Japan [1987b] in press). Second, disruptions of *CYR1* can be readily suppressed by high-copy *TPK* genes, but disruptions of *RAS* suppressed by these genes are temperature-dependent. We are exploring the hypothesis of alternate *RAS* function by both genetic and biochemical analyses.

CYR1 encodes adenylate cyclase. Its sequence and preliminary structural characterization were previously published (Kataoka et al., *Cell 43:* 493 [1985]). Our current effort is directed at determining how the activity of adenylate cyclase is regulated. As a first step, we have begun to purify this enzyme from *S. cerevisiae.* For this purpose, we have constructed a modified *CYR1* gene encoding a product with a novel amino-terminal domain. This domain consists of a peptide derived from an influenza virus protein, for which we have obtained monoclonal antibodies from R. Lerner and I. Wilson at the Research Institute of Scripps Clinic. A single-step affinity purification with elution by peptide has led to over 100-fold purification of yeast adenylate cyclase activity. This purified activity is still *RAS*-responsive. These studies should reveal whether there are other proteins that purify as part of the adenylate cyclase complex. Recent studies of the phosphodiesterases and protein kinases indicate that there is a negative feedback control of cAMP production (see below). We are testing to determine if part of this feedback control results in a physical modification of adenylate cyclase.

BCY1 encodes the regulatory subunit of the cAMP-dependent protein kinase (Toda et al., *Mol. Cell. Biol.* [1987a] in press), and its properties were reported in last year's Annual Report. Disruption of *BCY1*, like the introduction of the $RAS2^{val19}$ gene, induces severe aberrations in G_1 arrest, carbohydrate accumulation, carbon source utilization, germination, sporulation, and heat-shock resistance. Experiments described below indicate that the phenotypes of disruptions of *BCY1* appear to be mediated through its associated kinases. We have been unable to assign any role to *BCY1* other than its control of the *TPK* genes.

TPK1, TPK2, and *TPK3* encode the cAMP-dependent protein kinases (T. Toda et al., in prep.). Their properties were partly described in last year's report. More recently, we have obtained intragenic mutations in each of the *TPK* genes that suppress the heat-shock-sensitive phenotype of *BCY1* disruptions. Cells containing these TPK^w alleles and *bcy1* therefore have a kinase activity that is not responsive to fluctuations in cAMP. Yet such cells can arrest in G_1 upon starvation, can utilize nonfermentable carbon sources, can sporulate and germinate correctly, and can accumulate glycogen appropriately (S. Cameron et al., in prep.). Thus, the entire panoply of physiologic responses thought to be at least partly controlled by the cAMP effector system is performed normally despite the inability to modulate this effector system. Therefore, these processes must be under coordinate control, with the cAMP kinase system a contributing (but not exclusively determining) function. Most recently, study of the TPK^w genes has led to insights into the feedback control of cAMP production (see below).

PDE1 and *PDE2,* respectively, encode the low- and high-affinity cAMP phosphodiesterases of *S. cerevisiae* (Sass et al., *Proc. Natl. Acad. Sci. 83:* 9303 [1986]; J. Nikawa et al., in prep.). The *PDE2* gene was initially described last year. It has weak homology with several vertebrate phosphodiesterases. *PDE1,* recently isolated as a high-copy suppressor of $RAS2^{val19}$, has weak homology with phosphodiesterases of the slime mold, but no homology with *PDE2.* Thus, there are at least two distinct branches of phosphodiesterases in evolution.

Gene-disruption experiments with the phosphodiesterases have led to the surprising discovery of a rigorous feedback control of cAMP production. Strains lacking both *PDE* genes show only a modest twofold elevation of cAMP levels. However, strains lacking the *PDE* genes but containing the mutant $RAS2^{val19}$ gene show a 1000-fold in-

crease in cAMP. These results imply that there is a strong feedback control of cAMP generation in *S. cerevisiae,* which likely passes through the *RAS* proteins. Further experiments with the *TPK* genes indicate that feedback probably results from the activity of the cAMP-dependent protein kinase. In particular, activation of the kinase by disruption of *BCY1* depresses cAMP levels, whereas the presence of the *TPK*^{w} alleles, in the absence of wild-type *TPK* genes, causes a great elevation of cAMP levels. Work is in progress to characterize these interactions at the biochemical level.

SCH9 and other genes (e.g., *JUN1, SCH1,* and *SCH2*) that interact with the *RAS/CYR1* pathway have been identified. We have not yet fully characterized these genes at the nucleotide, genetic, or biochemical level. *SCH9* has the structure of a protein kinase and can perform many functions of the cAMP-dependent protein kinases.

Human Oncogenes

M. Wigler, C. Birchmeier, D. Young, G. Bolger, S. Sharma, J. Colicelli, G. Waitches, L. Rodgers, M. Riggs, K. O'Neil

We are studying two human oncogenes that we recently isolated: the *ros* and *mas* genes. These genes were isolated using a modification of the NIH-3T3 transfection assay, which identifies oncogenes by their ability to induce tumorigenicity (Fasano et al., *Mol. Cell. Biol. 4:* 1695 [1984]). It is now clear that this assay identifies normal human genes that have the potential to act as oncogenes when amplified, overexpressed, or rearranged as a secondary consequence of DNA transfer (Birchmeier et al., *Mol. Cell. Biol. 6:* 3109 [1986]; Young et al., *Cell 45:* 711 [1986]). These oncogenes map to the distal portion of chromosome 6q (Rabin et al., *Oncogene Res.* [1987] in press).

THE HUMAN *ROS* GENE

We previously described the isolation of two human oncogenes, called *mcf2* and *mcf3*, using the DNA cotransfer and tumorigenicity assay (Fasano et al., *Mol. Cell. Biol. 4:* 1695 [1984]). Nucleotide sequence analysis of *mcf3* cDNA clones indicated that the *mcf3* gene arose by rearrangement of a human gene homologous to the v-*ros* gene. It is likely that this rearrangement occurred during or after DNA transfer and was responsible for activating the oncogenic potential of *ros*. This gene, like its v-*ros* counterpart, contains a transmembrane domain and a carboxy-terminal domain that is homologous to the known tyrosine protein kinases. The rearrangement creating *mcf3* resulted in the loss of a putative extracellular domain. It is likely that the normal *ros* gene, like the normal counterpart of the v-*erb* and v-*fms* genes, is a growth factor or hormone receptor. We are currently attempting to obtain a full-length cDNA clone to help us identify the extracellular domain and the physiologic ligand of this receptor. Expression studies in progress clearly indicate that the *ros* gene is expressed in a very high proportion (80%) of human glioblastoma cell lines and hence might be a useful tumor cell marker for that cancer (Birchmeier et al., *Cold Spring Harbor Symp. Quant. Biol. 51:* 993 [1986]). We have recently identified a glioblastoma cell line with a deletion of the *ros* sequences encoding extracellular sequences (C. Birchmeier et al., in prep.).

THE HUMAN *MAS* GENE

We have continued the characterization of another human oncogene, which we call *mas.* This gene was isolated using the same methodology used for isolating the *mcf3* gene described above (Young et al., *Cell 45:* 711 [1986]). Comparison of the transforming locus with the placental locus, cDNA cloning and sequencing, and S1-nuclease-protection experiments have led to the following conclusions. Like *mcf3, mas1* was activated in NIH-3T3 cells during or after gene transfer by a DNA rearrangement, and, like *mcf3,* there is no evidence that *mas* was activated in the tumor cells from which it was ultimately derived. Unlike *mcf3,* the transforming *mas* gene is not rearranged within coding regions. Rather, its ability to transform cells appears to be entirely related to its high level of expression, a consequence of a rearrangement involving DNA 5′ to coding sequences. The normal *mas* gene, cloned from a human placental cosmid library, is only weakly transforming.

Two aspects of the *mas* gene make it of considerable interest as an oncogene. First, NIH-3T3 cells transformed with *mas* are highly tumorigenic and grow to high saturation densities but are not radically morphologically altered compared with normal NIH-3T3 cells. Second, the protein encoded by *mas* is very hydrophobic. Its hydropathy profile

closely resembles that of the visual rhodopsins, and the *mas* product is predicted to have seven transmembrane domains. In this respect, it is novel among cellular oncogenes. Our preferred hypothesis is that the *mas* protein, like rhodopsin, is a signal-transducing receptor that activates a GTP-binding protein. An understanding of the mechanism whereby *mas* transforms cells is likely to lead to new insights into growth-control mechanisms. We have been attempting to develop antibodies for the protein and to test putative ligands.

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HORMONAL AND DEVELOPMENTAL CONTROL OF GENE EXPRESSION

D.T. Kurtz W. Addison B. McDonald
D. Danna E. Louis

The rat α_{2u} globulin gene family has proved to be a valuable model system for the study of the hormonal, developmental, and tissue-specific control of gene expression in higher eukaryotes. α_{2u} was first described as the major protein present in the urine of male rats, but absent from the urine of female rats. Urinary α_{2u} globulin is produced in the liver, and the synthesis of hepatic α_{2u} is under complex hormonal control: Androgens, glucocorticoids, insulin, and growth hormone are all required for a normal level of hepatic α_{2u} globulin synthesis, and estrogens strongly repress the synthesis of this protein.

α_{2u} is produced in several other tissues in rats: Preputial gland, mammary gland, submaxillary gland, and lachrymal gland all produce α_{2u} globulin mRNA. α_{2u} is encoded by a multigene family, comprising 20–25 genes per haploid complement. It appears that different gene sets are being transcribed in the various tissues, and the hormonal control of the expression of the α_{2u} gene sets in these various organs is distinct.

INDUCTION OF α_{2u} GLOBULIN BY GLUCOCORTICOIDS

The induction of α_{2u} by glucocorticoid hormones has been extensively studied at the molecular level. In vivo, glucocorticoid induction occurs at the transcriptional level and is a secondary response to the hormone; i.e., protein synthesis is absolutely required for the induction of α_{2u} globulin mRNA by glucocorticoids. Virtually all of the studies in the past several years on the molecular mechanism of steroid hormone action have been on primary response genes (mouse mammary tumor virus, lysozyme, ovalbumin, etc.). Primary response is believed to involve the direct association of activated steroid receptor with target sites near the induced genes, resulting in increased transcription. Ongoing protein synthesis is not required. Secondary response almost certainly is the consequence of the hormone inducing the transcription of the mRNA for a regulatory protein(s). This mRNA must then be translated, and the resultant protein is responsible for the induction of the secondary response genes. The studies done on primary response genes in several laboratories over the past few years have resulted in a consensus as to the mode of action of these hormones: (1) Activated receptor does indeed bind to specific sequences near (or sometimes in) the target genes. The binding sites tend to contain the sequence –TGTTCT–. (2) Binding of activated receptor is necessary, but not sufficient, to induce transcription. The activated receptor must interact with other nuclear proteins to be effective. (3) Multiple binding sites for activated receptor occur near the target gene. Deletion or mutation of one of the sites will reduce the level of induction, but not abolish it. (4) These control regions tend to be enhancer-like, in that they are relatively position- and orientation-independent with respect to the promoter, and can act on heterologous promoters.

The studies done in this laboratory over the past few years on the α_{2u} globulin gene system are (with one exception discussed below) the only studies done at a molecular level on a secondary response gene. We have found some similarities, but some major differences, between the mode of induction of α_{2u} and that of primary response genes. Extensive linker-scanning mutagenesis of the 5′-flanking region of a cloned α_{2u} gene indicated that one precise region of DNA was absolutely required for glucocorticoid induction of α_{2u} mRNA in transfected L cells. One linker-scanning mutation (−137 to −125 relative to the cap site) completely abolished induction. Mutations adjacent to this sequence greatly reduced induction, whereas mutants anywhere else in the 5′-flanking region had no negative effect upon induction. It appears, at least for this example of secondary induction, that the hormone regulatory region is organized quite differently from those found in primary response genes, which are made of multiple copies of the regulatory sequence.

Curiously, a set of mutants farther upstream, between −210 and −180, actually increased the level of induction. We have termed this the "hyperinducible" region.

Initially, these results showed that the hexanucleotide –GGAACA–, which was in the middle of the sequence required for α_{2u} globulin induction, was in a similar position in the promoter of the rat α1-acid glycoprotein gene (α1-AGP), another rat-liver gene under secondary control by glucocorticoids. It has subsequently been shown that induction of α1-AGP by glucocorticoids requires the sequence –GGAACA– at −120 in the α1-AGP promoter. The sequence homology between the regulatory site in the α_{2u} promoter and the regulatory site in α1-AGP can be extended on either side of this hexanucleotide to encompass a 15-base sequence –ANGGAACANTNNTTG–. Of course, two genes are not sufficient to derive a consensus DNA sequence for secondary glucocorticoid induction, but the similarities between the two systems are striking.

NUCLEAR FACTORS THAT BIND TO THE α_{2u} GLOBULIN CONTROL REGION

DNase I protection studies ("footprinting") have demonstrated the presence of nuclear proteins from male rat liver that bind to two specific regions of the α_{2u} globulin promoter. One factor was found to bind to precisely the region that we had shown to be required for glucocorticoid induction. Furthermore, mutant genes that were poorly responsive or nonresponsive to hormones showed no binding to this factor. It was at first hoped that this protein was the regulatory protein whose transcription was being induced by the hormone; however, the level of this protein was unchanged in hypophysectomized rats with or without hormone treatment or in L cells with or without dexamethasone. This finding could be explained by several models. It is possible that this putative regulatory protein must

be modified to an active form by glucocorticoids. Alternatively, the protein may provide specificity to the regulatory system while another protein, induced by the hormone, activates transcription. The protein that binds to the glucocorticoid regulatory region has been found in virtually every tissue examined (male and female rat liver, rat kidney, and L cells).

A second binding protein has been detected that binds to sequences between −210 and −185. This partially overlaps with the "hyperinducible" region mentioned above. This protein seems to be found only in tissues that are competent to transcribe α_{2u} globulin: It is found in rat liver and in L cells, but not in rat kidney. It is striking that both footprints occur in regions of dyad symmetry; the upstream footprint occurs over the sequence −ATAtgTTTt-GCgAAAtgTAT− and the downstream footprint precisely covers the units of symmetry contained in the sequence −TGAcAtcGCCAAGTTN$_{17}$AAtc-CTTGGCTTCA−. The presence of dyad symmetry in regulatory regions is quite common in prokaryotes and correlates with a dimeric structure of the regulatory protein. Examples of eukaryotic regulatory proteins that bind to symmetric sequences are the GAL4 protein of yeast and a protein that binds to the chicken β-globin promoter.

Experiments designed to purify the factors that bind to the α_{2u} globulin promoter, as well as the cloning of their cDNAs, are currently under way.

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MOLECULAR BIOLOGY OF MOUSE DEVELOPMENT

D. Hanahan S. Alpert, V. Bautch, S. Efrat, L. Field, S. Grant, J. Hager, M. Pizzolato, J. Skowronski, S. Toda

During the past several years, the effort in mouse genetics at Cold Spring Harbor Laboratory has shifted to focus on the use of transgenic mice to study problems in control of gene expression and consequences of oncogene expression. Particular emphasis has been on using this approach to produce models of human diseases, notably certain types of cancer. The essence of transgenic mice is that they carry a specific new piece of DNA stably integrated into one of their chromosomes, and this new DNA is thus a heritable genetic element, which is capable of endowing a reproducible phenotype on the mice that inherit it. Transgenic mice are initially produced by microinjecting a solution of DNA into one of the pronuclei of a fertilized mouse egg, which is implanted into a foster mother and allowed to develop. The original transgenic mice are bred to found lineages, or families, of mice that carry the "transgene" as part of their genome. Transgenic mice are being used to study a number of specific cancers, including those of the insulin-producing pancreatic β cells, the heart atria, the skin, and the vascular endothelium. Targeted oncogene expression is also being exploited in studies of the immunological responses to transgene products and to cell lineage analyses. Other efforts are seeking to explore the prospects for antisense RNA inhibition of gene expression, and the consequences of expressing different cellular oncogenes in specific cell types, both individually and in genetic complementation experiments produced by mating different transgenic mice. The reports below summarize some of the projects that have progressed well during the past year and illustrate the breadth of the current efforts in molecular genetics studies using transgenic mice.

Self-tolerance and Autoimmunity in Transgenic Mice

S. Alpert, J. Skowronski, D. Hanahan
[in collaboration with T. Adams, University of Melbourne, Parkville, Australia]

The question of how organisms come to recognize self-proteins as such, thereby becoming immunologically nonresponsive, remains a central and enigmatic problem in immunology. Similarly, the mechanisms by which nonresponsiveness to self-proteins fail, resulting in autoimmune diseases, are also essentially undefined, although they most probably are closely related to the routes by which self-tolerance is established in the first place. The introduction of new genes into the mouse germ line results in the stable establishment of the transgene in a mouse chromosome, where it must now be considered self in a genetic sense. One would assume, therefore, that its gene product would be a self-antigen. We have investigated this question in the RIP-Tag lineages of transgenic mice and find that this is not always the case. The RIP-Tag transgene is composed of the rat insulin II gene 5′ regulatory region aligned to transcribe the early region of SV40. It is expressed exclusively in the pancreatic β cells of adult mice, which are the sole known source of insulin synthesis. Thus, expression is targeted to one specific cell type.

Four lineages have been examined for their immunological responses to the transgene product, large T antigen: RIP1-Tag2, -Tag3, -Tag4, and RIR-Tag1. Tolerance was addressed in two ways: (1) Serum was tested for the presence of autoantibodies to T antigen and (2) purified T antigen was injected in an attempt to elicit an immune response to it. Mice in the RIP-Tag3, -Tag4, and RIR-Tag lines showed serum antibodies to large T antigen. The frequency of autoantibodies was high, varying from 25% to 60% of the mice tested in these three lineages. In contrast, none of the RIP-Tag2 mice tested showed appreciable levels of autoantibodies to large T. The appearance of autoantibodies was found to precede tumor formation, which suggests that the immune response is not strictly a reaction to the appearance of solid tumors. The distinction between RIP-Tag2 and the other three lineages suggested that they differed in their self-tolerance. To address this directly, purified large T antigen was presented to mice from each lineage, as well as normal (nontransgenic) mice, in a standard immunization regimen of primary and secondary injections. Nontransgenic mice, and the mice from the RIP-Tag3, -Tag4, and RIR-Tag lineages, mounted a strong immune response to T antigen. In contrast, mice in the RIP-Tag2 lineage did not.

The conclusion from these two types of analyses is that mice in the RIP-Tag2 lineage are heritably tolerant to the transgenic self-antigen large T, whereas the other three lines are not. One consequence of this nontolerance is the appearance of autoantibodies, which is one characteristic of many autoimmune diseases. To address the heritable establishment of tolerance or nontolerance in these lines, we have examined the time course of the appearance of large T. In RIP-Tag2, large T appears on day 12 of embryogenesis, concomitant with insulin, and continues to be present in β cells thereafter (see following report). In contrast, no expression of T antigen is detected in neonatal mice of the RIP-Tag3 and -Tag4 lineages. Instead, scattered β cells expressing T antigen begin to appear at 8–12 weeks of age. This late onset of expression is provocative, since the expression is cell-type-specific to the β cells but not correctly regulated in development. It is possible that the random integration of the transgene in the original injected embryos resulted in the transgene being in chromosomal positions that restrict the accessibility of the gene during β cell development. This "position" must be a frequent one, given that the same effect appears in three independent lineages.

Since the period of immunological self-learning is thought to cover the last few days of embryogenesis in the mouse, as well as the first week or two after birth, one can propose that the RIP-Tag2 mice are self-tolerant to T antigen as a result of expression during this period, whereas the late onset of expression in the other lines occurs after the self-learning period is over. The failure to establish self-tolerance due to the late appearance of large T has a significant consequence. Autoantibodies to large T appear with high frequency, presumably as a result of the immune system viewing large T antigen as a foreign antigen. Mice with autoantibodies to large T also show lymphocyte infiltration of the islets of Langerhans, indicating that the immune system is seeking out the source of expression of this "foreign" antigen, which is actually a self-antigen that shows an aberrant pattern of expression. This observation suggests a mechanism of autoimmune disease: Delayed onset of expression of a protein results in a failure to establish self-tolerance and a consequent autoimmune response against it. We

will now address this question from several perspectives, in order to define further the characteristics of the tolerant state in the RIP-Tag2 lineage and the development of the autoimmune response in the nontolerant lines. One approach will be to present purified T antigen to neonatal nontolerant mice, to see whether antigen presentation during the "self-learning" period is sufficient to render these individuals tolerant to the T antigen when it subsequently appears in the β cells, thus obviating the autoimmune response. Success with this approach could be informative vis-à-vis the general concept of preventing autoimmune disease by immunizing with the autoantigen.

Cell Lineage Analyses of Pancreatic Endocrine Cells

S. Alpert, D. Hanahan [in collaboration with G. Teitelman, Cornell University Medical College]

The various cell types in the pancreas may originate from different stem cells or from common precursors. To examine this issue, we have sought to determine whether pancreatic cells can share common antigens during development. The ontogeny of the insulin-producing cells is being studied in developing transgenic mice by examining the appearance of cells expressing insulin itself, as well as the other pancreatic hormones, and tyrosine hydroxylase.

Tyrosine hydroxylase (TH), the first enzyme in the catecholamine biosynthetic pathway, has been used as a marker to study the development of the islets of Langerhans. In the adult mouse, TH is expressed in a few of the insulin-producing β cells in every islet. However, during development, TH is present in both the glucagon- and the insulin-containing cells (Teitelman et al., *Peptides 2:* 157 [1981]; *Dev. Biol.* [1987] in press). These findings suggest that progenitor cells that contain enzymes involved in catecholamine biosynthesis, and are present in the developing pancreatic duct, give rise to glucagon- and insulin-producing cells of adult islets.

The developing mouse pancreas appears as a diverticulum from the embryonic gut on day 10 of gestation (e10). The first bundles of exocrine tissue appear around this duct. Endocrine cells form around the pancreatic duct and produce the islets of Langerhans (Pictet and Rutter, *Handb. Physiol. 7:* 25 [1972]). In the mouse, cells with glucagon immunoreactivity have been noted on e10, insulin as early as e12 (Teitelman et al., *Dev. Biol.* [1987] in press), somatostatin on e17, and pancreatic polypeptide after birth (Teitelman et al., *Proc. Natl. Acad. Sci. 78:* 5225 [1981]). The TH cells appear alongside the duct and contain glucagon and/or insulin. Postnatally, TH cells are not found along the duct, but rather appear in a subset of the insulin-producing cells in the islets.

We have conducted an immunohistochemical analysis to clarify further the developmental lineage of the cells in the islets of Langerhans in RIP1-Tag2 mice. These mice harbor a hybrid gene consisting of 695 bp of the 5′-flanking region of the insulin gene fused to the protein-coding region for the SV40 large T antigen. Mice containing this gene express T antigen only in the β cells of the islets of Langerhans (Hanahan, *Nature 315:* 115 [1985]). If the transgene is regulated developmentally in the same manner as the insulin gene, we can use T antigen as a marker to investigate further the nature of the β cells' progenitor. In addition, we can determine if the portion of the insulin gene contained in our construct contains sufficient information for proper developmental regulation.

Cells containing T antigen first appear in the pancreas at e10 of development. Double-label experiments have shown that these cells can contain either glucagon or TH. (It is possible that some cells contain both glucagon and TH as well as T antigen, but the methods currently available cannot detect a "triple-labeled" cell.) At e12, cells that contain insulin and T antigen appear. Throughout development, all cells that express insulin also contain T antigen. However, the converse is not completely correct; all cells that contain T antigen do not stain for insulin. Instead, a few cells colabel for T antigen and somatostatin, or for T antigen and pancreatic polypeptide. These cells appear at e17 and at postnatal day 1 (p1), respectively. This result is further supported by the finding that cells which contain both glucagon and insulin appear at e12. The fact that T antigen coexpresses with markers for the other three islet cell types during development supports the possibility that a common precursor exists for all of the cells in the islets of Langerhans.

Although T antigen can coexpress with each of the islet cell types, it does not express in the acinar cells of the exocrine pancreas. Double-label experiments with T antigen and amylase have shown that throughout development, T antigen and this exocrine cell marker are always in separate cells.

At some point in postnatal development, T-antigen expression becomes limited to the insulin-producing cells. At p1, all of the insulin-producing cells, several of the glucagon-producing cells, and a few cells of the somatostatin and pancreatic polypeptides contain T antigen. The exact timing of segregation has yet to be determined, but it must be completed sometime between day 1 and day 14 of postnatal life, since, by this time, T antigen coexpresses only with insulin.

All of the studies described above were conducted on sections of pancreas. When sections of whole embryos were analyzed, T antigen was found to be expressed in the ventral part of the neural tube and in the neural crest. T-antigen expression in the neural tube begins at e9 (the time of neural tube formation) and has been followed through neural crest formation and up until e16. No insulin could be detected in these embryonic structures at any time. The fact that T antigen is present in both the non-β cells of the pancreas and in the neural tube and crest is somewhat puzzling. It is possible that insulin is present in these cells, but at levels too low to be detected by our methods. Radioimmunoassays will be conducted on the neural tissue of mouse embryos to detect low levels of insulin production. Alternatively, in these cell types, stable insulin transcripts may be produced but are not translated. RNA in situ experiments can determine the presence of insulin transcripts. It is also possible that insulin transcripts are produced but are not stable or that our hybrid construct has eliminated part of the region required for proper developmental regulation. We are currently trying to distinguish among these possibilities.

There are several theories that can explain the appearance of T antigen in the neural tube. The first is that neural and islet cells are phenotypically similar. Many neural markers are present in islet cells, including TH, phenyl *n*-methyl transferase, dopamine decarboxylase, γ-aminobutyric acid, neuron-specific enolase, 7B2, and PGP 9.5. It is possible that some of the same *trans*-activating factors are present in both neurons and islet cells. These factors may allow the neural proteins to be made in islet cells and islet cell markers to be expressed in neurons (e.g., somatostatin is expressed in some nerve cells). Alternatively, our construct could have omitted a repressor-binding site that is normally contained in the insulin 5′-flanking region. Normally, the repressor would prevent insulin expression in neural tissue.

Another hypothesis is that the islets of Langerhans are formed from the neural crest. Evidence against this ontogeny comes from experiments in which transplants of a quail neural crest into chick embryos did not result in quail cells populating the islets (Le Dourain, in *Gut Hormones,* Church-Livingstone, Edinburgh [1978]). Additionally, in vitro cultures of mouse embryos that have been enzymatically treated to remove the neural ectoderm can develop a pancreas (Pictet et al., *Science 191*: 191 [1976]). As Pearse has proposed, the possibility remains that an early migration of crest cells could populate the islets (Pearse and Takor-Takor, *Fed. Proc. 38*: 2288 [1979]). An additional analysis will be conducted on a second line of mice, RIP-Tag2, which also develops T-antigen-containing insulin-producing tumors at a relatively young age, to verify the cell lineage relationships. We will also look for T-antigen expression before e10 to determine whether there is any early migration of T-antigen-containing ectodermal cells.

β-cell Tumor Progression in Transgenic Mice: Identification of β Cells Containing Embryonic Antigens

S. Alpert, D. Hanahan [in collaboration with G. Teitelman and J. Lee, Cornell University Medical College]

The above report has raised the proposition that the endocrine cells of the pancreas arise from a common progenitor cell, perhaps one that contains TH. In addition to being expressed during development, TH is expressed in a few of the β cells of the adult mouse pancreas. Analyses of the neoplasia occurring in adult RIP1-Tag2 may address the questions of whether the hyperplasia of pancreatic islets is produced by proliferation of precursor cells and whether the few TH-containing cells could be this precursor population.

At 13 weeks of age, most pancreatic islets of the RIP1-Tag2 mice are hyperplastic and some have developed into solid insulinomas. When the animals were perfused and the tissue was processed for immunohistochemical staining, we found that, in most islets, all cells stained for insulin. The percentage of TH cells ranged from only a few percent to 100%, which is considerably greater than the normal 1% seen in a normal islet.

The presence of TH could be due to selective sur-

vival and proliferation of the few TH cells present in the islets before neoplasia. An alternative hypothesis is that all cells of the islet proliferate when stimulated by the oncogene and that, at a certain point, they regress to a more "immature" state and reexpress TH.

Transgenic mice of various ages were injected with [^{3}H]thymidine, and, after 3 hours, the animals were sacrificed and the pancreases were processed for immunohistochemistry and autoradiography. At all stages in postnatal development, all dividing cells contained insulin. Thus, it is unlikely that growth is due to the proliferation of an undifferentiated stem cell. Although the number of both TH-containing cells and proliferating cells increased throughout development, only rarely did a dividing cell in the islets contain TH. Thus, it is unlikely that TH marks an "embryonic" cell; rather, it appears that during islet growth, TH expression marks a postmitotic stage of adult β cells. Similar results with TH have been obtained in two other models of islet cell growth, the ob/ob mice and pregnant mice (G. Teitelman et al., pers. comm.). Only in embryos and in very advanced tumors can the TH cells divide. The mechanisms responsible for the sequence of phenotypic transformations of β cells during neoplasia and/or tumor formation have yet to be determined.

Characteristics of β-cell Tumor Formation

S. Efrat, D. Hanahan

A common observation in the studies of targeted oncogenesis in transgenic mice has been that only a small number of the target cells expressing the oncogene develop into solid tumors. Tumor formation in the RIP-Tag mice follows the same pattern: Of the several hundreds of islets in the pancreas, all of which express a potent oncogene, the SV40 large T antigen, only one to five develop into solid, vascularized tumors. This implies that although the oncogene product is necessary, it is not sufficient for tumor formation. Secondary events, or tumor progression factors, are likely to be involved in this process. Since in our system we are analyzing the consequences of oncogene activity in an entire animal, we have an opportunity of studying such events.

One possibility for a secondary event is a mutation that may cause activation of another oncogene or growth factor in a small number of β cells. Previously, we were not able to detect any activated oncogenes in the tumor DNA by the 3T3-cell transfection assay. Thus, our current approach is to examine tumor RNA for expression of known oncogenes by RNA blot analysis. Normal β cells, as well as β cells from transgenic mice in the pretumor stage, can then be examined for expression of those oncogenes that are expressed at high levels in the tumors, to determine which of them has been newly activated during tumor formation. To establish that a given candidate is indeed a cause, rather than a consequence, of tumor formation, we plan to use genetic complementation. The gene for the putative progression factor will be placed under the control of the insulin promoter to generate transgenic mice that express it in their β cells. These mice will be crossed with the RIP-Tag mice, which will result in mice expressing both T antigen and the putative progression factor in all their islets. The prediction is that in such mice, every islet will develop into a tumor.

So far, we have identified three groups of oncogenes that are expressed at high levels in the tumors: p53, members of the *myc* family, and an endogenous retrovirus. p53 is an unstable cellular protein that is present at very low levels in normal cells, but it is stabilized in the presence of T antigen and appears at high levels in cultured cells transformed by SV40. Northern analysis shows appreciable levels of p53 mRNA in the tumor cells. Using monoclonal antibodies directed against the large T and p53 proteins, we have performed immunohistological analysis on thin sections of pancreas of both fast and slow lineages (those that develop tumors and die between 12 and 20 weeks [fast] or 20 and 60 weeks [slow]). There is a complete correlation between the presence of large T and p53 in the β cells that are found in both islets and tumors. In the fast lineages, p53 appears early, concomitant with large T, whereas in the slow lineages, p53 is only detected relatively late, and not before T antigen is present. In all of the cases where T antigen is present, p53 is detected as well. p53 cannot be detected in the β cells of normal mice. Although this does not fit the simple criteria for a progression factor (since p53 is detected both in islets and in tumors), we nevertheless find it pertinent to conduct p53/T antigen genetic complementation experiments. We have already generated sev-

eral lineages of RIP-p53 mice, and these are currently being analyzed for expression.

Using probes for the human c-*myc* and N-*myc* genes, we have detected high levels of transcripts to each in the tumors. We do not yet have any information on their expression in islets. Transgenic mice harboring insulin-promoted mouse c-*myc* genes have already been generated. We have also detected transcripts in tumor RNA blots that hybridize to several retrovirus genomes, as well as to a cloned long terminal repeat fragment, a regulatory region shared by all retroviruses. This suggests that an endogenous provirus is expressed in the tumor cells and that it may have been activated in the process of tumor formation.

Polyomavirus Gene Expression in Transgenic Mice

V. Bautch, S. Toda, D. Hanahan [in collaboration with J. Hassel, McGill University, Montreal]

We are analyzing the effects of the early region of polyomavirus in transgenic mice. We plan to assess the expression of the polyomavirus early region promoter in the tissues of transgenic mice to determine the contribution of tissue-specific regulation to the overall processes of infection and tumorigenesis in mice. If the polyomavirus promoter is expressed in murine cell types for which no cellular promoter has been characterized, we may be able to target oncogene expression to unique cell types. In addition, we are also setting up a complementation analysis of the polyomavirus T antigens in the natural host to help elucidate the cooperating roles of these proteins in tumor formation. We are targeting expression of the polyomavirus T antigens to the β cells of the pancreas using the rat insulin II promoter to facilitate these complementation assays.

The permissive host for polyomavirus is the mouse, and normal routes of transmission result in a silent infection with no overt symptoms. However, newborn animals inoculated with high-titer virus preparations can develop tumors in a variety of tissues starting at 3 months of age. Polyomavirus contains a region expressed early during a lytic infection that is also required for transformation. The broad tissue distribution of polyomavirus-induced tumors suggests that many murine cell types are permissive for early region transcription.

Polyomavirus encodes three early region proteins, large T (100K), middle T (56K), and small T (22K) antigens. Several lines of evidence suggest that polyomavirus large T antigen (PyLT) contains an establishment function that immortalizes primary cells in culture and that polyomavirus middle T antigen (PyMT) contains an activity that morphologically transforms established cells, but not primary cells. These two proteins can cooperate with each other and with other oncogenes to transform primary cells. The function of polyomavirus small T antigen (PyST) in in vitro transformation is not well understood. The functions of the polyomavirus early proteins are less well defined in vivo, although all three T antigens appear to be involved in tumorigenesis in rats.

For these analyses, several genes have been injected into the pronucleus of one-cell mouse embryos. Table 1 summarizes the current progress in the most complete analyses, and the results are discussed below.

In addition to the lines described in Table 1, we have generated lines of mice carrying the insulin–polyomavirus T antigen constructs. None of these founder animals or their progeny have developed overt pathology at the age of 7.5 months. We are currently analyzing selected lines for expression of the polyomavirus T antigens by immunocytochemistry and RNA analysis.

TABLE 1 Status of Polyomavirus Transgenic Lines

Line	Construct	Copy no.	Age[a] (months)	Pathology
Py-1	Py/genomic	20–50	3.5	dead, bowel obstruction[b]
PyLT-1	PyLT(cDNA)	2–10	10.0	dead, lung/brain abnormal[b]
PyLT-2	PyLT(cDNA)	30–50	10.0	alive
PyMT-1A	PyMT(cDNA)	30–50	2.5	dead, hemangiomas
PyMT-1B	PyMT(cDNA)	2–10	7.5	dead, cause uncertain[b]
PyMT-2	PyMT(cDNA)	5–15	6.5	dead, hemangiomas

[a] Age of founder mouse at this writing or at death.
[b] Analysis is in progress to determine whether there is additional pathology.

We have recently obtained founder mice carrying polyomavirus-promoted SV40 T-antigen constructs. Because SV40 T antigen has led to pathology in all tissues to which expression has been targeted in transgenic mice, we hope that this gene will serve as a phenotypic "reporter" gene and that all tissues expressing the polyomavirus promoter will be affected. We have also obtained mice carrying a polyomavirus-promoted transformation-defective SV40 T antigen; we hope that this construct represents a nonlethal reporter gene that can be assayed in expressing tissues.

The PyLT and PyMT mice have been most extensively analyzed. Although PyLT mice show no pathology at this time (the recent death of one of the founder mice cannot be linked to expression of PyLT until tissue analysis is completed), the PyMT mice have developed fatal hemangiomas. One lineage has been analyzed in detail and will be described. It should be noted, however, that a different founder mouse also died with hemangiomas. This fact indicates that the pathology is not due to a position effect of integration but is most likely an intrinsic property of polyomavirus-promoted MT gene expression.

Hemangiomas are a proliferation of the endothelial lining of the blood vessels. Of 50 positive mice of the MT-1A lineage, 20 have died or have been sacrificed, and all had hemangiomas with no other overt pathology. No member of this lineage has lived beyond 4 months of age. Hemangiomas have been documented in most organs with a copious blood supply, including the liver, lung, and subcutaneous tissue. To document expression of the transgene, we performed a kinase assay on tissues. This assay takes advantage of the fact that PyMT complexes with cellular tyrosine kinases such as c-*src*, and this complex has autophosphorylation activity after immunoprecipitation. This assay showed that MT was expressed and complexed in tumor tissue and in several other organs (liver, spleen, testes, and lung), whereas no MT kinase activity was detected in kidney tissue from the same animal. We have recently been successful in passaging these tumors in vivo. This ability (1) indicates that the tumors are neoplasias and not just hyperplasias and (2) provides a source of tumor material for additional analysis.

These results pose several questions that we plan to address. The first is, What is the contribution of promoter specificity to the development of this very specific phenotype? The two extreme hypotheses are that the polyomavirus promoter may be expressed only in endothelial cells or that the promoter may be expressed in several different cell types, but only have some complementing activity necessary for transformation in endothelial cells. A direct test of promoter function using a high-sensitivity RNA protection assay is in progress, and preliminary results indicate that cell types other than endothelial cells express stable PyMT RNA.

The second question, which is a corollary of the first, is, Are there tissues containing PyMT that are not neoplastic? The presence of such cell types would indicate that some complementing activity necessary for transformation is missing in these cells. We are attempting in situ analysis using immunocytochemistry to determine if these cells exist.

The third question is, Does the coexpression of PyLT alter in any way the dominant phenotype associated with PyMT expression in these mice? We have started genetic complementation experiments by mating mice carrying the PyLT gene with mice carrying the PyMT gene. Several hybrid progeny have recently been obtained, and these will be monitored for alterations in phenotype. We are concurrently using the techniques described above to determine if the PyLT genes are being expressed. Some possible results are that PyLT expression will (1) suppress the development of hemangiomas due to PyMT expression, since PyLT can repress transcription from the polyomavirus promoter; (2) redirect development of neoplasms to epithelial tissues such as the parotid and mammary glands, since infection with polyomavirus leads to this pathology; and (3) have no effect on PyMT-induced hemangiomas.

Alpha-1-Antitrypsin Deficiency in Transgenic Mice

S. Grant, D. Hanahan [in collaboration with A. Pollard and J. Sorge, Scripps Clinic]

Alpha-1-antitrypsin (A1AT), the principal serum protease inhibitor, is a single polypeptide glycoprotein with a molecular weight of 52,000. It is synthesized and secreted by the hepatocyte into the circulation, whereby it readily diffuses through most tissues. Its principal action is to inhibit neutrophil elastase by forming a stable complex. In humans, a deficiency in A1AT allows neutrophil elastase to cleave elastin and other macromolecules compris-

ing the connective tissue matrix. The associated phenotype is primarily in the lung of adults as emphysema and less frequently in the liver of infants as cirrhosis.

A single autosomal allele is inherited from each parent, and the phenotype is expressed in an autosomal codominant pattern. The most common form of A1AT is type M, and the best-studied variant is type Z. Homozygotes for the Z allele occur once in 2000 people and have serum A1AT levels at 10–15% of the normal. The Z mutation arises from a G-A transition that converts glutamic acid at residue 52 to lysine. This mutation affects secretion of the protein rather than its specific activity.

Our goal is to produce a model of A1AT deficiency and study the expression of the human alleles in transgenic mice. The first step has been to create transgenic mice with the human genomic clones of the M and Z alleles. We have established two lineages of the M allele, both of which express the gene and secrete A1AT into serum. We have three lineages that contain the Z allele, and no abnormal phenotype has so far been observed.

The next step is to try to inhibit the expression of the mouse endogenous A1AT by expression of an antisense RNA, which is complementary to the mouse A1AT mRNA. A variety of experiments in cultured cells have suggested that antisense RNA can, under some circumstances, inhibit the expression of the mRNA to which it is complementary, presumably through a mechanism that involves annealing of the two RNAs. Two antisense constructs will be used initially; the mouse cDNA will be aligned in reverse (antisense) orientation in order to be transcribed under the direction of either mouse metallothionein or mouse albumin promoters, both of which are normally expressed at high levels in hepatocytes. The serum levels of A1AT will be assayed and related to any phenotype, with particular emphasis on lung and liver pathology. Mice with low levels of serum A1AT will be crossed with those mice carrying the human alleles to attempt to rescue the deficient phenotype.

The hepatic disease of Z-allele homozygotes is thought to be due to accumulation of A1AT in the Golgi. We will attempt to recapitulate this liver disease in mice by overexpressing the human Z allele with the mouse albumin promoter. Four lineages have been established and will be analyzed for levels of transgene expression, the intracellular localization of the Z protein, and development of liver pathology.

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GENETICS

For the past four decades, genetics has been used as an important tool of experimental biology at Cold Spring Harbor Laboratory. The combination of recombinant DNA technology and classical genetics gave rise to the field of molecular genetics. An important element of these approaches is the ability to isolate genes of interest, manipulate them in vitro, and return these constructs to the genome, usually at their original location. Thus, with this so called "reverse genetics," we can make mutations at will and then test their effect on a particular cellular phenomenon in vivo. In the past year, we have used these approaches on various systems such as cell-type determination and cell-cycle controls of both *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* and energy metabolism pathways of *Rhodobacter capsulata*. In other areas, we have continued our molecular studies of tobacco tissue culture and maize controlling elements.

During the past year, we witnessed a significant turnover of our colleagues in this area. Jim Hicks moved to Scripps Institute, California, Steve Dellaporta took a position at Yale University, Doug Youvan joined Massachusetts Institute of Technology, Pablo Scolnik moved to Dupont Research Facility, Clive Slaughter joined Howard Hughes Medical Institute at Dallas, and Steve Hinton moved to the Exxon Research facility at Trenton. We will miss them, and we wish them continued success and thank them for their contributions to Cold Spring Harbor Laboratory. Continuing with the tradition of our laboratory of efficient use of space, the newly vacated areas have already been assigned to new arrivals in the areas of molecular structure, yeast, and plant molecular biology.

YEAST GENETICS

A.J.S. Klar R. Cafferkey M. Kelly C.I.P. Lin G.P. Livi L. Miglio

Differentiated Parental DNA Strands Confer Developmental Asymmetry on Daughter Cells in Fission Yeast

A.J.S. Klar, R. Cafferkey, M. Kelly, L. Miglio

As demonstrated in Figure 1, the alternate alleles of the *mat1* locus, called *mat1-P* and *mat1-M,* interchange (switch) by a transposition-substitution event in which a replica of either the *mat2-P* or *mat3-M* donor "cassette" is transmitted to the *mat1* locus via a gene-conversion process. This genotypic change results in an interconversion between the P and M cell types of *Schizosaccharomyces pombe.*

Miyata and Miyata (*J. Gen. Appl. Microbiol. 27*: 365 [1981]) (Fig. 2) observed that between two sister cells, only one member (designated *Ps* or *Ms* in Fig. 2) produces a single switched cell in the next generation; i.e., only one grandchild is switched among the four progeny produced by two cell divisions of a single cell (the one-in-four rule). We want to determine how sister cells acquire developmentally different fates such that only one member of a pair of sister cells produces a switched progeny. At least two models come to mind. The first is that the ability to switch is inherited through the chromosome, and the second is that cytoplasmic (plus nuclear) factor(s) asymmetrically transferred from the mother cell influence switching in the next

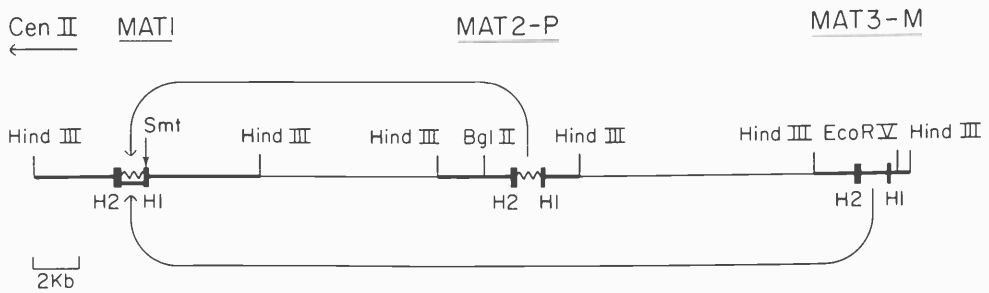

FIGURE 1 A portion of chromosome II showing the mating-type region. Smt indicates the site of the double-strand DNA break found in vivo.

generation. Other genetic studies suggested that the switching potential segregates in *cis* with the *mat1* locus. But why does only one among four descendants of a given chromosome switch? We may imagine a molecular model in which "Watson" and "Crick" strands of DNA are viewed as nonequivalent in their ability to acquire the developmental potential for switching. We may imagine that some sequence-specific DNA modification, such that only one specific chain (e.g., the Watson strand) at the *mat1* locus is modified (Fig. 3, asterisk). We further hypothesize that replication of the hemimodified chromosome generates sister chromatids, of which one is cut (the one possessing the old Watson chain, the left cell in Fig. 3) and the other is uncut. We know that the recombination required for switching is initiated by a double-strand break at *mat1*. On the basis of the model proposed here, the cell inheriting the broken chromosome is hypothesized to produce a single switched cell in the next generation. Although the specific details of the hypothesized "imprinting" event are not known, the key idea—of the segregation of DNA strands conferring developmental asymmetry to daughter cells—can be tested.

A molecular test of the strand-segregation model consists of determining the effect of cassette orientation on the switching of tandem cassette duplications of *mat1*. Both the strand-segregation model and the cytoplasmic segregation model predict that in the direct tandem duplication, a single cell could acquire the competence to switch both cassettes in the same cell cycle (Fig. 4A). In standard strains, since nearly 20% of *mat1* DNA is cut in vivo and the break persists throughout the length of the cell cycle, we can monitor at the molecular level the competence of a given cassette to switch by directly assaying for the presence of the double-strand break at that cassette by Southern blot analysis. More specifically, the chromosome of the cell designated *PsPs* in Figure 4A should have a double-strand cut at both cleavage sites to generate a 6.1-kb fragment. The predicted fragment having homology with the *mat1* sequence was found when the unrestricted DNA isolated from the strain with direct duplication was subjected to Southern analysis (Fig. 5b). This result does not distinguish between the models under consideration, but it does show that more than one cassette can become competent to switch in a single cell and within a single cell-division cycle.

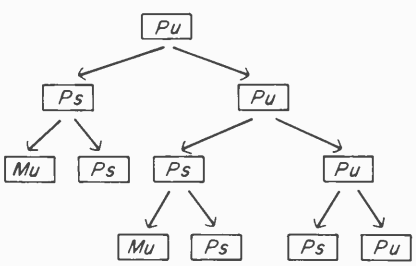

FIGURE 2 The Miyata and Miyata pattern of *mat1* switching in a cell lineage showing the switching rule of one-in-four related cells. The *u* designates cells that cannot produce a switched progeny, and the *s* designates those that can.

A specific physical prediction that distinguishes between these models is provided by the inverted duplication. The analogous 7.2-kb fragment in the inversely oriented duplication should be generated according to the cytoplasmic segregation model (the cut sites in this construction are 7.2 kb apart), but it should not be generated according to the strand-segregation model presented in Figure 3. As predicted in Figure 4B by the strand-segregation model, both strands of DNA should be modified (see top of the figure), one strand on one cassette and the other strand on the second cassette. Segregation of these strands should generate two daughter cells, one having a break in one cassette and the

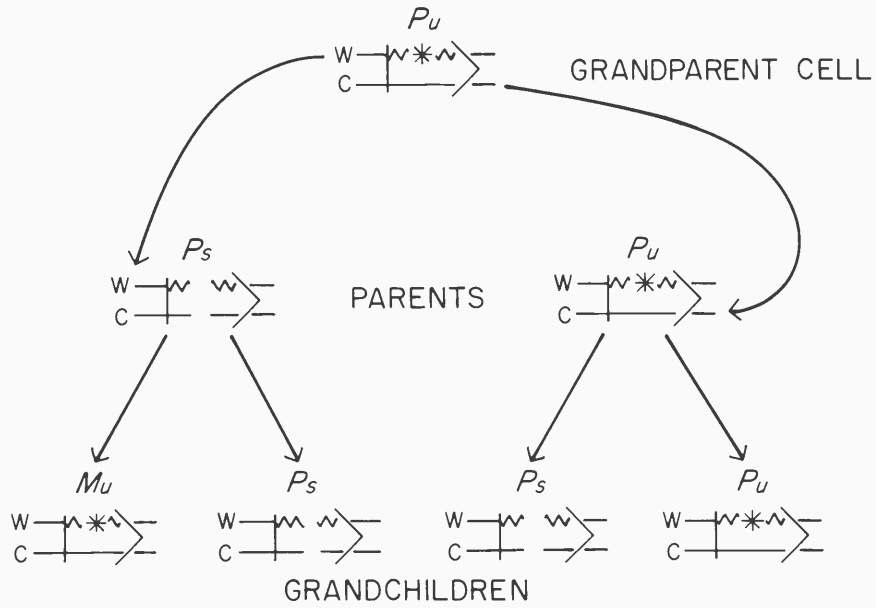

FIGURE 3 Strand-segregation model. Arrows originating from the grandparent cell indicate segregation of a particular DNA chain to the daughter cell. A gap in the continuity of the chromosome indicates the presence of a double-strand DNA break.

sister inheriting a break in the second cassette. In other words, the strand-segregation model predicts that a given cell should switch one or the other cassette, but never both cassettes, in a single cycle. As shown in Figure 5c, the 7.2-kb fragment is absent in the DNA isolated from a strain with the inverted duplication. More importantly, when the DNA was digested at sites flanking the duplication and then subjected to Southern analysis, we found that the observed level of break at either cut site is similar to that found in strains containing only one *mat1* cassette. Thus, in such a strain, two among four cells must be switchable. Therefore, the additional cell that acquires the capacity for switching must not be lacking critical cytoplasmic components required for switching. In conclusion, these results satisfy a key molecular prediction of the strand-segregation model and rule out the cytoplasmic segregation model. Thus, we conclude that the developmental asymmetry among sister cells of yeast is a consequence of inheriting nonequivalent parental DNA chains. The double-helical nature of the DNA molecule prompted the discoverers of that structure to make a well-known understatement, "It

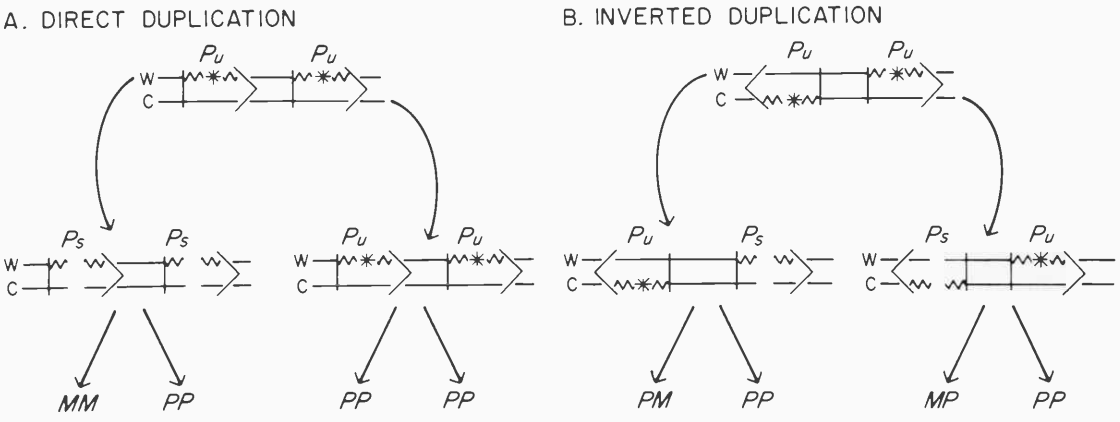

FIGURE 4 Predictions of the strand-segregation model with a direct (*A*) and an inverted (*B*) duplication of *mat1*.

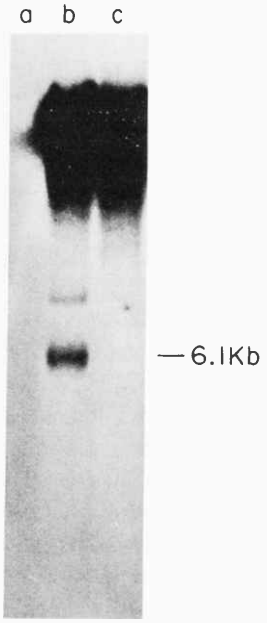

FIGURE 5 Southern blot analysis of DNA isolated from a strain with direct duplication (*b*) and inverted duplication (*c*). Undigested DNA was subjected to Southern blot analysis and was probed with the *mat1* sequence.

has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material." Our studies with yeast suggest that such a pretty molecule may also provide an inherent template for asymmetry among daughter cells in any biological system. For example, a modified strand or a preassembled transcriptionally competent complex may be delivered to only one daughter cell. Our future studies are directed toward understanding the molecular basis of chromosomal imprinting. To that end, *trans*- and *cis*-acting genetic elements that are known to affect switching are under investigation.

In a related study, we have addressed another interesting genetic property of this system. Egel (*Cold Spring Harbor Symp. Quant. Biol. 45:* 1003 [1981]) showed that *mat1* switching occurs only by obtaining information from the donor loci present on the same chromosome. Several other observations with this system suggest that an unusual organization of the *mat* region allows switching only in *cis* and also totally inhibits recombination between *mat2* and *mat3* genes, although these loci are located about 15 kb apart. When we meiotically crossed strains of opposite mating type containing deletions of *mat2* and *mat3* (designated *mat2,3*Δ::*LEU2*), each *mat1* allele gene in about 10% of the tetrads converted to the opposite allele. This was indicated by the generation of 3P:1M and 1P:3M tetrads. This *mat1* with *mat1* interaction occurs efficiently in meiosis when both donor loci are absent. We determined whether such an interaction would be blocked if the donor loci were present in *cis* to the *mat1* allele that is competent to gene-convert during meiosis.

For this purpose, we crossed a strain of genotype *mat1 mat2-P mat3-m*$^-$ (A strain) with one of genotype *mat1-M mat2,3*Δ::*LEU2* (B strain). The meiotic products were subject to tetrad analysis. The A strain has a mutation in the *mat3* cassette which, when transferred to *mat1*, allows the cell to mate as minus mating type; however, the zygotic cells will not undergo sporulation because of the mutation. A successful mating between these strains can only occur among *mat1-P*-containing cells of strain A and cells of strain B. Such a zygote will undergo sporulation. We found that in about 10% of the tetrads, the *mat1-P* allele gene-converted to the wild-type *mat1-M* allele. That is exactly the same frequency observed when *mat2* and *mat3* are absent. Thus, in meiosis, the opportunity for gene conversion by obtaining information from the donor loci does not preclude *mat1* interaction between homologs. Why meiotic behavior should be different from mitotic observations remains an interesting question for future studies.

Isolation of Genes Required for Mating-type Switching in Fission Yeast

R. Cafferkey, A.J.S. Klar

The switching of mating type in fission yeast requires the concerted action of at least ten SWI (switching) genes. Mutations in these genes either directly decrease the rate of mating-type switching or cause a reduction due to rearrangements at the mating-type expression locus (*mat1*). At the colony level, the phenotypes of *swi* mutations can be easily monitored by iodine staining. In a wild-type strain, cells of opposite mating type are generated at high frequency, and consequently mating and sporulation also occur at a high frequency. Since spore walls can be stained with iodine, a wild-type colony

turns black on exposure to iodine vapors. By reducing the rate of mating-type switching, *swi* mutations cause corresponding reductions in mating and sporulation and therefore show reduced iodine staining.

The mating-type-switching event is initiated by a double-strand break at the *mat1* locus. Three SWI genes (*swi1*$^+$, *swi3*$^+$, and *swi7*$^+$) are required for the generation of the break. Strains harboring these mutations have (1) a reduced level of double-strand breaks at *mat1,* (2) a correspondingly reduced rate of mating-type switching, and (3) a reduced rate of meiotic gene conversion at *mat1* in diploid cells. Using iodine staining as a screen, we have isolated the *swi3*$^+$ gene. An *S. pombe* genomic DNA library carried in the yeast/bacterial shuttle vector pDB248 was introduced into a strain of the genotype *swi3*$^-$ *leu1.32.* Approximately 50,000 *leu*$^+$ transformants were selected and exposed to iodine vapors. One *iodine*$^+$ transformant was identified, and a plasmid containing an 8.0-kb yeast DNA insert was recovered from this strain. Southern blot analysis was used to show that the yeast sequences in this plasmid were contiguous and present at a single copy number in the *S. pombe* genome. Linearization at a unique *Bgl*II site within the yeast sequences, followed by transformation, was used to target integration at the homologous chromosomal site. Genetic analysis demonstrated the absence of recombination between the integrated plasmid sequences and the *swi3* locus, thus establishing that the cloned sequences do contain the *swi3*$^+$ gene. Recently, H. Schmitt (pers. comm.) used a similar approach to clone the *swi1*$^+$ gene. We have also attempted the isolation of the *swi7*$^+$ gene. However, although a large number of transformants (>70,000) were examined, this attempt has thus far been unsuccessful.

Future experiments will be directed toward characterizing the structure and function of the *swi1*$^+$ and *swi3*$^+$ genes. Since all of the known *swi1* and *swi3* mutations are apparently leaky, gene-disruption experiments will be undertaken to determine the phenotypes of null mutations. The possibility that expression of these genes is cell-cycle-dependent will also be examined. Temperature-sensitive mutations of *swi1* and *swi3* proteins will be sought using in vitro mutagenesis of the cloned genes. Such mutations will be useful in determining the temporal order of protein action in the generation of the double-strand break at *mat1.*

Mutational Analysis of *mat1* Open Reading Frames

M. Kelly, A.J.S. Klar [in collaboration with D. Beach, Cold Spring Harbor Laboratory]

The *mat1* gene functions are required for mating and for meiosis and sporulation. An analysis of the DNA sequence revealed the presence of two open reading frames in each *mat1* allele. We have characterized transcripts covering the *mat1* locus and have found that each allele codes for two transcripts, which encompass the open reading frames. These transcripts are divergently transcribed from inside the cassette to the outside. Biological functions of these transcripts were defined by site-directed oligonucleotide mutagenesis of each coding region. Primarily nonsense *opal* mutations were generated. Phenotypic analysis of the mutations showed that two genes, one in each allele, are required for sporulation, whereas the other transcript of each allele is required for mating. Furthermore, it was found that those functions required for mating are expressed constitutively and those required for sporulation are expressed only under conditions of nutritional limitation.

Regulation of Silent Mating-type Gene Expression in *S. cerevisiae*: The Search for *SUM1*

G.P. Livi, A.J.S. Klar

Mating-type interconversion in the yeast *S. cerevisiae* involves a genetic rearrangement in which silent copies of α or **a** information, residing at the *HML* and *HMR* loci, respectively, are transposed to and expressed at *MAT.* The expression of *HML* and *HMR* is negatively regulated by the action of four unlinked *MAR* (or *SIR*) loci. Repression of *HM* gene transcription has been ascribed to the interaction of the *MAR/SIR* gene products, with *cis*-acting control sites that flank each locus. However, recent biochemical data suggest that the *MAR/SIR* gene products may not directly interact with these sites (Shore et al., *EMBO J. 6:* 461 [1987]).

To understand the molecular basis for this type of control, we have been studying the effect of the *sum1-1* mutation on *HM* gene expression. *sum1-1*

acts as an extragenic suppressor of mutations in *SIR2*, *SIR3*, and *SIR4* and may therefore define a gene more directly involved in regulating the expression of *HML* and *HMR* (Klar et al., *Genetics 111:* 745 [1985]). Northern blot analysis indicates that this suppression occurs at the level of *HM* gene transcription. The simplest genetic model to account for the involvement of the wild-type *SUM1* locus is one in which the *MAR/SIR* gene products negatively regulate *SUM1,* whose gene product, in turn, is required for *HM* gene expression. Consistent with this model is the fact that the *sum1-1* mutation exhibits no effect on *MAR/SIR* gene transcription.

Initial efforts to clone the *SUM1* gene have resulted in the isolation of two unique DNA sequences that complement the *sum1-1* mutation in vivo when carried on a high-copy-number plasmid. Each sequence codes for a single mRNA species. Neither sequence, however, contains the wild-type *SUM1* gene, as determined by selective integration and genetic mapping relative to *sum1-1.* Work is in progress to determine the effect of deleting each of these genes and to distinguish the *SUM1* gene clone from a new collection of complementing sequences derived from a centromere-based plasmid library.

Molecular Genetics of the *SUM* Genes of *S. cerevisiae*

C.I.P. Lin, G.P. Livi, A.J.S. Klar

The interconversion of budding-yeast mating type also involves a gene transposition event in which the silent information present at the *HML* and the *HMR* loci is transferred to the expressed *MAT* locus. The silent mating-type cassettes at *HM* loci are kept under negative transcriptional regulation by the action of four *trans*-acting *MAR/SIR* genes. We have previously identified an apparently positive regulatory gene, designated *SUM1* (*SU*ppressor of *M*ar); mutation of this gene results in the suppression of the *mar1-1* mutant allele. The *sum1* mutation keeps the *HM* loci unexpressed even in the *mar1* background. Interestingly, this suppressor also acted as the suppressor of a *mar1* deletion and a *mar2* deletion. It also apparently suppressed the *sir4* ochre mutation, but very weakly. Our recent results demonstrate that *sum1* does not suppress the *sir4* deletion mutation, and thus we do not believe that *sum1* acts as a suppressor of *sir4.*

To investigate possible interactions among the *MAR/SIR* genes and to identify new genes involved in regulating *HML* and *HMR* expression, suppressors of *mar2-1* were isolated. The two mutations thus identified have been designated *sum2-1* and *sum4-1.* These suppressors also suppress a deletion mutation of the *mar2* locus but fail to suppress a *mar1* deletion and a *sir4* deletion. Thus far in all of the *trans*-acting regulators identified, both *HML* and *HMR* are found to be coregulated regardless of whether the **a** or the α information resides there. The *sum2-1* and *sum4-1* mutations, however, strongly repress the expression of the **a** information at both *HML* and *HMR* loci but only weakly repress the expression of α cassettes at these same loci. This genetic analysis suggests that the gene products of *sum2* and *sum4* act downstream from the *MAR2* function but upstream of the *MAR1* and *SIR4* functions. The *SUM1* by this analysis can be placed following *MAR1* but before *SIR4.* The simple model that *MAR/SIR* and *SUM* genes transcriptionally regulate each other in a cascade is not supported, since the level of *MAR2* and *SIR4* is not affected by the *sum4* mutation. Further molecular analysis of these genes is needed to understand their role in silent cassette regulation.

The W and Z2 Sequence Difference between *HML* and *HMR* May Only Partially Regulate the Donor-choice Mechanism

A.J.S. Klar, L.M. Miglio

Our previous studies have shown that cells switch in a nonrandom fashion and that the direction of switching is dictated by the contents of the donor *HML* and *HMR* loci. Specifically, we found that *MAT*α cells preferentially choose *HMR* as the donor, whereas *MAT***a** cells preferentially choose *HML* as the donor. There seems to be a competition between the donors, since when the preferred donor is deleted, the remaining donor is used efficiently (Klar et al., *Cell 28:* 551 [1982]). An obvious feature that may affect the competition is the DNA sequence difference between *HML* and *HMR*. A 700-bp sequence (designated W) and a 90-bp sequence (designated Z2) are present both at *HML* and at *MAT*, but not at *HMR*. We have tested the possibility that the presence of W and Z2 sequences at *HML*, or their absence at *HMR*, plays a role in

the left-right donor-choice mechanism by replacing *HMR* with *HML*. A 5.4-kb *Xho*I fragment containing *HML* was inserted into a deletion of *HMR* that was constructed by combining the *Xho*I linker mutations nos. 51 and 296 (Abraham et al., *J. Mol. Biol. 176:* 307 [1984]). Such a strain with the cassette arrangement *HMLα-inc/HMLα-inc MAT***a**/*MATα-inc hmr*::*HMLα/hmr*::*HMLα HO/HO* was constructed. The **a** spores obtained from this strain were allowed to switch and mate with their α littermates. The resulting diploids will be either *MAT***a**/*MATα* or *MAT***a**/*MATα-inc*. In individual diploids, the presence of α or α-*inc* at *MAT* was distinguished by digesting the DNA isolated from these strains with *Hha*I endonuclease, which cuts α but not the α-*inc* DNA at a sequence present at the Y/Z junction.

With this procedure, we have found 65 cases where the *HML* was used and 10 cases where the *hmr*::*HML* donor was used. In our previous studies (a similar experiment with a strain containing wild-type *HMR*α), we found 47 cases where *HML* was used and only 5 cases where *HMR* was used (Klar et al., *Cell 28:* 551 [1982]). Thus, the W and Z2 sequence difference between *HML* and *HMR* only partially regulates the donor-choice mechanism.

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MOLECULAR GENETICS OF CELL-CYCLE CONTROL

D. Beach M. McLeod L. Brizuela
J. Potashkin G. Draetta
R. Booher M. Stein

During the past year, our research has remained concentrated in two main areas of interest: (1) the molecular genetics of the cell-division cycle and (2) the control of the transition from vegetative growth to sexual differentiation. The fission yeast, *Schizosaccharomyces pombe,* continues to be the major organism of study, but we have recently used a monoclonal antibody prepared against a yeast cell-cycle-control protein to identify its homolog in human cells. It is hoped that this finding will allow us to become directly involved in studies on the control of the division cycle in mammalian cells.

Control of Meiosis

M. McLeod, M. Stein, D. Beach

Major components of the pathway that controls the entry of yeast cells into meiosis and sporulation have been previously worked out by ourselves and investigators in other laboratories. The genes of the mating-type locus encode four gene products known as *matPi, Pc, Mi,* and *Mc*. All four genes appear to be required for meiotic initiation (M. Kelly, Yeast Genetics Section), which occurs only in h^+/h^- diploid cells. The *mat* genes act to control the transcription of an unlinked gene, *mei3*$^+$, that is expressed only during meiosis. Expression of this gene alone is sufficient to induce meiotic initiation. We believe that the product of the *mei3*$^+$ gene, a protein of 21 kD, acts to inhibit the product of the *ran1*$^+$ gene. The sequence of *ran1*$^+$ suggests that it encodes a protein kinase.

With the assistance of E. Harlow's laboratory (Protein Immunochemistry Section), monoclonal antibodies have been raised against the 21-kD *mei3*$^+$ gene product and the 52-kD *ran1*$^+$ gene product. The 21-kD protein was found only in meiotic cells, whereas the 52-kD *ran1*$^+$ gene product is present in

both vegetatively growing cells and meiotic cells. We are presently using the monoclonal antibodies to investigate the protein kinase activity of *ran1*$^+$ and to understand how it is controlled by *mei3*$^+$.

Two new mutants, *cgs1* and *cgs2*, that play a role in meiotic initiation have been isolated. The *cgs* mutants suppress the ability of the temperature-sensitive *ran2* mutants to undergo meiosis and sporulation; they are thus extragenic suppressors of *ran1* mutants. We have found that the *cgs1* mutant, which itself is meiotically defective, can be rescued by introduction of the gene of *Saccharomyces cerevisiae* encoding the regulatory subunit of cAMP-dependent protein kinase (*BCY1* gene, provided by M. Wigler's laboratory). This suggests that the *cgs1* gene may encode the fission yeast regulatory subunit. The nucleotide sequences of both the *cgs1* and *cgs2* genes are currently being determined.

Cell Cycle of Fission Yeast

G. Draetta, R. Booher, L. Brizuela, J. Potashkin, D. Beach

The product of the *cdc2*$^+$ gene is unique in being required during both the G_1 and the G_2 phases of the cell cycle and is still the major focus of our research. A series of 40 monoclonal antibodies against the 34-kD *cdc2*$^+$ gene product was prepared with the assistance of C. Bautista and E. Harlow (Protein Immunochemistry Section). These antibodies recognize the *cdc2* protein in a Western blot, and several were also effective in immunoprecipitation. We have been able to establish that the *cdc2* protein exists in at least six forms in yeast. Some of these forms represent different states of protein phosphorylation. We are presently attempting to identify the sites of phosphorylation.

Since the *cdc2* product is a protein kinase, we are interested in identifying its natural substrates. One possible candidate, the product of the *SUC1*$^+$ gene, has been tested. This gene was isolated 5 years ago by virtue of the ability of the plasmid-borne gene to rescue some, but not all, temperature-sensitive *cdc2* mutants. The gene was sequenced, mapped by S1 nuclease, and found to encode a product of 13 kD. The coding region of the gene was interrupted by two introns. The 13-kD product of the *Suc1*$^+$ gene was expressed at high levels in *Escherichia coli* and purified. We have not been able to find evidence that the *Suc1*$^+$ protein is a substrate of the *cdc2*$^+$ protein kinase in vitro. Further genetic screens for potential substrates of the *cdc2* protein kinase have been devised and have resulted in the isolation of many new mutants that are presently being characterized.

A new approach toward identification of proteins that associate with *cdc2* has recently been developed. We constructed an allele of *cdc2* (*cdc2Arg33*) that is mutated at the anticipated ATP-binding site. This "null allele" had an unexpected property. Overexpression of *cdc2Arg33* in a wild-type (*cdc2*$^+$) cell provoked a *cdc*$^-$ phenotype, whereas overexpression of the wild-type *cdc2*$^+$ gene caused no overt phenotype. We suspect that the product of *cdc2Arg33*, a protein that lacks kinase activity, competes with the wild-type gene product for a scarce activator of *cdc2*. The mutant allele might bind the activator, and in excess titrate out the activator, but of course not carry out its role in the cell cycle due to the *Arg33* mutation. We are currently attempting to identify sequences that, at high copy number, will rescue yeast strains expressing *cdc2Arg33*.

Identification of 34-kD Homolog of *cdc2*$^+$ Product in Human Cells

G. Draetta, J. Potashkin, D. Beach

The *cdc2*$^+$ gene of *S. pombe* is homologous to the *CDC28* cell-cycle "start" gene of *S. cerevisiae*. The gene products are protein kinases that have 62% overall amino acid sequence homology. Each gene is biologically active after introduction into the other yeast. Budding yeast and fission yeast are evolutionarily very distant, and since the *cdc2* and *CDC28* gene products are so similar, it seems likely that the cells of higher eukaryotes might contain an equivalent protein.

We have sought a human homolog of the 34-kD *cdc2*$^+$ gene product using monoclonal antibodies prepared against *cdc2*$^+$. First, each of 40 antibodies was tested for its capacity to recognize the *CDC28* gene product. Only two, J4 and JP4, cross-reacted with both the *cdc2* and *CDC28* proteins. We felt that these would be suitable antibodies with which to search for homologs in the cells of higher organisms. To our surprise, both the J4 and JP4 antibodies recognized a 34-kD protein in extracts of HeLa cells. The human 34-kD protein was identified both

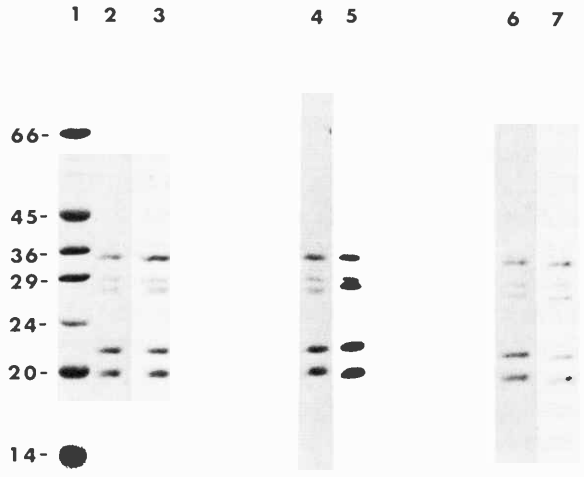

FIGURE 1 Proteolytic cleavage pattern of $p34^{cdc2}$, $p36^{CDC28}$, and human p34. (*1*) Standard markers with molecular weights indicated in kilodaltons. (2–7) *N*-chlorosuccinimide/urea cleaved proteins; (*2*) Coomassie-stained $p34^{cdc2}$; (*3*) ^{35}S-labeled $p34^{cdc2}$; (*4*) Coomassie-stained $p34^{cdc2}$; (*5*) ^{35}S-labeled $p36^{CDC28}$; (*6*) Coomassie-stained $p34^{cdc2}$; (*7*) ^{35}S-labeled human p34. Lanes *2* and *3*, *4* and *5*, and *6* and *7* are different visualizations (Coomassie blue or autoradiography) of single gel lanes. In each lane the uppermost band represents undegraded protein, and the smallest major band (20 kD) carries the entire amino-terminal two thirds of the polypeptide. The intervening three bands are, in each case, partial cleavage products.

in Western blots and by immunoprecipitation. Mapping of the tryptophan residues in the human protein by means of *N*-chlorosuccinimide/urea cleavage revealed complete conservation in the number and distribution of tryptophans between human p34, the *CDC28*, and the *cdc2*$^+$ gene products (Fig. 1).

It seems highly probable that the 34-kD protein from HeLa cells is a homolog of the *cdc2* and *CDC28* gene products of *S. pombe* and *S. cerevisiae*. We are presently testing whether the 34-kD protein has protein kinase activity and are investigating its properties in different growth states and at different stages of the cell cycle.

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SITE-SPECIFIC MUTAGENESIS

M. Zoller K. Johnson
L. Levin
J. Kuret

We are studying protein-protein interactions of yeast cAMP-dependent protein kinase. The experimental system of yeast is perfectly suited to the study of protein structure and function, since mutated proteins can be examined in the absence of the wild-type protein, and nonfunctional proteins can be analyzed using the power of yeast genetics. During the past year, we have been examining a number

of *Escherichia coli* expression systems in order to produce large amounts of protein for subsequent structural studies.

Structure-Function of Yeast cAMP-dependent Protein Kinase

M. Zoller, K. Johnson, L. Levin, J. Kuret

Our studies are aimed at an understanding of protein-protein interactions at the molecular level. cAMP-dependent protein kinase provides a system through which a number of questions can be addressed: (1) How does an enzyme, in this case a protein kinase, recognize its substrate? (2) What regions of the regulatory and catalytic subunits are important for association into the holoenzyme and how does cAMP disrupt this association? (3) How does phosphorylation change the structure and function of a protein? (4) What are the substrates in yeast?

A second area of research interest concerns the "new science" of protein engineering. Can existing proteins be modified to conduct new reactions, to eliminate undesirable reactions, or to operate under normally suboptimal conditions? The current approach has been largely through directed substitutions of amino acids at regions of the protein thought to be important. For example, protein engineering has successfully increased protein stability by introduction of disulfide bonds, has restricted substrate specificity in a protease, and has meticulously determined the importance of certain residues in binding. In the field of protein kinases, we are interested in understanding the specificity of the cAMP-dependent protein kinase for its substrates, in contrast to the tyrosine protein kinases of receptors and viral oncogene kinases. By using a genetic approach, we are trying to screen for a protein kinase with a new or restricted substrate specificity.

Catalytic Subunit (*TPK1, TPK2, TPK3*)

M. Zoller, K. Johnson, L. Levin, J. Kuret

We previously expressed *TPK1* in *E. coli* using the T7 phage promoter system. The protein was highly expressed, although we found that only about 10% of the total *TPK* protein was soluble. This, however, proved to be sufficient for use in phosphorylation studies, and thus we will soon begin purification. Upon the completion of sequencing of the other two related catalytic subunit genes, *TPK2* and *TPK3,* by T. Toda in M. Wigler's group (Genetics of Cell Proliferation Section), we began to express these genes as well. Genetic studies by S. Cameron and T. Toda demonstrated that any one of the three *TPK* genes was sufficient to maintain cell growth. Comparison of the putative protein sequences revealed that the major differences were in the amino-terminal regions. To compare the functions of the three kinases, we expressed *TPK2* and *TPK3*, as well as *TPK1*, in *E. coli.* It is possible that *TPK2* or *TPK3* expressed in *E. coli* will be more soluble than *TPK1* expressed in *E. coli.*

RESULTS

Monoclonal Antibodies to TPK1. *TPK1* was expressed in *E. coli*, and the protein was isolated in pure form by electroelution from an SDS-polyacrylamide gel. In collaboration with E. Harlow, with the help of C. Bautista (Protein Immunochemistry Section), the protein was used as antigen to develop monoclonal antibodies against *TPK1.* We obtained 17 monoclonal antibodies that recognized *TPK1* bound to nitrocellulose paper. Yeast cell extracts from strains containing just one of the three *TPK* genes were electrophoresed on an SDS-polyacrylamide gel and transferred electrophoretically to nitrocellulose. Western blot analysis revealed that the monoclonal antibodies raised against *TPK1* only recognized a protein in the *TPK1* strains. Preliminary evidence suggests that we could not detect *TPK2* or *TPK3* because the levels of these proteins are low compared with that of *TPK1*; activity studies in yeast by S. Cameron support this. With large quantities of *TPK2* and *TPK3* proteins expressed in *E. coli,* we will have *TPK2* and *TPK3* protein available for specific monoclonal antibody production.

Studies on the ATP-binding Site of TPK1. We previously constructed a mutant *TPK1* in which Lys-116 was changed to Arg-116. This resulted in the loss of catalytic activity, suggesting that charge alone was not sufficient for catalytic activity. A subsequent study demonstrated that the new Arg-116 protein could still bind to the regulatory subunit (*BCY1*) and was dissociated by the addition of

cAMP to the solution. This experiment was carried out by first binding *BCY1* to an anti-*BCY1* monoclonal antibody column and then passing ^{35}S-labeled *TPK1* through the column to form the holoenzyme on the column. Both the wild-type and Arg-116 *TPK1* proteins bound to the column, and upon addition of cAMP, the ^{35}S-labeled proteins were released.

Production of TPK2 *and* TPK3 *in* E. coli. The coding sequences of these genes were engineered into our T7 expression vector. These vectors have been modified from our earlier version by the addition of the F_1 origin sequences that turn these vectors into "phagemids" from which single-stranded DNA can be isolated for sequencing or oligonucleotide-directed mutagenesis. Our results indicate that the level of expression of *TPK2* and *TPK3* is approximately the same as that of *TPK1*. Similarly, the majority of the protein was insoluble. Activity analysis is now in progress.

FUTURE EXPERIMENTS

Purification of TPK1, TPK2, *and* TPK3. Recently, Jeff Kuret joined our laboratory as a postdoc; he had previously worked at the laboratory of P. Cohen, who is known for work on protein phosphorylation. Jeff and Lonny Levin will be responsible for developing a purification procedure for *TPK*. Pure catalytic subunit has never been purified from yeast, probably because the levels were low and there is a problem with proteolysis. Prior to the cloning of the *TPK* genes, the existence of three catalytic subunits was unknown. By using the *E. coli* strains, we can produce sufficient amounts of protein to characterize the three proteins fully. The availability of yeast strains that express and overproduce only one of the three *TPK*s (developed by M. Wigler's group) makes it possible to isolate the individual proteins from yeast. Expression of individual *TPK* genes using the ADH promoter should improve our yield of protein. The natural amino-terminal sequences of the three proteins are not yet known. Amino-terminal modification is also a question that remains to be answered.

Probing Protein-Protein Interactions between the Catalytic and Regulatory Subunits. We are interested in identifying regions within *TPK* and *BCY1* that are involved in the specific interaction between the two proteins. The approach for studying the catalytic subunit will be to use a genetic screen (developed in M. Wigler's laboratory) to identify suppressors of CYR^{ts} and RAS^{ts} phenotypes. These strains are characterized by a defect in the production of cAMP at 37°C. Among the possible suppressors will be activated catalytic subunits. Of these, some will be activated because of a defect in the association with regulatory subunit. They will be identified, sequenced, and biochemically analyzed for the ability to complex with *BCY1*. This analysis should yield a map of sites within the catalytic subunit that are involved in complex formation. Recently, we sequenced one of these suppressors and found that the defect is a single amino acid change of Thr-241 to Ala-241 in *TPK1*. This site is conserved in all cAMP-dependent protein kinases and was found to be phosphorylated in the bovine catalytic subunit. The region in which Thr-241 lies is thought to play a role in substrate interaction, and preliminary evidence indicates that this mutation does not affect catalytic activity. Current studies are in progress to test whether this mutant complexes with *BCY1*.

Phosphorylation of Specific Substrates of the Yeast Catalytic Subunit. To study the manner in which protein kinases recognize their substrates, it is important to identify natural substrates for *TPK*. The enzymes trehalase and fructose-1,6-bisphosphatase have been tentatively identified to be regulated by cAMP-dependent phosphorylation. Recently, R. Fletterick and co-workers (University of California, San Francisco) suggested that yeast glycogen phosphorylase was a substrate by inspection of its phosphorylation site (ArgArgLeuThr). We plan to obtain purified samples of these proteins to use as substrates for each *TPK*. In addition, these proteins may be used as reagents to probe enzyme-substrate interactions.

Cloning Kinase Substrates from Expression Libraries. The biological recognition sequence for cAMP-dependent phosphorylation has been characterized by both natural and synthetic substrates as ArgArgXaaSer(or Thr) or LysArgXaaXaaSer(or Thr). Generally, it is believed that this sequence is found in an exposed region of the natural substrate. We are interested in identifying other substrates and have developed a scheme to clone genes for other substrates from a yeast expression library. An expression library is plated out and prepared as for antibody screening. The filters

are incubated in buffer and [^{32}P]ATP, and an aliquot of *TPK1* is purified from *E. coli*. Clones expressing proteins with the proper recognition sequence will become phosphorylated and will be identified by autoradiography. This method provides direct access to the gene. Preliminary tests using known substrates and nonsubstrates have been encouraging. It is still not clear how frequently the kinase will phosphorylate a protein in vitro that is not a natural in vivo substrate. We hope that this will become a general method for identifying potential kinase substrates.

BCY1, the Regulatory Subunit of cAMP-dependent Protein Kinase

M. Zoller, K. Johnson, L. Levin, J. Kuret

BCY1 encodes the gene for the regulatory subunit of cAMP-dependent protein kinase. The protein sequence of the isolated regulatory subunit matched the coding sequence of the *BCY1* gene (T. Toda and co-workers). We were interested in structure-function relationships of this protein. The protein consists of three major domains: (1) an amino-terminal region, which maintains a dimer structure; (2) a hinge region, which is a major site for regulatory-catalytic (R-C) subunit interaction and contains a phosphorylatable substrate consensus sequence, ArgArgThrSerVal; and (3) a carboxy-terminal region, which consists of two tandemly repeated cAMP-binding domains. Previously, we overexpressed the *BCY1* protein in *E. coli,* purified the protein to homogeneity, and characterized the purified protein (K. Johnson and co-workers). The functional characteristics of the protein were indistinguishable from those of the natural yeast protein. In the absence of cAMP, the protein inhibited yeast *TPK1,* one of the three catalytic subunits from yeast. In addition, *BCY1* was phosphorylated by *TPK1.*

RESULTS

Monoclonal Antibodies to BCY1. The purified protein was used as antigen to produce monoclonal antibodies. From our initial fusion, only three positive clones were isolated, and all three clones reacted against *BCY1* produced in *E. coli.* A Western blot procedure was used to detect *BCY1* in yeast strains. A strain containing a multicopy plasmid with the *BCY1* gene showed an increased level of regulatory subunit, compared with a strain containing a single copy of *BCY1.* This analysis revealed that the protein in yeast migrated as a doublet on an SDS-polyacrylamide gel. The lower band migrated in coincidence with *BCY1* (*E. coli*-produced) and the upper band migrated 2000 daltons slower. This suggests that the protein in yeast undergoes posttranslational modifications. We previously demonstrated that phosphorylation of *BCY1* by *TPK1* in vitro did not change the mobility of the *E. coli*-produced protein. Efforts are under way to identify the nature and location of these modifications.

Mutations in the Hinge Region That Alter R-C Interaction and Phosphorylation of the Regulatory Subunit. The "hinge" region of the regulatory subunit is characterized by a substrate consensus sequence, ArgArgThrSerValSer, and is thought to be a major site for interaction between the regulatory and catalytic subunits. The role of phosphorylation in vivo is still unclear. To probe the importance of the hinge region in subunit-subunit interaction and the role of phosphorylation in the function of cAMP-dependent protein kinase, we systematically introduced amino acid substitutions into the *BCY1* protein. Our first mutations were aimed at Ser-145, the site of phosphorylation by catalytic subunit (ArgArgThr*Ser*ValSer). Using oligonucleotide-directed mutagenesis, we changed Ser-145 to Ala, Asp, Glu, Gly, Lys, Pro, and Thr. Each variant was expressed in *E. coli,* purified using a cAMP-agarose affinity column, and assayed for the ability to inhibit the yeast catalytic subunit (*TPK1*) in the absence of cAMP. Of these, the Ala, Gly, Glu, and Lys derivatives inhibited catalytic activity and presumably formed a complex with the catalytic subunit. Careful inspection of the data indicated that the Ala-145 and Gly-145 derivatives bound tighter to *TPK1* than to wild-type *BCY1* and that Glu-145 and Lys-145 derivatives bound less well to *TPK1* than to wild type. These results suggest that this region is a major site of R-C interaction, since single-amino-acid alteration can perturb stable complex formation.

We next assayed for the ability of each protein variant to be phosphorylated by *TPK1* in vitro. Of these, the Thr-145 derivative was phosphorylated to the same level as wild-type *BCY1,* and at approximately the same rate. The Lys-145 derivative was phosphorylated poorly. These results indicate that

the site of phosphorylation of the wild-type protein is Ser-145. The identification of the phosphorylated amino acid in the Lys-145 and Thr-145 derivatives is currently being determined in collaboration with D. Marshak (Protein Chemistry Section). It is known that the catalytic subunit can phosphorylate the sequence ArgArgXaaThr, and thus we predict that Thr-145 is phosphorylated in that derivative. The phosphorylated amino acid in the Lys-145 derivative could be either Thr-144 (ArgArg*Thr*Lys) or, possibly, Ser-147 (ArgArgThrLysVal*Ser*).

FUTURE EXPERIMENTS

In Vivo Role of Phosphorylation of BCY1. Having characterized seven different modifications at the phosphorylated serine, we will next probe the role of phosphorylation in the cell. This is an experiment that cannot be conducted in higher eukaryotic cells.

Identification of Amino Acids Involved in cAMP Binding. A comparison of the primary sequences of mammalian regulatory subunits, *E. coli* cap, and the yeast regulatory subunit revealed a number of conserved amino acids within the cAMP domains. Some of these residues have been shown to be involved in cAMP binding in cap by crystallographic analysis. We are interested in exploring the specific role of these conserved amino acids, as well as other regions that may be important and that cannot be identified by homology. The conserved amino acids can be systematically mutated and the properties of the resultant protein can then be studied. To identify the nonconserved regions that play a role in cAMP binding, we are using a random mutagenesis approach followed by a biochemical screen. Initially, we will pass the *BCY1* expression vector through a *mutD* strain that is deficient in replication error repair. The mutagenized plasmid will then be placed into the *E. coli* expression strain, and the colonies will be plated onto nitrocellulose. The filters will be prepared as for antibody screening, and ^{32}P-labeled cAMP will be added to the filters. The filters will then be washed and autoradiographed. Clones that can still bind cAMP will be detected on the autoradiogram. Mutants will then be isolated and subsequently screened for expression of *BCY1* and cAMP binding. Some of the mutants will be simply truncated proteins or unstable protein. We will conduct biochemical experiments to distinguish between the various mutants. Out of this analysis, we hope to obtain a map of positions that are important for cAMP binding. These data will complement crystallographic data obtained by J. Pflugrath and co-workers.

Localization of BCY1 *Protein in Yeast.* Monoclonal antibodies that recognize *BCY1* will be used to localize the protein in vivo. This will be accomplished by indirect immunofluorescence. A report on the mammalian cAMP-binding subunit localized the protein in the Golgi. Upon stimulation of adenyl cyclase, the localization of the regulatory subunit did not change; the catalytic subunit moved into the nucleus. It will be of interest to conduct this experiment in yeast to identify localization signals and to understand differences between the three *TPK* proteins.

Three-dimensional Structure of BCY1. We will be collaborating with J. Pflugrath and co-workers on determination of the three-dimensional structure of *BCY1*. The levels of protein produced by the *E. coli* expression system are now in the range needed to begin these studies. We will help with the expression and purification of the wild-type protein, as well as provide mutant proteins.

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PLANT GENETICS

S.L. Dellaporta M. Alfenito G. Giuliano C. Mitchell
A.C. Hiatt J. Chen P. Hinton D. Pollock
P. Scolnik P.S. Chomet B.A. Lowe J. Wood
M. Delannoy J. McIndoo

Ac Activity and DNA Modification

S.L. Dellaporta, M. Alfenito, J. Chen, P.S. Chomet, B.A. Lowe, C. Mitchell, J. Wood

During 1986, our research efforts continued to be focused on the biology and utility of the maize transposable element *Activator* (*Ac*). *Ac* has the ability to cycle from an active phase to an inactive phase. As reported last year, we have found a correlation between *Ac* activity and *Ac* DNA modification. We have extended these studies to show that this modification appears specific for *Ac* DNA, since flanking DNA sequences are not affected. This observation has allowed us to distinguish the active *Ac* element from several homologous cryptic *Ac*-like sequences found in the maize genome. Moreover, when genomic DNA is digested with methylation-sensitive restriction enzymes that do not cut within *Ac*, most, if not all, cryptic sequences remain in the high-molecular-weight fraction of DNA, whereas the active *Ac* element remains in the lower-molecular-weight fraction of DNA. Using this approach, we have cloned the *P* locus of maize by locating a genomic fragment in the hypomethylated DNA fraction containing *Ac* inserted into *P*.

MECHANISM OF *AC* TRANSPOSITION

We have analyzed the donor and target sites of *Ac* transposition from the *P* locus. Previous genetic analysis has indicated that the two mitotic daughter lineages that result from *Ac* transposition from *P* differ in their *Ac* constitution at the *P* locus. Both lineages, however, usually contain transposed *Ac* elements that map to the same genetic position. Using methylation-sensitive restriction enzymes and genomic blot analysis, we identified *Ac* elements at both the donor *P* locus and *Ac* target sites. Daughter lineages were shown to be mitotic descendants from a single transposition event. When both lineages contained *Ac* genetic activity, they both contained a transposed *Ac* element on identical genomic fragments independent of the genetic position of the target site. This indicates that in a majority of cases, *Ac* transposition takes place after replication of the donor locus but before completion of replication at the target site.

Other examples of somatic transposition of *Ac* from the *P* locus were analyzed where *Ac* activity was detected in only one of the two daughter lineages. In these cases, we did not detect the presence of the *Ac* element in the daughter lineage that lacks *Ac* activity. We are addressing the possibility that this lineage contains an inactive form of *Ac*. However, it appears that *Ac* has the ability to excise from *P* after replication and integrate into replicated target sites.

GENE-TAGGING EXPERIMENTS

We have performed several experiments to tag gene loci using maize transposable elements. As reported last year, we were able to clone the *R* locus of maize by transposon tagging with *Ac*. This past year, we attempted to mobilize *Ac* and *Mul*, another maize transposable element system, into genes involved in insect resistance (*bx*), gravitropism (*la*), and starch biosynthesis (*su*). Several mutations of *la* and *su* have been recovered, and we are presently confirming their inheritance by genetic analysis as well as their molecular nature by genomic blot analysis. We will begin molecular cloning experiments if these mutations are confirmed by these experiments. Another phase of this work has

been random insertional mutagenesis experiments. We have selected several hundred independent transpositions of *Ac* from gene loci on chromosome 1 (*P* locus), chromosome 9 (*Bz* and *Wx* loci), and chromosome 10 (*R* locus). This material, heterozygous for the newly transposed *Ac* element, was self-pollinated to obtain homozygous insertions. We are presently screening several thousand progeny for mutations segregating in this F2 population. Any mutations of interest will be cloned using *Ac* as a molecular probe.

Regulation of Polyamine Metabolism in Plant Cell Cultures

A.C. Hiatt, J. McIndoo, B.A. Lowe

Work on the regulation of polyamine metabolism in plants during the last year has focused on the involvement of polyamines in plant stress responses, regeneration of plants from cultured cells, gene regulation controlled by the rate of polyamine production, and purification of the enzymes responsible for polyamine synthesis.

POLYAMINES AND PLANT STRESS

The induction of polyamine production in plants exposed to many stress conditions is a well-characterized phenomenon that has been observed for more than 30 years. The use of plant cell cultures and mutants has enabled us to study the significance of this response to determine whether the increased synthesis of polyamines is a metabolic dead end or a response that enables the cell to withstand a particular adverse environmental condition. We have concentrated on low-pH stress, although many other stress conditions have the same effects (e.g., low potassium, high NaCl, high osmoticum, and desiccation). These studies were performed with tobacco cells in suspension culture growing in defined medium. We discovered that low-pH stress specifically induces the synthesis of only the first polyamine, putrescine (Fig. 1), and that this is specifically a decarboxylation of arginine. Increased synthesis of putrescine from or-

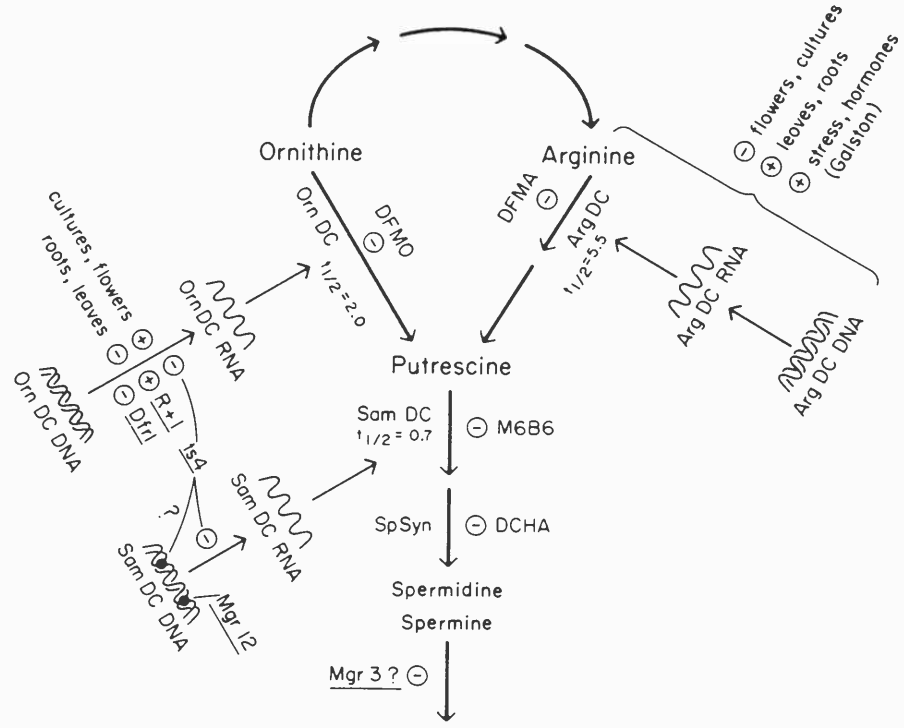

FIGURE 1 Polyamine pathway in tobacco. (OrnDC) Ornithine decarboxylase; (ArgDC) arginine decarboxylase; (SamDC) *S*-adenosylmethionine decarboxylase; (SpSyn) spermidine or spermine synthase; (DFMO) difluoromethylornithine; (DFMA) difluoromethylarginine; (MGBG) methylglyoxal-bis(guanylhydrazone); (DCHA) dicyclohexylammonium sulfate.

nithine was not observed. We also found that the increased conversion of arginine is an essential stress response, since addition of difluoromethylarginine (DFMA) during the response is lethal, whereas at neutral pH, DFMA has no effect on cell growth (Fig. 2). Finally, we also observed that the increased titer of putrescine during stress does not lead to increases in the other polyamines (spermidine or spermine), nor does it lead to increased conjugation of putrescine as an amide.

The polyamine conjugates are an interesting class of compounds because in the whole plant, they are only synthesized in abundance in the reproductive organs, especially the postfertilization ovary. Tobacco cell cultures also contain a substantial amount of polyamine conjugates (amides). During the past year, we have characterized the conjugation of polyamines in cell cultures of true polyamine mutants and have found that resistance to lethal inhibitors of free polyamine synthesis also results in abnormal polyamine conjugation, which, in turn, affects other metabolic pathways. In particular, the phenylpropanoid pathway in these mutants may be induced to produce large amounts of anthocyanin. This effect is due to abundant conjugation of polyamines to hydroxycinnamic acids, which are precursors to the anthocyanins, flavonoids, and lignin.

These observations prompted us to undertake a more direct approach toward understanding the metabolic function of the conjugates by measuring the involvement of conjugation in certain disease responses. In collaboration with the cell-culture group at Pioneer-Hi-Bred International, we have begun to study the biochemistry of certain disease responses in corn. In corn, infection by the fungus *Helminthosporium carbonum* results in a dramatic increase in synthesis of the phenylpropanoids from phenylalanine. This is true in both resistant and susceptible varieties of corn. The resistance response is characterized by rapid conversion of phenylpropanoids into antifungal phytoalexins. In

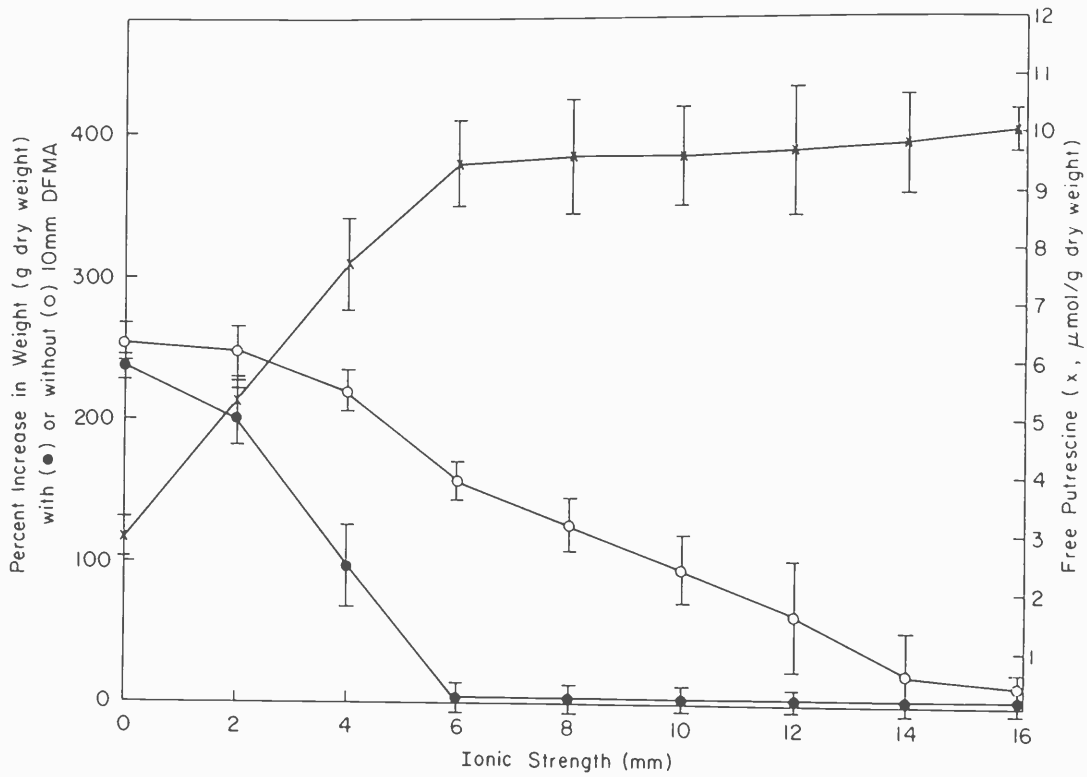

FIGURE 2 Effect of difluoromethylarginine and low pH on cell growth. Stationary-phase cultures were diluted fourfold into medium containing citrate-Tris (pH 3.5) of varying ionic strengths. An aliquot from each culture was taken to determine the initial dry weight. To half of the paired cultures was added 10 mM difluoromethylarginine. After 24 hr, an aliquot from each culture containing no difluoromethylarginine was taken to determine the intracellular free putrescine levels. The cultures were further allowed to grow for 7 days and were harvested to determine the increase in dry weight.

the susceptible response, however, these compounds are not synthesized and do not appear around the infection site; the fungus is then able to spread through the whole plant. Our efforts in the last few months have been directed toward trying to account for the induced phenylpropanoids and to determine if conjugation to polyamines subverts phytoalexin synthesis in susceptible varieties. Preliminary studies using purified fungal toxin and a resistant corn cell line have shown that the toxin alone specifically inhibits the flux of polyamines conjugated to the cell wall. These studies are currently being extended to include infection with whole fungus (both toxin-producing and non-producing) of susceptible and resistant varieties of corn in culture.

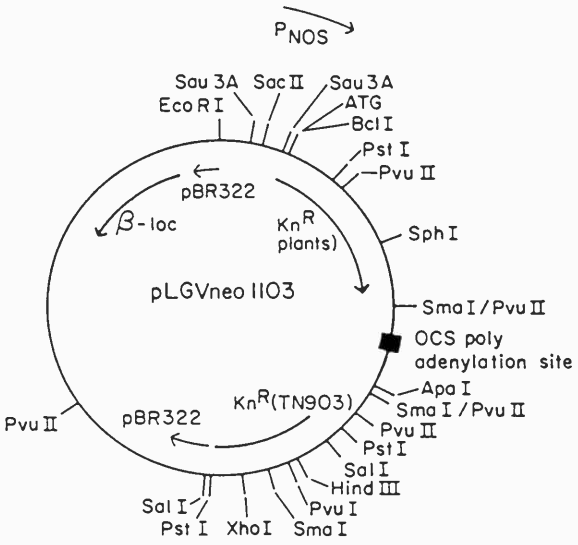

FIGURE 3 *Agrobacterium*-derived vector containing a plant promoter (P_{nos}) and a dominant selectable marker (Kn^R).

POLYAMINES AND DEVELOPMENT

The primary characteristic of tobacco cell lines that are resistant to inhibitors of polyamine synthesis is a failure to regenerate from culture into whole plants. In addition, plants that do regenerate and are developmentally abnormal are invariably slow to initiate the process of regeneration. However, this offers a way of isolating revertant cell lines by virtue of an enhanced rate of regeneration from a large population of cultured cells. Our results in the last year have shown that all revertants isolated by virtue of a restored ability to regenerate also have a restored ability to maintain the correct titer of free polyamines. One example is a difluoromethylornithine (DFMO)-resistant regeneration-deficient cell line that has a very low level of ornithine decarboxylase (OrnDC). The six revertants of this cell line isolated by virtue of restored initiation of regeneration also have restored levels of OrnDC and the wild-type levels of polyamines. Our results have suggested that there are a number of enzymes, in addition to the ones directly involved in biosynthesis, that control the polyamine titer. We have recently begun to try to isolate some of these genes by transforming regeneration-deficient polyamine mutants and selecting for restored regenerates. The transformation system we are using involves the use of *Agrobacterium tumefaciens*. An *Arabidopsis* genomic library in a bacterial plasmid was constructed in a disarmed *Agrobacterium* (Fig. 3); the resulting *Agrobacterium* recombinants are now being used to transform either leaf disks or protoplasts to select for kanamycin-resistant transformants with restored regeneration. *Arabidopsis* was used as the source of DNA because of the relatively small size of its genome, compared to that of tobacco.

Another approach to understand how production of polyamines may be involved in floral development involves the use of thin cell layer explants (TCL). In this system, floral stem sections at anthesis are induced to form either vegetative shoots, roots, or flower buds. It is not understood what determines the choice of developmental pathway in the cells of a TCL; however, any single TCL usually gives rise to only one type of structure. We have found that the production of polyamines, particularly from ornithine, is critical in giving rise to floral buds, whereas enhanced conversion of arginine to polyamines favors production of vegetative shoots. We are currently transferring the flowering polyamine mutants into TCL to see if a particular developmental abnormality of the parent plant (Table 1) is transferred in culture.

MOLECULAR GENETICS OF POLYAMINE REGULATION

Little is known about how polyamines control gene expression. To date, polyamine-regulated genes have not been well characterized from either animals or plants, with the exception of the murine OrnDC gene. In plants, it is known that many stress responses induce large increases in the titer of free polyamines. Thus, it is possible to isolate, by

TABLE 1 Whole Plant Phenotypes of Polyamine Synthesis Variants

Strain	Coral like	Very dwarf	Petal sepal	Extra petal	Petal anther	Stigma anther	Stamen ovule	Puzzle box
Class I								
Mgr5-RO[a]	X							
Mgr6-RO	X							
Mgr23-RO	X							
Class II								
Dfr1-RO		X						
ts4-RO		X						
Class III								X
Mgr15-RO		X						X
Mgr21-RO		X						
Class IV								
Mgr25-RO				X				
Rt1-RO					X			
Mgr3-RO							X	
Mgr3-F1[b]							X	
Mgr31-RO			X				X	
Mgr9-RO						X	X	
Mgr12-RO								
Mgr12-F1				X	X	X		
Mgr1-RO			X			X	X	
Mgr27-RO			X	X	X	X		

[a] RO, plants regenerated from culture.
[b] F1, progeny from a cross of an RO plant to wild type.

differential hybridization, cDNAs that are highly regulated by the polyamine titer, since a high percentage of mRNAs that are stress-induced are also very sensitive to the titer and ratio of intracellular polyamines. We have been interested in isolating these cDNAs to characterize the mechanism by which polyamines influence gene expression. Two dozen cDNAs were isolated that had greatly increased hybridization to mRNAs from stressed tobacco plants (both low pH and low potassium stress). One of these cDNAs has been shown by hybrid-selected translation and immunoprecipitation to be arginine decarboxylase (ArgDC). The abundance of ArgDC mRNA was greatly diminished when production of any polyamine was reduced in culture through feedback regulation by the addition of exogenous polyamines to the medium; ArgDC mRNA was also developmentally regulated, being found predominantly in leaves and much less in floral parts. Other cDNA probes have revealed developmentally regulated mRNAs or stress-regulated mRNAs, but not both. In general, the probes hybridized to near-single-copy genomic sequences. In contrast, ArgDC mRNA is complementary to approximately 50–100 genomic sequences that have extensive restriction polymorphisms. Of 24 stress-regulated cDNA probes used in Southern blots, only the ArgDC probe hybridized to numerous genomic copies; 18 of these sequences have thus far been cloned and partially mapped. Restriction polymorphisms suggest that they fall into four or five groups. One such genomic clone has been characterized in detail, and the promoter region and 5′-coding region have been isolated. The sequence immediately 5′ to the transcriptional start site is unremarkable; however, extensive sequencing of the entire 5′-untranslated region has not yet been completed. This promoter region has been reintroduced into a tobacco plant such that it produces a truncated ArgDC transcript and a 3′ octopine synthase polyadenylation signal and can thus be distinguished from the endogenous ArgDC mRNA. We are in the process of studying the effects of various stresses on transcription of the reintroduced sequence. Ultimately, we would like to determine which regulatory sequences are responsible for the transcriptional response to different types of stress. We also hope to understand why there are abundant, polymorphic ArgDC sequences in the tobacco genome and if this genetic organization is responsible for sensitivity of ArgDC to different environmental conditions.

Chloroplast Differentiation, Nuclear Gene Expression, and Transcription of Nuclear Genes in Tomato Plants

G. Giuliano, D. Pollock, P. Hinton, M. Delannoy, P. Scolnik [in collaboration with D. Spector, Cold Spring Harbor Laboratory]

Although the role of some environmental factors, such as light, in the regulation of plant gene expression has been extensively studied, little information exists on the action of other factors. That light is not the only factor controlling nuclear gene expression in plants is obvious from the fact that tissues such as roots, flowers, and fruits express certain nuclear genes at very low levels. In plant leaves, two typical examples of highly regulated genes are those coding for the small subunit (*ssu*) of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCase) and the chlorophyll-*a*/*b*-binding protein (*cab*). Both proteins are coded by small nuclear gene families, and the protein precursors are synthesized in the cytoplasm and then imported posttranslationally into the chloroplasts. A genetic approach to the study of the role of chloroplast differentiation in the regulation of nuclear gene expression is to utilize plants in which the development of these organelles is blocked by mutation.

Carotenoids are chloroplast membrane pigments whose basic function is to protect the photosynthetic membranes against the potentially harmful combination of light and oxygen. A mutant plant affected in carotenoid biosynthesis can suffer extensive photooxidative damage to its chloroplast membranes, but other cellular compartments seem to be relatively unaffected. Thus, plant carotenoid mutants constitute an attractive system for the study of nuclear-cytoplasmic interactions in plants. However, the problem with this approach is that this type of mutation is generally lethal due to the lack of photosynthesis. Seedlings develop using storage material, but after this supply is exhausted, plants die. Therefore, most studies with plant carotenoid mutants have been conducted on 1–3-week-old seedlings.

Our aim was to develop a system that would allow the study of gene expression in leaves of plants in which chloroplast differentiation is genetically blocked. We chose the tomato *ghost* mutant because previous reports indicated that this mutant is somatically unstable. Furthermore, among the dicots, tomato is one of the most attractive systems because of its well-developed genetics and the fact that it is transformable.

We germinated *ghost* seeds and observed that, consistent with the early observations of C. Rick, green sectors form in the *ghost* white leaves. Characterization of pigments in both types of tissues indicated that no colored carotenoids or chlorophyll accumulates in white sectors, whereas wild-type concentrations of both types of pigments are present in the white sectors. Electron microscopy was used to characterize the plastids in white and green leaves. Undifferentiated plastids, with little membrane structure, form in white leaves, but fully differentiated chloroplasts are observed in green tissue (Fig. 4). Thus, the somatic instability of the *ghost* mutation results in the restoration of carotenoid biosynthesis in the green sectors. The main function of carotenoids is to protect chlorophyll

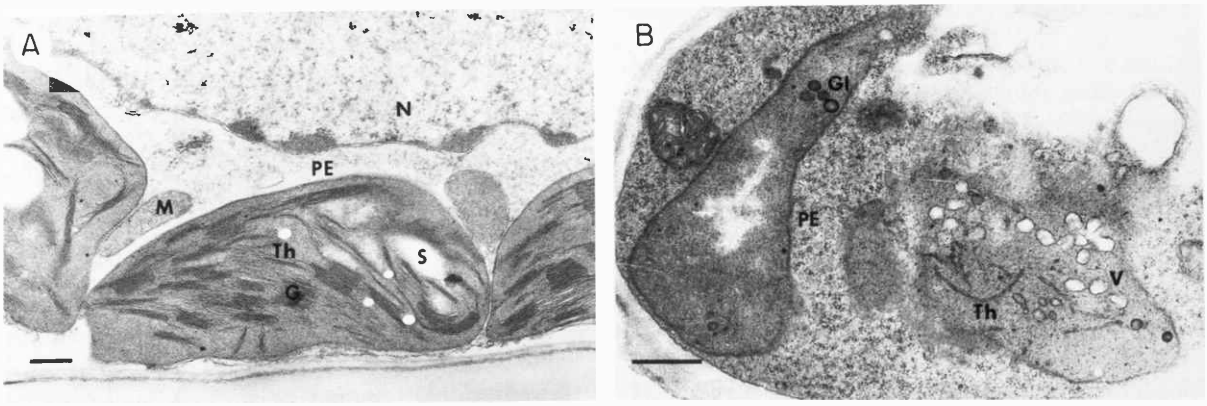

FIGURE 4 Plastids from leaves of tomato *ghost* plants grown in a growth chamber. (*A*) White sectors; (*B*) green sectors. (Gl) Globuli; (Th) thylakoids; (V) vacuole; (PE) plastid envelope; (M) mitochondrion; (G) grana; (S) starch; (N) nucleus. Bar, 0.5 μm. Magnifications: (*A*) 18,900×; (*B*) 11,900×.

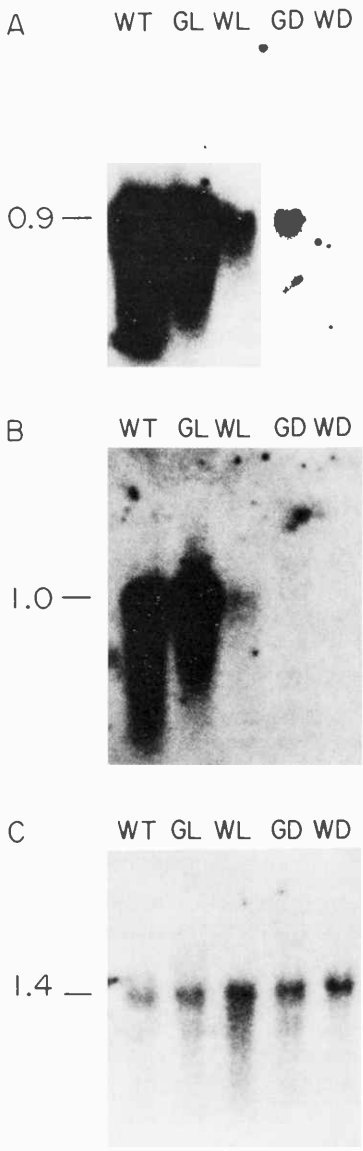

FIGURE 5 Northern blot analysis. Probes: (*A*) Small subunit of carboxylase; (*B*) chlorophyll-*a*/*b*-binding protein; (*C*) actin gene. (WT) Wild-type; (GL) green leaves of *ghost* plants grown in the light; (WL) white leaves of *ghost* plants grown in the light; (GD and WD) green and white leaves of *ghost* plants, respectively, grown in the light and placed in the dark for 2 days.

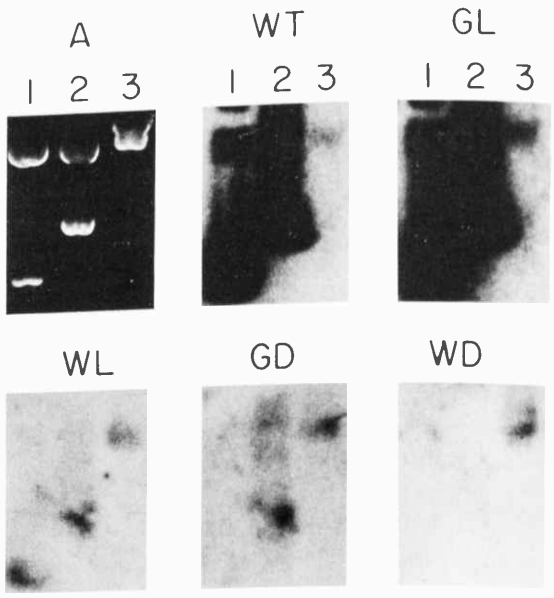

FIGURE 6 In vitro transcription experiment. Ethidium-bromide-stained gel containing plasmid DNA from *ssu* (lane *1*), *cab* (lane *2*), and actin (lane *3*) were blotted onto nitrocellulose and hybridized to RNA extracted from different tissues. See Fig. 5 for abbreviations.

against photooxidation, and thus chlorophyll accumulation in the green sectors is probably a secondary event due to the restoration of carotenoid biosynthesis. Green sectors are photosynthetic and can support the growth of a variegated *ghost* plant. Micropropagation through tissue culture was used to generate variegated *ghost* plants containing enough green leaves to support growth to maturity. Thus, we now have the first plant system in which the effects of a mutation in the carotenoid pathway can be monitored throughout development.

For our first study of gene expression in *ghost*, we obtained probes for two families of nuclear genes (*ssu* and *cab*) that code, respectively, for the chloroplast RuBPCase and chlorophyll-*a*/*b*-binding proteins. Northern probing of extracts from white and green *ghost* tissue from plants grown in the light shows that the mRNA levels for *ssu* and *cab* are similar to wild-type levels in *ghost* green tissue but are sharply reduced in *ghost* white tissue (Fig. 5). The influence of light on the expression of these two families can be studied by transferring the plants to the dark for 48 hours. A reduction in the mRNA levels of the two gene families can be observed in both white and green tissue (Fig. 5). Thus, even in the absence of colored carotenoids, light regulates the expression of these genes in mature *ghost* plants.

The decrease in mRNA levels observed in *ghost* white tissue could be due to transcriptional or post-transcriptional events. To differentiate between these two possibilities, we pulse-labeled mRNA in nuclei isolated from white and green tissue. The mRNAs were then extracted and hybridized to the

corresponding DNA probes immobilized on nitrocellulose filters. The results indicate (Fig. 6) that the primary effect of both light and colored carotenoids takes place at the transcriptional level.

We have demonstrated that maximum transcription of the two gene families studied requires both light and colored carotenoids. The mechanism of the carotenoid effect remains unknown. However, evidence gathered from different systems suggests that fully differentiated chloroplasts are required for the expression of some nuclear genes in plant leaves. A signal, of hitherto unknown nature, would be generated by the chloroplast and would act as a positive activator of nuclear gene expression. In vitro reconstitution systems currently under way should help characterize the effect of chloroplast components on transcription in isolated nuclei.

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PROKARYOTIC GENETICS

F. Daldal S. Cheng B. Naiman
E. Davidson S. Rook

Genetic Analysis of Electron- and Proton-transfer Pathways of the Photosynthetic Bacterium *Rhodobacter capsulatus*

F. Daldal, E. Davidson, B. Naiman,
S. Cheng, S. Rook

Facultative photosynthetic bacteria provide excellent systems for the study of various membrane complexes, such as the photosynthetic reaction center, ubiquinol:cytochrome *c* oxidoreductase-type redox-driven proton pumps, cytochrome oxidase, and ATP synthetase, all involved in microbial energy transduction. These organisms have versatile growth modes (Fig. 1) that enable the selection of mutants defective in only some of these complexes, which are viable through other pathways. Often, bacterial complexes are structurally simpler, but functionally, they are very similar to higher organisms. Some of these organisms, upon disruption, yield sealed membrane vesicles (chromatophores) containing all of the components of energy-transducing chains. Thus, in vitro studies of

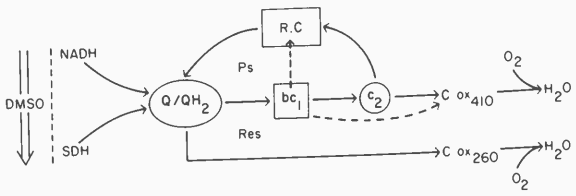

FIGURE 1 Various growth modes of *R. capsulatus*. (Ps) Photosynthesis in the absence of oxygen but in the presence of light; (Res) respiration; (DMSO) anaerobic growth in the presence of dimethylsulfoxide; (NADH and SDH) respiratory dehydrogenases; (Q/QH_2) quinone/quinol pool; (R.C) photosynthetic reaction center; (bc_1) ubiquinol:cytochrome c_2 oxidoreductase; (c_2) cytochrome c_2; (C ox_{410} and C ox_{260}) respiratory terminal oxidases. Dashed lines indicate newly discovered electron pathways.

the electron-transfer pathways using flash-activable "single-electron-turnover" techniques are possible. Furthermore, the electrochromic red shift of the carotenoid absorption spectrum (carotenoid bandshift) of photosynthetic bacteria is an invaluable tool for determining the transmembrane potential and for identifying electrogenic steps in electron flow through these complexes. Perhaps most importantly, some of these species are amenable to genetic analysis, and in particular, *Rhodobacter capsulatus* (formerly called *Rhodopseudomonas capsulata*) genetics is well developed.

Role of Cytochrome c_2 in Photosynthesis and Respiration

F. Daldal, E. Davidson, S. Rook

Cytochrome c_2 is an electron carrier common to photosynthetic and respiratory apparatuses of *R. capsulatus*. It takes electrons from the ubiquinol:cytochrome *c* oxidoreductase and donates them to the reaction center (in photosynthesis) or to the cytochrome oxidase (in respiration) (Fig. 1). To understand how this cytochrome interacts with various membrane complexes while carrying electrons, a multidisciplinary approach that we initiated 2 years ago was continued this year.

CYTOCHROME C_2 IN PHOTOSYNTHESIS

In the past, cytochrome c_2 was considered to be essential for photosynthesis and for the cytochrome c_2-dependent branch of respiration on the basis of the nonphotosynthetic phenotype of a mutant, MT113, which lacks c_2. However, our recent studies have shown that MT113 is not only defective in c_2, but also lacks all known *c*-type cytochromes, even though it can synthesize *b*-type cytochromes (E. Davidson et al., *Biophys. Biochem. Acta* [1987] in press). Therefore, to define the role of c_2 in vivo, we sought mutants missing only this protein. To isolate a c_2^- mutant, we first cloned the structural gene for c_2 (*cycA*) and determined its nucleotide sequence. Then, using the cloned gene, we constructed mutant alleles in vitro and introduced them into the chromosome of an otherwise wild-type strain. The mutants obtained were devoid of c_2 but, interestingly, they were proficient in photosynthesis and in respiration. Therefore, an alternative way(s), independent of c_2 and capable of reducing the oxidized *R. capsulatus* photosynthetic reaction center, must exist. This led us to the discovery of a c_2-independent photosynthetic electron pathway between the reaction center and the bc_1 complex (Fig. 1) (Daldal et al., *Proc. Natl. Acad. Sci. 83:* 2012 [1986]).

CHARACTERIZATION OF THE PHOTOSYNTHETIC ELECTRON PATHWAYS IN THE ABSENCE OF C_2

Study of a c_2^- mutant by flash spectroscopy, in collaboration with R. Prince (Exxon Corporation), established that in this new pathway, the electron donor to the reaction center is a membrane-bound *c*-type cytochrome, most likely cytochrome c_1 of the ubiquinol:cytochrome *c* oxidoreductase. This electron donation is very rapid (<100 μsec) but is only equal to approximately one fifth of that observed in the wild-type. Although this direct electron transfer from the bc_1 complex to the reaction center is low, it appears to be sufficient to support growth fully when high light intensity is available.

CHARACTERIZATION OF THE PHOTOSYNTHETIC ELECTRON PATHWAYS IN THE ABSENCE OF BOTH CYTOCHROMES C_2 AND C_1

To further previous studies, mutants missing only cytochrome c_1, as well as double mutants defective in both cytochromes c_1 and c_2, have been obtained. In contrast to c_2^- mutants, the c_1^- or c_1^-,c_2^- double mutants are deficient in photosynthesis, indicating the essential role of cytochrome c_1 during this pro-

cess. Although other *c*-type cytochromes are detectable in chromatophores derived from the double c_1^-,c_2^- mutant, they are not photooxidizable in vitro, suggesting that *c*-type cytochromes other than c_1 and c_2 are not involved in photosynthesis. This finding rules out the presence of other *c*-type cytochromes as an intermediate between the c_1 of the bc_1 complex and the reaction center. The electron donation from c_1 to the reaction center must therefore be direct. This is the first example of a direct electron transfer between two membrane complexes and thus we will continue its study in the future.

CYTOCHROME C_2 IN RESPIRATION

Analysis of the role of cytochrome c_2 in respiration is more complex because *R. capsulatus* has a branched respiratory pathway. One branch contains cytochrome c_2 and a cytochrome c_2-dependent cytochrome oxidase, and the alternate branch contains a cytochrome c_2-independent quinol oxidase (Fig. 1). Fortunately, mutants blocking either of these terminal oxidases exist, and thus a double mutant lacking cytochrome c_2 and also defective in quinol oxidase was constructed. This mutant can still grow by respiration and is sensitive to myxothiazol, a specific inhibitor of the bc_1 complex. Therefore, electron transfer from the bc_1 complex to cytochrome oxidase can also take place independently of c_2. Determination of the nature and the characteristics of the electron donor in this case is under way.

IN VITRO SITE-DIRECTED MUTAGENESIS OF CYTOCHROME C_2

Encouraged by the recent resolution of the three-dimensional structure of *R. capsulatus* c_2 by H. Holden and I. Rayment at the University of Arizona, we have initiated in vitro site-directed mutagenesis of this protein for relevant amino acid residues that are known to be involved in electron transfer. Our first attempt, in collaboration with Dr. Cusanovich at the University of Arizona, is directed at replacing the Lys-12 with an aspartic acid residue. This amino acid residue, situated near the water-exposed edge of the heme group, is thought to play an important role during electron transfer. A change from a basic residue to an acidic residue may drastically perturb electron donation rates and, hopefully, begin to reveal the underlying molecular rules of this process.

Genetic Analysis of the Structure and Function of the Ubiquinol:Cytochrome c_2 Oxidoreductase

E. Davidson, S. Rook, B. Naiman, F. Daldal

Ubiquinol:cytochrome *c* oxidoreductases (or bc_1 complexes) are membrane-bound, redox-driven proton pumps found in photosynthetic bacteria, plant chloroplasts, eukaryotic mitochondria, and many nonphotosynthetic bacteria. In *R. capsulatus*, this complex is common to photosynthetic and respiratory electron-transfer chains and consists of three subunits carrying prosthetic groups: Rieske Fe-S protein, cytochrome *b*, and cytochrome c_1. It oxidizes quinols generated by the photosynthetic reaction center or by respiratory dehydrogenases, and subsequently rereduces quinones. As a net result of these oxidation and reduction reactions, protons are translocated from the cytoplasmic side of the membrane to the periplasmic side, and the accompanying electrons are conveyed either back to the reaction center (in photosynthesis) or to cytochrome oxidase (in respiration) (Fig. 1).

ISOLATION OF THE STRUCTURAL GENES OF THE BC_1 COMPLEX COMPONENTS

This year, we have continued studies initiated last year on the electron pathways in and around the bc_1 complex. Earlier, using a nonphotosynthetic mutant with a lesion related to the bc_1 complex, we isolated by complementation the structural genes for the Rieske Fe-S protein (*petA*), cytochrome *b* (*petB*), and cytochrome c_1 (*petC*). On the plasmid obtained, these three genes were found located adjacent to each other, constituting an operon (*pet*). To determine the role of the bc_1 complex, mutations localized in each of these genes were obtained using the available clones. These bc_1^- mutants are nonphotosynthetic, indicating that a functional bc_1 complex is absolutely essential for this growth mode. However, as expected, they are able to grow by respiration via the alternate pathway. These results indicate that the branch point of the respiratory pathways must be before the bc_1 complex, most likely at the quinone pool (Fig. 1).

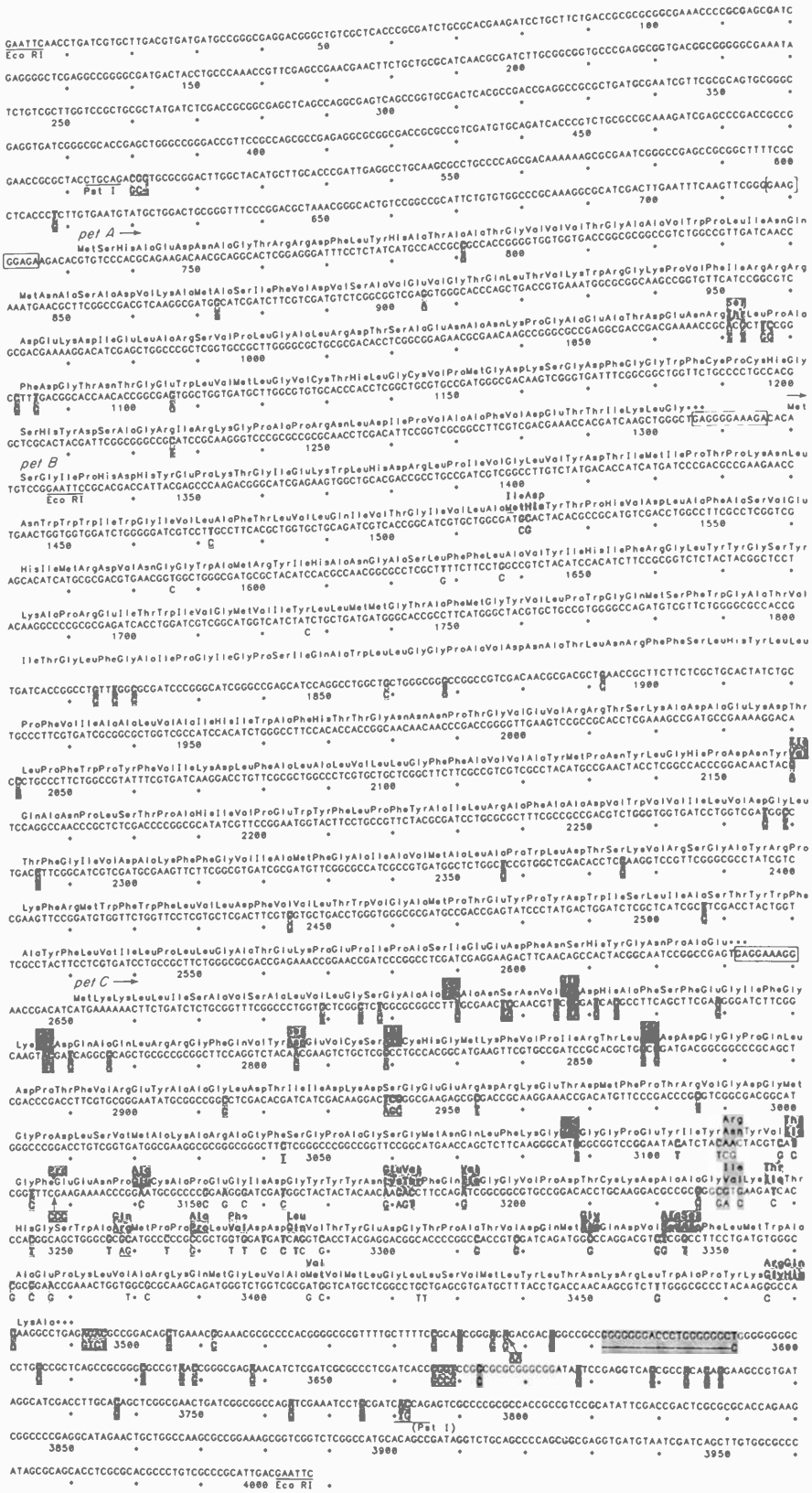

FIGURE 2 (*See facing page for legend.*)

GENETIC STRUCTURE OF *PET* OPERON

The approximately 4000-bp-long nucleotide sequence of the *pet* operon, consisting of the structural genes for Rieske Fe-S protein (*petA*), cytochrome *b* (*petB*), and cytochrome c_1 (*petC*), has been completed and is shown in Figure 2. The amino acid sequence deduced from it indicates that the Rieske Fe-S protein, cytochrome *b*, and cytochrome c_1, respectively, are 191, 437, and 279 amino acid residues long. They all present several regions of strong homology with the same proteins from various organisms. Furthermore, comparisons based on their hydropathy plots indicate an overall similarity in their spatial folding. The nucleotide sequence establishing the entire primary structure of the bc_1 complex now provides important basic knowledge for future studies on the structure-function relationship of the bc_1 complex, a redox-driven proton pump.

During these studies, we realized that an extraordinarily high homology at the nucleotide level existed between the bc_1 sequences derived from two distinct Rhodospirillaceae species, namely, the above-mentioned *pet* operon of *R. capsulatus* and the corresponding *fbc* operon of *Rhodopseudomonas sphaeroides* recently reported by N. Gabellini and W. Sebald. However, a detailed examination of the *R. sphaeroides* isolate used in earlier studies indicated that it is not a genuine *R. sphaeroides* strain, but rather a species closely related to *R. capsulatus*. Consequently, the *pet* operon of a bona fide *R. sphaeroides* strain has also been isolated and partially sequenced. This comparison established that *R. capsulatus* is currently the only organism from which the entire primary structure of the bc_1 complex is known.

SUBUNIT COMPOSITION OF *R. CAPSULATUS* BC_1 COMPLEX

The minimum number of the subunits required for a functional bc_1 complex is an important issue. To attack systematically this and other structural and functional questions related to the bc_1 complex, we have initiated the generation of specific monoclonal antibodies using the purified *R. capsulatus* complex provided by L. Dutton from the University of Pennsylvania. Using native complex, we have obtained a number of monoclonal antibodies recognizing specifically each one of the Rieske Fe-S protein, cytochrome *b*, and cytochrome c_1 subunits. Interestingly, although some of these antibodies interact only with denatured subunits, others appear to inhibit the function of the bc_1 complex, as tested by a cytochrome c_2-dependent quinol oxidase assay done in collaboration with L. Dutton's group. Using these antibodies, we have proved that the bc_1 complex of *R. capsulatus* contains only three subunits. The 10-kD subunit, earlier observed in purified complexes, does not seem to be necessary for the quinol oxidation reaction in vitro. Therefore, this complex appears to bind and process quinones and quinols without the need of a quinone-binding protein postulated to be present in some other organisms. Undoubtedly, these monoclonal antibodies will provide excellent tools for various future structural and functional studies.

STRUCTURALLY INTACT, BUT FUNCTIONALLY PERTURBED, BC_1 COMPLEX MUTANTS

Although our ongoing studies using null mutants have already begun to define the electron pathways in and around the bc_1 complex, we have realized that deeper insights into the structure-function relationship of the bc_1 complex could perhaps be obtained if mutants perturbed functionally, but assembling the complex properly, were available. One such mutant, R126, defective in only one of the two functional sites of the bc_1 complex, already exists and has been used to isolate the structural genes. To obtain other mutants of this type, we have begun to screen the effect of various bc_1 inhibitors, such as myxothiazol, stigmatellin, antimycin, UHDBT, and UHNQ, on the photosynthetic growth of *R. capsulatus*. These inhibitors have previously been studied only in vitro using chromatophores. We have found that myxothiazol and stigmatellin, specific for the quinol oxidation site, are potent inhibitors of photosynthetic growth in this species. For unknown reasons, antimycin and funiculosin, which affect the quinone reduction site, appear for the moment to be ineffective in vivo. Spontaneous mutants resistant to myxothiazol and stigmatellin have

FIGURE 2 Nucleotide and deduced amino acid sequence of the *pet* operon of *R. capsulatus* encoding the structural genes for the Rieske Fe-S protein (*petA*), cytochrome *b* (*petB*), and cytochrome c_1 (*petC*) subunits of the bc_1 complex. Boxed areas indicate the putative ribosome binding sites, and highlighted areas indicate the differences between the *pet* and *fbc* operons (see the text).

been obtained, and several were shown to map in the bc_1 gene cluster by a novel genetic tool, called "interposon tagging," that we have recently developed.

INTERPOSON TAGGING, A NEW TOOL FOR *R. CAPSULATUS* GENETICS

This tool is a natural extension of the "interposon mutagenesis" technique, developed by P. Scolnik, that we have been using to generate null mutants. In contrast to the mutagenesis technique, the tagging scheme takes advantage of a silent insertion, introduced in vitro in close proximity to the gene to be studied. It provides a selectable genetic marker for mapping using genetic crosses, and at the same time it tags the corresponding restriction fragments with a selectable marker for easy cloning of the studied mutations. With this technique, we have proved that the *aer126* mutation of R126 is located in the cytochrome *b* gene and have also shown that all of the mutations conferring myxothiazol resistance and most of the mutations conferring stigmatellin resistance are located in the *pet* operon. Several of these mutations have now been cloned, and determination of the corresponding nucleotide changes is now in progress.

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CELL BIOLOGY

The cell biology and biochemistry of mammalian growth control continue to be the major focus of the Cell Biology group. Over the past several years, we have evolved to five major sections encompassing oncogenes and growth factors, cytoskeletal structures, and stress proteins. As each group advances in its particular field, we find more and more overlapping principles that have increased the interactions within the group. This has allowed for synergistic collaborative efforts and speedy progress to be made. An account of some of the work carried out over the past year by the five subgroups is given below.

BIOLOGY OF TRANSFORMATION

J.R. Feramisco D. Bar-Sagi T. Kamata M.W. Wooten N.F. Sullivan J.C. Lamb K.T. Riabowol A. Fernandez-Solt N. Kronenberg C. Hallaran

Role of the *ras* Protein in Membrane Signal Transduction

D. Bar-Sagi, J.R. Feramisco

Essential to the understanding of the mechanism whereby *ras* proteins exert their effects on cell proliferation is the identification of molecular events that are directly modulated by these proteins. The mammalian *ras* family consists of three proto-oncogenes, Ha-*ras*, Ki-*ras*, and N-*ras*, each of which can acquire oncogenic properties by single missense mutations usually at either codon 12 or codon 61. The mutated forms of the *ras* genes are prevalent in human and rodent tumor cells and have been implicated in transformation in vitro and tumorigenesis in vivo.

Mammalian *ras* genes encode homologous 21-kD proteins that are membrane-associated guanosine triphosphate (GTP)-binding proteins. The proteins have an intrinsic low guanosine triphosphatase (GTPase) activity that, in certain cases, is impaired in the mutated oncogenic protein. The GTP hydrolytic activity is a common property of all known guanine-nucleotide-binding (G) proteins. Members of the G-protein family regulate the activities of their cellular target by a cycle of GTP binding and GTP hydrolysis. On the basis of an analogy between *ras* proteins and G proteins, it has been proposed that the reduction in GTPase activity that accompanies mutational activation of *ras* genes impairs the regulatory function of *ras* proteins, thereby leading to the derangement of cellular signals that control cell proliferation. Although the specific biochemical function of *ras* proteins has not as yet been identified, several studies have indicated that *ras* proteins may participate in the molecular events initiated by growth factors. Elucidation of the role of *ras* proteins in the acquisition of the transformed phenotype has been hampered by the pleiotropic nature of the transformation process. As has been shown previously, microinjection of the *ras* oncogene protein into quiescent cells results in the transient stimulation of proliferation. Therefore, the transforming properties of *ras* proteins are faithfully expressed in this assay. Furthermore, the microinjection approach offers the means by which oncogenic *ras* protein can be introduced abruptly into normal cells and therefore can allow identification of the immediate effects of *ras* proteins.

ROLE OF THE *RAS* PROTEINS IN CELL-SURFACE ACTIVITIES AND PHOSPHOLIPID TURNOVER

Confluent rat embryo fibroblasts (REF52) have a flattened polygonal shape. The cell-surface structure, as revealed by scanning electron microscopy, consists predominantly of short, slender extensions and small folds (Fig. 1). As early as 30 minutes after microinjection of the human Ha-*ras* oncogene pro-

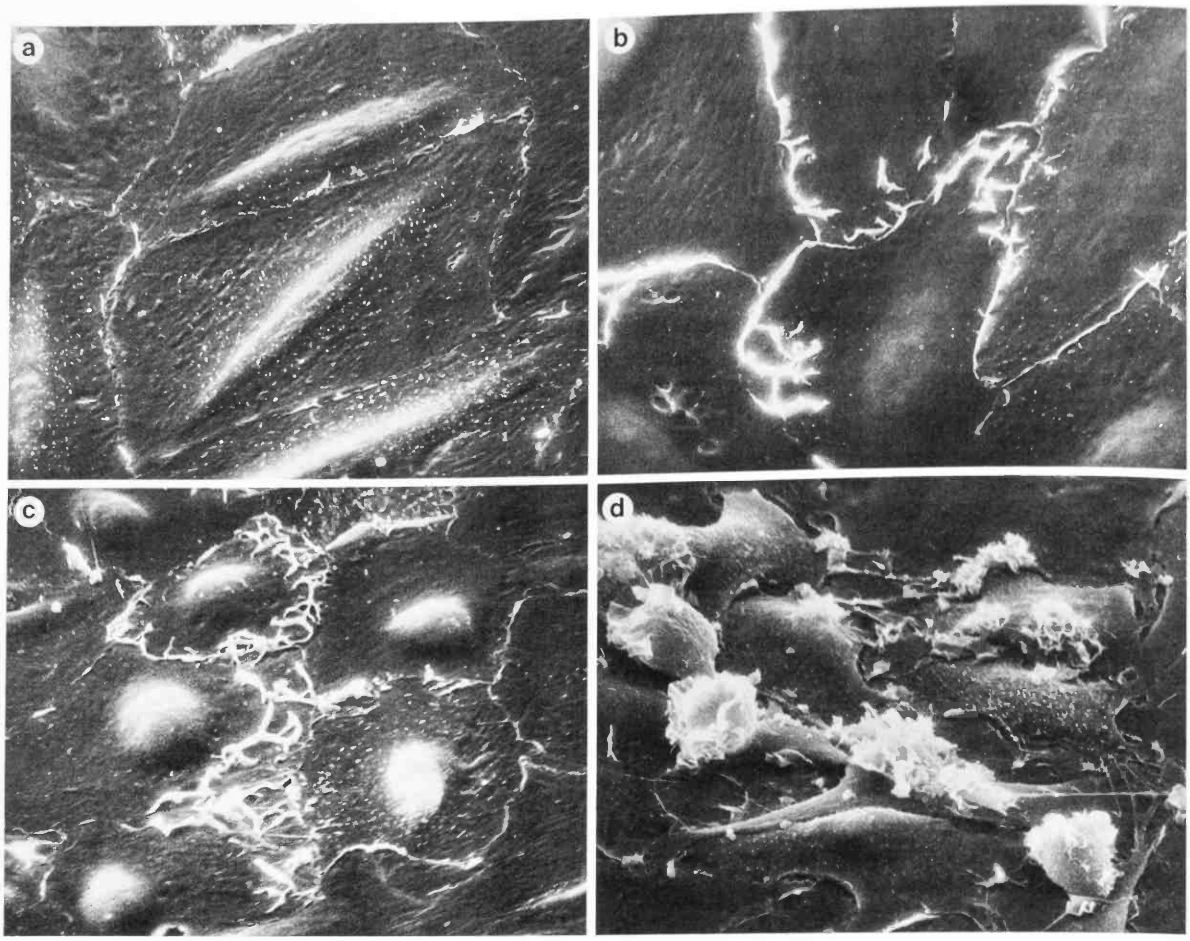

FIGURE 1 Scanning electron micrographs of REF52 cells injected with the *ras* oncogene protein. Cells were injected with either buffer alone (*a*) or with the *ras* oncogene protein (*b–d*). The time after injection was 30 min (*b*), 2 hr (*c*), and 10 hr (*a,d*).

tein, pronounced ruffling activity begins, as is indicated by the large lamellipodia that rise up along the periphery of the injected cells. Two hours after the cells are injected, large ruffles that form elaborate branching patterns are prominent on the dorsal surface. Ten hours after injection, the cells assume a partially rounded shape, and large regions of the membrane are now occupied by the surface ruffles. These sequential changes in surface morphology were reproducible both between different experiments and between the population of the injected cells in a given experiment. Microinjection of buffer alone or similar amounts of control protein (mouse immunoglobulin G, IgG) has no apparent effect on cell-surface morphology.

The occurrence of membrane ruffling is closely associated with pinocytotic activity. To test the effect of microinjection of the Ha-*ras* protein on fluid-phase pinocytosis, we monitored the uptake of fluorescein-conjugated dextran (FITC-dextran) by the injected cells. This substance meets the major criteria required for a marker of fluid-phase pinocytosis; it is readily soluble in aqueous medium, membrane-impermeable, stable within the intracellular milieu, and does not bind to the plasma membrane. Cells microinjected with the Ha-*ras* oncogene protein display a large number of intracellular vesicles containing the fluorescent marker, indicating a high rate of ongoing pinocytosis. The pinocytotic vesicles are mostly formed in small groups and are found predominantly along the inner margin of well-developed ruffled membranes (visible as a dark rim with many folds). In contrast, the level of pinocytotic uptake of FITC-dextran in cells that were microinjected with the control protein is very low and is comparable to that observed

in buffer-injected cells or uninjected cells. The stimulatory effect of *ras* oncogene protein on membrane ruffling and pinocytosis was not restricted to the particular cell type used because we found that microinjection of the protein into normal rat kidney cells similarly results in the enhancement of ruffling and pinocytosis. Comparison between the rates of uptake of FITC-dextran in *ras*-injected cells and in buffer-injected cells, as determined by the rate of formation of pinocytotic vesicles, has been done. The number of vesicles containing FITC-dextran increases linearly with time of exposure of cells to the fluorescent marker. These are the kinetics predicted for FITC-dextran uptake by fluid-phase pinocytosis. Furthermore, the rate of pinocytotic activity is ten times higher in cells that were injected with *ras* oncogene protein than in cells that were injected with buffer.

A common characteristic of cell-surface stimuli that trigger membrane-related events is their ability to alter phospholipid metabolism. We therefore decided to test whether the effects of *ras* proteins on membrane ruffling and pinocytosis are accompanied by changes in phospholipid metabolism. The phospholipid composition of the *ras*-injected cells was analyzed. The cells injected with buffer alone showed the same phospholipid composition as mock-injected cells. Injection of the *ras* oncogene protein had no apparent effect on the levels of ^{32}P-labeled phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidic acid at this level of resolution. Our attempts to quantify the levels of ^{32}P incorporated into the polyphosphoinositides PIP and PIP_2 were hampered by the very low net phosphorylation signal caused by the rapid turnover of the phosphates in these phospholipid species. Using an acidic TLC solvent system to analyze the lipid extracts, we have observed that the levels of lysophosphatidylcholine and lysophosphatidylethanolamine in *ras*-injected cells are reproducibly 1–1.5 times higher than in buffer-injected cells. This increase is detectable as early as 30 minutes after injection and becomes more apparent by 1 hour after injection. Lysophospholipids are the products of phospholipase A_2 activity. This enzyme catalyzes the hydrolysis of fatty acid ester bonds at the 2-position of 1,2-diacyl-*sn*-phosphoglyceride, thereby producing two products: free arachidonic acid and lysophospholipids. Therefore, the increase in lysophosphatidylcholine and lysophosphatidylethanolamine detected in cells injected with the *ras* oncogene protein is an indication that the activity of phospholipase A_2 was stimulated in these cells.

Activation of phospholipase A_2 accompanies several distinct types of responses triggered by ligands. The magnitude of the effect produced by microinjection of *ras* oncogene protein is similar to that observed upon ligand-induced activation of phospholipase A_2. The activity of the enzyme is calcium-dependent and leads to the release of arachidonic acid, which provides the precursor for the formation of prostaglandins and other metabolites. Free arachidonic acid, moreover, may partition into membranes and alter their structural properties by virtue of its effect on bilayer fluidity. Likewise, lysophospholipids, formed as a consequence of phospholipase A_2 activation, can exert profound effects on cell-surface organization by virtue of their detergent-like properties. Since membrane ruffling and pinocytosis are inevitably associated with dynamic changes in cell-surface properties, the activation of phospholipase A_2 by microinjection of the *ras* oncogene protein may be directly involved in the *ras*-induced stimulation of these cell-surface activities.

MICROINJECTION OF ANTIBODIES TO THE *RAS* ONCOGENE PROTEIN INHIBITS RUFFLING AND PINOCYTOSIS IN *RAS*-TRANSFORMED CELLS

F. McCormick of the Cetus Corporation, California, collaborated with us on this research. Normal rat kidney cells transformed by the v-Ki-*ras* oncogene (KNRK) (ATCC 1569) grow in culture as rounded cells that display numerous cell-surface ruffles, whereas normal rat kidney cells (NRK) grow as flat cells with fewer surface ruffles. Previous studies in our laboratories have shown that microinjection of neutralizing antibodies against the *ras* oncogene protein into KNRK cells induced the morphological reversion of these cells (Fig. 2). In a time-dependent manner, the injected anti-*ras* antibodies cause the KNRK cells to flatten, lose many of the cell-surface ruffles, and slow their growth. Injection of control IgGs had no effect on the morphology of these cells, and dilution of the immune IgG, so that less than 5×10^4 molecules of IgG were injected per cell, led to a loss of the morphological reversion as well.

Conversely, we have previously shown that microinjection of the human Ha-*ras* (T24) oncogene protein into normal rat cells causes the rapid appearance of cell-surface ruffles. Thus, it seems

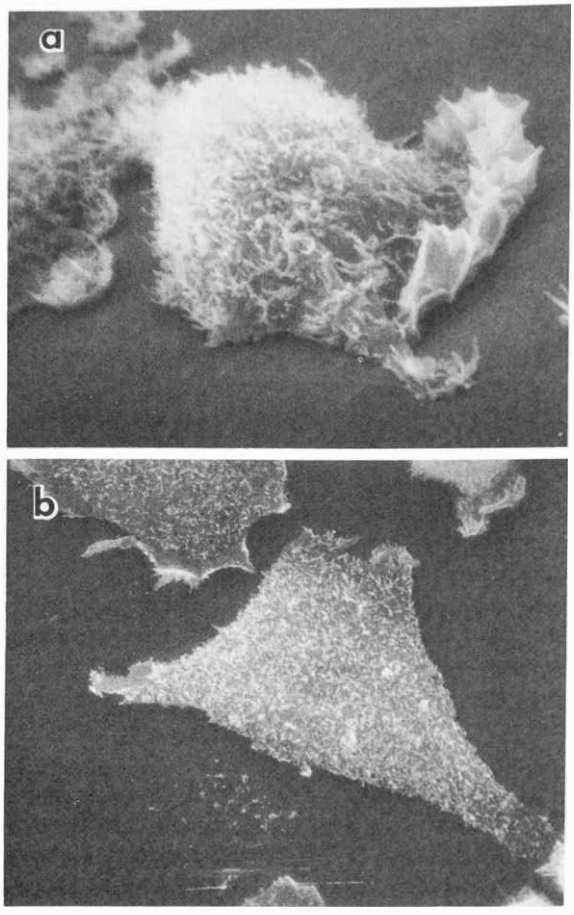

FIGURE 2 Morphological changes in KNRK cells following microinjection of anti-*ras* antibodies. KNRK cells growing in the presence of 10% calf serum were microinjected either with 5×10^5 molecules/cell of nonimmune IgG (*a*) or with 5×10^5 molecules/cell of mouse monoclonal anti-*ras* IgG (*b*). The mouse monoclonal anti-*ras* binds to both the proto-oncogenic and oncogenic forms of the *ras* protein. Cells were identified through the use of ink circles drawn on the bottom of the culture dish. The antibodies were dissolved in 5 mM sodium phosphate (pH 7.2) containing 70 mM sodium chloride. Ten hours postinjection, the cells were fixed and examined by scanning electron microscopy. Note the lack of surface ruffles in the cells injected with the anti-*ras* antibodies, whereas cells injected with control IgG retain the surface ruffles.

highly probable from these two sets of studies that the expression of the *ras* oncogene protein is somehow directly responsible for the enhanced cell-surface ruffling associated with the transformed cells.

One of the biological phenomena intimately associated with cell-surface ruffling is fluid-phase pinocytosis. KNRK cells, which display many surface ruffles, show a high level of uptake of horseradish peroxidase (HRP; used as a cytochemical marker of this activity by addition to the growth medium) into intracellular vesicles. The characteristics of this uptake of the HRP by the cells are similar to those that define fluid-phase uptake (i.e., the uptake is linear with time of incubation, proportional to the amount of HRP added to the medium, and not saturable with respect to the amount of HRP added to the medium). In a series of experiments in which we microinjected anti-*ras* antibodies into these cells prior to the measurement of fluid-phase pinocytosis, it was found that injection of the anti-*ras* IgG led to a decrease in the pinocytotic activity in the KNRK cells, which correlated with the cell-flattening response and the loss of cell-surface ruffles (Fig. 3). Again, the diminution of pinocytotic activity was dependent on the amount of immune IgG injected and was not seen with comparable amounts of injected nonimmune IgG.

Work from our laboratories and from others has shown that the *ras* oncogene protein obviates both the need for growth factors in the stimulation of proliferation in fibroblasts and the need for nerve growth factor in differentiation of PC-12 cells. Moreover, blockage of the function of the *ras* proto-oncogene protein in fibroblasts or PC-12 cells prevents the mitogenic or neurogenic effects of growth factors or nerve growth factor, respectively. Examination of the cell surface shows that enhanced membrane ruffling is an early change observed on fibroblasts in the mitogenic response to growth factors and on PC-12 cells in the neurogenic response to nerve growth factor. Together, these data support the idea that the *ras* proteins play a role in the signal transduction process of these distinct biological systems, and it is likely that membrane ruffling is an essential feature of the common biochemical reactions that are regulated by the *ras* proteins in these systems.

Influence of *ras* Protein Expression on Fluid-phase Endocytosis in Yeast

A. Fernandez-Solt, D. Bar-Sagi, J.R. Feramisco
[in collaboration with S. Powers, T. Toda, and M. Wigler, Cold Spring Harbor Laboratory]

As M. Wigler's laboratory has uncovered the regulation of cAMP metabolism in yeast by the *ras* proteins, they have generated many defined strains of yeast that have altered expression of the *ras* genes.

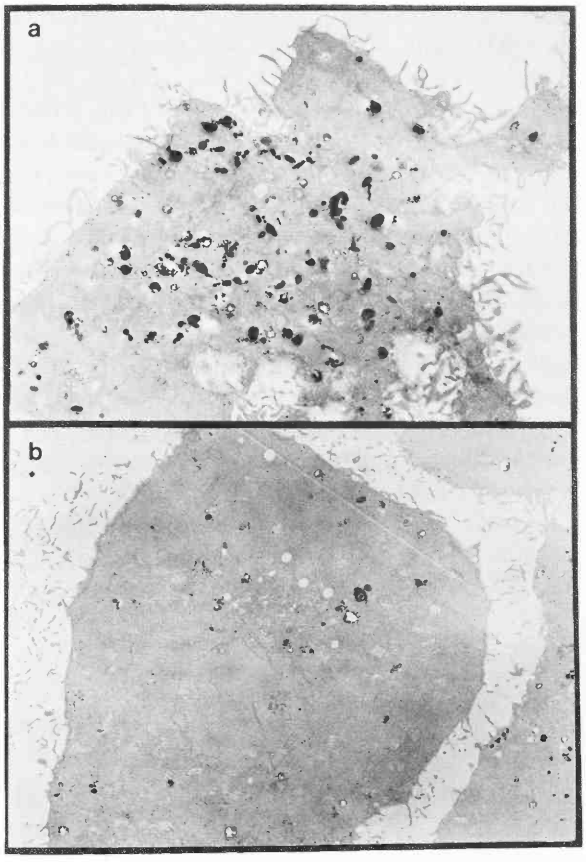

FIGURE 3 Inhibition of fluid-phase pinocytosis in KNRK cells by microinjection of anti-*ras* antibodies. KNRK cells were microinjected either with 5×10^5 molecules/cell of nonimmune IgG (*a*) or with a similar amount of anti-*ras* IgG (*b*). Ten hours postinjection, HRP was added to the medium (1 mg/ml). After a 30-min incubation period, the cells were washed, fixed, and processed to localize cell-bound enzyme with the diaminobenzidine hydrogen peroxide substrate mixture (dark, electron dense areas). Note the diminution of the uptake of HRP from the medium in the cell injected with the anti-*ras* antibodies, whereas cells injected with control IgG retain the high level of uptake of HRP.

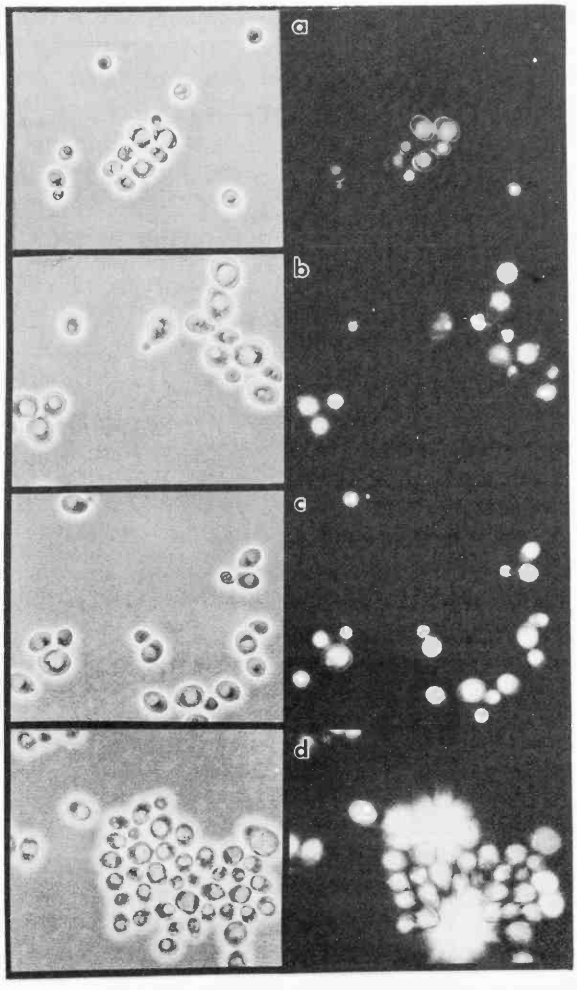

FIGURE 4 Endocytosis in the galactose-inducible *RAS2*^{val19} yeast strain. Yeast strain JR-281D-2-3 was grown overnight at 30°C in glucose medium (*a*), acetate medium (*b*), and acetate + galactose medium (*c*,*d*). Cells were then harvested and further incubated for 2 hr at 37°C with 6 mg/ml Lucifer yellow (in *d*, glucose was added). Cells were washed, mounted, and photographed using phase or fluorescence optics. Fluorescence pictures were shot with a constant exposure time of 40 sec. Growth of this strain in glucose alone represses the expression of *RAS2*^{val19} by 90%; growth in galactose induces *RAS2*^{val19} expression up to 500–1000 copies/cell.

We have undertaken a study of these strains to determine whether, in yeast, the *ras* proteins affect fluid-phase endocytosis as the proteins do in mammalian cells.

Fluid-phase endocytosis was measured in various yeast RAS1/RAS2 strains by means of the uptake of a fluorescent dye, Lucifer yellow carbohydrazine (LYch). These measurements reveal a positive relationship between *ras* gene expression and the level of endocytosis in the yeast cells. Although ras1$^-$/ras2$^-$ strains exhibited a very low level of endocytosis, strains expressing high levels of *RAS1* or *RAS2* showed high levels of endocytosis (Fig. 4).

We also found a strong effect of glucose on endocytosis in strains expressing mutated *RAS*, where the sugar stimulated endocytosis by 10–50-fold compared with its effect on wild-type strains. The glucose-induced enhancement of endocytosis appeared to be the result of a synergistic effect of *ras* and glucose on this biological activity. The effect of *ras* on endocytosis probably is not mediated through its previously demonstrated effect on adenylate cyclase, since strains lacking adenylate cy-

clase still showed normal levels of endocytosis. Further work is being carried out in order to determine more clearly the connection between the *ras* proteins and endocytosis in yeast.

Reduced Protein-kinase-C Activity in a *ras*-resistant Cell Line Derived from Ki-MSV-transformed Cells

T. Kamata, N.F. Sullivan, M.W. Wooten

We have examined phosphatidylinositol turnover and C-kinase distribution in a flat, cellular *ras*-resistant cell line (CII) derived from Kirsten murine sarcoma virus (Ki-MSV)-transformed NIH-3T3 cells (DT). This cell type has been shown to express high levels of the p21 Ki-*ras* gene product, yet it is resistant to the transforming effects of this protein. Our data indicate that CII cells have reduced levels of total C-kinase activity when compared with that of NIH-3T3 cells and do not retain the ability to phosphorylate the growth-associated 80-kD C-kinase substrate either in vivo or in vitro. Furthermore, the steady-state levels of diacylglycerol and the sum of inositol phosphates are elevated in DT cells, whereas in CII cells, these levels are reduced to an amount equivalent to that seen in NIH-3T3 cells. These data indicate a correlation between a protein-kinase-C-dependent pathway and resistance to transformation by *ras*.

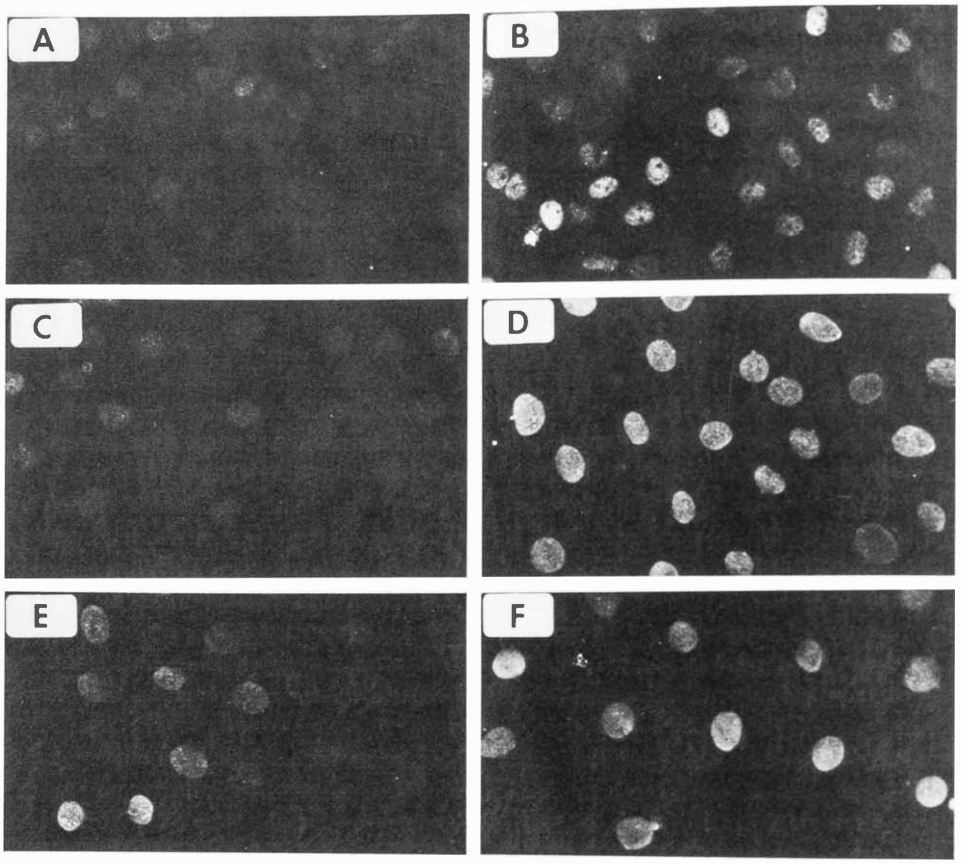

FIGURE 5 Immunofluorescence analysis of *fos* expression in stimulated and unstimulated fibroblast cells. Cells grown to near confluence on glass coverslips in DMEM and 10% serum were treated with medium containing 1% serum for 24 hr, followed by DMEM in the absence of serum for an additional 24 hr. Cells in panels *A* (parental), *C*, and *E* (v-*fos* sense transfectants) were then fixed in 3.7% formaldehyde. Parental 208F fibroblasts refed with DMEM and 10% serum (panel *B*) and cell lines containing v-*fos* sense constructs refed with DMEM containing 10^{-6} M dexamethasone (panels *D* and *F*) were incubated 60 min and fixed in formaldehyde. Coverslips were processed for immunofluorescence using affinity-purified anti-*fos* antibody. All fields were photographed using identical exposure times.

Cell Lines Inducible for the Production of *fos* Sense and Antisense RNA

K.T. Riabowol, J.R. Feramisco

Transcriptional induction of the c-*fos* proto-oncogene occurs within 5 minutes of mitogen stimulation in fibroblast cells and is the earliest known effect of growth factors on gene expression. Once transcribed, a wide variety of posttranslationally modified forms of *fos* proteins bind DNA with differing avidity. Cotransfection of v-*fos* constructs with several test promoters suggests that the v-*fos* gene either encodes or induces an activator of transcription. Although the function of *fos* is at present unknown, the low levels observed in quiescent cells, high transient amounts seen upon mitogen stimulation, and intermediate levels seen in cycling cells suggest a role in cell proliferation.

To test this idea, we have made steroid-inducible constructs containing the sense or antisense orientations of v-*fos* downstream from the mouse mammary tumor virus long terminal repeat. Transfection of rat 208F fibroblast cells has yielded several cell lines that show induction of *fos* sense or antisense RNA by Northern analysis. In addition, increased *fos*-specific nuclear fluorescence is seen in lines containing v-*fos* sense constructs upon addition of dexamethasone as shown in Figure 5, indicating production of v-*fos* p55. Staining of cells with affinity-purified *fos* antibody directed against a portion of the *fos* protein common to the viral and cellular forms demonstrates an increase of c-*fos* in serum-stimulated (panel B) versus quiescent (panel A) 208F cells. The remaining panels show similarly stained quiescent cell lines containing v-*fos* sense constructs in the absence (C and E) and presence (D and F) of 10^{-6} M dexamethasone.

The results of recent experiments in which 3T3 fibroblasts were transfected with steroid-inducible c-*fos* antisense constructs make the role of *fos* during the cell cycle unclear. Although one study reported that *fos* antisense RNA expression markedly reduced growth rate in unsynchronized cells, a subsequent report indicated that expression of *fos* antisense RNA resulted in a decreased ability of cells to reenter the cell cycle from a quiescent state but had no effect on rate of growth during the logarithmic phase of cell growth. We have not been able to demonstrate an effect of *fos* antisense RNA expression upon cell lines during logarithmic growth; however, its expression does inhibit [^{3}H]thymidine incorporation in quiescent cells stimulated with serum (Fig. 6A–D). Conversely, higher levels of [^{3}H]thymidine incorporation are seen in cell lines containing sense orientations of v-*fos* when quiescent cells are stimulated with low levels of serum (Fig. 6E–H). We are now in the process of determining the effect of different levels of v-*fos* p55 and *fos* antisense RNA on cell growth in several cell lines under a variety of growth conditions.

Cell Biology of cAMP-dependent Protein Kinase

J.C. Lamb, W.J. Welch, J.R. Feramisco [in collaboration with D. Glass, Emory University, and R. Adelstein and M. Conti, National Institutes of Health]

We have been interested in investigating the role of cAMP-dependent protein kinase (A-kinase) as a mediator of a number of cellular processes. Included among these are (1) the regulation of cell proliferation, (2) the control of cell shape, and (3) the modulation of cytoskeletal dynamics. We have approached this through a combination of biochemical, immunological, and pharmacological techniques, including microinjection of the purified kinase, antibodies to specific proteins, drugs, and hormones. Our previous studies have shown that elevating intracellular A-kinase activity through either the injection of the catalytic subunit or treatment of cells with defined drugs or hormones that raise intracellular cAMP results in dramatic changes in cell shape and the cytoskeleton. A closer analysis reveals that elevating A-kinase induces major rearrangements in the actin cytoskeleton and in the vimentin-containing intermediate filaments but does not apparently significantly reorder the third major cytoskeletal network, the microtubules. In our investigations, we have also identified a number of changes in the levels of protein phosphorylation that accompany these alterations in cell shape and the cytoskeleton. The most prominent changes are associated with the intermediate filament protein, vimentin, which shows an increase of five- to eightfold with respect to background levels of phosphorylation, and the nuclear lamins, a group of proteins that appear to be closely associated with the intermediate filaments. It therefore appears that the major rearrangement of the intermediate filament is associated with

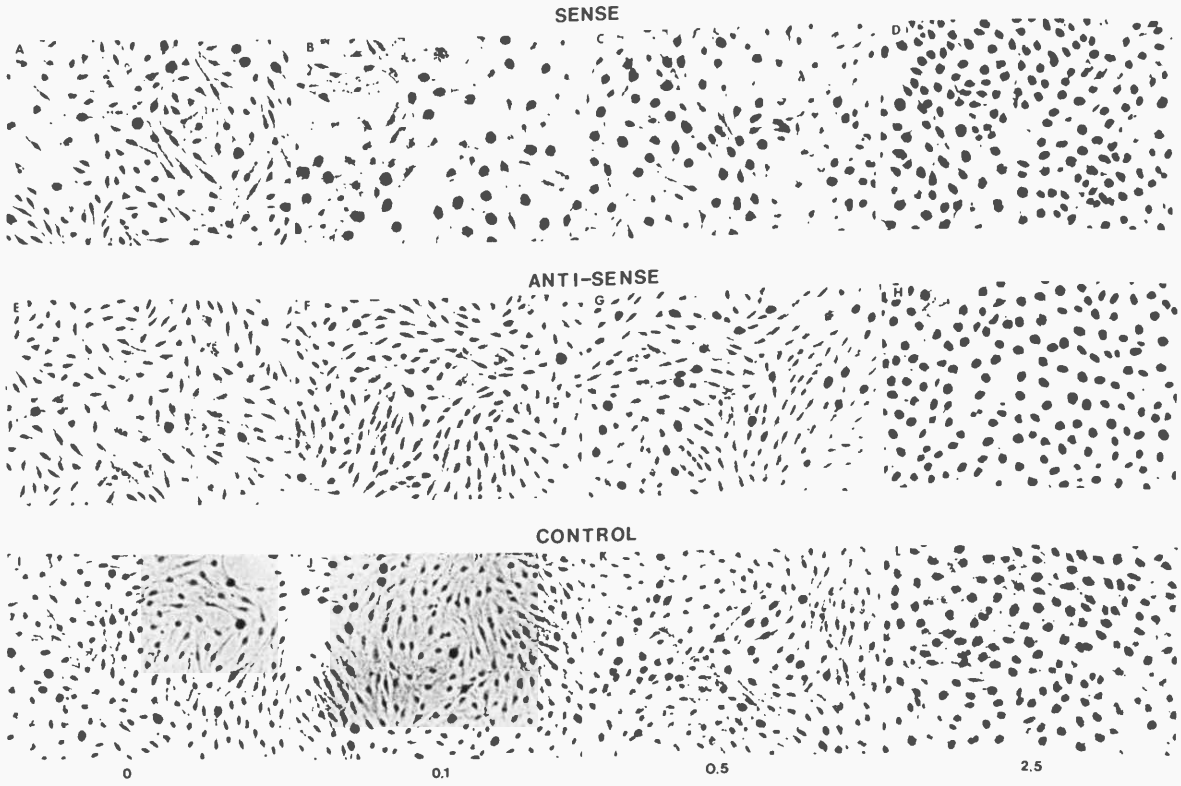

FIGURE 6 DNA synthesis in the *fos* cell lines in response to serum. Cells grown and serum-starved as outlined in Fig. 5 were refed with DMEM containing 1 μCi/ml [^{3}H]thymidine, 10^{-6} M dexamethasone, and 0% serum (*A,E,I*), 0.1% serum (*B,F,J*), 0.25% serum (*C,G,K*), or 2.5% serum (*D,H,L*) and grown for 24 hr. Cells were then fixed and processed for emulsion autoradiography.

changes in the phosphorylation of the intermediate filament protein subunits.

Our work this year has concentrated on a number of other changes in protein phosphorylation caused by the elevation of A-kinase activity, notably, those associated with the reorganization of the microfilaments. In this case, we have identified significant changes in the phosphorylation levels of two microfilament-associated proteins, myosin light chain and myosin light-chain kinase. Previously, a number of authors have suggested that actin microfilament assembly in cells may be regulated through the differential phosphorylation of these two proteins, which would, in turn, modulate the avidity of actin and myosin (two integral proteins in microfilaments). Indeed, in smooth-muscle cells, there appears to be a good correlation between the levels of light-chain phosphorylation, myosin light-chain kinase activity, and muscle tension. It has been proposed that this system is regulated through the action of A-kinase, which inactivates myosin light-chain kinase through phosphorylation. In our present studies, we have shown, through the microinjection of A-kinase, that phosphorylation of myosin light-chain kinase and the accompanying reduction in myosin light-chain phosphorylation correlate with the depolymerization of the microfilaments. These data support the idea that such a mechanism exists for the regulation of microfilament assembly in living cells.

As an alternative approach, we have directly inactivated myosin light-chain kinase in living cells by injecting an affinity-purified antibody directed against myosin light-chain kinase. In these experiments (Fig. 7), we have shown that the injected antibodies cause the disassembly of the actin microfilaments. This effect is specific since control antibodies do not show the same effect. These results support the potential mechanism in which cells regulate microfilament assembly by modulating the activity of myosin light-chain kinase. At present, we are investigating the role of this mecha-

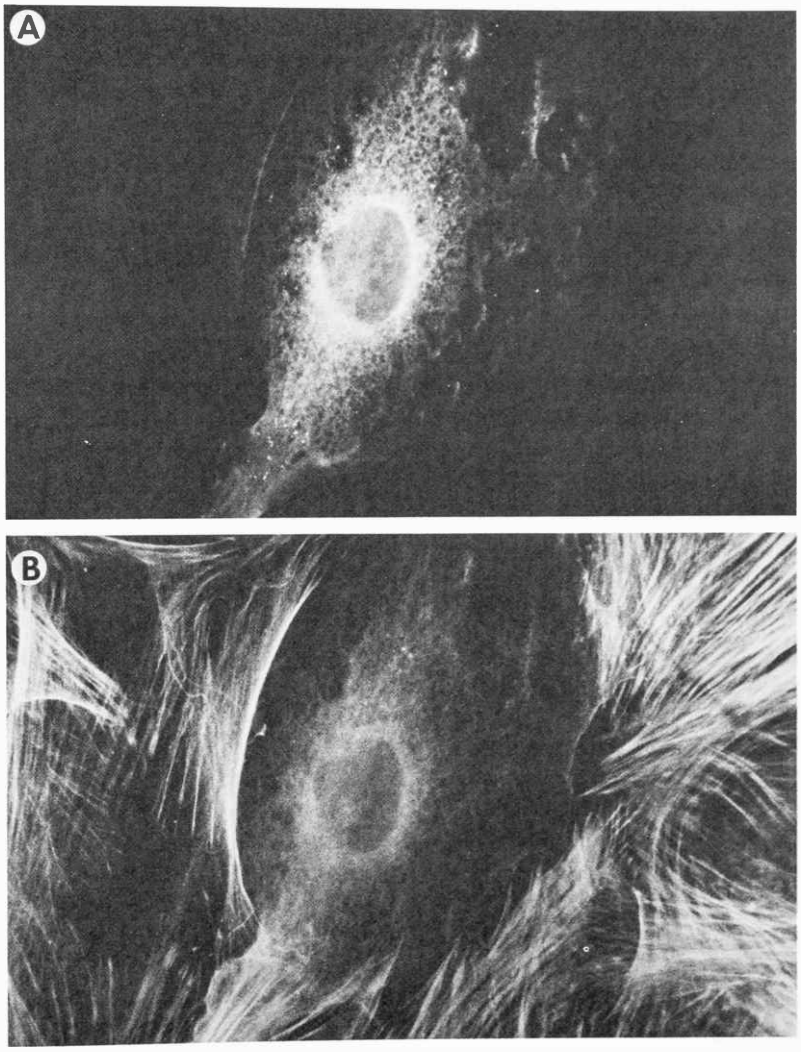

FIGURE 7 Effect of microinjecting affinity-purified anti-myosin light-chain kinase on the distribution of the microfilaments. Rat embryo fibroblasts grown on coverslips were microinjected with 0.1 mg/ml of affinity-purified rabbit polyclonal antibody directed against myosin light-chain kinase. Sixty minutes after microinjection, the cells were fixed and stained for the distribution of the injected antibody with FITC-conjugated goat anti-rabbit antibody or rhodamine phalloidin, a cyclopeptide specific for F actin. (*A*) Distribution of injected antibody; (*B*) distribution of the microfilaments.

nism in actin microfilament assembly in cells during the normal rearrangements seen in the microfilament networks during such events as cell division.

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MAMMALIAN STRESS RESPONSE: A CELLULAR DEFENSE MECHANISM

W.J. Welch P. Arrigo M. Mulcahy
L. Mizzen G. Blose
J.P. Suhan N. Kronenberg

In the last year, considerable progress has been made in defining the biological changes that occur in cells experiencing physiological stress. Work from our own laboratory, as well as from other laboratories, has demonstrated that the stress proteins fall into two major families, the so-called glucose-regulated proteins and the heat-shock proteins, and that both families exhibit considerable interrelated properties. For example, members of both families appear to be immunologically related, in addition to sharing certain biochemical properties, including the binding of ATP. Hence, what we learn regarding one member of the stress protein family will likely prove useful in our understanding of the properties of the other members of the stress protein family.

Most of our efforts continue to focus on the biology and biochemistry of the individual stress proteins. We have now succeeded in purifying six of the seven major stress proteins and have prepared either monoclonal or polyclonal antibodies against each. Using these reagents, we have succeeded in characterizing some of the properties of the individual stress proteins and have determined their intracellular locations, both in the normal cell and in cells experiencing physiological stress (for a summary of these data, see Table 1). Thus, the major task remaining is to determine the biochemical functions of the individual stress proteins and their putative roles in cellular protection. As discussed below, we have begun to realize some success in this difficult endeavor.

This year, I am pleased to welcome two new scientists. Patrick Arrigo arrived from the University of Geneva in early spring as a visiting scientist and has already succeeded in purifying and partially characterizing the small 28,000-dalton mammalian stress protein. While in Geneva, Patrick participated in many of the first biochemical experiments examining the small heat-shock proteins of *Drosophila melanogaster* and therefore brings with him considerable experience in analyzing the small mammalian stress protein. Lee Mizzen, a postdoctoral fellow, arrived from the University of Western Ontario and has already made considerable progress in dissecting some of the biological and biochemical parameters involved in the mechanism by which cells protect themselves during exposure to physiological stress. A third addition to our staff was Mary Mulcahy. A recent graduate of the State University of New York at Binghamton, she arrived during the winter and has proved invaluable in helping with our biochemical studies.

Purification and Characterization of the Small 28,000-dalton Mammalian Stress Protein

P. Arrigo, J.P. Suhan, W.J. Welch

We have begun to pay considerable attention to the properties and structure of the small 28-kD mammalian stress protein. This protein has often been overlooked, since it contains very few methionine residues and therefore requires metabolic labeling

with [^{3}H]amino acid mixtures for easy visualization. Previous work from our laboratory had shown that the 28-kD protein is heavily phosphorylated, with such phosphorylation increasing in quiescent cells following exposure to various growth factors, tumor promoters, or agents (e.g., ionophores) that interfere with calcium homeostasis (Welch, *J. Biol. Chem. 260:* 3058 [1985]). This result, along with previous observations implicating a role for the small heat-shock proteins in normal developmental and differentiation processes in *Drosophila* and yeast, indicates that the 28-kD protein may serve an essential role in normal cellular growth processes and that phosphorylation may serve in regulating its function.

During the past year, we have carried out a number of biochemical fractionation studies aimed at determining the intracellular locale of the 28-kD protein in cells before, during, and after recovery from physiological stress. Early after its synthesis, we find that the majority of the 28-kD protein is present within the nuclear fraction of the heat-treated cells. Because the intermediate-filament cytoskeletal network collapses around the nucleus, we are not yet certain whether the 28-kD protein is actually present within the nucleus or whether it copurifies with nuclei owing to an "entrapment" within the collapsed filaments. Once we have obtained antibodies specific for the 28-kD protein, we should be able to pinpoint its precise location in the cells through indirect immunofluorescence analysis. During recovery from heat-shock treatment, we find that most of the 28-kD protein relocates within the soluble cytoplasmic fraction. Accompanying its redistribution into the soluble portion of the cells is a corresponding decrease in its phosphorylation. Hence, we are investigating whether phosphorylation of the 28-kD protein might be involved in regulating its intracellular locale.

Despite an increased synthesis after heat shock, the absolute amount of the 28-kD protein that accumulates in the cell is not as great as that observed for most of the other stress proteins. Consequently, purification of the protein has been difficult, and only recently have we succeeded in purifying the protein to apparent homogeneity. A key step in the purification involves gel filtration, since the protein appears to have an unusually large native molecular mass of approximately 500,000 daltons or greater. Hence, we have developed a rapid three-step purification of the protein and can purify approximately 1 mg of the protein from 8–10 liters of suspension HeLa cells in a matter of a few days (see Fig. 1). Preliminary data utilizing a variety of biophysical techniques indicate that the 28-kD protein exists as a well-defined particle easily visible in the electron microscope. Efforts to characterize further the properties and possible function of this very interesting stress protein are currently under way.

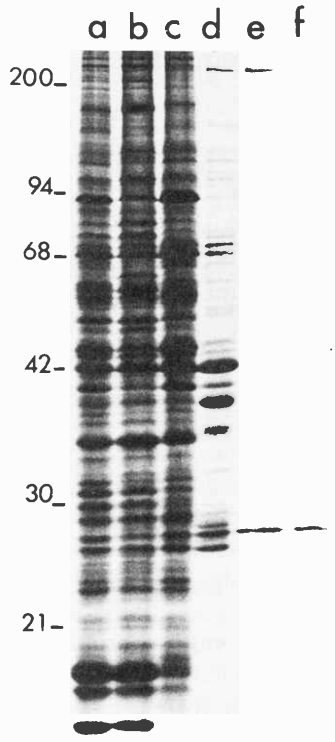

FIGURE 1 Steps in the purification of the small 28,000-dalton mammalian heat-shock protein. HeLa cells, growing in suspension, were heat-shock-treated at 43°C for 90 min and then allowed to recover at 37°C for 8 hr. The cells were collected, washed, and lysed by dounce homogenization in hypotonic buffer. The cell lysate was centrifuged at 15,000*g*, the supernatant was applied to a hydroxyapatite column, and the column was washed with a buffer containing 30 mM K_2HPO_4. The material eluting off the column and containing the majority of 28-kD protein was concentrated and directly applied to a gel-filtration column. The peak fractions of the 28-kD protein eluting from the column were pooled and directly applied to a DEAE-cellulose ion-exchange column, and the 28-kD protein was eluted using a linear gradient of NaCl. Shown in the figure are the protein profiles of each step of the purification as analyzed by SDS-PAGE. (*a*) Heat-shocked cell lysate; (*b*) 15,000*g* pellet; (*c*) 15,000*g* supernatant; (*d*) material eluting off the hydroxyapatite column; (*e*) peak fraction of the 28-kD protein eluting off the gel filtration column; (*f*) purified 28-kD protein eluting off the DEAE-cellulose column.

Purification and Characterization of the Glucose-regulated Stress Proteins

W.J. Welch

The synthesis of three of the mammalian stress proteins is markedly affected by changes in extracellular levels of glucose or calcium. For example, when incubated in culture medium containing little or no glucose or calcium, cells respond by increasing the synthesis of proteins (M_r = 80,000 and 100,000) and correspondingly decreasing the production of the 90-kD heat-shock protein. Upon refeeding with glucose or calcium, the cells now respond in the opposite manner, decreasing the production of the 80-kD and 100-kD proteins and increasing the synthesis of the 90-kD protein. Interestingly, this inverse regulatory relationship also occurs in cells following anoxia (oxygen starvation): increased 80-kD and 100-kD protein synthesis and a corresponding decreased 90-kD protein synthesis. Following reoxygenation of the cells, there is a decreased production of the 80-kD and 100-kD proteins and an increase in the 90-kD protein, as well as an increase in production of some of the other stress proteins.

In addition to their reciprocal regulation in the cell, in the last year, we and other investigators have learned that the glucose-regulated proteins (GRPs) appear to be similar to the heat-shock proteins (HSPs). Specifically, DNA sequence information, along with biochemical and immunological studies, has demonstrated that the 80-kD GRP is highly related to the 70-kD HSP family and that the 100-kD GRP is homologous to the 90-kD HSP. All of the proteins, however, appear to be located at different sites within the cell. Hence, it is likely that all of the stress proteins, be they GRPs or HSPs, are performing similar functions within the cell, albeit in different intracellular compartments.

This year, we succeeded in purifying to homogeneity the two major (80-kD and 100-kD) GRPs. Purification of the 90-kD HSP was accomplished a few years earlier (Welch and Feramisco, *J. Biol. Chem. 257:* 14949 [1982]). The 80-kD and 100-kD proteins, located in the endoplasmic reticulum and Golgi, respectively, are extracted from the particulate fraction with nonionic detergents and can be partially purified by ion-exchange chromatography. As a final purification step, we have taken advantage of two pertinent biochemical properties that we have determined for the proteins. First, we have shown that the 80-kD protein, like the 70-kD HSPs, binds ATP, and we have utilized affinity chromatography on agarose columns containing covalently linked ATP as a final purification step for the protein (Welch and Feramisco, *Mol. Cell. Biol. 5:* 1229 [1985]; W. Welch, in prep.). In the case of the 100-kD protein, we have shown it to be a glycoprotein and have purified the protein to homogeneity via columns containing a covalently linked lectin, concanavalin A (Welch et al., *J. Biol. Chem. 258:* 7102 [1983]; W. Welch, in prep.). Using the purified proteins, we plan to compare further the structural and biochemical properties of the 80-kD and 100-kD GRPs and determine which domains are homologous to the corresponding 70-kD and 90-kD HSPs, respectively. In addition, we are examining how the two GRPs are situated (e.g., topography) within the endoplasmic reticulum and Golgi apparatus.

A Function for the Major Stress Protein in Stabilizing Macromolecular Complexes?

L. Mizzen, W.J. Welch

Much of our work has and will continue to focus on the biology of the 72-kD mammalian stress protein. This follows from the facts that the 72-kD protein (1) represents the most highly induced protein in cells experiencing physiological stress, (2) appears to be highly conserved among different organisms, and (3) appears to be integral to the protection of the cell and the acquisition of thermotolerance (discussed below). Consequently, we believe that the key to understanding how cells defend against physiological stress will ultimately lie in our dissecting the function of the 72-kD protein.

Our previous studies have resulted in the purification, characterization, and determination of the intracellular locale of the 72-kD protein before, during, and after recovery from physiological stress. We have found that the 72-kD protein exhibits an avid affinity for ATP and have exploited this property to develop a rapid two-step purification of the protein using affinity chromatography on agarose columns containing covalently linked ATP (Welch and Feramisco, *Mol. Cell. Biol. 5:* 1229 [1985]). Using biochemical fractionation and immunological procedures, we have shown that shortly after induction of the stress response, the

majority of the 72-kD protein accumulates within the nucleus, most notably within the nucleolus (Welch and Feramisco, *J. Biol. Chem. 259:* 4501 [1984]). Using high-resolution immunoelectron microscopy, we find most of the protein present within that region of the nucleolus involved in the assembly of ribosomes and other small ribonucleoproteins (Welch and Suhan, *J. Cell Biol. 103:* 2035 [1986]). This locale of the 72-kD protein is quite interesting considering that nucleolar function is impaired (i.e., production of rRNA and assembly of new ribosomes) and that the organization of the nucleolus is severely perturbed after heat-shock treatment. We and others have therefore suggested that the 72-kD protein plays a role in the repair of such nucleolar abnormalities and thereby facilitates the recovery of normal nucleolar function during recovery from heat-shock treatment.

During the later periods of recovery from stress, most of the 72-kD protein begins to accumulate within the cytoplasm, with a portion of the protein associating with unusually phase-dense structures that appear to consist of large aggregates of denatured protein (Welch and Suhan, *J. Cell Biol. 103:* 2035 [1986]). In addition, we find some of the 72-kD protein colocalized with ribosomes, reminiscent of its codistribution within that region of the nucleolus involved in ribosomal assembly. As discussed below, we believe that this association with the ribosomes is integral to the proper functioning of the translational machinery in the stressed cell.

Two previous observations have suggested an approach to examine the function of the 72-kD protein. First, it has been shown that cells can acquire resistance to severe heat-shock treatment simply by prior exposure of the cells to a mild sublethal heat-shock challenge. This phenomenon, referred to as acquired thermotolerance, appears to be dependent on the production of the heat-shock proteins during the initial heat exposure. Along with this acquisition of resistance, it has been shown that the cells can also acquire a resistance to translational inhibition. Specifically, if mammalian cells are brought to a rather high temperature (e.g., 45°C for 30 min), overall translation is severely inhibited and does not return until the cells have recovered at 37°C for a number of hours. If, however, the cells are presented with a mild heat shock (e.g., 43°C for 1 hr), allowed to recover and synthesize the stress proteins, and then presented with the more severe (45°C for 30 min) heat-shock treatment, translation patterns are not nearly so inhibited (see Fig. 2).

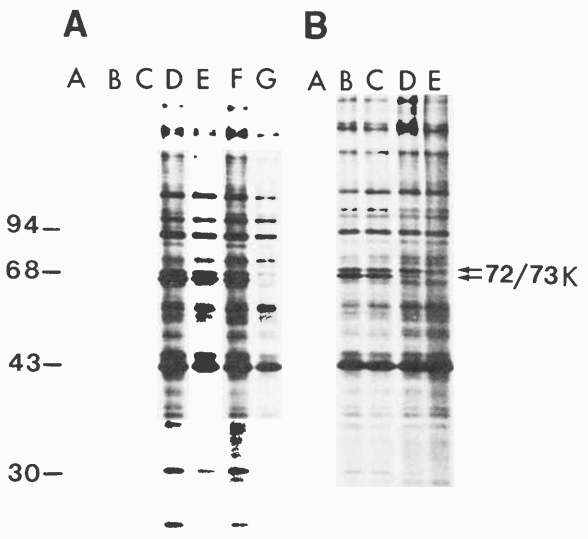

FIGURE 2 Protection of translation in the thermotolerant cell. (Panel *A*) Rat embryo fibroblasts, growing on 35-mm dishes, were heat-shock-treated at 45°C for 30 min, returned to 37°C, and pulse-labeled with [^{35}S]methionine for 1 hr at various times after heat shock. After labeling, the cells were harvested, and the labeled proteins were analyzed by SDS-PAGE. Shown is a fluorogram of the gel. Labeling times after heat shock: (*A*) 0–1; (*B*) 2–3; (*C*) 4–5; (*D*) 6–7; (*E*) 8–9; (*F*) 12–13; (*G*) 14–15 hr. (Panel *B*) Rat embryo fibroblasts, growing on 35-mm dishes, were heat-shock-treated at 43°C for 1 hr and then returned to 37°C. Following their recovery at 37°C for 12 hr, the cells were again heat-shock-treated at 45°C for 30 min, returned to 37°C, and pulse-labeled with [^{35}S]methionine, and the labeled proteins were analyzed as described above. Labeling times after heat shock: (*A*) 0–1; (*B*) 2–3; (*C*) 4–5; (*D*) 6–7; (*E*) 8–9 hr. Note the increased translational capacity of the cells made "thermotolerant" by the prior heat-shock treatment.

Hence, since we have demonstrated a colocalization of the 72-kD protein with the translational machinery, we are pursuing the possibility that such an association may be responsible for the development of translational thermotolerance.

We think that the 72-kD protein probably has a similar role in the stabilization of other macromolecular complexes in the cell; i.e., similar to its role in protecting the translational machinery, the 72-kD protein may serve a rather promiscuous role in protecting other protein containing complexes, such as those involved in transcription and/or heterogeneous nuclear RNA (hnRNA) processing. This is suggested since most of the agents that induce the stress response are potent protein denaturants that probably perturb protein function. Complexes that contain multiple protein components would therefore be large targets for such protein

denaturants, and hence there might occur an inhibition of many cellular activities. Consistent with this idea are the results of Yost and Lindquist (*Cell 45:* 185 [1986]), who have shown that rather severe heat-shock treatment of *Drosophila* cells results in diminished hnRNA processing. However, if the cells are first made thermotolerant, the inhibition of hnRNA processing is less pronounced. Thus, like its possible role in protecting the translational machinery, the 72-kD protein may also allow the thermotolerant cell to process new mRNA transcription correctly. This proposed general protective role of the 72-kD protein seems plausible, since (1) such high levels of the protein are produced after stress, (2) the protein is observed to distribute throughout much of the cytoplasm and nucleus, and (3) many cellular activities can be protected simply by inducing the synthesis of the 72-kD protein (as well as that of the other stress proteins) prior to a severe heat-shock treatment, which, by itself, usually results in a diminishment of these different cellular activities. Hence, using a combination of in vitro and in vivo techniques, we are designing experiments aimed at addressing this possible function of the 72-kD protein in protecting different cellular machineries after heat-shock treatment.

A Family of ATP-binding Stress Proteins

W.J. Welch

A key to understanding their possible function was the observation that a number of mammalian stress

TABLE 1 Characterization and Localization of the Stress Proteins

Stress protein ($\times 10^{-3}$)	Modification	Location		Remarks
		37	42	
Major				
28	phosphorylation	n.d.	n.d.	increased phosphorylation to growth factors, tumor promoters, or calcium perturbations; partial homology with lens α crystallins; native molecular mass, ≥400,000 daltons
72	methylation	–	cytoplasmic nuclear nucleolar	most highly induced stress protein; binds nucleotides (ATP) and perhaps RNA; portion colocalizes with ribosomes; expression in primates at 37°C and cell-cycle-regulated; elevated expression in human cells after transformation
73	methylation	cytoplasmic	cytoplasmic nuclear (nucleolar?)	homology with 72 kD; portion present with cycled microtubules/intermediate filaments; homology with clathrin-coated vesicle uncoating ATPase
90	phosphorylation methylation	cytoplasmic	cytoplasmic (nuclear?)	abundant cellular phosphoprotein with multiple isoelectric forms; increased phosphorylation in response to dsDNA; transient association with tyrosine kinases; present in steroid hormone receptor complex; synthesis repressed following glucose/calcium deprivation
110		nucleolar	nucleolar cytoplasmic	present in nucleolar fibrillar regions; sites of pol III transcription
Minor				
80	phosphorylation ADP-ribosylation	endoplasmic reticulum	endoplasmic reticulum	increased synthesis following glucose/calcium deprivation; homology with immunoglobulin heavy-chain binding protein; binds ATP and immunologically related to 72-kD/73-kD stress proteins
100	phosphorylation glycosylation	Golgi plasma membrane	Golgi plasma membrane nuclear	increased synthesis during glucose/calcium deprivation; portion associated with 90-kD stress protein; shows homology with hsp 90

n.d. indicates not determined.

proteins exhibit avid binding of ATP in vitro (Welch and Feramisco, *Mol. Cell. Biol. 5:* 1229 [1985]). Specifically, the 72-kD and 73-kD heat-shock proteins and the 75-kD and 80-kD glucose-regulated proteins have all been shown to bind ATP, but they appear to function within different intracellular compartments: The 72-kD and 73-kD proteins are present within the cytoplasm, nucleus, and nucleolus; the 80-kD protein is present within the endoplasmic reticulum; and the 75-kD protein is located within the mitochondria. Moreover, cDNA sequence analysis, as well as immunological data, has now demonstrated that ATP-binding proteins in this family are structurally related, perhaps via a conserved domain involved in their ability to bind nucleotides. Domains in the proteins that are unique probably account for the different intracellular compartmentalization of the individual proteins.

Many workers in the field are leaning toward the idea that this family of related stress proteins serve similar functions within their different locales. Moreover, this general function appears to involve an association with other macromolecules, with a subsequent hydrolysis of ATP. In the case of the constitutive and abundant 73-kD protein, evidence has implicated it serving a role in the ATP-dependent dissociation of clathrin coats, which envelope lipid vesicles involved in endo- and exocytosis. As discussed above, we think that the highly stress-induced 72-kD protein may serve some ATP-dependent protective and/or reparative role involved in stabilizing and/or restoring macromolecular complex activity. Finally, H. Pelham's laboratory (MRC) has provided evidence that the 80-kD glucose-regulated stress protein is identical to a previously described protein involved in the ATP-dependent solubilization of denatured and/or aggregated IgG heavy-chain molecules within the endoplasmic reticulum. We are pursuing the possibility that the related, 75-kD glucose-regulated protein may serve a role similar to that of the 80-kD protein, but within its intracellular compartment, the mitochondrion.

We will continue to examine and compare the physical and biological properties of this family of ATP-binding stress proteins. Specifically, with respect to their physical properties, we plan to map the domains within the proteins involved in ATP binding and/or determine those domains common to the proteins that underlie their immunological relatedness. In addition, using the purified proteins and their corresponding antibodies, we are examining possible substrates and associated proteins involved in stress-protein-dependent ATP hydrolysis. We are confident that what we learn regarding one member of the family will prove useful in dissecting the functions of the other members.

Antibody Production

W.J. Welch, L. Mizzen, P. Arrigo, N. Kronenberg

As we purify more stress proteins and determine which molecules they interact with in the cell, we also continue to produce both polyclonal and monoclonal antibodies to these various proteins. Special emphasis this year has been placed on obtaining antibodies to the small 28-kD stress protein. In addition, we have immunized mice with a number of the other purified stress proteins and hope to isolate specific hybridomas to each over the next few years. Finally, in the last year, we completed our characterization of a number of monoclonal antibodies to the 72-kD and 73-kD stress proteins. We have used them extensively and have provided them to over 50 different investigators throughout the world.

The Stress Response in the Transformed Cell

L. Mizzen, J. Lamb, J.R. Feramisco, W.J. Welch

In collaboration with J. Mendecki at the Montefiore Hospital in the Bronx, we have begun to examine the effects of hyperthermic treatment of tumors within mice. Using a laser to direct microwaves to the tissue, we have succeeded in raising the temperature of the tumor to greater than 42°C. Using two-dimensional gel electrophoresis and immunological assays, we have demonstrated that such treatment results in a vigorous production of the stress proteins. More importantly, in our preliminary studies, we have achieved considerable regression of the tumor, in some cases after only a single l-hour hyperthermic treatment. Obviously, we are continuing these efforts; we are examining which tumors may respond better and are looking for agents that, when coupled with the hyperthermic treatment, result in a synergistic killing effect. Indeed, we have now found a class of agents that appear to increase the killing effects of heat dramatically. Current ef-

forts are under way to determine the underlying mechanism that allows for such a synergistic killing effect.

Coupled with these studies is our intent to step up studies designed to address why transformed cells grown in vitro appear to be highly sensitive to heat-shock treatment as compared with their normal cell counterparts. Using a variety of normal and transformed cells, we want to determine whether there is a difference in either the amount, locale, modification, and/or regulation of the stress proteins between the two different cell types. Although little was accomplished in this endeavor during the past year, we hope to bring on additional help in the upcoming year to examine more closely this differential sensitivity of transformed cells to heat-shock treatment and to exploit this observation in the in vivo hyperthermic treatment of tumors in animals.

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MOLECULAR BIOLOGY OF THE CYTOSKELETON

D.M. Helfman S. Cheley E. Kuismanen S. Erster L.A. Finn J.C. Lamb

Role of Tropomyosin in Cytoskeleton Organization and Cell Shape

S. Cheley, J.C. Lamb, J.R. Feramisco, D.M. Helfman

Transformation of cells in tissue culture results in a variety of cellular changes, including alterations in cell growth, adhesiveness, motility, morphology, and organization of the cytoskeleton. Morphological changes are perhaps the most readily apparent feature of transformed cells. These changes are clearly associated with the cytoskeleton. Recent studies using comparative two-dimensional protein gel analysis of normal and transformed cells have revealed that of the numerous cytoskeletal proteins, tropomyosin expression is selectively altered in transformed cells. These alterations in tropomyosin expression appear to correlate well with the rearrangement of microfilament bundles and the morphological alterations observed in transformed cells.

We have used an established rat embryonic 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. Of the

five tropomyosin (TM) isoforms present in cultures of normal rat fibroblasts (e.g., REF52 cells), only TM-1 is absent in the adenovirus-transformed Ad5D.4A cells. These cells are characterized by a somewhat rounded morphology and by the absence of microfilament bundles (stress fibers). 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. To address this question directly, we examined the effects of "forced" expression of the TM-1 isoform on cell shape and the cytoskeleton in transformed Ad5D.4A cells. To introduce TM-1 into living fibroblasts, we microinjected in-vitro-synthesized mRNA encoding TM-1. A full-length cDNA encoding TM-1 was subcloned into Sp6 plasmids for the generation of TM-1 mRNA. Microinjection of TM-1 mRNA into Ad5D.4A cells led to cell spreading and concomitant restoration of actin microfilament bundles. These results were blocked by pretreatment of the cells with cycloheximide. Cell spreading and formation of the microfilament bundles were not seen after microinjection with truncated forms of TM-1 mRNA, nor after microinjection of mRNA encoding two other fibroblast tropomyosin isoforms normally expressed in transformed cells. Our results support the hypothesis that selective loss of a major tropomyosin isoform may be responsible for the changes in cell shape and microfilament organization that accompany transformation.

Analysis of Sequences Involved in Internal Splice-site Selection of the TM-1 Gene

D.M. Helfman, L.A. Finn

Recently, we reported that rat fibroblast TM-1 and skeletal-muscle β-TM are expressed from a single gene by alternative RNA splicing and polyadenylation (Helfman et al., *Mol. Cell. Biol. 6:* 3582 [1986]). The tropomyosin gene has 11 exons and spans approximately 10 kb (Fig. 1). Sequences common to all mRNAs expressed from this gene were found in exons 1 through 5 (amino acids 1–188) and exons 8 and 9 (amino acids 214–257). Exons 6 and 11 are specific for fibroblast mRNA (amino acids 189–213 and 258–284, respectively), whereas exons 7 and 10 are specific for skeletal-muscle mRNA (amino acids 189–213 and 258–284, respectively). In addition, exons 10 and 11 contain the entire 3′-untranslated sequence including the polyadenylation site. Although the gene is also expressed in smooth muscle (stomach, uterus, and vas deferens), only the fibroblast-type mRNA can be detected in these tissues. Furthermore, S1-nuclease and primer-extension analyses indicated that all mRNAs expressed from this gene are transcribed from a single promoter. The molecular basis for the tissue-specific expression is currently unknown.

To study which sequences 5′ and 3′ of the tissue-specific exons contain the necessary information for alternative RNA splicing, a series of DNA constructions (minigenes) was constructed and transfected into cultured cells. The RNA products were then characterized by S1-nuclease-protection analysis. One simple construct we have used contains sequences from exon 5 through exon 9. To express the cloned regions of the tropomyosin gene, these sequences were ligated to a plasmid containing the SV40 early promoter and poly(A) site. Expression of this minigene in rat fibroblasts and L6 myoblasts resulted in an RNA product containing the fibroblast-specific exon. Thus, the product contains exons 5, 6, 8, and 9 (see Fig. 1). We are currently trying to express this minigene in appropriate cell types (e.g., fused myoblasts) that represent skeletal muscle to determine if the skeletal-muscle-specific sequences (exon 7) will be utilized. In the future, we hope to develop in vitro systems to study the factor(s) responsible for tissue-specific splicing.

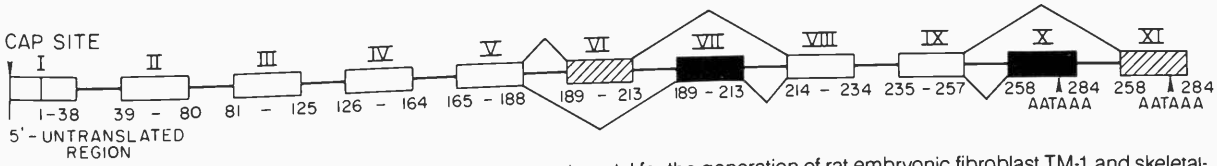

FIGURE 1 Schematic diagram of the tropomyosin gene and model for the generation of rat embryonic fibroblast TM-1 and skeletal-muscle β-tropomyosin mRNAs by alternative RNA processing. Open boxes represent common exons, hatched boxes represent fibroblast or smooth-muscle exons, closed boxes represent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

In Vivo Analysis of 3′-proximal Sequences Involved in Splicing and Polyadenylation

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

We have begun to study the 3′-end processing events of the TM-1 gene, which require both alternative exon selection and utilization of the appropriate poly(A) site (Fig. 1). To localize the *cis*-acting sequence elements required for these processes, a number of tropomyosin DNA minigene constructions were generated. These minigenes are initially being transfected into COS cells, and the products analyzed by S1-nuclease-protection experiments. We eventually plan to express the minigenes in appropriate cell types (fibroblasts and myotubes) to determine if the RNAs expressed from these constructions are processed in a tissue-specific manner that reproduces the in vivo events. Deletion analysis indicates that more than 100 bp 3′ of the fibroblast poly(A) site are required for cleavage and polyadenylation. Tropomyosin minigene constructions have also been prepared to localize sequences 5′ of the alternatively spliced exons that are involved in tissue-specific expression of these exons. A series of BAL-31 deletions have been constructed in the large intron (~2.8 kb) separating exon 9 (common exon) and exon 10 (skeletal-muscle-specific exon). Preliminary results indicate that removal of most of the internal sequences (2.2 kb) from this large intron does not affect the processing products observed in vivo by transient expression. We hope that these experiments will determine the minimal intron sequences required for tissue-specific RNA processing. Ideally, we will be able to study the sequence requirements for processing in vivo and reproduce the effects in vitro in order to identify possible *trans*-acting factors responsible for the tissue-specific RNA processing.

Identification of Promoter Sequences of the TM-1 Gene

E. Kuismanen, D.M. Helfman

We have begun to define those sequences within the 5′-flanking regions of the TM-1 gene that are required for transcription. DNA sequence analysis revealed that the promoter region is G-C-rich, with very few A-T sequences. A sequence resembling a TATA box, TTTTA, is located 19 bp upstream of the cap site. In contrast to most of the characterized eukaryotic genes, no sequence resembling a CCAAT box is found upstream of the putative TATA-like sequence. Further analysis revealed the occurrence of two putative Sp1-binding sites (CCGCCC or GGGCGG). These sequence motifs are contained in many other cellular and viral promoters and are thought to function by interacting with the positively acting transcription factor Sp1. The first occurrence of an Sp1-binding site is 38 nucleotides upstream of the cap site, and the second sequence is located 148 nucleotides upstream of the cap site. In addition, this second putative Sp1-binding site is flanked on each side by an 8-bp direct repeat, CCGAGGGG. The functional significance, if any, of this repeat remains to be established. In addition, using gel mobility shift and footprint analyses, we have identified a number of regions in the promoter that appear to interact with DNA-binding proteins.

To define functionally the sequences in the promoter needed for transcription, a number of constructions have been prepared using the promoter region of the tropomyosin gene ligated to the chloramphenicol acetyltransferase gene (*cat*). Preliminary experiments indicate that expression of *cat* requires less than 600 bp upstream of the site of transcription initiation. In addition, expression from the tropomyosin promoter was found to be rather weak, but it could be increased significantly (>tenfold) when the SV40 enhancer element was ligated to the plasmid. These results suggested that the tropomyosin gene may require an enhancer element for expression. In preliminary experiments using an enhancer-trap construct containing the tropomyosin promoter and *cat* gene, we identified a putative enhancer element in the structural part of the tropomyosin gene. Work is currently under way to define precisely which sequences in the tropomyosin gene apparently behave like an enhancer element.

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CELL BIOLOGY OF THE NUCLEUS

D.L. Spector M.R. Delannoy

The overall aims of our laboratory are (1) to identify specific nuclear regions with specific functions and to understand how these functional domains are organized and how they interact with each other during the cell cycle, and (2) to elucidate the subnuclear distribution of the *myc* oncogene protein as a basis for subsequent biochemical analyses aimed at determining the functional associations of this nuclear protein.

Identification of a Nucleoplasmic Reticulum: The Putative Heterogeneous Nuclear RNA Processing Machinery

D.L. Spector

In previous studies (Spector et al., *Biol. Cell 49:* 1 [1983]; Spector, *Biol. Cell 51:* 109 [1984]; Smith et al., *J. Cell Biol. 101:* 560 [1985]), we have examined the organization of the nucleoplasm using monoclonal and polyclonal antibodies that recognize seven distinct nuclear proteins. Of particular interest was the colocalization of (1) a monoclonal anti-Sm antibody that recognizes a 28-kD protein associated with U1, U2, U4/U6, and U5 small nuclear ribonucleoprotein particles (snRNPs); (2) autoantibodies against U1 or U2 snRNPs; and (3) CRLM, a polyclonal antibody raised against a rat liver nuclear matrix preparation that recognizes two major EDTA- and RNase-sensitive nuclear-matrix-associated proteins of 107 kD and 103 kD, respectively. All of the proteins recognized by these antibodies colocalized within a discrete nuclear domain that we have named the nucleoplasmic reticulum on the basis of its network-like organization and location within the cell nucleus (Figs. 1a and 2). The nucleoplasmic reticulum contains at least two macrocomponents, nuclear protein clusters (NPCs) and linkers, which are the connections between the NPCs (Fig. 1a). We have observed such a network to be a common component of all eukaryotic cell and tissue types thus far examined. This snRNP-enriched nuclear region does not colocalize with the bulk of nuclear DNA (Fig. 3) or RNA polymerase II and is resistant to DNase and high-salt (2 M NaCl) extraction. These data suggest that the nucleoplasmic reticulum may not be directly associated with the transcriptional regions of the nucleoplasm. Alternatively, the concentration of snRNPs within the nucleoplasmic reticulum raises the possibility that this nuclear region is involved in heterogeneous nuclear RNA (hnRNA) processing. Along this line, we have shown that the inhibition of hnRNA synthesis by the drug 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) causes a marked alteration in the organization of the nucleoplasmic reticulum. After drug treatment, the NPCs appear more rounded and uniform in shape (compare a and b in Fig. 1) and their fluorescence intensity is increased, whereas the linkers are less evident (Fig. 1b). Therefore, the linkers may represent elements being transported from NPC to NPC or from NPC to the nuclear envelope, where they may be extruded into the cytoplasm as mature mRNP particles. These data raise the possibility

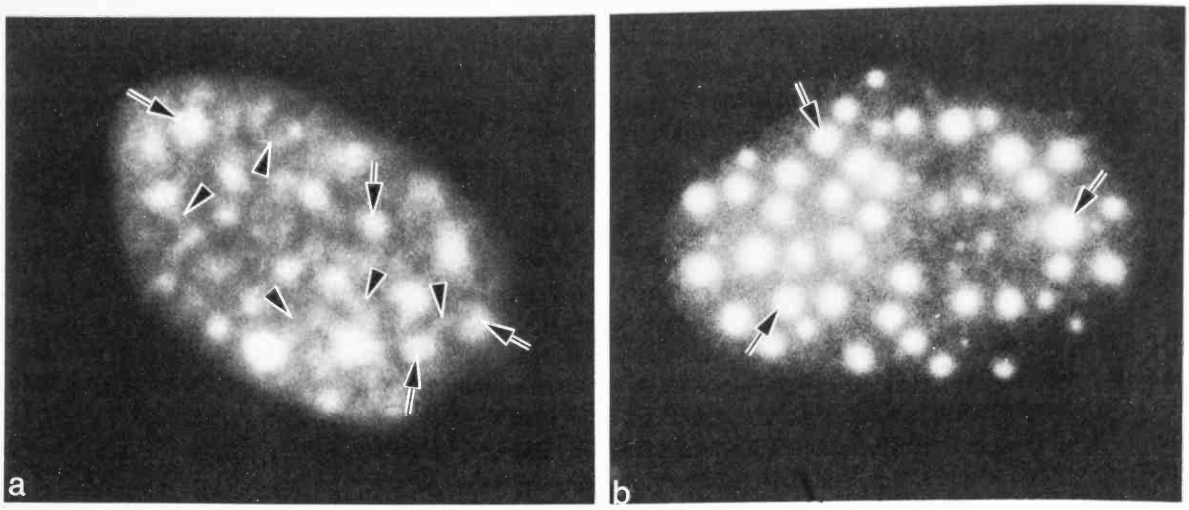

FIGURE 1 (*a*) Immunofluorescence of the nucleoplasmic reticulum in a PtK2 cell nucleus immunostained with the anti-Sm antibody. The heterogeneously shaped NPCs (arrows) are connected via linkers (arrowheads) to form a reticulum. Numerous NCPs and linkers may be observed depending on the plane of focus. (*b*) When hnRNA synthesis was inhibited by the drug DRB (25 μg/ml, 1 hr), the NCPs assumed a more uniform, rounded shape (arrows) and increased in fluorescence intensity, whereas the linkers were less evident. Magnification, 2820×.

that the nucleoplasmic reticulum is a dynamic structure involved directly in hnRNA processing and/or in the transport of processing intermediates and spliced messages within the nucleoplasm.

Our current studies are intended to characterize this nuclear region biochemically and ultrastructurally. We are specifically interested in isolating the nucleoplasmic reticulum and in determining its protein and nucleic acid constituents. Using nucleic acid probes, we will evaluate the in situ spatial orga-

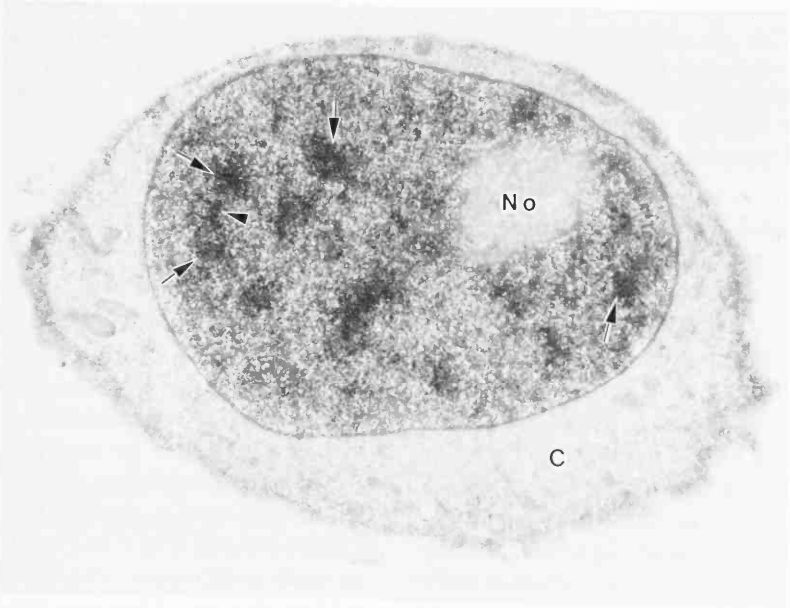

FIGURE 2 Immunoelectron microscopic localization of the nucleoplasmic reticulum stained with the anti-Sm antibody and peroxidase-conjugated second antibody. Both NPCs (arrows) and linkers (arrowheads) may be observed in this thin section (100 nm). The cytoplasm (C) and nucleolus (No) did not exhibit immunoreactivity (no poststaining). Magnification, 4047×.

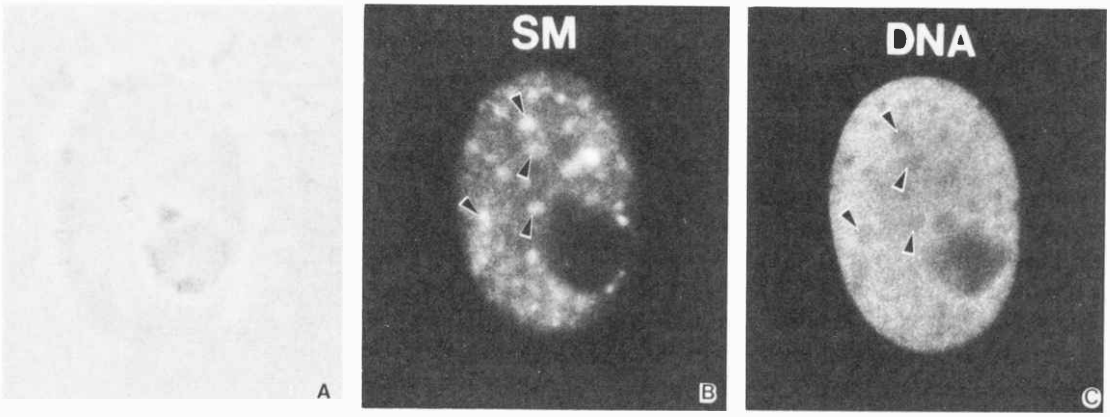

FIGURE 3 Double-label immunofluorescent images with the anti-Sm (*B*) and anti-DNA (*C*) antibodies showing that the regions of the nucleoplasm that contain the nucleoplasmic reticulum are exclusive of the nuclear regions that contain the bulk of DNA. Compare arrowheads in *B* and *C*. Magnification, 1000×.

nization of specific genes and determine whether the pre-mRNAs for certain genes or gene families are processed in specific macromolecular complexes (NPCs).

Colocalization of the *myc* Oncogene Protein and snRNP Particles

D.L. Spector, M.R. Delannoy, N.F. Sullivan [in collaboration with R.A. Watt, Molecular Oncology Group, Smith, Kline and French Laboratories]

In an attempt to examine the nuclear associations of the *myc* oncogene protein, we examined its subnuclear distribution in situ by immunofluorescence microscopy in quail cells (Q8) nonproductively transformed with the avian myelocytomatosis virus MC29. The MC29 virus encodes an 875-amino-acid c-*myc*-related protein of approximately 110 kD ($p110^{gag\text{-}myc}$ or v-*myc*). The amino-terminal portion of the protein (454 amino acids) is derived from the retroviral *gag* gene, whereas the carboxy-terminal portion of the protein is not of retroviral origin and specifies 5 amino acids from the 5′-untranslated region and 416 amino acids from the coding sequence of the avian c-*myc* gene (Reddy et al., *Proc. Natl. Acad. Sci. 80:* 2500 [1984]). Antibodies used in this study have been raised against the recombinant c-*myc* protein synthesized in and purified from *Escherichia coli* (Watt et al., *Mol. Cell. Biol. 5:* 448 [1985]).

The nuclear distribution of v-*myc* appeared as a speckled pattern that occupied a portion of the nucleoplasm (Fig. 4c). This protein-distribution pattern was identical regardless of the fixative used (methanol, acetone, glutaraldehyde, or paraformaldehyde). Since the distribution of v-*myc* resembled that which has been reported for snRNPs (Spector et al., *Biol. Cell 49:* 1 [1983]), Q8 cells were further examined by triple-label fluorescence using (1) a monoclonal anti-Sm antibody that recognizes the 28-kD protein associated with U1, U2, U4/U6, and U5 snRNPs (Lerner et al., *Proc. Natl. Acad. Sci. 78:* 2737 [1981]), (2) a polyclonal antibody that recognizes the v-*myc* protein (p110) in quail cells (Watt et al., *Mol. Cell. Biol. 5:* 448 [1985]), and (3) Hoechst stain, a fluorochrome that binds nonintercalatively to double-stranded DNA. Both antigens (Sm and v-*myc*) colocalized within the nucleus in irregularly shaped clusters (Fig. 4c–d), which formed a network, as determined by serial sections, throughout the nucleoplasm (Fig. 5c–g). The nucleoli and cytoplasm were devoid of immunoreactivity (Fig. 4c–d). The nuclear regions immunostained by both of these antibodies were exclusive of those regions that exhibited reactivity with Hoechst stain (Fig. 4b). Hoechst staining localized concentrations of DNA in the perinucleolar region as well as in several patches throughout the nucleoplasm (Fig. 4b). To determine if the colocalization of the v-*myc* protein and snRNPs was due to interactions between the *gag* or *myc* portions of v-*myc*, immunofluorescence was performed on neuroendocrine cells of a human colon adenocarcinoma (COLO 320). In COLO 320 cells, the c-*myc* gene is amplified and expressed at elevated levels, and the c-*myc* protein produced is not associated with a retroviral *gag* protein (Alitalo

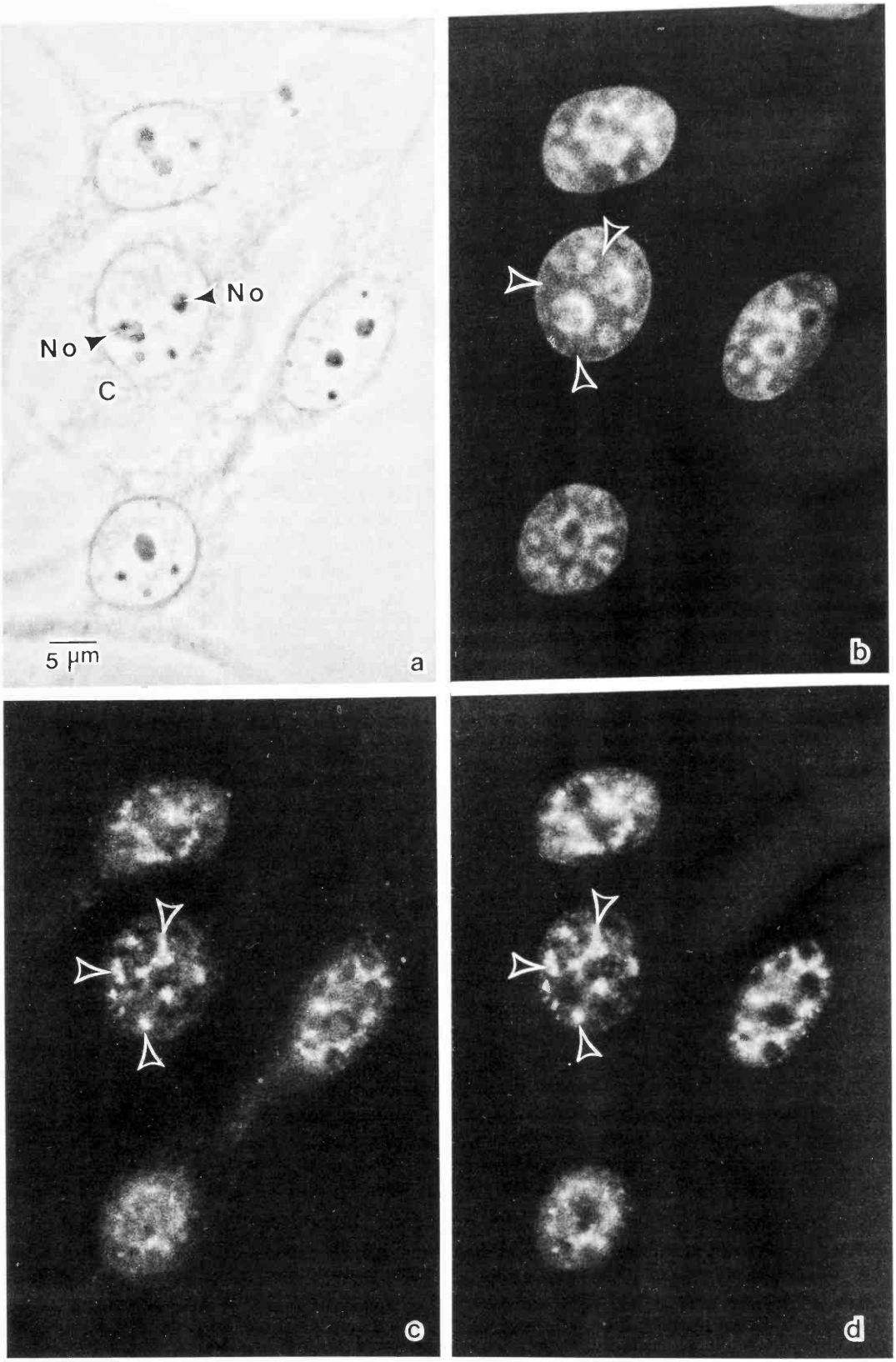

FIGURE 4 *(See facing page for legend.)*

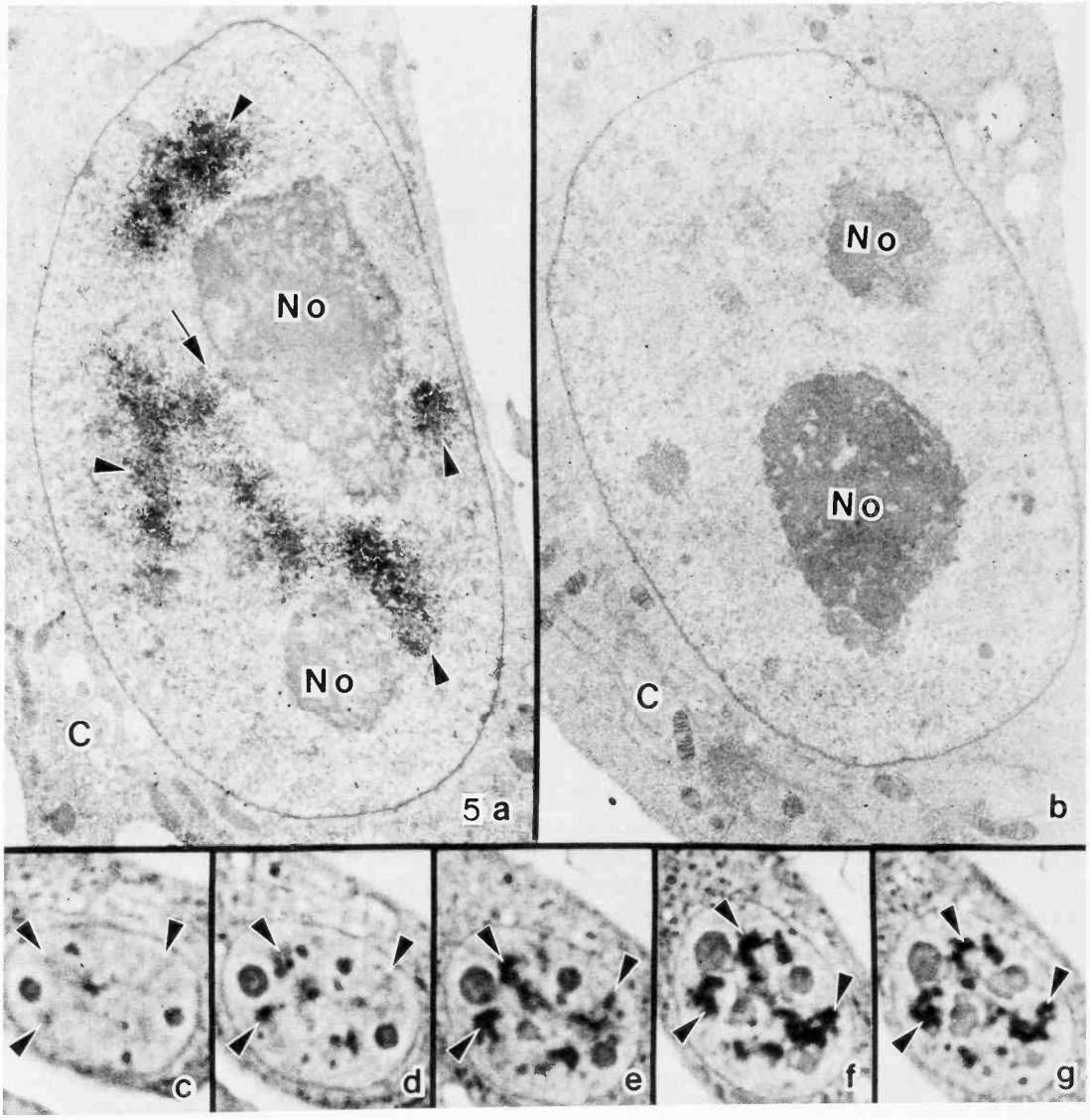

FIGURE 5 (*a*) Immunoelectron microscopic sublocalization of v-*myc* in quail (Q8) cells. The immunostained regions (arrowheads) identified a branched network traversing the nucleoplasm. The network appeared to come into contact with the nucleolar surface (arrow) but was rarely observed to contact the nuclear lamina and/or envelope. Neither the nucleoli (No) nor cytoplasm (C) was immunostained (no poststaining). (*b*) Cells immunolabeled with rabbit preimmune sera in place of the primary anti-c-*myc* antibody did not exhibit immunostaining (no poststaining). (*c–g*) Adjacent serial sections (200 nm) showing continuity of the nuclear regions immunostained with the anti-c-*myc* antibody by light microscopy (follow arrowheads through series of pictures). Magnifications: (*a*) 13,800×; (*b*) 10,000×; (*c–g*) 1500×.

FIGURE 4 Phase-contrast (*a*) and triple-label fluorescence (*b–d*) of quail (Q8) cells immunolabeled with anti-Sm (*d*), anti-c-*myc* (*c*) antibodies, and Hoechst stain for DNA (*b*). Both snRNPs and v-*myc* colocalized in a speckled staining pattern within the nucleoplasm (arrowheads in *c,d*). The bulk of nuclear DNA as visualized by Hoechst stain (*b*) was exclusive of the Sm and v-*myc* immunostained regions (compare arrowheads in *b*, *c*, and *d*). The nucleoli (No) and cytoplasm (C) did not stain with the anti-Sm and anti-c-*myc* antibodies (*c,d*). Magnification, 1520×.

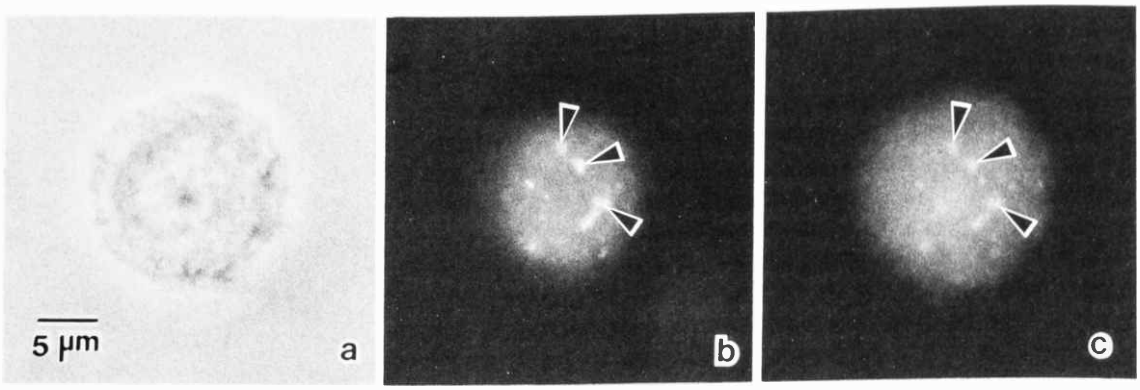

FIGURE 6 Phase-contrast (*a*) and double-label immunofluorescence (*b,c*) of COLO 320 cells immunolabeled with anti-Sm (*b*) and anti-c-*myc* (*c*) antibodies showing the colocalization of c-*myc* and snRNPs (compare arrowheads in *b* and *c*). Magnification, 1500×.

et al., *Proc. Natl. Acad. Sci. 80:* 1707 [1983]). Similar to the results obtained for v-*myc*, a colocalization of c-*myc* and snRNPs was observed in COLO 320 cells (Fig. 6), strongly suggesting that the colocalization of *myc* and snRNPs is not mediated by the retroviral *gag* protein.

Data from several laboratories over the last few years has contributed to the elucidation and morphological characterization of a nuclear region with concentrations of snRNP particles as well as several other nonhistone nuclear proteins (Sharp et al., *Am. J. Med. 52:* 148 [1972]; Deng et al., *J. Cell Biol. 91:* 654 [1981]; Spector et al., *Biol. Cell 49:* 1 [1983]; Smith et al., *J. Cell Biol. 101:* 560 [1985]; Nyman et al., *J. Cell Biol. 102:* 137 [1986]). The fact that these proteins preferentially associate with specific nuclear regions rather than being diffusely distributed suggests that a particular function or set of functions of which these proteins are a part, may be localized to this region of the nucleoplasm. On the basis of previous studies showing (1) a concentration of snRNPs to be present within this region (Spector et al., *Biol. Cell 49:* 1 [1983]), (2) the bulk of nuclear DNA to be absent from this region (Smith et al., *J. Cell Biol. 101:* 560 [1985]), (3) the inability of DNase I and high-salt extraction (2 M NaCl) to alter the protein distribution patterns (Spector et al., *Biol. Cell 49:* 1 [1983]), and (4) the sensitivity of the distribution pattern of proteins in this region to drugs that inhibit hnRNA synthesis, it is most likely that the functions associated with this nuclear region include one or more aspects of hnRNA processing such as splicing and/or polyadenylation, of which both have been shown to require snRNPs. The colocalization between the *myc* protein and snRNPs raises the possibility that the *myc* protein may have a direct or indirect involvement in hnRNA processing. Alternatively, both the *myc* protein and the hnRNA-processing machinery may be anchored to a common nuclear region and serve different functions. For example, the *myc* protein has been shown to bind DNA in a nonspecific manner (Donner et al., *Nature 296:* 262 [1982]), and recently, it has been implicated in the elongation of nascent chains during DNA replication (Studzinski et al., *Science 234:* 467 [1986]). Thus, several intriguing possibilities currently exist for the functional role of the *myc* protein. Experiments are in progress to distinguish between these possibilities.

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QUEST LABORATORY/TWO-DIMENSIONAL GEL BIOTECHNOLOGY RESOURCE

J.I. Garrels C. Chang M. Hannaford S. Scheib
B.R. Franza H. Sacco P. Myers G. Mak
M.E. Lambert J. Kos G. Blose R. Cardinale
J. Ross

The building of databases derived from analysis of two-dimensional gels is an ongoing activity in our laboratory. This activity has now progressed from a prototype effort, coupled with intensive software development, to a routine operation based on a mature software system. Our facility now supports the development of several databases and allows other investigators at Cold Spring Harbor Laboratory and from other institutions to interact easily with them. We maintain an annotated spot map for each of our databases, and we support efforts to identify more of the protein spots for each of our databases. Most importantly, the databases are proving useful in research.

As our databases have developed, our ways of thinking about them have developed as well. They obviously contain much information about the regulation of protein synthesis, turnover, and modification in defined experimental systems that is not otherwise accessible, but the information cannot all be digested at once. Much will not be relevant until more proteins are identified and until more critical experiments are done. Conventional two-dimensional gel analysis, without a database, gives a broad but uninterpretable view of the cell. A database has both breadth (many spots) and depth (many experiments). It is the depth features that make the database useful. In any particular study, an investigator may decide there is no need to report or discuss the many changes seen on two-dimensional gels; this information is stored away safely in the computer for future use. The database will later be used (1) to find out what is already known about particular proteins of interest, (2) to judge whether the known proteins are among the most significant changes in the investigator's system, and (3) to select a manageable number of unknown proteins for future study. Once proteins for study have been selected, the database may guide the investigators in efforts to purify the protein, to obtain antibodies, or to obtain the gene.

In this report, we describe the databases that are being built and how they have been used. We focus on a few of the known proteins, including proliferating cell nuclear antigen (PCNA) and a coregulated protein of 85 kD, which are objects of study in each mammalian database. These and other markers, such as the heat-shock proteins and the major cytoskeletal proteins, are readily identified in all mammalian species and serve to unify our understanding. In fact, the proteins that are shared by many cell types and that have highly characteristic modes of regulation may be among the most important to understand, because these can most readily indicate altered physiological states. As we complete the studies of virally transformed cells, and begin detailed analysis of oncogene-transformed cells, we expect to understand more clearly some of the altered states and to focus on the pathways that are targets of the transforming genes.

Core Databases

J.I. Garrels, B.R. Franza

THE RAT DATABASE

The database for REF52 cells and related rat cell lines is being developed as a tool to detect and understand cellular changes of protein synthesis, degradation, and modification associated with transformation, and the regulation of proliferation. This database now contains data for 155 samples, most of which contain more than 2000 quantitated and accurately matched proteins. Approximately 80 of the proteins now have names that are referenced in the published literature, and several hundred more have been identified as nuclear, cytoskeletal, or mitochondrial.

Our current studies are focusing on a set of transformation-induced proteins, including PCNA, a PCNA-related satellite spot, and a coregulated protein of 85 kD ("85K"), and a set of growth-

sensitive proteins, including the tropomyosin isoforms, vimentin, vinculin, and caldesmon. Figure 1 reveals what the present database has to say about these proteins. It contains data from experiments to compare normal REF52 cells with a variety of virally transformed derivatives, experiments to study the effects of growth to saturating density in each line, and experiments to study the response of several lines to serum stimulation of quiescent cells. The graphs show that PCNA and "85K" are induced by the same transformed lines, that both are proliferation- and serum-sensitive in normal REF52 cells, and that both are largely unregulated in transformed cells. Since PCNA is known to be an accessory protein for DNA replication (see DNA Synthesis Section), we now plan to study the 85-kD protein, in collaboration with B. Stillman, to determine whether it too has a role in DNA replication.

Conversely, the tropomyosin forms 1 and 2 are elevated in confluent cells but are repressed by transformation or serum stimulation (in normal cells). Another cytoskeletal protein, caldesmon, has been identified on our gels using antibodies obtained from Fumio and Shigeko Matsumura. Cal-

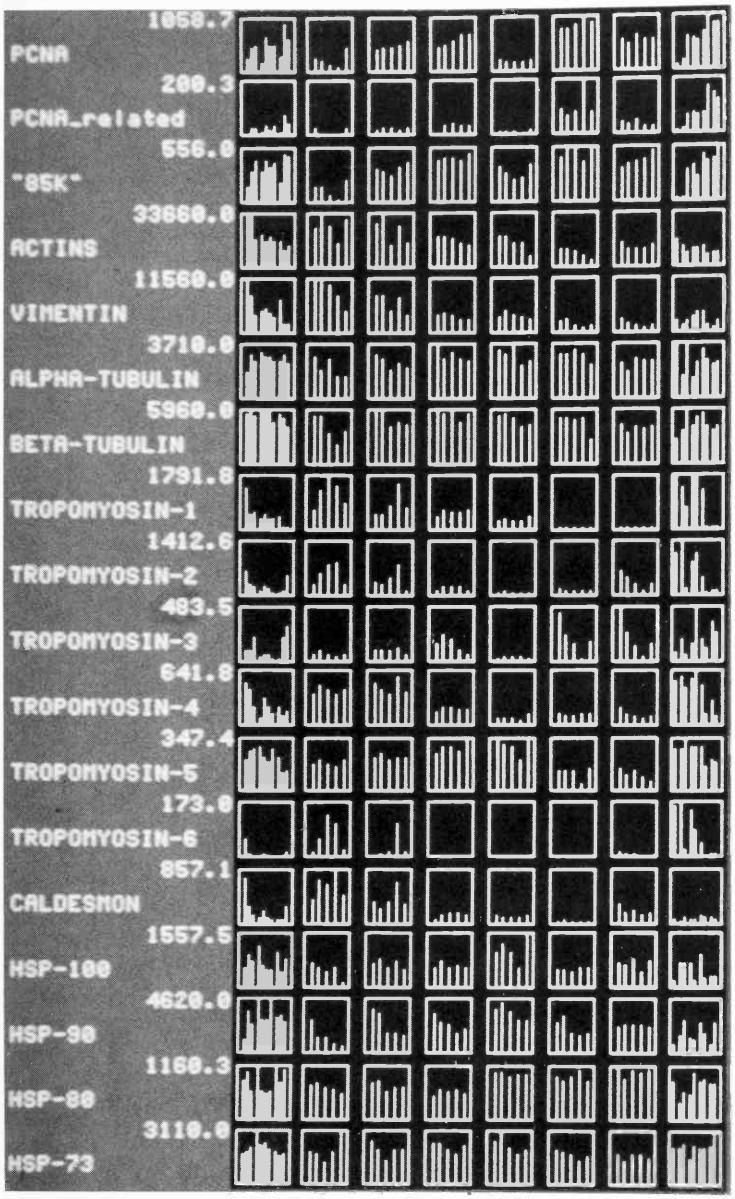

FIGURE 1 Database analysis of selected known proteins. Each graph shows the quantitative analysis of one protein in one experiment. Each row of graphs presents data for one protein as named at the left; each column presents data for a different experiment as described below. A vertical bar is plotted for each sample analyzed by a two-dimensional gel; the height of the bar shows the amount of radioactive methionine incorporated into the protein during the labeling interval, relative to the incorporation into all proteins. The full-scale height (indicated at the left as parts per million) is the same for all graphs in a row. The experiment in column 1 is a comparison of normal REF52 cells (first bar in each graph) to nine transformed derivatives. Bars 2–7 are SV40-transformants (WT2, AG2, NU2, WT6, AG6, and NU6), bar 8 is a Ki-MSV transformant, and bars 9 and 10 are adenovirus transformants (A5D and A5W). WT2 and WT6 are minimal SV40 transformants, AG2 and AG6 are anchorage-independent lines, and NU2 and NU6 are fully tumorigenic lines. The experiments in columns 2–7 show the rates of protein synthesis during growth to confluence followed by refeeding in REF52 cells (2), WT2 cells (3), AG2 cells (4), NU2 cells (5), A5D cells (6), and A5W cells (7). Bars 1, 2, and 3, in each graph show cells at days 2, 3, and 4 during proliferative growth, bar 4 shows cells at confluence on day 6, and bar 5 shows day 6 cells, 15 hours after being refed with fresh medium and serum. Column 8 shows the specific effect of fresh serum on serum-deprived cells. The first 3 bars are normal REF52 cells labeled (1) without refeed, (2) 1–4 hr after stimulation, and (3) 21–24 hr after stimulation. The second 3 bars show the same treatment for WT6 cells, and the last 3 bars show the same treatment for A5D cells.

desmon has a pattern of regulation very much like that of tropomyosin 1, except that it is not completely repressed in adenovirus-transformed cells.

One method to determine the significance of these changes relative to all changes occurring in the cell is to use a correlation plot (Fig. 2). All proteins are plotted as points on a logarithmic scale. On the *x*-axis, one pair of samples (normal vs. SV40-transformed cells) are compared, and on the *y*-axis, another pair of samples (normal vs. adenovirus-transformed cells) are compared. A correlation between the responses to SV40 and adenovirus is apparent, and the positions of the spots of interest (marked by special symbols) within the distribution of all changes can be readily noted. PCNA and "85K" are among the most significantly induced proteins, and the tropomyosin isoforms represent some of the largest decreases of protein synthesis due to transformation.

Other proteins induced by both viruses might be of great interest; in particular, there may be proteins that share overall patterns of regulation with PCNA and "85K." To learn more about these other induced proteins, we ask the computer to show us all the proteins within a box we have drawn on the screen (box in upper right of Fig. 2). The computer then shows us where each of these proteins is on the two-dimensional gel image and allows us to display their database graphs (Fig. 3). We see that there is at least one other protein that is regulated in the same manner as PCNA and "85K." Of course, the correlation plots are useful for analyzing many other combinations of samples and for selecting proteins with other regulatory patterns. With these software tools, interesting proteins from any group of gels can be selected, and quantitative information from the database can be displayed within a few minutes.

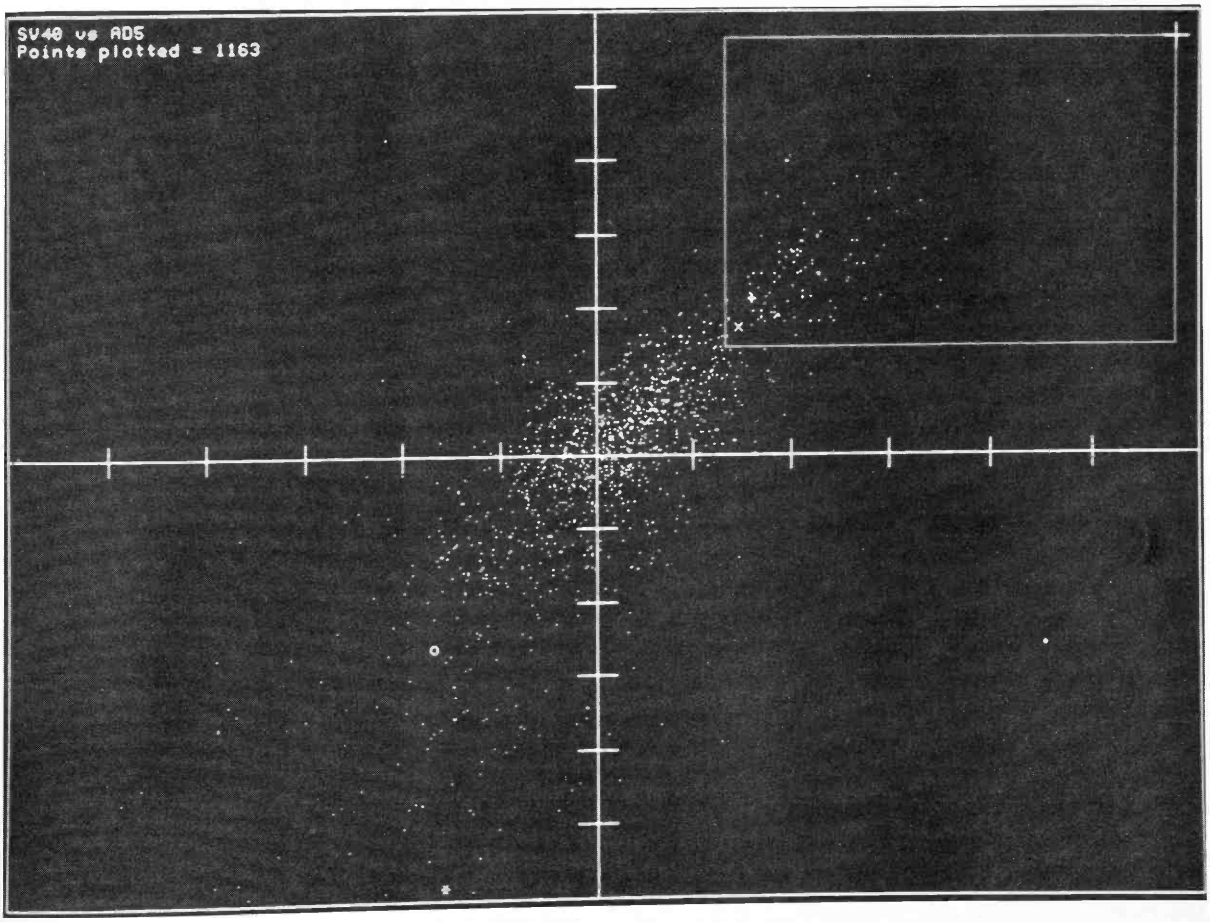

FIGURE 2 Correlation analysis of the effects of SV40 and adenovirus-induced transformation. Each protein detected in REF52, WT6, and A5D was plotted as a point showing its relative rate of synthesis in WT6 (*x*-axis) and A5D (*y*-axis) relative to REF52. Each axis is a logarithmic scale divided by factors of 2. The correlation of the proteins increased and decreased by each type of transformation is clear. Points marked by special symbols are PCNA (+), 85K (×), tropomyosin 1 (*), and tropomyosin 2 (O).

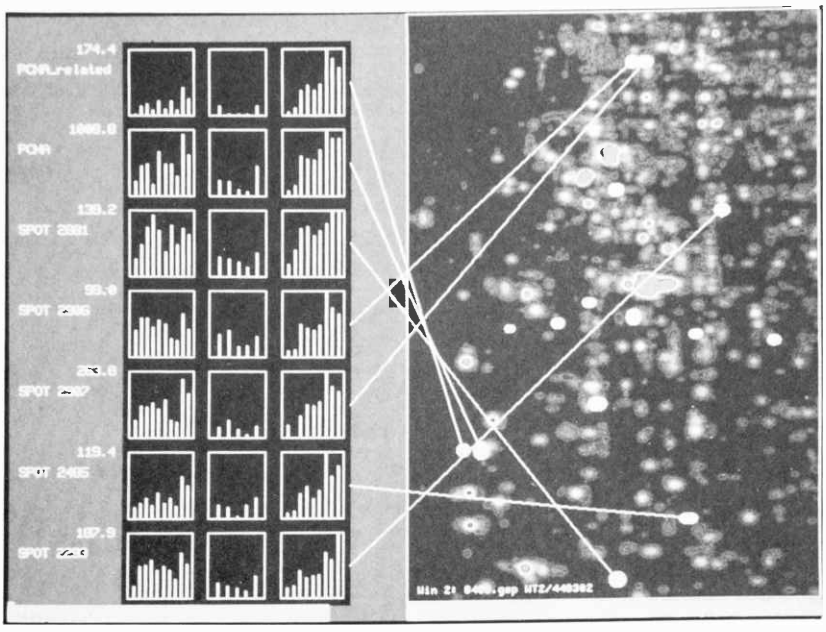

FIGURE 3 Proteins of interest selected from correlation analysis. Proteins that increase in both SV40-transformed cells and adenovirus-transformed cells were selected using the box shown in Fig. 2. The proteins from this box are highlighted on the two-dimensional gel image, and database graphs for experiments 1, 2, and 8 are displayed for some of these. Note that several have overall patterns of regulation similar to those of PCNA and 85K.

Many other comparisons have been made using the current database, including comparisons of (1) reproducibility of transformation by SV40 in several different cell line isolates, (2) cell lines independently selected for anchorage-independence or tumorigenicity, (3) cells transformed by DNA tumor viruses and an RNA tumor virus, and (4) density and serum-stimulation responses of normal versus transformed cells. Other controls (not shown here) include reproducibility of a given sample analyzed on multiple gels and the reproducibility of labeling in clones of REF52 cells. This work (J.I. Garrels and B.R. Franza, submitted) shows the existence of a rigorous and self-consistent database containing more than 200,000 separate spot quantitations; it shows new data of interest for proteins that have already been identified and has also identified some new proteins of interest for each type of comparison. Future additions to the REF52 database will focus on cells transformed by oncogenes.

THE HUMAN LYMPHOBLAST DATABASE

We have not directly pursued the assembly of a database for any human cell type. However, as a consequence of some specific experiments on human B and T lymphoblasts that utilized two-dimensional gel analysis, we have derived some intriguing results, and we see as a logical outcome of these studies the initiation of the development of a human lymphoblast database.

The analysis of cell lines established in tissue culture by B. Clarkson and co-workers at the Memorial Sloan-Kettering Cancer Center represents a study of B lymphoblasts derived from patients diagnosed as having chronic myelogenous leukemia (CML). These lymphoblasts are unique in that they represent either normal diploid cells or cells carrying the 9;22 translocation marker of CML isolated from the same individual at different stages of the disease process. Such "matched pairs" are being utilized in an attempt to define a set of cellular proteins that are uniquely altered in the cells bearing the chromosomal rearrangement. In addition to comparisons made between cells grown under normal tissue-culture conditions, the matched pairs are being subjected to the stress of elevated temperature to determine if inducible responses in the normal cells are altered in the translocation-positive cells. At this time, it appears that a small number of cellular proteins are reproducibly altered in the translocation-positive cells, and indeed the re-

sponse to stress highlights these differences. Further sets of matched pairs are being analyzed to confirm these initial observations.

An integral part of the development of a human lymphoblast database would be the identification of the products of the histocompatibility genes. As a result of a collaboration with R. De Mars (University of Wisconsin-Madison Medical School), we have been able to identify products of the HLA class I and class II genes. The project was initiated to study the ability of the two-dimensional gel system to detect quantitative alterations in proteins encoded by the HLA genes in cell lines previously documented to have mutations in such genes. De Mars has developed a unique family of B-lymphoblast cell lines, all derived from one parental diploid lymphoblast line. These cells have deletions in the region of chromosome 6 that encodes HLA class I and class II proteins. A combination of two-dimensional gel analysis of whole-cell lysates and immunoprecipitations utilizing antibodies that recognize the class I and class II gene products has been utilized, resulting in the identification of such proteins and their entry into the database for further analysis. An interesting future aspect of this study involves the ability of the database algorithms to detect proteins, other than the class I and class II gene products, that have been altered as a result of the chemically induced mutagenesis of their respective genes. Such analysis may point out proteins that always change whenever a class I or class II gene product is altered and therefore may suggest some linkage with the HLA loci.

In collaboration with S. Josephs, F. Wong-Staal, M. Popovic, and R.C. Gallo (NIH/NCI Laboratory of Tumor Cell Biology) and D. Zagury and colleagues (Pierre-Marie Curie University, Paris), we have begun the analysis of the effects of the expression of a strain of human T-lymphotropic virus type III (HTLV-III) on normal human T lymphoblasts and on the human T-lymphoblast cell line H9. Initial results of these studies demonstrate that the synthesis of one of the viral protein products, referred to as p24, is altered, depending on the proliferation status of the cell. Normal, freshly isolated T lymphoblasts show minimum production of p24 when infected by virus, during a time course when the same cells infected by HTLV-III and simultaneously stimulated by the lectin, phytohemagglutinin (PHA), show substantial production of this viral protein. Interestingly, in the same experiment, exposure of the same cells to the mitogen interleukin-2 (IL-2), concomitant with infection with the virus, did not result in detectable p24 production. When the immortalized cell line H9 is infected with the same virus, production of p24 occurs readily within the first 24 hours, suggesting that cellular processes mediated by exposure of normal T lymphoblasts to PHA are constitutive in H9 cells. By using the database system to follow the response of these different cell types to the conditions mentioned above, we have also been able to extract information on the behavior of the cellular proteins PCNA and "85K" mentioned earlier in the rat database studies. We have found that normal human T lymphoblasts stimulated with either PHA or IL-2 show a coordinate induction in the synthesis of PCNA and "85K." In addition, the infection of such cells with HTLV-III results in induction of synthesis of these two proteins. Extensive time-course and cell-cycle studies on whole populations of T lymphoblasts, as well as on subpopulations of T lymphoblasts, after exposure to lectins, mitogens, and/or virus are planned to elucidate the nature of such responses.

Other studies on the response of cells to HTLV-III have suggested to us that certain extracellular stimuli and intracellular events (e.g., as mediated by the expression of a gene, such as the adenovirus type-5 early region 1A gene) would modulate the expression of genes controlled through the viral long terminal repeat (LTR) sequences in an apparently positive or negative manner, depending on the specific experimental conditions. Given our interest in actually determining the proteins involved in such regulatory processes, we are developing an assay system to detect cellular and/or viral gene products that interact either directly with putative regulatory nucleic acid sequences or indirectly with proteins already associated with such sequences. This assay system is designed to utilize the two-dimensional gel separation of proteins, and its use is meant to add to the depth of the database. In addition, it will provide a sensitive, quantitative means of monitoring such complex interactions both in whole-cell systems and in subcellular fractions shown to maintain the ability to mimic whole-cell activities such as transcription.

We are now beginning to compare these various experiments within the database structure and look forward to the ability, for example, to point to the quantitative alteration in a class I gene product in a normal T lymphoblast stimulated by PHA and simultaneously infected with HTLV-III. Such intra-

database investigations point to the real future of cellular protein analysis utilizing the database system described herein.

THE MOUSE DATABASE

We are constructing a mouse embryo database by analyzing carefully staged early embryos provided by D. Solter's laboratory (Wistar Institute). Conditions for labeling of early embryos have been established and several series of gels have been run. Dr. Solter and colleagues will perform a full analysis and interpretation of the data during visits to our laboratory in 1987. These early stages of development will complement our earlier studies with L. Silver and M. Krangel on postimplantation stages of development.

We have collaborated with D. Hanahan (see Molecular Biology of Mouse Development) to build a database for his studies of transgenic mice. In the samples of pancreatic islet cells expressing the SV40 T antigen, we have identified the proliferation-sensitive markers PCNA and "85K." These and other proteins mark the progression of preneoplastic cells to a fully neoplastic phenotype, indicating that the mouse system will have parallels with the other transformation system under study.

THE YEAST DATABASE

The yeast database has been initiated with J. Warner (Albert Einstein College of Medicine) as project coordinator and with major input from the laboratories of C. McLaughlin (University of California, Irvine) and M. Wigler (Cold Spring Harbor Laboratory). McLaughlin has carried out an analysis of strain diversity, finding that common laboratory strains are easily compared, although some more distant strains are highly divergent. He has also placed on our maps the names of 20 enzymes, based on earlier work from his laboratory and that of H. Boucherie (University of Bordeaux).

The plan for the yeast database is to add more physiological experiments and to identify many more proteins. Future experiments will include the dependency of the pattern on carbon source; the rates of labeling and turnover; comparison of a, α, and diploid strains; and analysis of the temperature-sensitive RNA splicing mutants, RNA2. More proteins will be identified by soliciting antibodies from the yeast community and, if possible, by plasmid overexpression.

A Protein Database for the Study of Inducible Responses to DNA Damage

M.E. Lambert, J.I. Garrels

The REF52 database now also contains experiments designed to study cellular responses elicited in cells after exposure to different classes of DNA-damaging agents, including chemical carcinogens and irradiation. The objective of these studies is to identify specific markers for inducible responses to different classes of DNA damage and to study the involvement of these proteins in DNA repair processes, regulation of growth arrest, and cellular transformation. These studies have now revealed that PCNA is a DNA-damage-inducible protein and is expressed at an elevated level after exposure of the DNA to a variety of different agents, including the metabolically activated form of benzo[a]pyrene, BPDE, and the alkylating agent MNNG. This induction can be elicited rapidly after exposure of either exponential or quiescent confluent cultures of REF52 and is correlated with long patch DNA-repair synthesis. Studies with biochemical inhibitors of DNA replication and repair further suggest that the triggering of this induction is itself partially dependent on hydroxyurea- and aphidicolin-sensitive metabolic pathways. Experiments are in progress (in collaboration with M. Mathews and B. Stillman), using permeabilized cell systems and subcellular DNA-repair assays, to characterize the potential involvement of PCNA in unscheduled DNA synthesis and to investigate the regulation of this induction at both the transcriptional and translational levels. In addition to PCNA, we have also identified a unique family of DNA-damage-inducible proteins that are rapidly induced by either the protein synthesis inhibitor cycloheximide or by the DNA polymerase inhibitor aphidicolin. Preliminary studies indicate that a related family of proteins is also induced in human fibroblasts by similar agents and that these proteins are related to major histocompatibility class I genes.

Changes in Gene Expression during Proliferation and Immortalization of Nonestablished Rat Kidney Epithelial Cells

M.E. Lambert, J.I. Garrels [in collaboration with N. Sullivan, M. Quinlan, and T. Grodzicker, Cold Spring Harbor Laboratory]

We have used the QUEST (quantitative electrophoresis technologies) system to study changes in gene expression during proliferation and immortalization of nonestablished rat kidney epithelial (RKE) cells, derived from the same inbred strain of rat (F344) as is REF52. Addition of conditioned media from cultures of RKE cells infected with adenovirus-5 12S to fresh cultures of noninfected RKE cells has previously been shown to result in continuous cellular DNA synthesis and proliferation (see Adenovirus Genetics Section). In metabolic labeling studies of proteins synthesized after addition of this factor, we have now observed pleiotropic effects on cellular gene expression starting within the first 24 hours. These include an increase in the expression of PCNA (cyclin), as well as a coregulated 85K protein, and a decrease in expression of tropomyosin I and II. In addition, further specific polypeptide subsets have been identified that are either specifically increased or decreased in their level of expression. We have also studied changes in cellular protein expression associated with the immortalization of RKE cells. Fresh cultures of RKE cells were treated with single low doses of the chemical carcinogen MNNG (0.1 μg/ml) and then selected in the presence of the proliferation factor for periods of up to 2 weeks. Further selection, for preneoplastic enhanced growth variants (EGVs), was imposed by growth in 5% serum for an additional 2 weeks. Both spontaneous and MNNG-induced EGVs have been analyzed, and all were found to have decreased expression of several major cytokeratin epitopes as well as decreased expression of tropomyosin I and II. Using the QUEST system, we have distinguished between changes in gene expression that are associated, in this system, with either induced proliferation per se or commitment to continuous proliferation (cellular immortalization).

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Curran, T., L. Sambucetti, K. Rubino, B.R. Franza, and J.I. Morgan. 1987. c-*fos* induction: A possible nuclear switch in growth regulation. In *ICSU Proceedings of the Miami Winter Symposium.* (In press.)

Franza, B.R., L. Sambucetti, D.R. Cohen, and T. Curran. 1987. Two-dimensional gel analysis of c-*fos*, Fos-related protein, and cellular proteins complexed with c-*fos*. *Oncogene* (in press).

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Josephs, S., R. Sadie, V. Heisig, L.J. Seigel, M.J. Renan, B.R. Franza, and F. Wong-Staal. 1987. HTLV III LTR-directed gene expression: Sublocalization of the critical regulatory elements. *Nucleic Acids Res.* (Submitted.)

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Lambert, M.E., I.B. Weinstein, and J.I. Garrels. 1987. Specific cellular proteins are induced by DNA damage in rodent cells: Studies using the QUEST system. (In preparation.)

Lambert, M.E., J.F. McDonald, P.B. Fisher, and I.B. Weinstein. 1987. Expression of *copia* in transfected mammalian cells is inducible by genomic stress. *Nucleic Acids Res.* (Submitted.)

Lambert, M.E., M. Quinlan, T. Grodzicker, J. Garrels, and N. Sullivan. 1987. Changes in gene expression during proliferation and immortalization of non-established rat kidney epithelial cells: Development of a protein database. (In preparation.)

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Shen, W.P.V., T.H. Aldrich, G. Venta-Perex, B.R. Franza, and M.E. Furth. 1987. Expression of normal and mutant *ras* proteins in human acute leukemia. *Oncogene* (in press).

NEUROSCIENCE

The Neuroscience Program at Cold Spring Harbor Laboratory focuses on the functional development of neurons. We are particularly interested in understanding the differentiation of neurons in the brain, including the seat of higher cognitive functions, the cerebral cortex. We face many mysteries in studying neuronal development: What causes neurons to stop cell division after migrating to specific locations in the brain? What stimulates neurons to grow the long axonal and dendritic fibers that connect nerve cells together? What induces electrical excitability and neurotransmitter production in neurons? These are a few of the broad questions we address in our research. To answer such questions, we employ a multidisciplinary approach using modern methods of cell biology, molecular genetics, biochemistry, and physiology. The environment at Cold Spring Harbor Laboratory provides a unique setting for such research as we draw on all the scientific resources to support our efforts.

NEURONAL GROWTH AND DIFFERENTIATION

D.R. Marshak J.C. Figueiredo
L.O. Goodwin
R. Tonner

Last year, we described the purification and characterization of a new neuronal growth factor, neurite extension factor (NEF) (Kligman and Marshak, *Proc. Natl. Acad. Sci. 82:* 7136 [1985]). This factor is a protein that causes embryonic neurons from chick cerebral cortex to elaborate processes in serum-free, defined media. NEF appears to have some specificity for the central nervous system over the peripheral nervous system. If we can uncover the mechanism of action of neuronal growth and differentiation factors such as NEF, we might discover new ways to approach degenerative neurological diseases, such as Alzheimer's disease. Our ultimate goal is to use neuronal growth factors or their genes for therapeutic intervention in such degenerative diseases.

In Press, Submitted, and In Preparation

Marshak, D.R. 1987. Structural analysis of proteins of the nervous system. In *Modern methods of protein chemistry* (ed. J. L'Italien). Plenum Press, New York. (In press.)

Marshak, D.R. and B.A. Fraser. 1987. Structural analysis of brain peptides. In *Brain peptides* (ed. Krieger et al.), pp. 13–54. John Wiley and Sons, New York. (In press.)

COLD SPRING HARBOR MEETINGS

51st COLD SPRING HARBOR SYMPOSIUM ON QUANTITATIVE BIOLOGY

Molecular Biology of *Homo sapiens*

May 28 – June 4

314 participants

Thirteen years marked the time between the discovery of the double helix in 1953 and the elucidation of the genetic code in 1966. A similar interval has now passed since the development by Cohen and Boyer of a simple procedure for the cloning of selective DNA fragments. The scientific advances made possible by the subsequent modification and elaboration of these original cloning procedures now amaze, stimulate, and increasingly often overwhelm us. Facts that until recently were virtually unobtainable now flow forth almost effortlessly. Most excitingly, the frenetic pace of these new discoveries, instead of marking the impending end of a glorious moment of learning, gives every indication of opening up scientific frontiers that will take hundreds if not thousands of years to explore thoroughly.

This new era of enlightenment is nowhere more apparent than in our newfound ability to study ourselves at the molecular level. So it was very easy to chose the title for this, our 51st Symposium. By focusing on "The Molecular Biology of *Homo sapiens*," we would be chosing a topic that most certainly will be returned to over and over during the second 50 Symposium years. In contrast, only once in our first 50 Symposium years did our discussions focus exclusively on ourselves (Human Genetics in 1964).

Happily, this year's Symposium marked the first year of use of our new facility built expressly for the holding of meetings, the very wonderful Oliver and Lorraine Grace Auditorium. The formal dedication ceremonies occurred on June 1st, during the Symposium, with the main dedicatory speech delivered by Daniel Koshland, editor of *Science* magazine and Professor of Biochemistry at the University of California, Berkeley.

In organizing the program for this Symposium, with Marcello Siniscalco, we have benefited from the thoughtful advice of many colleagues, in particular Paul Berg, Sir Walter Bodmer, David Botstein, Michael Brown, Charles Cantor, Thomas Caskey, Joseph Goldstein, Lee Hood, Tom Maniatis, Joseph Sambrook, and Allan Wilson. Many more potential speakers were suggested than could be included in this our most intense Symposium yet attempted. The final program comprised 123 speakers, with most of the audience of 314 staying virtually to the end. Sir Walter Bodmer, a participant of our 1964 Symposium, gave an incisive introductory talk, and Tom Caskey, a participant of our 1966 Genetic Code Symposium, gave the very able summarizing remarks. Near the conclusion, at a special session presided over by Walter Gilbert and Paul Berg, the feasibility of beginning soon a total sequencing of the human genome was discussed. This is a topic that generates diverse reactions, many of which were clearly expressed during the discussion.

This meeting was supported in part by the Banbury Fund; the Department of Energy; Lucille P. Markey Foundation; National Cancer Institute/National Institutes of Health; The National Science Foundation; and the Rita Allen Foundation.

Welcoming Remarks: J.D. Watson

Introduction: W.F. Bodmer, Imperial Cancer Research Fund: Human genetics—The molecular challenge.

SESSION 1 HUMAN GENE MAP (1)

Chairman: V. McKusick, Johns Hopkins Hospital

McKusick, V.A., Johns Hopkins University, Baltimore, Maryland: The gene map of *Homo sapiens*—Status and prospectus.

Gray, J.W., van den Engh, G., Trask, B., Lucas, J., Pinkel, D., Van Dilla, M., Fuscoe, J., Yu, L., Lawrence Livermore National Laboratory, Livermore, California: Chromosome classification and purification using flow cytometry and sorting.

Pinkel, D.,[1] Gray, J.W.,[1] Trask, B.,[1] van den Engh, G.,[1] Fuscoe, J.,[1] van Dekken, H.,[1,2] [1]Lawrence Livermore National Laboratory, Livermore, California; [2]Radiobiological Institute, TNO, The Netherlands: Cytogenetic analysis by in situ hybridization with fluorescently labeled nucleic acid probes.

Deaven, L.,[1] Van Dilla, M.,[2] [1]Los Alamos National Laboratory, New Mexico; [2]Lawrence Livermore National Laboratory, Livermore, California: Construction of human chromosome-specific DNA libraries from flow-sorted chromosomes.

SESSION 2 HUMAN GENE MAP (2)

Chairman: D. Botstein, Massachusetts Institute of Technology

White, R., Julier, C., Leppert, M., Nakamura, Y., O'Connell, P., Woodward, S., Lathrop, G.M., Lalouel, J.-M., Howard Hughes Medical Institute, Salt Lake City, Utah: Construction of human genetic linkage maps. I. Progress and perspectives.

Lalouel, J.-M., Lathrop, G.M., White, R., Howard Hughes Medical Institute, University of Utah Medical Center, Salt Lake City: Construction of human genetic maps. II. Methodological issues.

Lander, E.,[1,2,3] Botstein, D.,[2] [1]Whitehead Institute for Biomedical Research, [2]Dept. of Biology, Massachusetts Institute of Technology, [3]Harvard University, Cambridge, Massachusetts: Mapping complex genetic traits in humans—New methods using a linkage map of RFLPs.

Cantor, C.R., Smith, C.L., Dept. of Genetics and Development, Columbia University, College of Physicians & Surgeons, New York, New York: Approaches to physical mapping of the human genome.

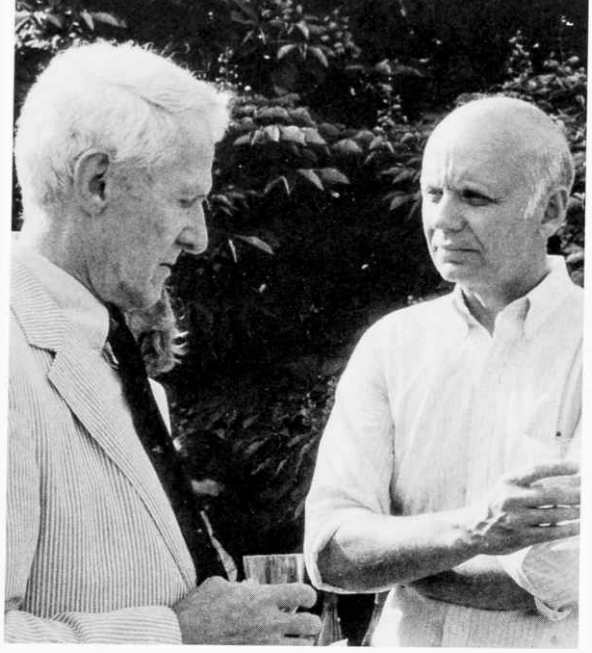
Weissman, S.M.,[1] Lawrance, S.K.,[1] Srivastava, R.,[1] Chroney, M.J.,[1] Rigas, B.,[1] Vasavada, H.,[1] Gillespie, G.A.,[1] Smith, C.,[2] Cantor, C.,[2] Collins, F.S.,[3] [1]Dept. of Human Genetics, Yale University School of Medicine, New Haven, Connecticut; [2]Dept. of Human Genetics, Columbia University, New York, New York; [3]Dept. of Internal Medicine, University of Michigan Medical School, Ann Arbor: The human MHC—Approaches to characterization of large regions of DNA.

Lehrach, H., Poustka, A., Pohl, T., Barlow, D., Craig, A., Zehetner, G., Frischauf, A.-M., EMBL, Heiledberg, Federal Republic of Germany: Molecular approaches to mammalian genetics.

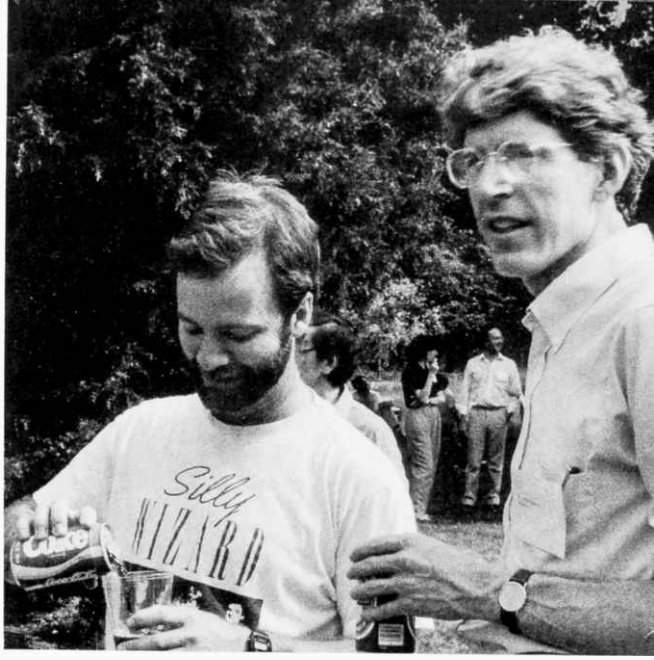
Antonarakis, S.E.,[1] Warren, A.C.,[1] Wong, C.,[1] Metaxotou, C.,[2] Chakravarti, A.,[3] [1]Dept. of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland; [2]Dept. of Pediatrics, Athens University School of Medicine, Greece; [3]Dept. of Biostatistics, University of Pittsburgh, Pennsylvania: Reduced recombination rate in chromosomes 21 which undergo nondisjunction in trisomy 21.

V. McKusick, F. Ruddle

G. Attardi, M. Siniscalco, A. Wilson



M. Witunsky, G. M. Browne



R. Myers, T. Maniatis

SESSION 3 HUMAN CANCER GENES (1)

Chairman: G.F. Vande Woude, NCI-Frederick Cancer Research Facility

Park, M., Dean, M., Kaul, K., Gonzatti-Haces, M., Iyer, A., Robins, T., Blair, D., Vande Woude, G.F., NCI-Frederick Cancer Research Facility, Frederick, Maryland: The human *met* oncogene is a member of the tyrosine kinase family.

Toyoshima, K.,[1] Semba, K.,[1] Akiyama, T.,[2] Ikawa, S.,[1] Yamamoto, T.,[1] [1]Institute of Medical Science, University of Tokyo, [2]Meiji College of Pharmacy, Japan: c-*erbB*-2 gene encodes a receptor-like protein with tyrosine kinase activity.

Barbacid, M., Sukumar, S., Martin-Zanca, D., Katzav, S., Mitra, G., NCI-Frederick Cancer Research Facility, Frederick, Maryland: *ras* and *trk*—Two transforming genes of human tumors.

Wigler, M., Fasano., O., Birnbaum, D., Birchmeier, C., Young, D., Cold Spring Harbor Laboratory, New York: Tumorigenicity assays for human protooncogenes.

Shimizu, K.,[1] Nakatsu, Y.,[1] Oh-uchida, M.,[1] Nomoto, S.,[1] Sekiguchi, M.,[2] [1]Dept. of Biology, Faculty of Science, [2]Dept. of Biochemistry, Faculty of Medicine, Kyushu University, Fukuoka, Japan: Structure of the activated c-*raf*-1 gene from human stomach cancer.

Gonzalez, F.J., Jaiswal, A.K., Nebert, D.W., NCI, National Institutes of Health, Bethesda, Maryland: P450 genes—Structure, evolution, regulation, and relationship in cancer.

SESSION 4 HUMAN GENE MAP (3)

Chairman: L. Hood, California Institute of Technology

Hood, L.,[1] Concannon, P.,[1] Klein, M.,[3] Lai, E.,[1] Siu, G.,[1] Strauss, E.,[1] Pickering, L.,[2] [1]California Institute of Technology, Pasadena; [2]T-Cell Sciences, Inc., Cambridge, Massachusetts; [3]Toronto Cancer Institute, Canada: T-cell receptor genes—Organization, diversification, and rearrangements.

Mak, T.W., Yoshikai, Y., Iwamoto, I., Minden, M., Ohasi, P., Caccia, N., Toyonaga, B., Ontario Cancer Institute and Dept. of Medical Biophysics, University of Toronto, Canada: Genes of the T-cell antigen receptors in normal and malignant T cells.

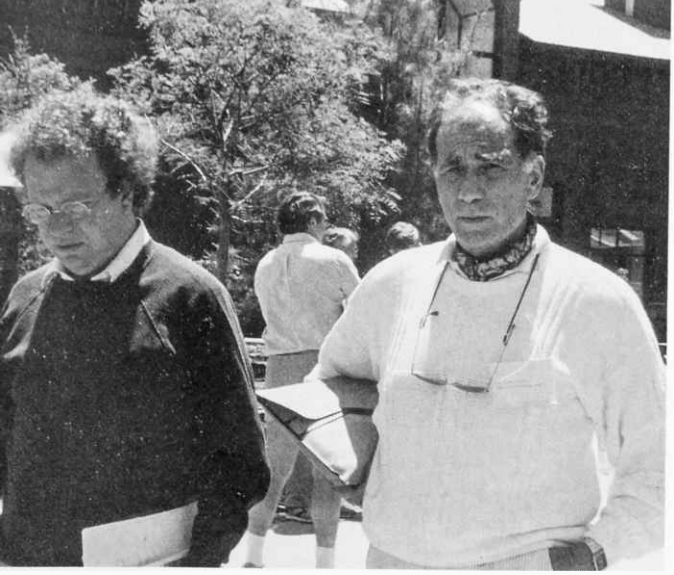
Strominger, J.L., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Human histocompatibility antigens—Structure, genetics, and regulation of expression.

Mach, B., Rollini, P., Berdoz, J., Berte, C., Reith, W., Gorski, J., Dept. of Microbiology, University of Geneva Medical School, Switzerland: Human Ia antigens—Complexity, polymorphism, and regulation.

McDevitt, H.O., Bell, J.I., Depts. of Medicine and Medical Microbiology, Stanford University, California: Polymorphism in the class II region of the human major histocompatibility complex.

Kohonen-Corish, M.R.J., Dunckley, H., Reid, M.A., Serjeantson, S.W., John Curtin School of Medical Research, Australian National University, Canberra: HLA class II RFLPs are haplotype specific.

Hoover, M.L., Capra, J.D., Black, K., University of Texas Health Science Center, Dallas: Insulin-dependent diabetes mellitus is associated with polymophic forms of the T-cell receptor β-chain gene.



J. L. Mandel, P. Chambon

D. Goeddel, D. Botstein

SESSION 5 GENETIC DIAGNOSIS (1)

Chairman: P.L. Pearson, University of Leiden

Bell, G., Bell, J., Bates, G., Davies, K.A., Estavill, X., Farrall, M., Kruyer, H., Law, H.Y., Lench, N., Scambler, P., Stanier, P., Wainwright, B., Watson, E., Williamson, R., Cystic Fibrosis Genetics Research Group, St. Mary's Hospital Medical School, University of London, England: The cystic fibrosis locus.

Donis-Keller, H., Barker, D., Knowlton, R., Schumm, J., Braman, J., Dept. of Human Genetics, Collaborative Research, Inc., Lexington, Massachusetts: Highly polymorphic RFLP probes as diagnostic tools.

Tsui, L.-C., Buchwald, M., Dept. of Genetics, Hospital for Sick Children, Toronto, Canada: Mapping of the cystic fibrosis locus on chromosome 7.

Dean, M.,[1] Park, M.,[1] Woodward, S.,[2] White, R.,[2] Vande Woude, G.F.,[1] [1]NCI-Frederick Cancer Research Facility, Frederick, Maryland; [2]University of Utah, Salt Lake City: The *met* oncogene locus is tightly linked to cystic fibrosis.

Davies, K.E.,[1] Forrest, S.,[1] Kenwrick, S.,[1] Patterson, M.,[1] Paulsen, K.,[2] Smith, T.,[1] Wilson, L.,[1] Lavenir, I.,[1] Ball, S.,[1] [1]Nuffield Dept. of Clinical Medicine, Oxford, England; [2]Institut für Humangenetik und Anthropologie, John Radcliffe Hospital, Freiburg, Federal Republic of Germany: Molecular characterization of X-linked diseases.

Worton, R.B., Ray, P.N., Bodrug, S., Thompson, M.W., Dept. of Genetics and Research Institute, Hospital for Sick Children and Dept. of Medical Genetics, University of Toronto, Canada: Analysis of an X-autosome translocation responsible for X-linked muscular dystrophy.

Kunkel, L., Monaco, A., Colletti, C., Bertelson, C., Division of Genetics, Children's Hospital, Boston, Massachusetts: Molecular genetics of Duchenne muscular dystrophy.

Pearson, P.L., Genetics Dept. State University of Leiden, The Netherlands: Carrier detection and gene analysis of inherited muscular dystrophies.

Gusella, J.F.,[1] Gilliam, T.C.,[1] Tanzi, R.E.,[1] MacDonald, M.E.,[1] Cheng, S.,[1] Wallace, M.,[2] Haines, J.,[2] Conneally, P.M.,[2] Wexler, N.S.,[3] [1]Massachusetts General Hospital and Dept. of Genetics, Harvard University, Boston; [2]Dept. of Medical Genetics, Indiana University, Indianapolis; [3]Dept. of Neurology, Columbia University, New York, New York: Molecular genetics of Huntington's disease.

SESSION 6 HUMAN EVOLUTION (1)

Chairman: L.L. Cavalli-Sforza, Stanford University

Cavalli-Sforza, L.L., Dept. of Genetics, Stanford University, California: Contributions of DNA studies to the problem of the history of human ethnic groups.

Andrews, P., Dept. of Paleontology, British Museum, London, England: Fossil evidence on human origins and dispersal.

Attardi, G., Chomyn, A., Mariottini, P., Division of Biology, California Institute of Technology, Pasadena: Seven unidentified reading frames of human mitochondrial DNA encode subunits of the respiratory chain NADH dehydrogenase.

Stoneking, M.,[1] Bhatia, K.,[2] Wilson, A.C.,[1] [1]Dept. of Biochemistry, University of California, Berkeley; [2]Institute of Medical Research, Goroka, New Guinea: More on mitochondrial DNA and human evolution.

Pääbo, S., Dept. of Cell Research, University of Uppsala, Sweden: Molecular genetic investigations of ancient human remains.

Doolittle, R.F., Feng, D.F., Johnson, M.S., McClure, M., Dept. of Chemistry, University of California, San Diego, La Jolla: The relationship of human protein sequences to those of other organisms.

G. P. Georgiev, M. Singer

E. Wagner, J. Sorge

SESSION 7 DRUGS MADE OFF HUMAN GENES 1: CLOTTING ANTI-CLOTTING FACTORS

Chairman: E. Davie, University of Washington

Sadler, J.E.,[1] Shelton-Inloes, B.B.,[1] Sorace, J.M.,[1] Titani, K.,[2] [1]Howard Hughes Medical Institute and Dept. of Medicine, Washington University, St. Louis, Missouri; [2]Dept. of Biochemistry, University of Washington, Seattle: Cloning of cDNA and genomic DNA for human Von Willebrand factor.

Long, G.L., Lilly Research Laboratories, Indianapolis, Indiana: Structure and evolution of the human protein C gene.

Berkner, K.,[1] Busby, S.,[1] Davie, E.,[2] Hart, C.,[1] Insley, M.,[1] Kisiel, W.,[3] Kumar, A.,[1] Murray, M.,[1] O'Hara, P.,[1] Woodbury, R.,[1] Hagen, F.,[1] [1]ZymoGenetics, [2]Dept. of Biochemistry, University of Washington, Seattle; [3]Dept. of Pathology, University of New Mexico, Albuquerque: Isolation and expression of cDNAs encoding human factor VII.

Adamson, R., Amphlett, G., Bologna, M., Dorner, A., Foster, W.B., Hewick, R., Israel, D., Kamen, R., Kaufman, R., Pittman, D., Schmidli, B., Smith, K., Szklut, P., Toole, J., Wang, J., Wasley, L., Genetics Institute, Inc., Cambridge, Massachusetts: Recombinant human factor VIII.

Lawn, R.M.,[1] Capon, D.J.,[1] Gitschier, J.,[1] Tuddenham, E.G.D.,[2] Vehar, G.A.,[1] Wion, K.L.,[1] Wood, W.I.,[1] [1]Genentech, Inc., South San Francisco, California; [2]Royal Free Hospital School of Medicine, London, England: Molecular biology of hemophilia.

Vehar, G.A., Keyt, B., Spellman, M., Hancock, W., Builder, S., Genentech, Inc., South San Francisco, California: Production of human tissue plasminogen activator using recombinant DNA technology.

Stump, D., Lijnen, H.R., Collen, D., Center for Thrombosis and Vascular Research, University of Leuven, Belgium: Biochemical and biological properties of single-chain urokinase-type plasminogen activator.

SESSION 8 DRUGS MADE OFF HUMAN GENES 2: ANTI-CANCER AGENTS

Chairman: R.A. Flavell, Biogen Research Corp.

Finter, N.B., Ball, G.D., Fantes, K.H., Johnston, M.D., Lewis, W.G., Wellcome Laboratories, Beckenham, England: Interferon production from human cell cultures.

Taniguchi, T.,[1] Fujita, T.,[1] Hatakeyama, M.,[1] Yamada, G.,[1] Minamoto, S.,[1] Shibuya, H.,[1] Ohashi, T.,[1] Yamanishi, K.,[1] Mori, H.,[1] Hardy, R.R.,[1] Matsui, H.,[2] Hamuro, J.,[2] Uchiyama, T.,[3] [1]Osaka University, Osaka; [2]Ajinomoto, Co., Inc., Yokohama; [3]Kyoto University, Japan: Molecular biology of the interleukin-2 system.

Fiers, W.,[1] Brouckaert, P.,[1] Devos, R.,[1] Fransen, L.,[2] Leroux-Roels, G.,[1] Remaut, E.,[1] Suffys, P.,[1] Tavernier, J.,[2] Van der Heyden, J.,[2] Van Roy, F.,[1] [1]Laboratory of Molecular Biology, State University, [2]Biogent, Ghent, Belgium: Lymphokines and monokines in anticancer therapy.

Goeddel, D., Aggarwal, B., Palladino, M.A., Pennica, D., Sugarman, B., Shepard, H.M., Wong, G.H.W., Genentech, Inc., South San Francisco, California: Tumor necrosis factor—Gene structure, expression, and biological properties.

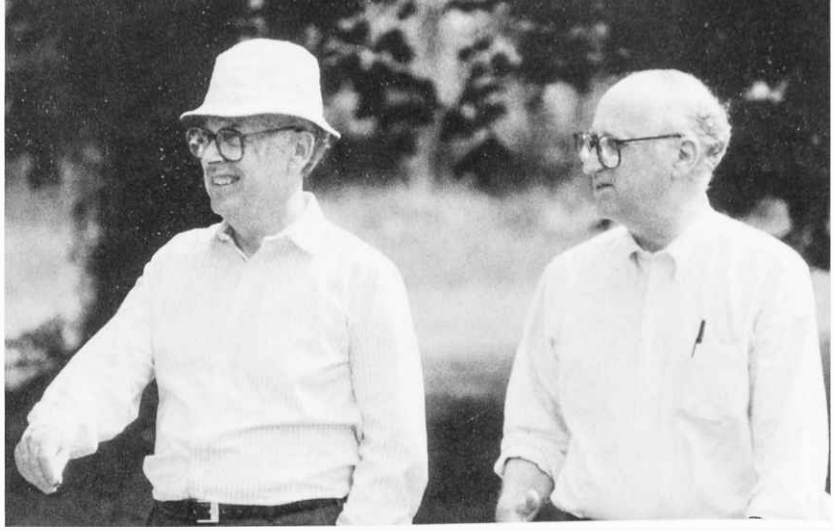
Nedospasov, S.A.,[1] Shakhov, A.M.,[1] Turetskaya, R.L.,[1] Mett, V.A.,[1] Georgiev, G.P.,[1] Korobko,V.G.,[2] Dobrynin, V.N.,[2] Filippov, S.A.,[2] Bystrov, N.S.,[2] Boldyreva, E.F.,[2] Chuvpilo, S.A.,[2] Chumakov, A.M.,[2] Ovchinnikov, Y.A.,[2] [1]Institute of Molecular Biology, [2]Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, Moscow: Tandem arrangement of tumor necrosis factor and lymphotoxin genes in human genome.

Cerami, A., Rockefeller University, New York, New York: Cachectin—A macrophage protein that induces a catabolic state in infected animals.

Lomedico, P.T., Kilian, P.L., Gubler, U., Stern, A.S., Chizzonite, R., Hoffmann-La Roche Inc., Roche Research Center, Nutley, New Jersey: Interleukin-1 molecular biology.

Cate, R.L.,[1] Donahoe, P.K.,[2] [1]Biogen Research Corp., Cambridge, [2]Dept. of Surgery, Massachusetts General Hospital and Harvard Medical School, Boston: Development of Mullerian inhibiting substance as a potential anticancer drug.

H. Lebrach, P. L. Pearson



J. D. Watson, D. Koshland

SESSION 9 GENETIC DIAGNOSIS (2)

Chairman: T. Maniatis, Harvard University

Wallace, R.B., Dept. of Molecular Genetics, Beckman Research Institue of the City of Hope, Duarte, California: Use of oligonucleotide probes for diagnostic purposes.

Mullis, K., Faloona, F., Scharf, S., Saiki, R., Horn, F., Erlich, H., Dept. of Human Genetics, CETUS Corp., Emeryville, California: Specific enzymatic amplification of DNA in vitro.

Myers, R.M.,[1] Lerman, L.S.,[2] Maniatis, T.,[3] [1]Dept. of Physiology, University of California, San Francisco; [2]Genetics Institute, Cambridge, Massachusetts; [3]Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Detection and localization of single base mutations in total genomic DNA.

Lerman, L.,[1] Myers, R.,[2] Lumelsky, N.,[3] Maniatis, T.,[4] [1]Genetics Institute, Cambridge, Massachusetts; [2]Dept. of Physiology, University of California, San Francisco; [3]Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts; [4]Dept. of Biology, Yale University, New Haven, Connecticut: Helix stability and genetic analysis.

Woo, S.L.C., Howard Hughes Medical Institute, Dept. of Cell Biology and Institute of Molecular Genetics, Baylor College of Medicine, Houston, Texas: Molecular basis of phenylketonuria and potential somatic gene therapy.

Lebo, R., Chehab, F., Lau, Y.F., Kan, Y.W., Howard Hughes Medical Institute and Dept. of Medicine, University of California, San Francisco: Analysis of normal and abnormal human genomes with flow-sorted chromosomes.

Latt, S.,[1] Lalande, M.,[1] Donlon, T.,[1] Wyman, A.,[2] Rose, E.,[1] Shiloh, Y.,[1] Korf, B.,[1] Saki, K.,[1] Kang, J.,[1] Stroh, H.,[1] Harris, P.,[1] Bruns, G.,[1] Kaplan, L.,[1] [1]Children's Hospital, Harvard Medical School, Boston; [2]Massachusetts Institute of Technology, Cambridge: DNA-based detection of chromosome deletion and amplification—Diagnostic and mechanistic significance.

SESSION 10 DRUGS MADE OFF HUMAN GENES 3: GROWTH FACTORS

Chairman: G.J. Todaro, Oncogen, Seattle

Todaro, G.J.,[1] Twardzik, D.,[1] Purchio, T.,[1] Eiferman, R.,[2] Schultz, G.,[2] [1]Oncogen, Seattle, Washington; [2]Dept. of Biochemistry, University of Louisville, Kentucky: Tumor growth factors and vaccinia virus growth factors—Role in epithelial wound healing.

Derynck, R., Lindquist, P., Rosenthal, A., Bringman, T., Winkler, M., Goeddel, D.V., Genentech, Inc., South San Francisco, California: Transforming growth factors-α and -β—Precursor structures and biological activities.

Fiddes, J.C.,[1] Whang, J.L.,[1] Mergia, A.,[1] Friedman, J.,[1] Tumolo, A.,[1] Hjerrild, K.A.,[1] Gospodarowicz, D.,[2] Abraham, J.A.,[1] [1]California Biotechnology, Inc., Mountain View; [2]Cancer Research Institute, University of California, San Francisco: Isolation and characterization of clones encoding basic fibroblast growth factors.

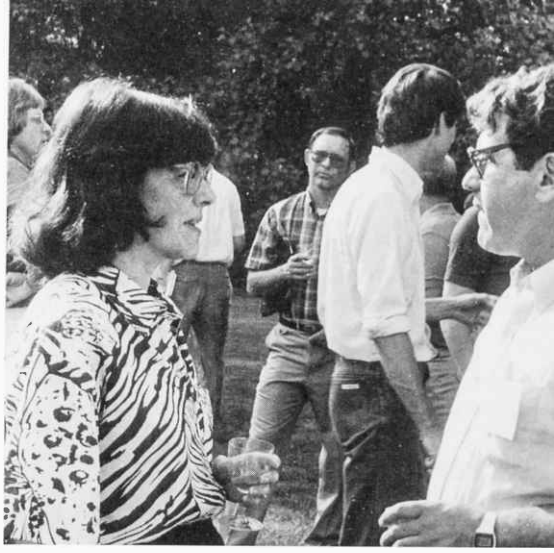
Seeburg, P.H., Genentech, Inc., South San Francisco, California: Human growth hormone—From clone to clinic.

Ralph, P., Warren, K., Weaver, J., Kawasaki, E., Ladner, M., McConlogue, L., Mark, D., White, T., Cetus Corp., Emeryville, California: Cloning of the cDNA and biological properties of human macrophage growth factor, CSF-1.

Donahue, R., Clark, S., Genetics Institute, Cambridge, Massachusetts: In vivo studies with recombinant human GM-CSF.

Browne, J.K., Cohen, A.M., Egrie, J.C., Lai, P.H., Lin, F.K., Strickland, T., Stebbing, N., Amgen, Thousand Oaks, California: Erythropoietin—Gene cloning, protein structure, and biological properties.

R. Williamson, N. Wexler



T. Grodzicker, G. Vande Woude

SESSION 11 HUMAN EVOLUTION (2)

Chairman: M. Singer, National Cancer Institute

Singer, M., Skowronski, J., NCI, National Institutes of Health, Bethesda, Maryland: The abundant LINE-1 family of repeated DNA sequences in mammals—Genes and pseudogenes?

Deka, N., Paulson, K.E., Sawada, I., Willard, C., Schmid, C.W., Dept. of Chemistry, University of California, Davis: I. Properties of a human transposon-like sequence. II. Evolution of the primate α-globin gene cluster and its interspersed Alu repeats.

Bernardi, G., Bernardi, G., Institut Jacques Monod, Paris, France: The human genome and its evolutionary context.

Hill, A.V.S.,[1] Flint, J.,[1] Bowden, D.K.,[2] Oppenheimer, S.J.,[3] Weatherall, D.J.,[1] Clegg, J.B.,[1] [1]MRC, University of Oxford, John Radcliffe Hospital, England; [2]Dept. of Anatomy, Monash University, Victoria, Australia; [3]Dept. of Tropical Paediatrics, Liverpool School of Tropical Medicine, England: Population genetics of α-thalassemia and the malaria hypothesis.

Shen, C.-K. J., Marks, J., Shaw, J.P., Hu, W.S., Ayres, M., Shen, C., Dept. of Genetics, University of California, Davis: Recent developments in the study of novel new members and reconstruction of human α-thalassemia-2 genotypes in monkey cells.

Kazazian, H.H.,[1] Antonarakis, S.E.,[1] Wong, C.,[1] Youssoufian, H.,[1] Boehm, C.D.,[1] Huang, S.-Z.,[1] Goff, S.,[2] Orkin, S.H.,[2] [1]Dept. of Pediatrics, Johns Hopkins School of Medicine, Baltimore, Maryland; [2]Dept. of Pediatrics, Harvard Medical School, Boston, Massachusetts: Comparisons of β-thalassemia and hemophilia A mutations—New lessons from a giant gene.

SESSION 12 RECEPTORS (1)

Chairman: R. Axel, Columbia University College of Physicians & Surgeons

Axel, R.,[1,2] Maddon, P.,[1] [1]Dept. of Biochemistry, [2]Howard Hughes Medical Institute, Columbia University College of Physicians & Surgeons, New York, New York: The T cell-surface protein, T4—Possible roles in the cellular immune response and in the pathogenesis of AIDS.

Ullrich, A.,[1] Riedel, H.,[1] Lee, J.,[1] Gray, A.,[1] Coussens, L.,[1] Dull, T.,[1] Waterfield, M.,[2] Verma, I.,[3] Williams, L.,[4] Schlessinger, J.,[5] [1]Genentech, Inc., South San Francisco, California; [2]Imperial Cancer Research Fund, London, England; [3]Salk Institute, La Jolla, California; [4]University of California, San Francisco; [5]Weizmann Institute of Science, Rehovot, Israel: Molecular features involved in cell-surface receptor function and their role in oncogenesis.

Ruddle, F.H., Miskimins, W.K., Roberts, M.P., Dept. of Biology, Yale University, New Haven, Connecticut: Organization and expression of the human transferrin receptor gene.

Greene, W.C., NCI, National Institutes of Health, Bethesda, Maryland: How human T lymphocytes grow—A molecular analysis of the human interleukin-2 receptor.

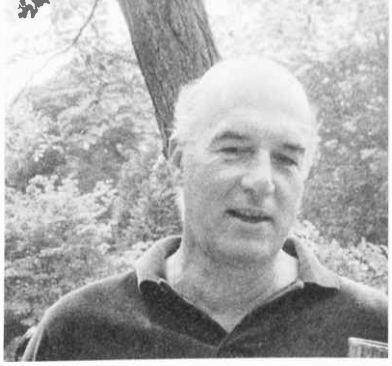
Honjo, T., Shimizu, A., Kondo, S., Sabe, H., Ishida, N., Kinoshita, M., Saito, U., Suzuki, N., Kanamori, H., Matsunami, N., Yaoita, Y., Dept. of Medical Chemistry, Kyoto University Faculty of Medicine, Japan: Expression and function of the human interleukin-2 receptor.

Chambon, P., Green, S., Krust, A., Kumar, V., Walter, P., Devic, M., Govindan, M.V., Stropp, U., CNRS, INSERM, Strasbourg, France: Structure and function of estrogen and glucocorticoid receptors.

Evans, R.M.,[1] Weinberger, C.,[1] Hollenberg, S.,[1] Giguere, V.,[1] Rosenfeld, M.G.,[2] [1]Howard Hughes Medical Institute, Salk Institute, [2]University of California School of Medicine, San Diego: Structure and expression of human glucocorticoid receptor cDNA—A *trans*-acting factor related to the c-*erbA* proto-oncogene family.

P. Berg

M. Capecchi



C. Basilico

SESSION 13 HUMAN GENE MAP (4)

Chairman: M. Siniscalco, Memorial Sloan-Kettering Cancer Center

Mandel, J.L.,[1] Arveiler, B.,[1] Camerino, G.,[2] Hanauer, A.,[1] Heilig, R.,[1] Koenig, M.,[1] Oberlé, I.,[1] [1]CNRS, INSERM, Strasbourg, France; [2]Dipartimento de Genetica e Microbiologia, Universita di Pavia, Italy: Genetic mapping of the human X chromosome—Linkage analysis of the q27-qter region, including the fragile X locus and isolation of expressed sequences.

Goodfellow, P.N.,[1] Goodfellow, P.J.,[1] Wolfe, J.,[2] Banting, G.,[1] Pym, B.,[1] Pritchard,[1] Darling, S.M.,[1] [1]ICRF, [2]Dept. of Human Genetics, University College, London, England: Molecular genetics of *MIC2*—An X and Y chromosome-located expressed gene.

Rouyer, F., Simmler, M.C., Vergnaud, G., Johnsson, C., Weissenbach, J., INSERM, CNRS, Institut Pasteur, Paris, France: The pseudoautosomal region of the human sex chromosomes.

Page, D.C., Brown, L., Bieker, K., Pollack, J., Whitehead Institute, Cambridge, Massachusetts: Sex reversal—Deletion mapping the male-determining function of the Y chromosome.

Cooke, H.J., Smith, B.A., MRC Mammalian Genome Unit, Edinburgh, Scotland: Variability at the telomeres of the human X/Y pseudoautosomal region.

Casanova, M.,[1] Seboun, E.,[1] Leroy, P.,[1] Disteche, C.,[2] Magenis, E.,[3] Bishop, C.,[1] Fellous, M.,[1] [1]INSERM, Institute Pasteur, Paris, France; [2]Dept. of Pathology, University of Washington, Seattle; [3]Dept. of Medical Genetics, Oregon Health Sciences University, Portland: Molecular biology of Y chromosome and anomalies of sex determination in man.

de la Chapelle, A., Dept. of Medical Genetics, University of Helsinki, Finland: Sex reversal—Genetic and molecular studies on 46,XX and 45,X males.

SESSION 14 HUMAN CANCER GENES (2)

Chairman: C.M. Croce, Wistar Institute

Croce, C.M., Wistar Institute, Philadelphia Pennsylvania: Molecular genetics of human B- and T-cell neoplasia.

Rabbitts, T.H., Baer, R., Bulawela, L., Mengle-Gaw, L., Medical Research Council, Cambridge, England: T- and B-cell receptor gene rearrangements and lymphoid tumor etiology.

Le Beau, M.M.,[1] Espinosa, R.,[1] Yang, F.,[2] Schneider, C.,[3] Larson, R.A.,[1] Rowley, J.D.,[1] Diaz, M.O.,[1] [1]University of Chicago, Illinois; [2]Dept. of Cellular and Structural Biology, University of Texas Health Science Center, San Antonio; [3]Laboratoire Européen de Biologie Moléculaire, Heidelberg, Federal Republic of Germany: Juxtaposition of transferrin and transferrin receptor genes in inv(3) and t(3;3) in acute nonlymphocytic leukemia.

Groffen, J.,[1] Stam, K.,[1] Heisterkamp, N.,[1] de Klein, A.,[2] Grosveld, G.,[2] [1]Oncogene Science, Inc., Mineola, New York; [2]Erasmus University, Rotterdam, The Netherlands: Oncogene activation by chromosomal translocation in chronic myelocytic leukemia.

Alt, F.W., DePinho, R., LeGouy, E., Zimmerman, K., Nisen, P., Dept. of Biochemistry and Biophysics, Columbia University College of Physicians & Surgeons, New York, New York: The *myc* family of cellular oncogenes.

Leder, P., Chung, J., Halazonetis, T., Sarid, J., Schmidt, E., Dept. of Genetics, Harvard Medical School, Boston, Massachusetts: Studies on the regulation and function of the human c-*myc* gene.

Spector, D.L.,[1] Sullivan, N.F.,[1] Watt, R.A.,[2] [1]Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; [2]Smith Kline & French Laboratories, Swedeland, Pennsylvania: Association of the *myc* oncogene protein with snRNPs—Is *myc* involved in RNA processing?

Verma, I.M., Van Beveren, C., Mitchell, R., Kruijer, W., Deschamps, J., Neijlink, F., Molecular Biology and Virology Laboratory, Salk Institute, San Diego, California: Proto-oncogenes *fos* and *fms*.

U. Franke

I. Verma

S. Aaronson

SESSION 15 GENE THERAPY (1)

Chairman: P. Berg, Stanford University

Thomas, E.D., Fred Hutchinson Cancer Research Center, Seattle, Washington: Marrow transplantation and gene therapy.

Bernstein, A.,[1] Dick, J.,[1] Magli, C.,[2] Phillips, R.A.,[2] [1]Mt. Sinai Hospital Research Institute, [2]Hospital for Sick Children Research Institute, Toronto, Canada: Gene transfer into hematopoietic stem cells – Implications for gene therapy.

Wagner, E.,[1] Paige, C.,[2] Keller, F.,[2] [1]European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany; [2]Basel Institute for Immunology, Switzerland: Gene transfer into multipotent hemopoietic stem cells.

Gregg, R.G., Smithies, O., University of Wisconsin, Madison: Targeted modification of human chromosomal genes.

Capecchi, M.R., Thomas, K.R., Dept. of Biology, University of Utah, Salt Lake City: High-frequency targeting of genes to specific sites in the mammalian genome.

Groner, Y., Elroy-Stein, O., Bernstein, Y., Dafni, N., Levanon, D., Danciger, E., Neer, A., Dept. of Virology, Weizmann Institute of Science, Rehovot, Israel: Molecular genetics of Down's syndrome – Overexpression of transfected human Cu/Zn-superoxide dismutase gene and the consequent physiological changes.

Miller, A.D., Hock, R.A., Palmer, T.D., Dept. of Molecular Medicine, Fred Hutchinson Cancer Research Center, Seattle, Washington: Transfer of genes into human somatic cells using retroviral vectors.

Orkin, S.H.,[1,2] Williams, D.W.,[1,2] Lim, B.,[1] Dexter, M.,[3] Mulligan, R.C.,[4] [1]Division of Hematology, Children's Hospital, [2]Howard Hughes Medical Institute, Harvard Medical School, Boston, Massachusetts; [3]Christie Hospital, Manchester, England; [4]Whitehead Institute, Massachusetts Institute of Technology, Cambridge: Retrovirus-mediated transfer and expression of human adenosine deaminase sequences in hematopoietic cells.

SESSION 16 RECEPTORS (2)

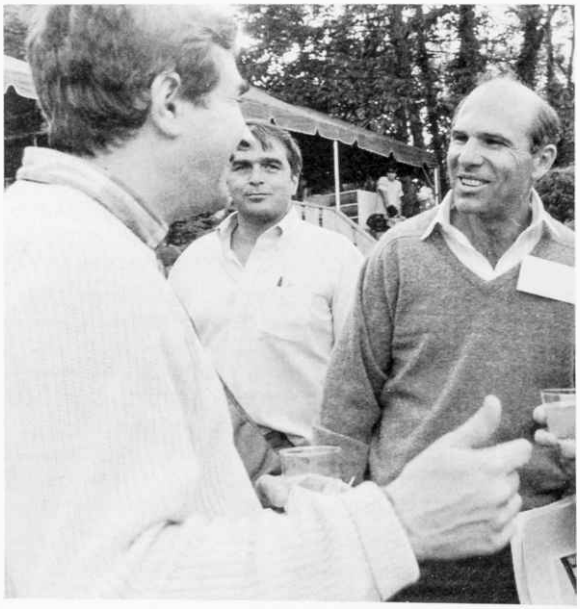
Chairman: M.J. Goldstein, University of Texas

Russell, D.W., Brown, M.S., Goldstein, J.L., Dept. of Molecular Genetics, University of Texas Health Science Center, Dallas: Molecular genetics of the human low-density lipoprotein receptor.

Motulsky, A.G., Deeb, S., Failor, A., Walter, D., Brunzell, J., Albers, J., Depts. of Medicine and Genetics, University of Washington, Seattle: Molecular genetics of apolipoproteins and coronary heart disease.

Mahley, R.W., Gladstone Foundation Laboratories, Depts. of Pathology and Medicine, University of California, San Francisco: Cellular molecular biology of lipoprotein metabolism – Characterization of lipoprotein receptor–ligand interactions.

Rutter, W., University of California, San Francisco: The genes for human insulin and its receptor – Self specific synthesis and transmembrane signalling.



A. Beaudet, R. White, J. Minna

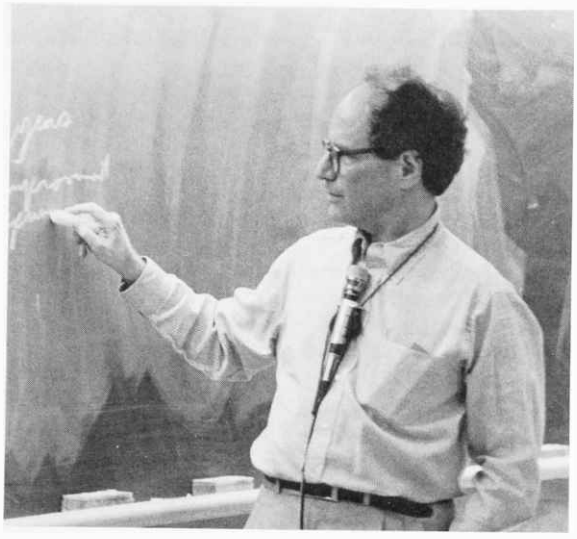
SESSION 17 SEQUENCING OF THE HUMAN GENOME

Discussion led by **Walter Gilbert,** Harvard University and **Paul Berg,** Stanford University

SESSION 18 HUMAN CANCER GENES (3)

Chairman: J. Sambrook, University of Texas Health Science Center

Bootsma, D., van Duin, M., Hoeijmakers, J., Westerveld, A., Yasui, A., Dept. of Cell Biology and Genetics, Erasmus University, Rotterdam, The Netherlands: Identification of DNA repair genes in the human genome.



W. Gilbert

Francke, U., Dept. of Human Genetics, Yale University School of Medicine, New Haven, Connecticut: Mapping of genes involved in growth control.epShows, T.B., Dept. of Human Genetics, Roswell Park Memorial Institute, Buffalo, New York: The chromosome 11 gene map; genes and markers in growth, development, and cancer.

Cavenee, W.,[1,2] Hansen, M.,[1] Scrable, H.,[2] Koufos, A.,[2] [1]Ludwig Institute for Cancer Research, McGill University, Montreal, Canada; [2]Dept. of Microbiology and Molecular Genetics, University of Cincinnati College of Medicine, Ohio: Molecular genetics of human familial cancer.

Housman, D.,[1] Glaser, T.,[1] Gerhard, D.S.,[1] Bruns, G.,[2] Jones, C.,[3] [1]Massachusetts Institute of Technology, Cambridge; [2]Dept. of Pediatrics, Harvard Medical School, Boston, Massachusetts; [3]University of Colorado Medical Center, Denver: Genetic analysis of the Wilms' tumor-aniridia region of human chromosome 11p.

Minna, J., Battey, J., Brooks, B., Carmichael, J., Cuttitta, F., DeGreves, J., Gu, J., Ihde, D., Lebacqu-Verheyden, A.M., Linnoila, I., Johnson, B., Mulshine, J., Nau, M., Sausville, E., Seifter, E., Vinocour, M., Gazdar, A., NCI, National Institutes of Health, Bethesda, Maryland: Molecular and cellular biology of human lung cancer.

Aaronson, S.A., Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, Maryland: Human oncogenes involved in the growth factor activated pathways of cellular proliferation.

SESSION 19 GENE THERAPY (2)

Chairman: D.W. Martin, Jr., Genetech, Inc.

Martin, D.W., Jr., Williams, S.R., McIver, S., Genentech, Inc., South San Francisco, California: The molecular basis of an inherited immunodeficiency disease and of its potential cure.

Kan, Y.W.,[1] Ho, Y.S.,[1] Palese, P.,[2] Norton, G.,[2] Cheung, M.C.,[1] Chang, J.,[1] [1]Howard Hughes Medical Institute and Dept. of Medicine, University of California, San Francisco; [2]Dept. of Microbiology, Mt. Sinai Medical Center, New York, New York: Expression and function of suppressor tRNA genes in mammalian cells.

Sorge, J., Beutler, E., Scripps Clinic and Research Foundation, La Jolla, California: Correction of the defect of Gaucher disease fibroblasts and lymphoblasts by retroviral-mediated gene transfer.

Yee, J.-K.,[1] Jolly, D.,[2] Respess, J.,[1] Friedmann, T.,[1] [1]Dept. of Pediatrics, University of California School of Medicine, San Diego, La Jolla; [2]Lab Horomones INSERM, Paris, France: Gene transfer and expression by a transcriptionally inactivated retroviral vector containing an internal promoter.

Caskey, C.T., Chang, S., Belmont, J., Henkel-Tigges, J., Wager-Smith, K., Institute for Molecular Genetics and Howard Hughes Medical Institute, Baylor College of Medicine, Houston, Texas: Retroviral-mediated gene transfer of human HPRT and ADA.

Anderson, W.F.,[1] Kantoff, P.,[1] Eglitis, M.,[1] McLachlin, J.,[1] Karson, E.,[1] Zwiebel, J.,[1] Nienhuis, A.,[1] Karlsson, S.,[1] Blaese, R.M.,[1] Kohn, D.,[1] Gilboa, E.,[2] Armentano, D.,[2] Gillio, A.,[3] Bordignon, C.,[3] O'Reilly, R.,[3] [1]National Institutes of Health, Bethesda, Maryland; [2]Princeton University, New Jersey; [3]Memorial Sloan-Kettering Cancer Center, New York, New York: Gene transfer and expression in nonhuman primates using retroviral vectors.

Beaudet, A.L.,[1,2] Jackson, M.J.,[1] Wood, P.A.,[1] O'Brien, W.E.,[1,2] [1]Dept. of Pediatrics, Baylor College of Medicine, [2]Howard Hughes Medical Institute, Houston, Texas: The human argininosuccinate synthetase locus—Gene regulation and potential for gene therapy.

Mulligan, R.C., Whitehead Institute for Biomedical Research and Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Gene transfer–A potential new therapy for the treatment of genetic disease.

Summary: T. Caskey, Baylor College of Medicine

MEETINGS

G-Proteins and Signal Transduction

April 30–May 4

ARRANGED BY

Henry R. Bourne, University of California, San Francisco
Alfred G. Gilman, University of Texas Health Science Center
John Northup, University of Calgary
Michael Wigler, Cold Spring Harbor Laboratory

196 participants

The 1986 meeting on G-Proteins and Signal Transduction was the first of its kind at Cold Spring Harbor Laboratory. Its focus was on those processes of signal transduction (hormonal and sensory) that are mediated by guanine-nucleotide-binding proteins. Scientific presentations included analysis of the receptor molecules, such as the visual rhodopsins and the β-adrenergic receptor, the G-proteins themselves, and G-protein-like molecules of the *ras* family, and the effector systems, such as adenylate cyclase and phospholipases. The major themes of the meeting were the overall similarity of very diverse signaling systems; the molecular structure of receptors and G-proteins; the diversity of G-proteins; the role of GTP in regulating phospholipases; and the structure and function of the *ras* proteins and their similarity to G-proteins. The highlights of the meeting included reports of the primary structure of various G-proteins; the primary structure of the β-adrenergic receptor and its similarity to rhodopsin and to a newly discovered oncogene; and evidence that phosphatidyl inositol is regulated by GTP binding proteins.

This meeting was supported in part by the National Science Foundation; National Institute of General Medical Sciences and Child Health and Human Development/National Institutes of Health.

SESSION 1 THE PRIMARY STRUCTURE OF G PROTEINS

Chairmen: **M. Simon,** California Institute of Technology
A. Gilman, University of Texas Health Science Center

Robishaw, J.D., Graziano, M.P., Smigel, M.D., Gilman, A.G., Dept. of Pharmacology, University of Texas Health Science Center, Dallas: Analysis of cDNAs for $G_{s\alpha}$ and G_{β}.

Itoh, H.,[1,2] Kozasa, T.,[1] Nakafuku, M.,[1] Fukui, Y.,[1] Yamamoto, M.,[1] Nagata, S.,[1] Nakamura, S.,[1] Katada, T.,[2] Ui, M.,[2] Kaziro, Y.,[1] [1]Institute of Medical Science, University of Tokyo; [2]Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan: Cloning of cDNAs coding for G proteins from rat brain and function of the *ras* gene in *Schizosaccharomyces pombe*.

Fong, H.K.W.,[1] Hurley, J.B.,[2] Doolittle, R.F.,[3] Simon, M.I.,[1] [1]Biology Division, California Institute of Technology, Pasadena; [2]Howard Hughes Medical Institute, Seattle, Washington; [3]Dept. of Chemistry, University of California, San Diego, La Jolla: Repetitive segmental structure of the transducin β-subunit and homology with the yeast *CDC4* gene.

Bourne, H.R., Sullivan, K., Masters, S.B., Dept. of Pharmacology, University of California, San Francisco: G protein α-chains—Structure versus function.

Nukada, T., Tanabe, T., Sugimoto, K., Takahashi, H., Noda, M., Numa, S., Depts. of Medical Chemistry and Molecular Genetics, Kyoto University Faculty of Medicine, Japan: Primary structures of the α- and β-subunits of G proteins deduced from the cDNA sequences.

Michel, T.,[1,4] Winslow, J.W.,[1,4] Smith, J.A.,[2,5] Seidman, J.G.,[3] Neer, E.J.,[1,4] Depts. of [1]Medicine, [2]Pathology,

[3]Genetics, Harvard Medical School, Boston; [4]Cardiovascular Division, Brigham and Women's Hospital; [5]Dept. of Molecular Biology and Pathology, Massachusetts General Hospital: cDNA cloning of the GTP-binding protein, α, and of a novel G protein, α_h.

Bray, P.,[1] Carter, A.,[2] Kamholz, J.,[3] Puckett, C.,[3] Spiegel, A.,[2] Nirenberg, M.,[1] [1]NHLBI, [2]NIADDK, [3]NINCDS, National Institutes of Health, Bethesda, Maryland: Human cDNA clones for α-subunits of G_s and an unidentified G protein.

SESSION 2 THE REGULATION AND STRUCTURE OF ADENYLATE CYCLASE

Chairmen: **D. Storm,** University of Washington
R. Lefkowitz, Duke University Medical Center

Ross, E.M., Brandt, D.R., May, D.C., Dept. of Pharmacology, University of Texas Health Science Center, Dallas: Regulation of G_s function by β-adrenergic receptors in reconstituted phospholipid vesicles.

Lefkowitz, R.J., Benovic, J.L., Strasser, R.H., Caron, M.G., Duke University Medical Center, Durham, North Carolina: Molecular and regulatory properties of adenylate cyclase-coupled adrenergic receptors.

Birnbaumer, L., Codina, J., Mattera, R., Sunyer, T., Toro, M.J., Baylor College of Medicine, Houston, Texas: Functional and structural properties of N_s and other N proteins.

Smigel, M., Dept. of Pharmacology, University of Texas Health Science Center, Dallas: Purification and properties of bovine brain adenylate cyclase.

Minocherhomjee, A., Rosenberg, G., Shattuck, R., Storm, D.R., Dept. of Pharmacology, University of Washington, Seattle: Comparison between calmodulin-sensitive adenylate cyclases purified from bovine brain and *Bordetella pertussis*.

Peterkofsky, A.,[1] Gazdar, C.,[1] Liberman, E.,[1] Miller, D.,[2] Reddy, P.,[1] [1]National Institutes of Health, Bethesda, Maryland; [2]New York State Institute of Basic Research in Developmental Disability, Staten Island: Regulation of *E. coli* adenylate cyclase (AC) activity by factors with shared functions.

Klein, P., Theibert, A., Vaughan, R., Devreotes, P., Dept. of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland: Receptor G-protein interactions and *Dictyostelium* development.

Kataoka, T.,[1,2] Broek, D.,[1] Field, J.,[1] Wigler, M.,[1] [1]Cold Spring Harbor Laboratory, New York; [2]Dept. of Physiology and Biophysics, Harvard Medical School, Boston, Massachusetts: Interaction of *RAS* proteins with yeast adenylate cyclase.

Smith, R.F., Choi, K.-W., Tully, T., Quinn, W.G., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Deficient protein kinase C activity in *turnip*, a *Drosophila* learning mutant.

SESSION 3 POSTER SESSION

Allen, T.J.A., Baker, P.K., Dept. of Physiology, King's College, London, England: Serotin-sensitive cyclic nucleotide levels in the perfused axon.

Amons, R., Jansen, G.M.C., Maessen, G.D.F., Maassen, J.A., Moller, D.W., Dept. of Biochemistry, University of Leiden, The Netherlands: Structure and properties of *Artemia* and human EF-1.

Anand-Srivastava, M.B., Srivastava, A.K., Clinical Research Institute of Montreal, Canada: Protein kinase C modulates adenylate cyclase activity in rat brain striatum. Possible involvement of G_i-guanine nucleotide regulatory protein.

Anderson, G.R., Farkas, B.K., Dept. of Cell and Molecular Biology, Roswell Park Memorial Institute, Buffalo, New York: Guanine nucleotide regulation of a transformation-associated lactate dehydrogenase.

Aub, D.L., Frey, E.A., Sekura, R.D., Cote, T.E., Dept. of Pharmacology, USUHS, Bethesda, Maryland: Coupling of the TRH receptor to phospholipase C by a GTP-binding protein distinct from N_s or N_i.

Berta, P.,[1] Le Peuch, C.J.,[1] Cavadore, J.C.,[1] Alouf, J.E.,[2] Haiech, J.,[1] [1]Centre de Recherches de Biochimie Macromoléculaire, CNRS, INSERM, Montpellier; [2]Unité des Antigènes bactériens, CNRS, Institute Pasteur, Paris, France: Involvement of GTP in the regulation of cultured vascular smooth muscle cell response to 5-hydroxytryptamine.

Bigay, J., Deterre, P., Pfister, C., Chabre, M., Laboratory Biophysique Moléculaire et Cellulaire, Grenoble, France: Activation of transducin by fluoride and aluminum: AlF_4^- mimicks the γ-phosphate of GTP in the binding site of $T\alpha$·GDP.

Bojanic, D., Fain, J.N., Dept. of Biochemistry, University of Tennessee, Memphis: Regulation of [^{3}H]vasopressin binding to liver membranes and detergent extracts by a guanine nucleotide.

Bokoch, G.M.,[1] Sklar, L.A.,[1] Button, D.,[1] Smolen, J.E.,[2] [1]Scripps Clinic and Research Foundation, La Jolla, California; [2]Mott Hospital, University of Michigan, Ann Arbor: Regulation of ligand–receptor dynamics by guanine nucleotides–Rapidly interconverting states for the neutrophil formyl peptide receptor.

Braun, A.P., Sulakhe, P.V., University of Saskatchewan College of Medicine, Saskatoon, Canada: Distribution and characteristics of guanine nucleotide binding proteins in bovine heart.

Corrèze, C.,[1] d'Alayer, J.,[1] Coussen, F.,[1] Berthillier, G.,[1] Deterre, P.,[2] Monneron, A.,[1] [1]Dept. de Biologie Moléculaire, Institute Pasteur, Paris; [2]Laboratoire de Biophysique Moléculaire, Grenoble, France: Interference of anti-β antibodies with adenylate cyclase activity in brain membranes.

Dixon, R.A.F.,[1] Kobilka, B.K.,[4] Strader, D.J.,[3] Benovic, J.L.,[4] Dohlman, H.G.,[4] Frielle, T.,[4] Bolanowski, M.A.,[4]

Bennett, C.D.,[2] Mumford, R.A.,[3] Slater, E.E.,[3] Sigal, I.S.,[1] Caron, M.G.,[4] Lefkowitz, R.J.,[4] Strader, C.D.,[3] Depts. of [1]Virus and Cell Biology Research, [2]Medicinal Chemistry, Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania; [3]Dept. of Biochemistry, Merck Sharp and Dohme Research Laboratories, Rahway, New Jersey; [4]Howard Hughes Medical Institute, Depts. of Medicine, Biochemistry, and Physiology, Duke University Medical Center, Durham, North Carolina: Peptide mapping and production of antibodies to the mammalian $\beta 2$-adrenergic receptor.

Dudycz, L.W., Kelleher, D.J., Johnson, G.L., Wright, G.E., University of Massachusetts Medical Center, Worcester: Ability of N^2-(*p*-*n*-butylphenyl) derivatives of guanine nucleotides to bind and activate bovine transducin.

Fain, J.N.,[1] Litosch, I.,[2] [1]Dept. of Biochemistry, University of Tennessee, Memphis; [2]Dept. of Pharmacology, University of Miami School of Medicine, Florida: Hormonal activation of phosphoinositide breakdown in the presence of guanine nucleotides using cell-free systems from blowfly salivary glands.

Fitzgerald, T.J., Uhing, R.J., Exton, J.H., Howard Hughes Medical Institute and Dept. of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, Tennessee: Evidence for a vasopressin receptor–GTP binding protein complex.

Guerrero, I.,[1] Pellicer, A.,[1] Green, S.,[2] Green, L.A.,[2] Burstein, D.,[1] [1]Dept. of Pathology, [1]Dept. of Pharmacology, New York University Medical Center, New York: Activated and highly expressed normal N-*ras* gene induces neuronal differentiation in PC12 rat pheochromocytoma cells and in sublines nonresponsive to nerve growth factor.

Guillon, G.,[1] Kirk, C.J.,[2] [1]CNRS, INSERM, Montpellier, France; [2]Dept. of Biochemistry, Birmingham, England: GTP-dependent activation of PIP2 phosphodiesterase by vasopressin in WRK1 cells–Studies with intact cells and partially purified membranes.

Hamm, H.E.,[1] Deretic, D.,[1] Hatta, S.,[1] Bennett, N.,[2] [1]Dept. of Physiology and Biophysiology, University of Illinois College of Medicine; [2]Laboratory of Biophysiology, CNRG, Grenoble, France: Monoclonal antibodies to the photoreceptor G protein which block its light activation–Antigenic sites and mechanism of action.

Hingorani, V., Ho, Y.-K., Dept. of Biological Chemistry, University of Illinois Health Sciences Center, Chicago: Chemical modification of bovine transducin by fluorescein-5′-isothiocyanate.

Homburger, V., Audigier, Y., Brabet, P., Bockaert, J., Rouot, B., CNRS, INSERM, Montpellier, France: Immunolike reactivity against the GTP-binding protein subunit α-o in neuronal and nonneuronal tissues from different species.

Jacquemin, C.,[1] Thibout, H.,[1] Lambert, B.,[2] Correze, C.,[1] [1]INSERM, Unité de Recherche sur la Glande Thyroide et la Régulation Hormonale, Bicetre; [2]Dept. of Biochemistry, Faculté des Science, Reims, France: Autonomous regulation of adenylate cyclase in rat adipocytes–Endogeneous ADP-ribosylation of G_s under the influence of adenosine.

Lad, R.,[1] Gerfen, C.,[2] Milligan, G.,[3] Gierschik, P.,[4] Spiegel, A.,[1] [1]NIADDK, [2]NIMH, National Institutes of Health, Bethesda, Maryland; [3]Dept. of Biochemistry, University of Glasgow, Scotland; [4]Dept. of Pharmacology, University of Heidelberg, Federal Republic of Germany: Immunohistochemical localization of guanine nucleotide binding proteins in rat retina.

SESSION 4 SIGNAL TRANSDUCTION. I. HORMONE ACTION

Chairmen: **P. Sternweis,** University of Texas Health Science Center
J. Northup, University of Calgary

Perkins, J.P., Dept. of Pharmacology, Yale University School of Medicine, New Haven, Connecticut: Ligand-induced uncoupling of the functional interaction of β-adrenerigic receptors and G_s.

Orellana, S.T., Brown, J.H., University of California, San Diego, La Jolla: Phorbol ester inhibits guanine nucleotide-regulated inositol phosphate formation in membrane and permeabilized preparations of 1321N1 astrocytoma cells.

Bauer, S., Gierschik, P., Grandt, R., Jakobs, K.H., Pharmakologisches Institute der Universität Heidelberg, Federal Republic of Germany: Phosphorylation of G proteins by intracellular signal-controlled protein kinases.

Valentine-Braun, K., Fraser, E.D., Hollenberg, M.D., Northup, J.K., Dept. of Pharmacology, The University of Calgary, Canada: Purification and properties of the major EGF-kinase substrate of human placental membranes.

Tota, M.R., Rosenbaum, L.C., Peterson, G.L., Schimerlik, M., Dept. of Biochemistry and Biophysics, Oregon State University, Corvallis; Atrial muscarinic receptor—Characterization and interaction with guanine nucleotide binding proteins.

Sternweis, P.C., Florio, V.A., Dept. of Pharmacology, University of Texas Health Science Center, Dallas: GTP-dependent regulatory proteins and their interaction with muscarinic-cholinergic receptors.

Breitwieser, G.E., Lapointe, J.Y., Szabo, G., Dept. of Physiology and Biophysics, University of Texas Medical Branch, Galveston: Muscarinic activation of K^+ conductance is mediated by a guanine nucleotide binding protein in the heart.

Fischer, J.B., Schonbrunn, A., Laboratory of Toxicology, Harvard School of Public Health, Boston, Massachusetts: Bombesin receptors are coupled to a G protein not sensitive to cholera or pertussis toxin.

Gomperts, B.D., University College London, England: N_P and N_E—Two sites of GTP control in stimulus-secretion coupling.

SESSION 5 SIGNAL TRANSDUCTION. II. Sensory Systems

Chairman: **H. Bourne,** University of California Medical Center, San Francisco

Jones, D., Barbosa, E., Reed, R., Dept. of Molecular Biology and Genetics, Johns Hopkins School of Medicine, Baltimore, Maryland: G proteins of the olfactory system.

Corson, D.W.,[1,2] Fein, A.,[1,2] Payne, R.,[1] [1]Marine Biological Laboratory, Woods Hole; [2]Dept. of Physiology, Boston University School of Medicine, Massachusetts: Chemical excitation of quantal events in *Limulus* photoreceptors.

Selinger, Z.,[1] Devary, O.,[1] Blumenfeld, A.,[1] Heichal, O.,[1] Minke, B.,[2] Depts. of [1]Biological Chemistry, [2]Physiology, The Hebrew University of Jerusalem, Israel: Light-activated GTP hydrolysis and breakdown of phosphoinositides in *musca* eye membranes.

Lerea, C.L.,[1] Somers, D.E.,[1]Klock, I.B.,[2] Bunt-Milam, A.H.,[2] Hurley, J.B.,[1] [1]Howard Hughes Medical Institute, [2]Dept. of Opthalmology, University of Washington, Seattle: Identification of a transducin (T_c) specifically expressed in bovine cone outer segments.

Kühn, H., Wehner, M., Institute für Neurobiologie, Jülich, Federal Republic of Germany: Interaction between photoactivated rhodopsin and transducin.

Gillespie, P.G., Charbonneau, H., Zelus, B., Beavo, J.A., Dept. of Pharmacology, University of Washington, Seattle: Subunit structure and functions of photoreceptor cyclic GMP phosphodiesterase(s).

Deterre, P.,[1] Bigay, J.,[1] Robert, M.,[1] Pfister, C.,[1] Kühn, H.,[2] Chabre, M.,[1] [1]Dept. of Molecular and Cellular Biology, CNRS, Grenoble; [2]Institute für Neurobiologie der Julich, Federal Republic of Germany: Activation mechanism of cGMP phosphodiesterase by transducin—Characterization of the complex formed by phosphodiesterase inhibitor and transducin α-subunit.

H. R. Bourne

SESSION 6 G PROTEINS AND PHOSPHOLIPID METABOLISM

Chairmen: **M. Gershengorn,** Cornell University School of Medicine
J. Putney, Medical College of Virginia

Ui, M., Okajima, F., Katada, T., Murayama, T., Kurose, H., Dept. of Physiological Chemistry, Hokkaido University, Sapporo, Japan: G proteins serving as the substrate of pertussis toxin-catalyzed ADP-ribosylation mediate receptor-linked adenylate cyclase inhibition and inositol phospholipid breakdown.

Putney, J.W., Jr., Taylor, C.W., Merritt, J.E., Rubin, R.P., Division of Cellular Pharmacology, Medical College of Virginia, Richmond: Receptor regulation of inositol trisphosphate production in exocrine glands.

Harden, T.K., Hepler, J.R., Dept. of Pharmacology, University of North Carolina School of Medicine, Chapel Hill: Evidence for the involvement of a guanine nucleotide regulatory protein that is not G_i in muscarinic receptor-stimulated phosphoinositide hydrolysis in 1321N1 human astrocytoma cells.

Williamson, J.R., Coll, K.E., Mah, S., Hansen, C., Dept. of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia: Hormone-receptor coupling to phospholipase C and production of inositol phosphates in rat liver.

Gershengorn, M.C., Dept. of Medicine, Cornell University Medical College and New York Hospital, New York, New York: Calcium, inositol trisphosphate, and lipids in signal transduction by thyrotropin-releasing hormone in pituitary GH cells—Evidence for involvement of a novel G protein.

Wojcikiewicz, R.J.H., Kent, P.A., Fain, J.N., Dept. of Biochemistry, University of Tennessee, Memphis: Evidence that receptors which activate phospholipase-C interact with a G protein that is not G_i or G_s.

Blackmore, P.F., Bocckino, S.B., Exton, J.H., Howard Hughes Medical Institute and Dept. of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, Tennessee: Role of a guanine nucleotide binding protein in the hormone-induced hydrolysis of hepatic phosphotidylinositol 4,5-P_2 —Formation of *myo*-inositol 1,3,4,5-P_4.

Haslam, R.J., Hrbolich, J.K., Dept. of Pathology, McMaster University, Hamilton, Ontario, Canada: Activation of phospholipase C in washed platelet membranes by GTPγS and by agonists added with GTP.

Paris, S., Pouyssegur, J., Centre de Biochimie, CNRS, Nice, France: Evidence of a role for a GTP-binding protein in thrombin-induced activation of phosphoinositide hydrolysis and Na^+/H^+ exchange in hamster fibroblasts.

SESSION 7 RAS/G-LIKE PROTEINS

Chairmen: **D. Gallwitz,** University of Marburg
M. Wigler, Cold Spring Harbor Laboratory

Jurnak, F.,[1] Xuong, N.,[2] Hamlin, R.,[2] [1]Dept. of Biochemistry, University of California, Riverside; [2]Dept. of Physics, University of California, San Diego: The GOP site in *E. coli* elongation factor Tu at 2.7 Å resolution.

Kjeldgaard, M., Nyborg, J., la Cour, T., Clark, B.F.C., Dept. of Chemistry, Arhus University, Denmark: A model for the three-dimensional structure of the G binding site in GDP/GTP-binding proteins.

Gallwitz, D., Brökel, R., Molenaar, C.M.T., Rauh, A., Wagner, P., Schmitt, H.D., Institut für Physiologische Chemie I, Universität Marburg, Lahnberge, Federal Republic of Germany: The *ras*-related YPT gene product in yeast—A GTP-binding protein involved in cytoskeletal function.

Madaule, P.,[1] Myers, A.,[2] Axel, R.,[1,3] [1]Howard Hughes Medical Institute, [2]Dept. of Biological Sciences, [3]Dept. of Biochemistry, Columbia University, New York, New York: The p21 *rho* of *Saccharomyces cerevisiae*.

Evans, T., Fraser, E.D., Brown, M.L., Northup, J.K., Dept. of Pharmacology, University of Calgary, Canada: The purification and properties of GTP-binding proteins from human placental membranes.

Chardin, P., Tavitian, A., INSERM, Faculté de Médecine, Paris, France: *ral*: A new *ras*-related gene.

Lowe, D., Delwart, E., Capon, D., Goeddel, D., Dept. of Molecular Biology, Genentech Inc., South San Francisco, California: R-*ras*, a new member of the *ras* proto-*onc* gene family with intrinsic GTPase activity.

Young, D., Waitches, G., Birchmeier, C., Fasano, O., Wigler, M., Cold Spring Harbor Laboratory, New York: A human oncogene encoding a protein with a structure similar to rhodopsin.

SESSION 8 POSTER SESSION

Levine, M.A.,[1] Ahn, T.G.,[1] Van Dop, C.,[1] Kaufman, K.,[1] Smallwood, P.,[1] Bourne, H.R.,[2] Sullivan, K.,[2] [1]Johns Hopkins University, Baltimore, Maryland; [2]University of California, San Francisco: Molecular basis for genetic deficiency of αGs in pseudohypoparathryoidism Ia.

Liao, Y.C., Chen, E., Levinson, A.D., Dept. of Molecular Biology, Genentech Inc., South San Francisco, California: Primary structure of α-subunit of human G protein that stimulates adenylate cyclase—Homology with transducin, *ras*, and elongation factors.

Litosch, I., Dept. of Pharmacology, University of Miami School of Medicine, Florida: Guanosine 5′-(3-0-thio)trisphosphate activates phospholipase C-mediated breakdown of exogenous phosphatidylinositol-4,5-bisphosphate substrate in rat cerebral cortical membranes.

Lombardo, B., Anglade, G., Monneron, A., Dept. de Biologie Moléculaire, Institut Pasteur, Paris, France: Immunolocalization of β-subunits in brain sections.

Longabaugh, J.P.,[1] Vatner, D.E.,[2] Vatner, S.F.,[2] Homcy, C.J.,[1] [1]Cellular and Molecular Research Laboratory,

Massachusetts General Hospital, Boston; [2]New England Regional Primate Research Center, Southborough: Cholera toxin promoter ADP ribosylation in dogs with congestive heart failure.

McMahon, K.K., Dept. of Pharmacology, University of South Carolina School of Medicine, Columbia: Functional characteristics and pertussis toxin ADP-ribosylation patterns of a 39K G protein in fetal, neonatal, and adult rat heart.

Melchiore, S., Mitchell, C., Vary, J., Ho., Y.-K., Dept. of Biological Chemistry, University of Illinois Health Sciences Center, Chicago: *Bacillus megaterium* spore proteins that cross-react with transducin β-subunit antibodies.

Mochly-Rosen, D., Koshland, D.E., Jr., Dept. of Biochemistry, University of California, Berkeley: Enzymatic properties of protein kinase C.

Nambi, P., Whitman, M., Aiyar, N., Crooke, S.T., Dept. of Molecular Pharmacology, Smith Kline and French Laboratories, Philadelphia, Pennsylvania: Induction of functional inhibitory guanine nucleotide regulatory protein (G_i) in canine kidney cell line by sodium butyrate.

Parmeggiani, A., Anborgh, P.A., Canceill, D., Crechet, J.B., Jacquet, E., Jensen, M., Merola, M., Mortensen, K.K., Swart, G.W.M., Laboratoire de Biochimie, École Polytechnique, Palaiseau, France: Properties of the guanine nucleotide binding of EF-Tu.

Polonis, V.R.,[1] Anderson, G.R.,[2] Doyle, D.J.,[1] [1]State University of New York, [2]Roswell Park Memorial Institute, Buffalo, New York: P^1,P^4-DI(guanosine-5′)tetraphosphate (Gp_4G) is a competitive inhibitor of v-*ras*-Ha p21 autophosphorylation.

Pribilla, I. Free University of Berlin, Institute of Biochemistry, Federal Republic of Germany: Inhibition of dopaminergic agonist binding to striatal D_2-receptors by the action of protein kinase C.

Ranu, R.S., Dept. of Microbiology, Colorado State University, Fort Collins: Eukaryotic initiation factor eIF-2 as a G protein.

Rasenick, M.M., Hatta, S., Marcus, M.M., Dept. of Physiology and Biophysics, University of Illinois College of Medicine, Chicago: Guanine nucleotide exchange among GTP-binding proteins which regulate neuronal adenylate cyclase.

Rosenthal, W., Sezer, O., Rudolph, U., Koesling, D., Cetin, E., Yajima, M., Schultz, G., Pharmakolog, Institut der Freien Universität, Berlin, Federal Republic of Germany: Involvement of guanine nucleotide binding proteins (N proteins) in the regulation of intracellular calcium in human neutrophil leukocytes (PMNs).

Simon, M., Oosawa, K., Mutoh, N., Kaplan, N., Biology Division, California Institute of Technology, Pasadena: Genetic and biochemical analysis of signalling by bacterial chemoreceptors.

Stadel, J.M., Poksay, K.S., Nakada, M.T., Crooke, S.T., Smith Kline & French Laboratories, Philadelphia, Pennsylvania: Sodium butyrate modulates the β-adrenergic receptor–adenylate cyclase complex of 3T3-L1 fibroblasts.

Tripp, M.L.,[1] Bouchard, R.A.,[1] Piñon, R.,[1] Meisenhelder, J.,[2] Hunter, T.,[2] [1]Dept. of Biology, University of California, La Jolla; [2]Molecular Biology and Virology Laboratory, Salk Institute, San Diego: Developmentally regulated phosphoproteins in *Saccharomyces cerevisiae* – Positive and negative regulation by environmental signals.

Taylor, C.W., Merritt, J.E., Rubin, R.P., Putney, J.W., Jr., Dept. of Pharmacology, Medical College of Virginia, Richmond: Receptor coupling to phospholipase C in exocrine pancreas and parotid gland.

Vatner, D.E.,[1] Vatner, S.F.,[2 3] Fujii, A.M.,[2 3] Homcy, C.J.,[1] Harvard Medical School, [1]Massachusetts General Hospital, [2]Brigham and Women's Hospital, Boston; [3]New England Regional Primate Research Center, Southborough, Massachusetts: Decreased muscarinic receptors and inhibition of adenylate cyclase in dogs with heart failure.

Wehrmann, M.,[1] Ehbrecht, H.-J.,[1] Wittinghofer, F.,[2] Pingoud, A.,[1] [1]Zentrum Biochemie, Medizinische Hochschule Hannover; [2]Max-Planck-Institute für Medizinische Forschung, Heidelberg, Federal Republic of Germany: The v-Ha-*ras* oncogene product p21 cross-reacts immunologically with the *E. coli* EF-Tu.

Weiss, E.R., Johnson, G.L., Dept. of Biochemistry, University of Massachusetts Medical Center, Worcester: Characterization of antibodies raised against synthetic peptides corresponding to cytoplasmic domains of rhodopsin.

SESSION 9 RAS PROTEINS

Chairmen: **R. Firtel,** University of California, San Diego
J. Feramisco, Cold Spring Harbor Laboratory

Birchmeier, C., Wigler, M., Cold Spring Harbor Laboratory, New York: Maturation of amphibian oocytes is induced by microinjected *ras* protein.

Bar-Sagi, D., Feramisco, J.R., Cold Spring Harbor Laboratory, New York: Rapid induction of cell-surface activities in quiescent fibroblasts following microinjection of H-*ras* proteins.

Stacy, D.W., Smith, M.R., Roche Institute of Molecular Biology, Nutley, New Jersey: C *ras* activity and transformation by separate viral oncogenes.

Furth, M.E.,[1] Swanson, M.E.,[2] Aldrich, T.A.,[1] Elste, A.M.,[2] Greenberg, S.M.,[2] Schwartz, J.H.,[2] [1]Memorial Sloan-Kettering Cancer Center, New York, New York; [2]Howard Hughes Medical Institute, Columbia University, New York, New York: Abundant expression of a *ras* protein in *Aplysia* neurons.

Firtel, R.A.,[1] Reymond, C.D.,[1] Gomer, R.H.,[1] Thiebert, A.,[2] Devreotes, P.,[2] Nellen, W.,[1] [1]Dept. of Biology, University of California Chemistry; [2]Johns Hopkins Medical School, Baltimore, Maryland: *ras* gene regulation and function in *Dictyostelium*.

Sigal, I.S.,[1] Gibbs, J.B.,[1] Smith, G.M.,[1] D'Alonzo, J.S.,[1] Schultz, L.D.,[1] Socher, S.H.,[1] Wolanski, B.,[1] Jurnak, F.,[2] Scholnick, E.M.,[1] [1]Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania; [2]University of California, Riverside: Structure-function of Ha-*ras*.

Hall, A.,[1] Marshall, C.J.,[1] McCormick, F.,[2] McKay, I.,[1] Milley, R.,[2] Paterson, H.,[1] Trahy, M.,[2] [1]Institute of Cancer Research, Chester Beatty Laboratories, London, England; [2]Cetus Corp., Emeryville, California: Biochemical consequences of N-*ras* activation.

Fasano, O., De Vendittis, E., Zahn, R., Vitelli, A., European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: Role of nucleotides and Mg ions in the regulation of the function of yeast and human *ras* proteins.

Toda, T., Powers, S., Kataoka, T., Wigler, M., Cold Spring Harbor Laboratory, New York: Alternate RAS pathway in yeast—Activation of adenylate cyclase is not only function of yeast *RAS*.

Powers, S.,[1] Broek, D.,[1] Field, J.,[1] Wigler, M.,[1] Michaelis, S.,[2] Santa Anna, S.,[2] Herskowitz, I.,[2] [1]Cold Spring Harbor Laboratory, New York; [2]University of California, San Francisco: Effect of fatty acid acylation on *RAS* function.

Eukaryotic Transposable Elements

May 7–May 11

ARRANGED BY

Gerald R. Fink, Whitehead Institute for Biomedical Research
Gerald M. Rubin, University of California, Berkeley
Stephen L. Dellaporta, Cold Spring Harbor Laboratory
James Hicks, Cold Spring Harbor Laboratory

124 participants

This meeting brought together workers on yeast, *C. elegans*, *Drosophila*, plants and vertebrates. The unifying theme was the molecular biology of transposable elements in each of these systems. Over 85 abstracts were presented on elements ranging from the Ty of yeast, the TCl of *C. elegans*, the P and copia of *Drosophila*, the Spm and Ac of maize, and retroviruses of vertebrates. Much of the discussion focused on the regulation of expression of transposable elements. The expression of these elements appears to be under the control of genes both within the elements and elsewhere in the genome. The expression of the genes within the transposable elements has profound effects upon the genes into which the elements have inserted as well as the ability of the element to transpose. Several reports suggested that DNA modification was related to the stability of a transposable element.

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SESSION 1 GENE REGULATION BY TRANSPOSABLE ELEMENTS. I.

Chairman: G.S. Roeder, Yale University

Coney, L.R., Roeder, G.S., Dept. of Biology, Yale University, New Haven, Connecticut: Control of *HIS4* expression by Ty elements.

Kapakos, J., Farabaugh, P., Dept. of Microbiology, University of Connecticut Health Center, Farmington: Interactions between the Ty917 enhancer and silencer sites and the upstream promoter element in regulating adjacent gene expression.

Clare, J., Kapakos, J., Farabaugh, P., Dept. of Microbiology, University of Connecticut Health Center, Farmington: Transcription by yeast transposable elements and that of adjacent genes is controlled by both enhancer and silencer sites.

Errede, B.,[1,2] Company, M.,[1] Hutchison, C.A. III,[2,3] Teague, M.A.,[1] Connell, L.B.,[2] [1]Dept. of Chemistry, [2]Curriculum of Genetics, [3]Dept. of Microbiology and Immunology, University of North Carolina, Chapel Hill: Yeast cell type regulation of Ty1 enhancer function.

Kingsman, S.M., Mellor, J., Fulton, A., Rathjen, P., Wilson, W., Kingsman, A.J., Dept. of Biochemistry, University of Oxford, England: Multicomponent expression signals in the yeast transposon Ty.

Van Arsdell, S., Thorner, J., Dept. of Biochemistry, University of California, Berkeley: Yeast transposable element *sigma* functions as a hormone-inducible promoter.

Fedoroff, N.V., Kingsbury, J.A., Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: Isolation of the maize *Spm* element and characterization of the *a-m2* alleles.

Schwarz-Sommer, Z., Tacke, E., Saedler, H., Max-Plank-Institut für Züchtungsforschung, Köln, Federal Republic of Germany: Sequence comparison of states of *a1-m1* suggests a model of *Spm* (*En*) action.

Kim, H.Y., Raboy, V., Schiefelbein, J., Furtek, D., Nelson, O., Dept. of Genetics, University of Wisconsin, Madison: Transcription of defective *Spms* in *bz-m13* and change of state derivatives in maize.

Gierl, A., Pereira, A., Cuypers, H., Schwarz-Sommer, Z., Saedler, H., Max-Planck-Institut für Züchtungsforschung, Köln, Federal Republic of Germany: Molecular analysis of *En/Spm* transposable element system of *Zea mays*.

SESSION 2 FAMILIES OF TRANSPOSABLE ELEMENTS

Chairman: G. Fink, Whitehead Institute for Biomedical Research

Kermicle, J., Alleman, M., Laboratory of Genetics, University of Wisconsin, Madison: Analysis of tissue-specific differences among maize *R* alleles by transposable element mutagenesis and recombination.

Hoffman-Liebermann, B.,[1] Liebermann, D.,[1] Cohen, J.B.,[2] Kedes, L.K.,[2] Cohen, S.N.,[1] [1]Dept. of Genetics, [2]The MEDIGAN Project, Dept. of Medicine, Stanford University School of Medicine, California: TU elements—A heterogeneous family of modularly structured eukaryotic transposons.

Fawcett, D.H., Lister, C.K., Kellett, E., Finnegan, D.J., Dept. of Molecular Biology, University of Edinburgh, Scotland: Structure of the I factor controlling I-R hybrid dysgenesis in *Drosophila melanogaster*.

Spence, S., Gilbert, D., Jenkins, N., Copeland, N., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Acquisition, structure, and expression of newly acquired ecotropic proviruses in SWR/J-RF/J hybrid mice.

Gerats, A.G.M., Dept. of Genetics, Free University, Amsterdam, The Netherlands: Transposable elements in *Petunia hybrida*.

Rhodes, P.R., Vodkin, L.O., Plant Molecular Genetics Laboratory, USDA, Beltsville, Maryland: Highly structured borders characterize the Tgm1 element of soybean.

Cornu, A., Farcy, E., INRA, Dijon, France: Genetics of transposition at anthocyanin loci in petunia.

Yang, W.K.,[1] Ou, C.Y.,[1] Chang, L.Y.,[2] Myer, F.E.,[1] Yang, D.M.,[1] Roberson, L.E.,[1] [1]Biology Division, Oak Ridge National Laboratory; [2]Dept. of Microbiology, University of Tennessee, Knoxville: Expression of three distinct families of related endogenous retroviral gene elements in normal mouse tissues.

Salamini, F.,[1] DiFonzo, N.,[2] Marotta, R.,[2] Motto, M.,[2] [1]Max-Plank-Institut für Züchtungsforschung, Köln, Federal Republic of Germany; [2]Istituto Sperimentale per la Cerealicoltura Via Stezzano, Bergamo, Italy: Stability of components of the *o2-m(r)-Bg* system of maize controlling elements.

SESSION 3 GENE REGULATION BY TRANSPOSABLE ELEMENTS. II.

Chairman: P. Schedl, Princeton University

Tsubota, S., Schedl, P., Dept. of Biology, Princeton University, New Jersey: Characterization of control mutations generated by the mobilization of P elements.

Voelker, R.A., Sterling, J., National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Genetic and molecular characterization of suppressor of *sable* in *Drosophila melanogaster*.

Parkhurst, S., Corces, V., Dept. of Biology, Johns Hopkins University, Baltimore, Maryland: Retroviral elements and transcriptional suppression in *Drosophila*.

Zachar, Z., Chou, T.-R., Bingham, P.M., Dept. of Biochemistry, State University of New York, Stony Brook: Molecular analysis of an allele-specific suppressor locus in *Drosophila*.

Strand, D.,[1] Lambert, M.,[2] Franza, B.R.,[2] McDonald, J.,[1] [1]Dept. of Genetics, University of Georgia, Athens; [2]Cold Spring Harbor Laboratory, New York: Molecular analysis of the transcriptional effects of insertion of a *copia* element into the *Adh* locus of *Drosophila melanogaster*.

O'Hare, K.,[1] Roiha, H.,[2] Rubin, G.M.,[2] [1]Dept. of Biochem-

istry, Imperial College of Science and Technology, London, England; [2]Dept. of Biochemistry, University of California, Berkeley: P transposable element insertions at the *singed* and *white* loci of *Drosophila melanogaster*.

Mount, S.M., Rubin, G.M., Dept. of Biochemistry, University of California, Berkeley: Reversion and suppression of the *copia* insertion mutation *white-apricot* in the fruit fly *Drosophila melanogaster*.

Horak, I., Baumruker, T., Wirth, T., Gehe, C., Institut für Virologie und Immunobiologie der Universität Würzburg, Federal Republic of Germany: Tissue-specific expression of mouse retrotransposons LTR-IS/MuRRS.

Goodenow, M., Hayward, W.S., Memorial Sloan-Kettering Cancer Center, New York, New York: Insertional activation of the c-*myc* gene by avian leukosis virus—Integration and deletion of the provirus.

SESSION 4 CONTROL OF TRANSPOSITION AND EXCISION. I. MAINLY *DROSOPHILA*

Chairman: G.M. Rubin, University of California, Berkeley

Rubin, G.M., Laski, F.A., Rio, D.C., Dept. of Biochemistry, University of California, Berkeley: P-element transposition in *Drosophila*.

Mullins, M.C., Rio, D.C., Rubin, G.M., Dept. of Biochemistry, University of California, Berkeley: Definition of the *cis* sequences necessary for P-element transposition.

Simmons, M., Raymond, J., Boedigheimer, M., Dept. of Genetics and Cell Biology, University of Minnesota, St. Paul: The influence of nonautonomous P elements on manifestations of hybrid dysgenesis in *Drosophila melanogaster*.

Robertson, H.M., Engels, W.R., Laboratory of Genetics, University of Wisconsin, Madison: P-element regulation in *Drosophila melanogaster*—A sensitive assay of the repressor.

Engels, W., Robertson, H., Dept. of Genetics, University of Wisconsin, Madison: Cytotype regulation of P elements in the *Drosophila* germ line.

Lim, K.J.,[1] Judd, B.H.[2] [1]Dept. of Biology, University of Wisconsin, Eau Claire; [2]National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Mobility of a retrovirus-like transposable element *gypsy* in two laboratory strains of *Drosophila melanogaster*.

Walbot, V., McLaughlin, M., Warren, C., Dept. of Biological Sciences, Stanford, University, California: Inactivation and reactivation of the mutator transposable element system in maize assayed by somatic instability of *bronze2-Mu1*.

Baker, B.J.,[1] Fedoroff, N.,[2] Schell, J.,[1] [1]Max-Planck-Institut für Züchtungsforschung, Köln, Federal Republic of Germany; [2]Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: Transposition of maize controlling element *Ac* in tobacco.

SESSION 5 POSTER SESSION

Sol, K., Lapoine, M., MacLeod, M., Nadeau, C., DuBow, M.S., Dept. of Microbiology and Immunology, McGill University, Montreal, Canada: A cloned fragment of HeLa DNA containing consensus sequences of satellite II and III DNA hybridizes with *Drosophila* P element and with the 1.8-kb family of human *KpnI* fragments.

Deka, N., Paulson, K.E., Willard, C., Schmid, C.W., Dept. of Chemistry, University of California, Davis: A transposon-like element in human DNA.

Furano, A.V., Robb, F.T., Robb, S.M., D'Ambrosio, E., National Institutes of Health, Bethesda, Maryland: Members of the rat long interspersed repeated DNA family (LINE or L1 family) are mobile DNA elements.

Potter, S.S.,[1] Bourekas, E.,[1] Lamb, A.,[2] Lloyd, J.,[2] McNeish, J.,[1] Pine, D.,[1] Smyth-Templeton, N.,[1] [1]Children's Hospital Research Foundation, Division of Basic Science Research, Cincinnati, Ohio; [2]Wesleyan University, Middletown, Connecticut: Phylogenetic screening for transposable elements, insertion of *Drosophila* transposable elements into transgenic mice, and the nucleotide sequence of the loop of a complete foldback element.

Carlson, J.E., Kemble, R.J., Dept. of Plant Biology, Allelix, Inc., Mississauga, Ontario, Canada: The corn elements *Ac* (nuclear) and S2 (mitochondrial) share peptide sequence homology with viral reverse transcriptases.

Heidler, S.A., Liebman, S.W., Dept. of Biological Sciences, University of Illinois, Chicago: Transposition of the yeast Ty element into all regions of a gene.

Ruan, K., Emmons, S., Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Excision of the *Caenorhabditis elegans* transposable element Tc1 can be precise or imprecise.

Garon, C.F., Jansen, L.L., Rocky Mountain Laboratories, NIAID, Hamilton, Montana: Molecular cloning and characterization of inverted repeat sequences in human placental DNA.

Shell, B., Szurek, P., Dunnick, W., Dept. of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor: Interruption of immunoglobulin heavy-chain switch regions by insertion of transposon-like element MS3 in murine plasmacytoma P3.

Kim, B.D., Dept. of Plant Sciences, University of Rhode Island, Kingston: Four-stranded DNA—An intermediate of site-specific recombination.

Dreyfus, D.H., Emmons, S.W., Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Properties of a polymorphic foldback family in *Caenorhabditis elegans*.

Coupland, G.,[1] Baker, B.,[2] Fedoroff, N.,[3] Schell, J.,[2] Starlinger, P.,[1] [1]Institut für Genetik, University of Cologne; [2]Max-Planck-Institut für Züchtungsforschung, Köln, Federal Republic of Germany; [3]Carnegie Institution of Washington, Baltimore, Maryland: An assay for *Ac* excision in tobacco.

Kunze, R., Starlinger, P., Institut für Genetik, University of Cologne, Federal Republic of Germany: Studies on the expression of transposable element *Ac*.

Lechelt, C.,[1] Laird, A.,[2] Starlinger, P.,[1] [1]Institut für Genetik, University of Cologne, Federal Republic of Germany; [2]State Dept. of Science, Canberra, Australia: The P locus of *Zea mays*.

Merckelbach, A., Starlinger, P., Institut für Genetik, University of Cologne, Federal Republic of Germany: The aberrant *Ds* element in the *Adh1 2F11* allele

Müller-Neumann, M., Fusswinkel, H., Starlinger, P., Institut für Genetik, University of Cologne, Federal Republic of Germany: Studies on the expression of the *Zea mays* controlling element activator at the protein level

SESSION 6 CONTROL OF TRANSPOSITION AND EXCISION. II. WORMS AND YEAST

Chairman: P. Anderson, University of Wisconsin

Anderson, P., Collins, J., Eide, D., Saari, B., Dept. of Genetics, University of Wisconsin, Madison: Insertion and excision of the nematode transposable element Tc1.

Levitt, A., Ruan, K., Nelson, K., Emmons, S., Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Regulation of transposable elements in *Caenorhabditis elegans*.

Moerman, D.G., Mori, I., Benian, G.M., Waterston, R.H., Dept. of Genetics, Washington University, St. Louis, Missouri: Genetic and molecular studies of the transposable element Tc1 in *Caenorhabditis elegans*.

Rose, A.M., Harris, L.J., Rattray, B., Babity, J.M., Dept. of Medical Genetics, University of British Columbia, Vancouver, Canada: Tc1 transposition in Bristol strains of *Caenorhabditis elegans*.

Garfinkel, D.J., NCI-Frederick Cancer Research Facility, Frederick, Maryland: The transpositional competence of Ty elements in yeast.

Clare, J., Farabaugh, P., Dept. of Microbiology, University of Connecticut Health Center, Farmington: The gene encoding the reverse transcriptase of Ty elements is expressed by translational frameshifting.

Rothstein, R., Chrebet, G., Beniaminowitz, A., Wallis, J.W., Dept. of Genetics and Development, Columbia University College of Physicians & Surgeons, New York, New York: Genetic control of delta recombination.

Kingsman, A.J., Adams, S., Mellor, J., Malim, M., Kingsman, S.M., Dept. of Biochemistry, University of Oxford, England: Proteins and particles of yeast transposon Ty.

SESSION 7 MUTATIONS CAUSED BY TRANSPOSABLE ELEMENTS IN PLANTS

Chairman: D.S. Robertson, Iowa State University

Robertson, D.S., Dept. of Genetics, Iowa State University, Ames: Genetic analysis of putative deletions induced by the *Mu* system of maize.

Freeling, M., Sundaresan, V., Chen, C.-H., Oishi, K., Vayda, M., Lillis, M., Dept. of Genetics, University of California, Berkeley: The *Mu* transposons of maize.

Bennetzen, J.L., Dept. of Biological Sciences, Purdue University, West Lafayette, Indiana: Genetic and molecular properties of the *Mu* transposable element system of maize.

Chandler, V.,[1] Rivin, C.,[2] [1]Institute of Molecular Biology, University of Oregon, Eugene; [2]Dept. of Botany and Plant Pathology, Oregon State University, Corvallis: Molecular properties of endogenous *Mu* elements in maize.

Chourey, P.S.,[1,2] Still, P.E.,[2] DeRobertis, G.A.,[2] [1]USDA-ARS, [2]University of Florida, Gainesville: Altered temporal and/or spatial expression of sucrose synthetase genes in McClintock's *sm-m6258* and *Ac*-induced revertant alleles in maize.

Dennis, E.S.,[1] Taylor, B.H.,[1] Peterson, T.A.,[1] Howard, E.A.,[1] Walker, J.A.,[1] Gerlach, W.L.,[1] Sachs, M.M.,[1] Peacock, W.J.,[1] Schwartz, D.,[3] Beach, L.,[4] [1]CSIRO Division of Plant Industry, Canberra, Australia; [2]University of Indiana, Bloomington; [3]Washington University, St. Louis, Missouri; [4]Pioneer Hi-Bred Seeds, Johnston, Iowa: The Ac/Ds controlling element system of maize.

Döring, H.-P., Nelsen, B., Tillmann, E., Institut für Genetik, University of Cologne, Federal Republic of Germany: *Ds*-mediated rearrangements at the *sh* locus of maize.

Coen, E., Martin, C., Robbins, T., Mackay, S., Hudson, A., Almeida, J., Carpenter, R., John Innes Institute, Norwich, England: Novel patterns of plant gene expression arise by transposable element excision and rearrangement.

Sommer, H., Bonas, U., Hehl, R., Krebbers, E., Piotrowiak, R., Saedler, H., Max-Plank-Institut für Züchtungsforschung, Köln, Federal Republic of Germany: Transposable elements at the *nivea* locus of *Antirrhinum majus*.

SESSION 8a USES OF TRANSPOSONS

Chairman: P.A. Peterson, Iowa State University

Peterson, P.A.,[1] Wienand, U.,[2] Paz-Ares, J.,[2] Weydemann, U.,[2] Niesbach-Klösgen, U.,[2] Martin, W.,[2] Saedler, H.,[2] [1]Dept. of Agronomy, Iowa State University, Ames; [2]Max-Plank-Institut, Köln, Federal Republic of Germany: Four decades of studies with the 9.4-kb enhancer transposable element lead to the cloning of four genes in *Zea mays*.

Shepherd, N.S.,[1] Schwarz-Sommer, Z.,[2] Sorrentino, J.,[1] Mattes, M.,[1] [1]E.I. du Pont de Nemours & Co., Wilmington, Delaware; [2]Max-Planck Institut fur Züchtungsforschung, Köln, Federal Republic of Germany: Unstable alleles of the *A1* gene of *Zea mays*.

Williamson, V.M., Paquin, C.E., Walton, J., ARCO Plant Cell Research Institution, Dublin, California: Cloning of the *Adh4* gene of yeast by Ty tagging.

SESSION 8b EVOLUTION AND DISTRIBUTION OF P ELEMENTS

Chairman: P.A. Peterson, Iowa State University

Petes, T.D.,[1] Goebl, M.,[2] Vincent, A.,[1] Stamenkovich, D.,[1] [1]Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois; [2]Dept. of Genetics, University of Washington, Seattle: Genetic factors influencing the spread of transposable elements in the yeast genome.

Langley, C.H., National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Population genetics of *copia*-like elements.

Saedler, H., Schwarz-Sommer, Z., Max-Plank-Institut für Züchtungsforschung, Köln, Federal Republic of Germany: Plant transposable elements and their role in evolution.

Bucheton, A., Simonelig, M., Crozatier, M., Vaury, C., Pélisson, A., Laboratoire de Génétique, Université de Clermont-Ferrand, Aubière, France: Intraspecific and interspecific distribution of sequences homologous to the I factor involved in *IR* hybrid dysgenesis in *Drosophila melanogaster*.

Georgiev, G.P., Gerasimova, T.I., Mizrokhi, L.J., Ilyin, Y.V., Institute of Molecular Biology, USSR Academy of Sciences, Moscow: Transposition explosions and transposition memory in *Drosophila melanogaster*.

SESSION 9 INTERMEDIATES AND ENZYMES

Chairman: J. Strathern, NCI-Frederick Cancer Research Facility

Varmus, H.E.,[1,2] Mossie, K.G.,[1] Hagino-Yamagishi, K.,[2] Donehower, L.,[2] Basu, S.,[2] Depts. of [1]Biochemistry and Biophysics, [2]Microbiology, University of California, San Francisco: Characterization of Mo-MLV integration and putative retrotransposon intermediates in *Drosophila* and yeast.

Strathern, J.N., Raveh, D., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Physical intermediates generated during the mating-type switch in *Saccharomyces cerevisiae*.

Skalka, A.M.,[1] Duyk, G.,[2] Alexander, F.,[1] Cobrinik, D.,[2] Soltis, D.,[1] Leis, J.,[2] [1]Dept. of Molecular Oncology, Roche Institute of Molecular Biology, Nutley, New Jersey; [2]Dept. of Biochemistry, Case Western Reserve University, Cleveland, Ohio: Integration of avian retroviral DNA—Biochemical studies.

Klar, A.J.S., Cold Spring Harbor Laboratory, New York: Developmental asymmetry among daughter cells is a consequence of inheriting nonequivalent parental DNA chains in fission yeast.

Grandgenett, D., Institute for Molecular Virology, St. Louis University, Missouri: Nuclease mechanism of the avian retrovirus pp32 integrase.

Goff, S.P., Roth, M.J., Tanese, N., Colicelli, J., Dept. of Biochemistry and Molecular Biophysics, Columbia University College of Physicians & Surgeons, New York, New York: Analysis of the integration site and integrase function of the Moloney murine leukemia virus.

Flavell, A., Brierley, C., Dept. of Biochemistry, University of Dundee, Scotland: Structure and origin of extrachromosomal *copia* elements.

Veluthambi, K., Jayaswal, R.K., Hallberg, C., Gelvin, S.B., Dept. of Biological Sciences, Purdue University, West Lafayette, Indiana: Molecular genetics of *Agrobacterium tumefaciens*—Plant interactions.

Greenblatt, I.M., Depts. of Molecular and Cell Biology, University of Connecticut, Storrs: *Ac* transposition from *P* in maize.

Chen, J., Greenblatt, I., Dellaporta, S.L., Cold Spring Harbor Laboratory, New York: Molecular analysis of *Ac* transposition from *P* locus in maize.

Ilyin, Y.V., Arkhipova, I.R., Mazo, S., Institute of Molecular Biology, USSR Academy of Sciences, Moscow: Reverse transcriptase intermediates in transposition of *mdg* elements in *Drosophila melanogaster*.

I. Mori, K. Nelson

RNA Processing

May 14–May 18

ARRANGED BY

Robert P. Perry, Institute for Cancer Research, Philadelphia
Hugh D. Robertson, Rockefeller University
Susan M. Berget, Baylor College of Medicine

322 participants

The RNA Processing spring meeting brought together researchers interested in all aspects of the maturation of primary transcripts into mature functional RNA molecules, as well as the processing reactions involved in forming transfer, messenger, and ribosomal RNAs in bacteria, yeast, and higher cells.

The five-day program included two full poster sessions and eight sessions on RNA catalyzed reactions, structure and mechanisms, tRNA processing, rRNA processing, mRNA splicing, mRNA processing, and metabolism, small RNAs and RNPs, and Poly(A) and 3′ ends.

This meeting was supported in part by the National Institute of General Medical Sciences/National Institutes of Health and the National Science Foundation.

SESSION 1 RECOGNITION SIGNALS FOR mRNA SPLICING

Chairman: C. Weissmann, University of Zurich

Aebi, M., Hornig, H., Weissmann, C., Institut für Molekularbiologie I, Universität Zürich, Switzerland: Sequence requirements for splicing of pre-mRNA of higher eukaryotes.

Ohshima, Y., Tatei, K., Kitayama, H., Takemura, K., Mayeda, A., Institute of Biological Sciences, University of Tsukuba, Japan: Sequence-specific binding to 5′ and 3′ splice sites by an snRNP fraction.

H. Robertson, R. Cole, W. Filipowicz

Reed, R., Maniatis, T., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: A role for exon sequences and splice site proximity in splice site selection.

Zhuang, Y., Chabot, B., Weiner, A.M., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Design and construction of a suppressor U1 snRNA.

Zillman, M., Robberson, B., Berget, S.M., Dept. of Biochemistry, Baylor College of Medicine, Houston, Texas: U1, U2, and U4/6 snRNPs are required for ordered assembly of the active spliceosome.

Black, D.L., Steitz, J.A., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Evidence for the involvement of the snRNP containing the U4 and U6 RNAs in premessenger splicing.

Noble, J., Chaudhuri, M., Prives, C., Manley, J.L., Dept. of Biological Sciences, Columbia University, New York, New York: Alternative splicing path-ways of SV40 early pre-mRNA in vitro.

Cellini, A., Rossi, J., Dept. of Molecular Genetics, Beckman Research Institute of the City of Hope, Duarte, California: Intron-encoded features governing the choice of branch site during pre-mRNA splicing in yeast.

Pikielny, C.,[1] Rosbash, M.,[2] Depts. of [1]Biochemistry, [2]Biology, Brandeis University, Waltham, Massachusetts: A high-resolution assay of spliceosome assembly and identification of three snRNAs associated with the yeast spliceosome.

Freistadt, M.S.,[1] Cross, G.A.M.,[1] Branch, A.D.,[2] Robertson, H.D.,[2] Laboratory of [1]Molecular Parasitology, [2]Genetics, Rockefeller University, New York, New York:

Analysis of the mini-exon precursor RNA of *Trypanosoma brucei* and some possible in vivo processing intermediates.

Guthrie, C., Riedel, N., Simmons, T., Parker, R., Siliciano, P., Dept. of Biochemistry and Biophysics, University of California, San Francisco: Yeast contain an unexpected diversity of snRNAs, many of which are dispensable for growth.

SESSION 2 mRNA SPLICING: RNA INTERMEDIATES

Chairman: P. Perlman, Ohio State University

Solnick, D., Dept. of Molecular Biophysics and Biochemistry, Yale University Medical School, New Haven, Connecticut: Does pre-mRNA have secondary structure in vivo?

Ruskin, B., Nelson, K., Shneider, N., Green, M.R., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Effects of RNA sequence and secondary structure on RNA–factor interactions and splicing in vitro.

Hornig, H., Aebi, M., Weissmann, C., Institut für Molekularbiologie I, Universität Zürich, Switzerland: Rabbit β-globin pre-mRNA splicing—No evidence for a functionally significant base-pairing interaction between the 5′ splice region and the branch site.

Plotch, S.J., Krug, R.M., Memorial Sloan-Kettering Cancer Center, New York, New York: In vitro splicing of influenza viral NS1 mRNA and NS1-β-globin chimeras—Possible mechanisms for the control of viral mRNA splicing.

Thompson-Jäger, S., Duchêne, M., Löw, A., Domdey, H., Genzentrum der Ludwig-Maximilians-Universität München, Federal Republic of Germany: Structural features required in the yeast actin mRNA-precursor for efficient splicing.

Adami, G.R., Carmichael, C.G., Dept. of Microbiology, University of Connecticut Health Center, Farmington: Mutants in the polyoma virus late leader may define a minimum exon length for splicing and mRNA accumulation.

Furdon, P., Kole, R., Dept. of Pharmacology and Lineberger Cancer Research Center, University of North Carolina, Chapel Hill: Effects of exon sequences on in vitro splicing of human β-globin pre-mRNA.

Gattoni, R., Schmitt, P., Keohavong, P., Stévenin, J., INSERM, CNRS, Institut de Chimie Biologique, Strasbourg, France: In vitro systems promote alternative splicing of natural adenovirus E1A transcripts.

Lang, K.M., Spritz, R.A., University of Wisconsin, Madison: In vitro splicing pathways of pre-mRNAs containing multiple IVSs.

Hartmuth, K., Barta, A., Institute of Biochemistry, University of Vienna, Austria: Evidence for unusual branch point selection in in vitro splicing of human growth hormone pre-mRNA.

Posters:

Adam, S.A., Nakagawa, T., Woodruff, T.K., Dreyfuss, G., Dept. of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, Illinois: Isolation and expression of the gene for the mRNA poly(A) binding protein.

Aloni, Y.,[1] Rosnekov, O.,[1] Seiberg, M.,[1] Kessler, M.,[1] Levine, A.J.,[2] [1]Dept. of Genetics, Weizmann Institute of Science, Rehovot, Israel; [2]Dept. of Molecular Biology, Princeton University, New Jersey: Eukaryotic RNA polymerase II can prematurely terminate transcription at precise sites that resemble a prokaryotic termination signal.

Altuvia, S., Oppenheim, A., Dept. of Molecular Genetics, Hebrew University-Hadassah Medical School, Jerusalem, Israel: RNase III positively regulates translation of the phage lambda CIII gene.

Andrews, P.G.,[1] Walter, P.,[2] Kole, R.,[1] [1]Dept. of Pharmacology and Lineberger Cancer Research Center, University of North Carolina, Chapel Hill; [2]Dept. of Biochemistry and Biophysics. University of California, San Francisco: Autoimmune sera recognize the 68-kD subunit of the signal recognition particle.

Ares, M., Jr., Dept. of Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Connecticut: Structure and function of yeast U2 RNA.

Arrigo, A.-P.,[1,2] Simon, M.,[2] Darlix, J.-D.,[3] [1]Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; [2]Dept. of Molecular Biology, University of Geneva, Switzerland; [3]CNRS, University Paul Sabatier, Toulouse, France: Characterization of a 20S RNP particle ubiquitous from yeast to man.

Baas, P.D., Bovenberg, R.A.L., van de Meerendonk, W., Institute of Molecular Biology and Medical Biotechnology and Laboratory for Physiological Chemistry, State University of Utrecht, The Netherlands: A model for the processing pathway of the primary transcript of the human calcitonin gene.

Barta, A., Hartmuth, K., Institute of Biochemistry, University of Vienna, Austria: In vitro splicing of a plant pre-mRNA in a HeLa cell nuclear extract.

Been, M.D., Cech, T.R., Dept. of Chemistry and Biochemistry, University of Colorado, Boulder: Specific base changes that affect splice site selection of the self-splicing intron in *Tetrahymena* nuclear pre-rRNA.

Bhat, B., Brady, H., Wold, W., Institute for Molecular Virology, St. Louis University School of Medicine, Missouri: Region E3 of adenovirus—Deletion mutants that alter differential RNA processing.

Bohmann, D.,[1] Tebb, G.,[2] Keller, W.,[1] Mattaj, I.W.,[2] [1]DKFZ, German Cancer Research Center; [2]EMBL, Heidelberg, Federal Republic of Germany: Transcription factors interacting with a *Xenopus* U2 snRNA gene promoter.

Bringmann, P., Reuter, R., Winkelmann, G., Bochnig, P., Lührmann, R., Max-Plank-Institut für molekulare Genetik, Otto-Varburg-Laboratories, Berlin, Federal Republic of Germany: Structure of U snRNPs and their assembly into spliceosomes.

Browner, M.F., Lawrence, C.B., Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas: Probing mRNA secondary structure in native particles.

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SESSION 3 mRNA SPLICING: ACTIVE PROTEIN COMPLEXES

Chairman: M. Mathews, Cold Spring Harbor Laboratory

Lin, R.-J., Lustig, A., Abelson, J., Division of Biology, California Institute of Technology, Pasadena: In vitro analysis of temperature-sensitive mutations affecting mRNA splicing in yeast.

Last, R., Anthony, J., Maddock, J., Woolford, J., Carnegie-Mellon University, Pittsburgh, Pennsylvania: Analysis of yeast gene products necessary for mRNA splicing.

Bindereif, A., Green, M.R., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Characterization of RNP complexes generated during pre-mRNA splicing in vitro.

Konarska, M., Sharp, P.A., Center for Cancer Research and Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Electrophoretic analysis of splicing complexes.

Krämer, A., Frendewey, D., Baier, T., Frick, M., Keller, W., Division of Molecular Biology, Institute of Cell and Tumor Biology, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Analysis of the involvement of snRNPs, protein factors, and ATP in the pre-mRNA splicing reaction.

Perkins, K.K., Furneaux, H.M., Arenas, J., Young, B., Freyer, G.A., Hurwitz, J., Molecular Biology Program, Memorial Sloan-Kettering Cancer Research Center, New York, New York: mRNA splicing using isolated fractions from HeLa cells.

Choi, Y.D.,[1] Grabowski, P.J.,[2] Sharp, P.A.,[2] Dreyfuss, G.,[1] [1]Dept. of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, Illinois; [2]Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Role for hnRNPs in RNA splicing.

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SESSION 4 RNA 3′ END FORMATION/POLYADENYLATION

Chairman: J. Alwine, University of Pennsylvania

Gil, A., Proudfoot, N.J., Sir William Dunn School of Pathology, University of Oxford, England: In vivo mRNA 3′ end formation of mutant rabbit β-globin clones containing synthetic sequences.

Zhang, F., Cole, C.N., Dept. of Biochemistry and the Molecular Genetics Center, Dartmouth Medical School, Hanover, New Hampshire: Linker scanning and internal deletion analysis of the poly(A) region of the HSV-1 *tk* gene defines essential sequences and optimal spatial requirements for polyadenylation.

Kessler, M.M., Beckendorf, R.C., Westhafer, M.A., Nordstrom, J.L., Dept. of Biochemistry and Biophysics, Texas A & M University and Texas Agricultural Expt. Station, College Station: Analysis of the SV40 early transcript and a synthetic poly(A) signal.

Wickens, M., Bardwell, V., Conway, L., Sheets, M., Stephenson, P., Zarkower, D., Dept. of Biochemistry, University of Wisconsin, Madison: Regions of SV40 late pre-mRNA that specify accurate and efficient cleavage and polyadenylation.

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Gallinaro, H., Sittler, A., Jacob, M., CNRS, INSERM, Faculté de Médecine, Strasbourg, France: Splicing in vivo of the transcripts from early region 3 of adenovirus 2.

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Ruohola, H.,[1] Baker, S.M.,[2] Parker, R.,[3] Platt, T.,[1] [1]Dept of Cell Biology, Yale University, New Haven, Connecticut, [2]Dept. of Biochemistry, University of Rochester Medical Center, New York; [3]Dept. of Biochemistry and Biophysics, University of California, San Francisco Orientation-dependent 3′ end formation—*cyc1* transcription terminator fragments inserted in the actin intron of yeast

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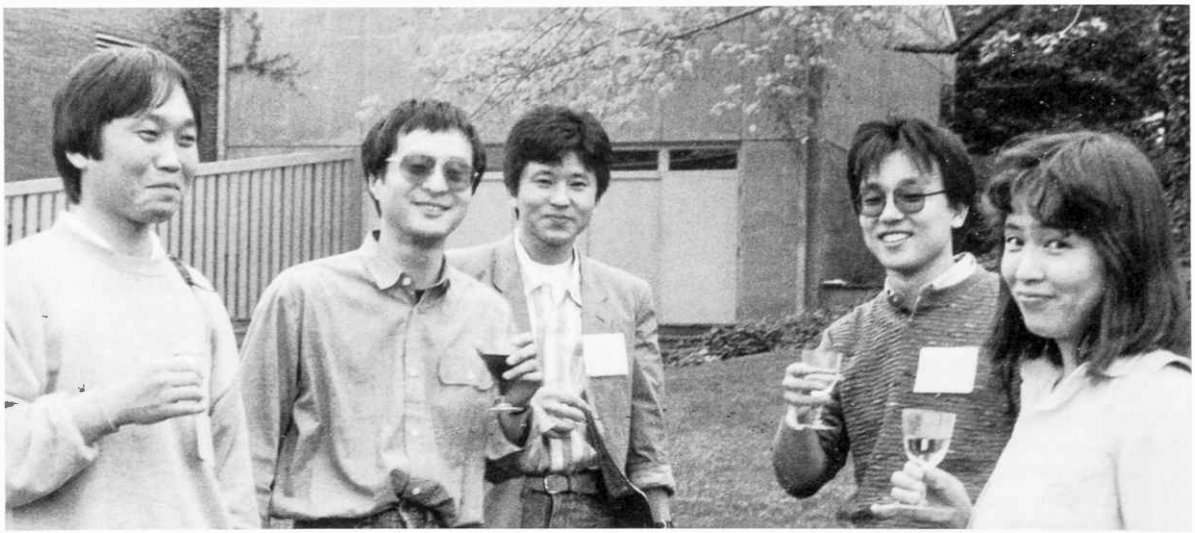

H. Sakamoto, T. Toda, K. Umesono, T. Kochi, S. Toda

R. Sperling, M. Mathews, M. Deutscher

SESSION 5 RNA PROCESSING AND TURNOVER AS A MODE OF REGULATION

Chairman: M. Rosbash, Brandeis University

Laski, F.A., Rio, D.C., Rubin, G.M., Dept. of Biochemistry, University of California, Berkeley: *Drosophila* P-element transposition is regulated at the level of mRNA splicing.

Peterson, M.L., Perry, R.P., Institiute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania: Regulated production of μ_m and μ_s mRNA – The importance of linkage and intron length.

Shaw, G., Kamen R., Genetics Institute, Cambridge, Massachusetts: Selective degradation of mRNAs mediated by a sequence in the 3′ noncoding region.

Svensson, C., Akusjärvi, G., Dept. of Medical Genetics, Uppsala University, Biomedical Center, Sweden: Defective RNA splicing in the absence of adenovirus VA RNA_I.

Caffarelli, E., Gehring, C., Fragapane, P., Cutruzzolà, F., Bozzoni, I., Centro Acidi Nucleici, CNR, Roma, Italy: Splicing of ribosomal protein transcripts in *Xenopus laevis* oocytes.

Dabeva, M.A., Warner, J.R., Dept. of Cell Biology, Albert Einstein College of Medicine, Bronx, New York: A yeast ribosomal protein regulates the splicing of the transcript of its own gene.

Ross, J., Kobs, G., Peltz, S.W., Brewer, G., McArdle Laboratory for Cancer Research and Dept. of Pathology, University of Wisconsin, Madison: Turnover of mRNA in cell-free extracts.

Yost, H.J., Lindquist, S.L., Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois: RNA splicing is interrupted by heat shock and rescued by heat-shock protein synthesis.

SESSION 6 CATALYTIC RNA

Chairman: P. Sharp, Massachusetts Institute of Technology

Zaug, A.J., Cech, T.R., Dept. of Chemistry and Biochemistry, University of Colorado, Boulder: Discovery of a new RNA enzyme.

Burke, J.,[1] Kerker, B.,[1] Tierney, W.,[1] Williamson, C.,[1] Zaug, A.,[2] Cech, T.,[2] [1]Dept. of Chemistry, Williams College, Williamstown, Massachusetts; [2]Dept. of Chemistry and Biochemistry, University of Colorado, Boulder: Functions of highly conserved pairing and nonpairing sequences in self-splicing of the *Tetrahymena* rRNA intervening sequence.

Hall, D.,[1] Povinelli, C.,[1] Ehrenman, K.,[2,3] Pedersen-Lane, J.,[2] Belfort, M.,[2] [1]School of Applied Biology, Georgia Tech, Atlanta; [2]Wadsworth Laboratories, New York State Dept. of Health, [3]Dept. of Microbiology and Immunology, Albany Medical College: Nondirected splicing-defective mutations in the intron of the phage T4 *td* gene.

Peebles, C.L.,[1] Romiti, S.L.,[1] Perlman, P.S.,[2] Jarrell, K.A.,[2] [1]Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania; [2]Dept. of Genetics, Ohio State University, Columbus: Alternative reactions of a type II self-splicing intron.

Tabak, H.F.,[1] Van der Horst, G.,[1] Kamps, L.,[1] Arnberg, A.C.,[2] Van der Veen, R.,[1] Grivell, L.A.,[1] [1]Laboratory of Biochemistry, University of Amsterdam; [2]Laboratory of Biochemistry, State University of Groningen, The Netherlands: Intron-catalyzed formation of interlocked RNA circles.

Altman, S., Baer, M., Gold, H., Guerrier-Takada, C., Lawrence, N., Vioque, A., Dept. of Biology, Yale University, New Haven, Connecticut: Enzymatic cleavage of RNA by RNA.

Pace, N.R., Reich, C., Olsen, G.J., Waugh, D.S., James, B.D., Liu, J., Pace, B., Gardiner, K.J., Marsh, T.L., Dept. of Biology, Indiana University, Bloomington: The *Bacillus subtilis* RNase P

Chang, D.D., Clayton, D.A., Dept. of Pathology, Stanford University School of Medicine, California: A site-specific endoribonuclease involved in the priming of mitochondrial DNA synthesis at the heavy-strand origin – The enzymatic activity requires a novel ~130-nucleotide RNA species.

SESSION 7 RIBOSOMAL RNA PROCESSING

Chairman: A. Lambowitz, St. Louis University School of Medicine

Garriga, G., Lambowitz, A., Dept. of Biochemistry, St. Louis University School of Medicine, Missouri: Protein-dependent splicing of a group I intron in RNP preparations and by soluble fractions.

Tanner, N.K., Cech, T.R., Dept. of Chemistry and Biochemistry, University of Colorado, Boulder: A guanosine binding site required for cyclization of the self-splicing IVS RNA from *Tetrahymena thermophila*.

Uhlenbeck, O.C., Wu, H., Milligan, J., Sampson, J., Dept. of Chemistry and Biochemistry, University of Colorado, Boulder: Structure:Function relations in RNA.

Robertson, H.D., Benenfeld, B.J., Niebling, K., Branch, A.D., Rockefeller University, New York, New York: Local tertiary structure at an RNase III cleavage site as defined by ultraviolet light-induced cross-linking.

Wollenzien, P.L., Dept. of Biochemistry, St. Louis University School of Medicine, Missouri: Structure of *E. coli* 16S rRNA–RNA sites in contact with the 3′ terminal region identified by psoralen cross-linking and reverse transcription.

Rairkar, A., Rubino, H., Lockard, R.E., Dept. of Biochemistry, Cornell University Medical College, New York, New York: The secondary structure of rabbit 18S ribosomal RNA determined from biochemical and phylogenetic data.

Kass, S.,[1] Craig, N.,[2] Sollner-Webb, B.,[1] [1]Johns Hopkins University School of Medicine, Baltimore; [2]University of Maryland Baltimore County, Catonsville: The primary processing events of mouse and human rRNA are analogous and directed by conserved sequences.

Mishima, Y., Katayama, M., Mitsuma, T., Ogata, K., Dept. of Biochemistry, Niigata University School of Medicine, Japan: Processing of mouse rRNA in a cell-free system.

Posters:

Plunkett, G., III, Echols, H., Dept. of Molecular Biology, University of California, Berkeley: The role of RNA processing and degradation in retroregulation of bacteriophage lambda *int* gene expression.

Porter, T.N., Berget, S.M., Wilson, J.H., Dept. of Biochemistry, Baylor College of Medicine, Houston, Texas: In direct competition, splicing predominates over polyadenylation.

Raziuddin, Braaten, D.C., Schlessinger, D., Dept. of Microbiology and Immunology, Washington University School of Medicine, St. Louis, Missouri: Transfected mouse rDNA is expressed from a new upstream start site.

Reddy, R., Henning, D., Das, G., Dept. of Pharmacology, Baylor College of Medicine, Houston, Texas: U6 RNA, a capped snRNA, is transcribed by RNA polymerase III.

Reyes, V.M., Strobel, M., Abelson, J., Division of Biology, California Institute of Technology, Pasadena: tRNA splicing in *Saccharomyces cerevisiae*.

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Thomas, J.R., Little, R., Braaten, D.C., Powers, J., Schlessinger, D., Dept. of Microbiology and Immunology, Washington University School of Medicine, St. Louis, Missouri: Nucleolar organization assessed by DNase I resistance of rDNA spacers bound to the nucleolar matrix.

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Walstrum, S.A., Uhlenbeck, O.C., Dept. of Chemistry and Biochemistry, University of Colorado, Boulder: Determining the substrate specificity of a self-splicing RNA using modified oligoribonucleotides.

West, D., Belfort, M., Maley, G., Maley, F., New York State Dept. of Health, Albany, New York: In vivo and in vitro expression of an intron-deleted phage T4 *td* gene.

Westaway, S., Phizicky, E., Abelson, J., Division of Biology, California Institute of Technology, Pasadena: *Saccharomyces cerevisiae* tRNA ligase—Characterization of the gene.

Whitelaw, E., Proudfoot, N.J., Sir William Dunn School of Pathology, University of Oxford, England: Transcriptional termination is linked to 3′-end processing in the human α2-globin gene.

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Yancey, S.D., Arraiano, C.M., Kushner, S.R., Dept. of Genetics, University of Georgia, Athens Analysis of mRNA turnover in *Escherichia coli*.

Yang, U.-C., Flint, S.J., Dept. of Molecular Biology, Princeton University, New Jersey Transport of transcripts of integrated viral and inducible genes in adenovirus-infected cells.

Zeitlin, S., Parent, A., Silverstein, S., Efstratiadis, A., Columbia University, New York, New York Pre-mRNA splicing and the nuclear matrix

SESSION 8 TRANSFER RNA PROCESSING

Chairman: W. Filipowicz, Friedrich Miescher Institute

Clark, M.W., Abelson, J., Division of Biology, California Institute of Technology, Pasadena: The cellular location of tRNA ligase in *Saccharomyces cerevisiae*.

Willis, I.,[1] Söll, D.,[1] Greer, C.L.,[2] [1]Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut; [2]Dept. of Biological Chemistry, College of Medicine, University of California, Irvine: Inefficient, imprecise, and independent splice-site cleavage of mutant tRNA precursors by the *Saccharomyces cerevisiae* splicing endonuclease.

Belford, H.G., Greer, C.L., Dept. of Biological Chemistry, College of Medicine, University of California, Irvine: Stable complex formation in tRNA splicing in vitro.

Knapp, G., Dept. of Microbiology, University of Alabama, Birmingham. Analysis of the yeast tRNA splicing endonuclease and its interactions with wild-type and mutant pre-tRNAs.

Castaño, J.,[1] Ornberg, R.,[2] Tobian, J.,[1] Zasloff, M.,[1] [1]NICHD, [2]NIADDK, National Institutes of Health, Bethesda, Maryland: Purification and characterization of a 5′ pre-tRNA processing nuclease from *Xenopus laevis* ovaries.

Rosen, A., Daniel, V., Dept. of Biochemistry, Weizmann Institute of Science, Rehobot, Israel: Transcription and precursor processing of a rat $tRNA^{Glu}$ gene.

Hai, T.-Y, Reilly, R.M., RajBhandary, U.L., Dept. of Biology,

T. Martin, G. Brawerman, R. Perry

Massachusetts Institute of Technology, Cambridge: The role of the TψC loop in tRNA structure and processing—Analyses of mutants in the TψC loop of the *Escherichia coli* SuIII $tRNA^{Tyr}$.

Roe, B.A., Wilson, R.K., Dept. of Chemistry, University of Oklahoma, Norman: Studies on the role of ms^2i^6A in *Escherichia coli* $tRNA^{Phe}$—Aminoacylation kinetics and codon-reading properties.

Buzayan, J.M., Hampel, A., Gerlach, W.L., Bruening, G., Dept. of Plant Pathology, College of Agriculture and Environmental Sciences, University of California, Davis: Autolytic processing and subsequent spontaneous ligation of RNAs that are complementary to a plant virus satellite RNA.

Tabler, M., Tsagris, M., Günther, I., Sänger, H.L., Max-Planck Institut für Biochemie, Martinsried, Federal Republic of Germany: Sequence requirements for the cleavage and circularization of oligomeric linear potato spindle tuber viroid RNA.

RNA Tumor Viruses

May 20–May 25

ARRANGED BY

Robert Eisenman, Fred Hutchinson Cancer Research Center
Anna M. Skalka, Roche Institute of Molecular Biology

353 participants

The 1986 RNA Tumor Virus meeting brought together over 400 researchers with interests ranging from clinical medicine to molecular genetics. The meeting reflected three major currents in the RNA tumor virus field: retroviral structure and replication, the biology of human retroviruses, and the structure and function of retroviral and cellular oncogenes. Each of these areas tended to draw upon one another. Studies on retroviral replication focused on the structure and mechanism of action of the viral endonuclease in mediating integration of viral DNA and on the interaction of retroviral structural proteins with nucleic acids. An important concept concerned the prevalence of ribosomal frameshifting in generating and regulating the quantities of viral gene products. Progress was reported in identifying a viral RNA structural motif that may promote frameshifting.

Considerable attention was devoted to the mechanisms regulating retroviral gene expression both by cell-type-specific factors and by factors encoded within

the viral genome. The theme of *trans*-activation was particularly apparent in studies of the human retroviruses. Of potential importance was the finding of an RNA from a new open reading frame within the HIV (AIDS retrovirus) *trans*-activator region, whose product may play a role in relieving repression of HIV structural gene expression. Virus-encoded *trans*-activating factors appear likely to not only affect viral genes, but probably modulate expression of cellular genes as well, and thus their action may have important consequences for the host cell. Studies on HIV showed that this virus infects B cells as well as T cells and neuronal cells.

Nearly half of the presentations at the meeting concerned viral and cellular oncogenes. Sessions dealt with oncogene proteins related to growth factors and their receptors and oncogenes whose products function in the nucleus and cytoplasm. Highlights included the findings that inhibition of phosphorylation at the carboxy terminus of the c-*src* activates its kinase activity, a transformation phenotype induced by v-*src* is related to splicing of a cryptic intron, the protein encoded by *myc* can be found in association with snRNPs, and the function of the v-*fms*/CSF-1 receptor product may involve activation of phospholipase C.

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SESSION 1 MOLECULAR BIOLOGY OF REPLICATION AND PROVIRAL INTEGRATION

Chairmen: **J. Coffin,** Tufts University School of Medicine
H. Robinson, Worcester Foundation

Vijaya, S., Steffen, D.L., Robinson, H.L., Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Retroviral integrations map near regions of open chromatin structure.

Shih, C.-C., Coffin, J.M., Tufts University School of Medicine, Boston, Massachusetts: Analysis of avian retrovirus integration sites.

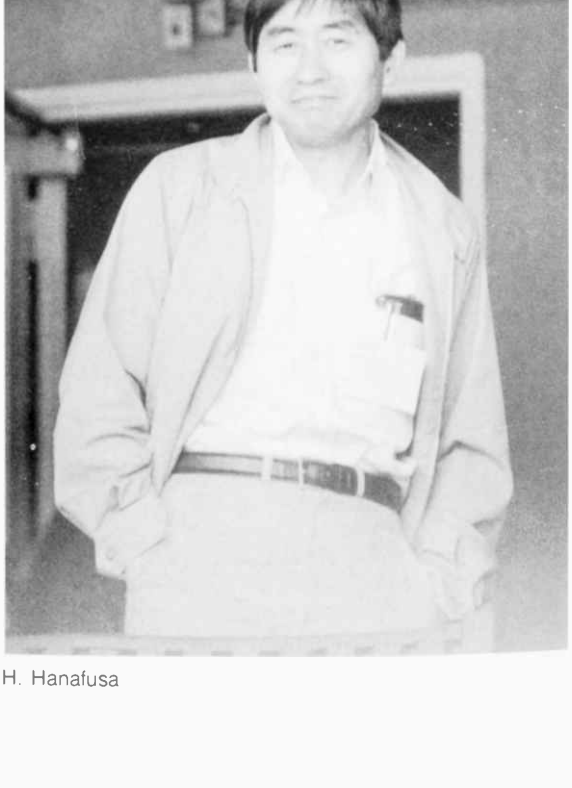
Soltis, D.,[1] Cobrinik, D.,[2] Terry, R.,[1] Leis, J.,[2] Skalka, A.M.,[1] [1]Dept. of Molecular Oncology, Roche Institute of Molecular Biology, Nutley, New Jersey; [2]Dept. of Biochemistry, Case Western Reserve University, Cleveland, Ohio: Active avian retroviral *pol* gene proteins expressed in *E. coli*.

Roth, M.J., Tanese, N., Okada, A., Goff, S.P., Dept. of Biochemistry and Molecular Biophysics, Columbia University College of Physicians & Surgeons, New York, New York: Expression of the 3′ terminal region of the *pol* gene of Mo-MLV in bacteria and animal cells.

Levin, J.G.,[1] Hu, S.C.,[1] Court, D.,[2] Zweig, M.,[3] Rein, A.,[4] [1]NICHD, [2]NCI, National Institutes of Health, Bethesda, Maryland; [3]PRI, [4]NCI-Litton Frederick Cancer Research Facility, Frederick, Maryland: A disulfide-linked reverse transcriptase–endonuclease complex in MLV particles.

Ramsey, C.A., Luk, K.-C., Panganiban, A.T., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: DNA–DNA interaction is required for SNV DNA integration; the integrase can mediate insertion of nonretroviral DNA in *trans*; integrase produced in *E. coli* has DNA binding activity.

Katz, R.A., Skalka, A.M., Roche Institute of Molecular Biology, Roche Research Center, Nutley, New Jersey: Genetic and biochemical analysis of mutations in the ASLV



H. Hanafusa

pol and *pol-env* overlap regions – Implications of *pol* proteolytic processing and *env* splicing.

Cobrinik, D.,[1] Katz, R.,[2] Terry, R.,[2] Skalka, A.M.,[2] Leis, J.,[1] [1]Case Western Reserve University, Cleveland, Ohio; [2]Roche Institute of Molecular Biology, Nutley, New Jersey: LTR junction sequences required for in vitro ASLV *pol*-endo cleavage and virus production.

Miller, C.K., Temin, H.M., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Analysis of spontaneously arising revertants of nontransforming *rel*-containing viruses.

Jacks, T.,[1] Majors, J.,[2] Luciw, P.,[3] Varmus, H.E.,[1] [1]Depts. of Biochemistry and Microbiology, University of California, San Francisco; [2]Dept. of Biological Chemistry, University of Washington School of Medicine, St. Louis, Missouri; [3]Chiron Research Laboratories, Chiron Corp., Emeryville, California: Analysis of ribosomal frameshifting in retroviral gene expression.

Gloger, I., Panet, A., Hebrew University-Hadassah Medical School, Jerusalem, Israel: Regulation of the readthrough of the *gag-pol* genes in Mo-MLV-infected cells by glutamine starvation.

SESSION 2 MOLECULAR BIOLOGY OF REPLICATION/RETROVIRAL VECTORS

Chairmen: **V. Vogt,** Cornell University
S. Hughes, NCI-Frederick Cancer Research Facility

Miernicki-Steeg, C., Southard, L., Vogt, V.M., Section of Biochemistry, Molecular and Cellular Biology, Cornell University, Ithaca, New York: Binding of avian sarcoma and leukosis virus p19^{gag} to nucleic acids and membranes.

Leis, J.,[1] Phillips, N.,[1] Fu, X.,[1] Tuazon, P.,[1] Traugh, J.,[2] [1]Case Western Reserve University School of Medicine, Cleveland, Ohio; [2]University of California, Riverside: RNA binding properties of the minor nucleocapsid protein pp19 of AMV – Possible regulation by phosphorylation.

Fu, X.,[1] Katz, R.,[2] Skalka, A.,[2] Leis, J.,[1] [1]Case Western Reserve University School of Medicine, Cleveland, Ohio; [2]Roche Institute of Molecular Biology, Nutley, New Jersey: Site-specific mutations of the major nucleocapsid protein pp12 of RSV (Pr-C).

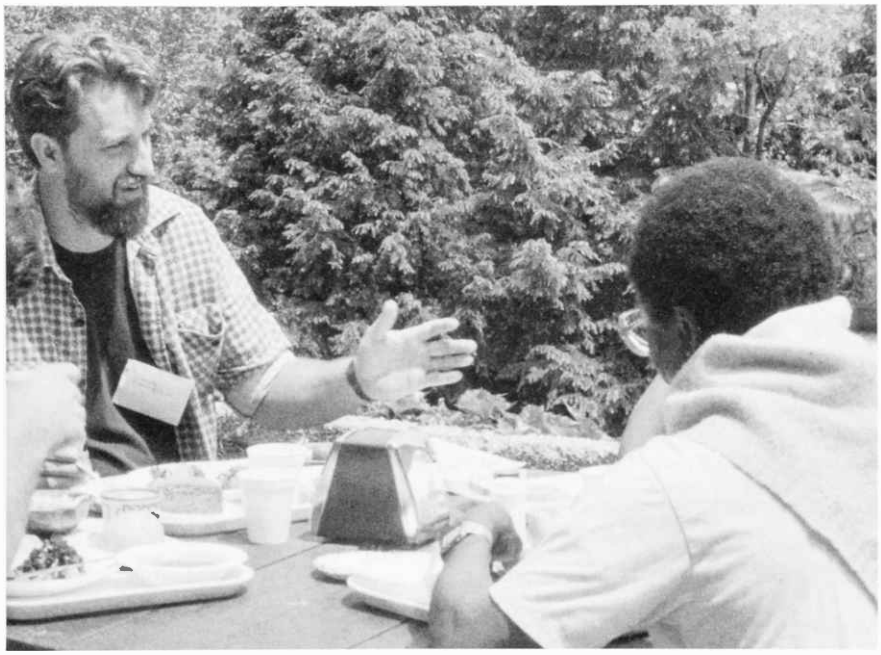
Gorelick, R.J.,[1] Karpel, R.K.,[2] Henderson, L.E.,[1] Oroszlan, S.,[1] [1]NCI-Frederick Cancer Research Facility, Frederick, Maryland; [2]Dept. of Chemistry, University of Maryland, Catonsville: Interaction of murine viral proteins with single-stranded polynucleotides.

Cunningham, J., Tseng, L., Scadden, D., Howard Hughes Medical Institute and Dept. of Medicine, Brigham and Women's Hospital, Boston, Massachusetts: Transfer of the gene encoding the murine ecotropic virus receptor into nonpermissive cells.

Booth, S.C., To, R.Y.-L., Neiman, P.E., Fred Hutchinson Cancer Research Center, Seattle, Washington: Inhibition of retroviral replication by anti-sense RNA.

Emerman, M., Temin, H.M., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Biochemical analysis of gene suppression in retrovirus vectors.

Petropoulos, C.J.,[1] Ordahl, C.P.,[2] Hughes, S.H.,[1] [1]NCI-Frederick Cancer Research Facility, Frederick, Maryland; [2]Dept. of Anatomy, University of California, San Francisco: Tissue-specific expression of chicken actin promoters in retroviral vectors.



S. Hughes

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Yu, S.F.,[1] Kantoff, P.W.,[2] Giglio, A.,[3] Eglitis, M.A.,[2] Armentano, D.,[1] McLachlin, J.R.,[2] O'Reilly, R.,[3] Karlsson, S.,[2] Nienhuis, A.,[2] Gilboa, E.,[1] Anderson, F.W.,[2] [1]Dept. of Molecular Biology, Princeton University, New Jersey; [2]NCI, National Institutes of Health, Bethesda, Maryland; [3]Sloan-Kettering Institute, New York, New York: Transfer and expression of genes in primates using retroviral-mediated gene transfer.

von Ruden, T.,[1] Kantoff, P.A.,[2] Kohn, D.A.,[2] Armentano, D.,[1] Blaese, R.M.,[2] Gilboa, E.,[1] Anderson, F.W.,[2] [1]Dept. of Molecular Biology, Princeton University, New Jersey, [2]NCI, National Institutes of Health, Bethesda, Maryland: Correction of adenosine deaminase deficiency in human T and B cells using retrovirus mediated gene transfer.

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SESSION 3 POSTER SESSION

Bacheler, L., Fels Research Institute, Temple University School of Medicine, Philadelphia, Pennsylvania: Transcriptional activity of integration sites for Mo-MLV.

Schulz, A.,[1] Hess, N.,[2] Friedrich, R.,[1] [1]University of Freiburg, [2]Heinrich Pette Institute, University of Hamburg, Federal Republic of Germany: Construction of an infectious Fr-MLV containing a selectable marker.

Furkes, R.,[1] Evans, R.R.,[2] Levin, J.G.,[3] Yang, W.K.,[2] [1]University of Tennessee, Oak Ridge Graduate School of Biomedical Science, [2]Biology Division, Oak Ridge National Laboratory, [3]NICHD, National Institutes of Health, Tennessee: Identification of endonucleases in Gross passage A strain MLV.

Quinn, T., Mumm, S., Grandgenett, D., St. Louis University, Institute for Molecular Virology, Missouri: Avian retrovirus pp32 protein—In vitro, in situ, and in vivo analysis.

Sharmeen, L., Cywinski, A., Taylor, J., Fox Chase Cancer Center, Philadelphia, Pennsylvania: The priming of retrovirus plus-strand DNA synthesis.

Tanese, N., Roth, M.J., Goff, S.P., Dept. of Biochemistry and Molecular Biophysics, Columbia University College of Physicians & Surgeons, New York, New York: Analysis of retroviral *pol* gene products by expression of fusion proteins in *E. coli*.

Panet, A.,[1,2] Baltimore, D.,[1] [1]Whitehead Institute for Biomedical Research and Massachusetts Institute of Technology, Cambridge; [2]Hebrew University-Hadassah Medical School, Jerusalem, Israel: Characterization of DNA binding proteins and endonuclease activities in Mo-MLV.

Krump-Konvalinkova, V.,[1] Armentano, D.,[1] Hwang, L.,[2] Gilboa, E.,[1] [1]Dept. of Molecular Biology, Princeton University, New Jersey; [2]Wistar Institute of Anatomy and Physiology, Philadelphia, Pennsylvania: Alternative splicing within the retroviral intron—A model for regulation of RNA processing.

Stoltzfus, C.M., Berberich, S.L., Dept. of Microbiology, University of Iowa, Iowa City: Sequences between the *env* and *src* genes influence splicing efficiency of RSV RNA.

Wills, J.W.,[1] Weldon, R.A.,[1] Hunter, E.,[2] [1]Dept. of Microbiology, Oklahoma State University, Stillwater; [2]Dept. of Microbiology, University of Alabama, Birmingham: Expression of the RSV *gag* gene in mammalian cells with an SV40 vector.

Hecht, S.J., Coffin, J.M., Tufts University School of Medicine, Boston, Massachusetts: Expression and processing of the retroviral *gag* protein in *E. coli*.

Panganiban, A., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: In vivo suppression of the murine virus *gag* amber codon is due to an intrinsic *cis*-acting feature of the viral RNA, suppression can occur in many vertebrate cell types and does not require *trans*-acting viral gene products.

Moore, R., Brookes, S., Dixon, M., Smith, R., Dickson, C., Peters, G., Imperial Cancer Research Fund Laboratories, London, England: Complete nucleotide sequence and protein coding potential of MMTV and observations on the poison sequence.

Rhee, S., Clayson, E., Huxiong, H., Hunter, E.: Dept. of Microbiology, University of Alabama, Birmingham: Mutations within the the *gag* gene of MPMV affect precursor cleavage and infectious virus assembly.

Méric, C., Spahr, P.-F., Dept. of Molecular Biology, University of Geneva, Switzerland: Mutations in RSV *gag* protein p12 which suppress infectivity but not RNA packaging.

Prats, A.C., Darlix, J.L., Centre de Recherche de Biochimie et de Génétique Cellulaires du CNRS, Toulouse, France: Unique interactions between nucleic acid binding protein p10 and genomic RNA in MLV virions.

Darlix, J.L., Prats, A.C., Centre de Recherche de Biochimie et de Génétique Cellulaires du CNRS, Toulouse, France: Involvement of retroviral bipartite ribosome binding site and nucleic acid binding protein in the control of viral RNA translation and packaging.

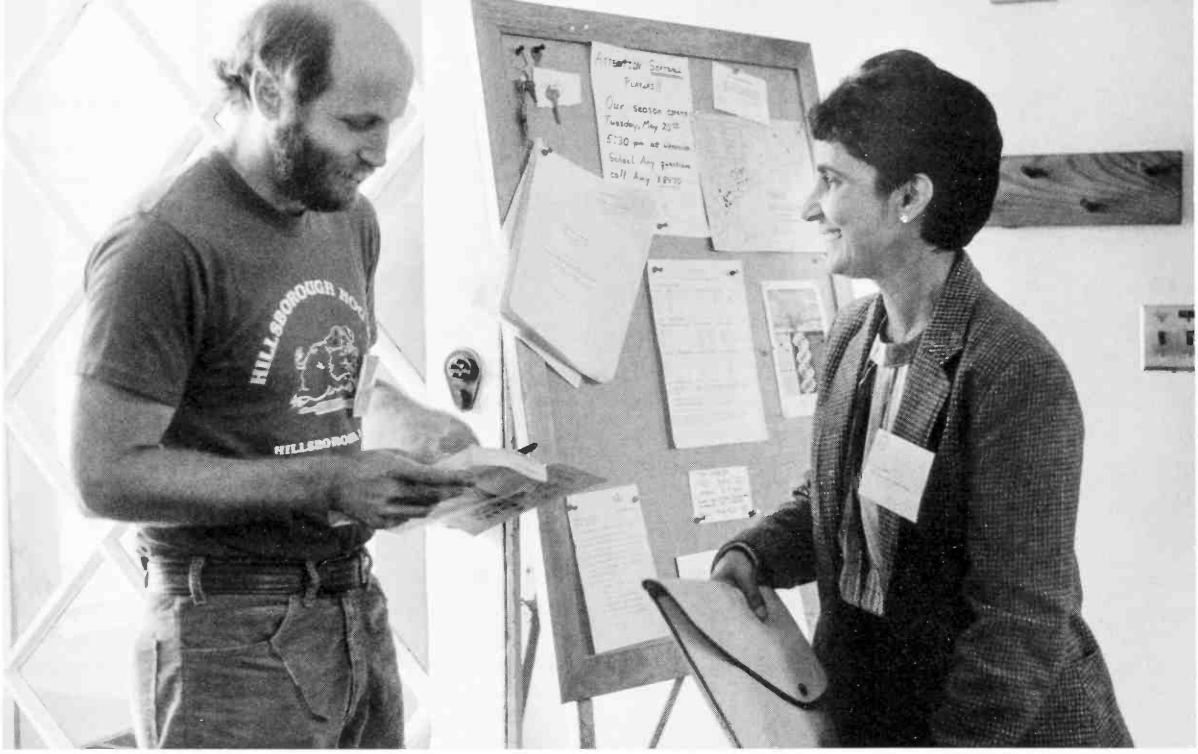
Embretson, J.E., Temin, H.M., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Sequences required in *cis* between the REV-A primer binding site and the 3′ end of the encapsidation sequence.

Bova, C., Olsen, J., Swanstrom, R., Dept. of Biochemistry and Cancer Research Center, University of North Carolina, Chapel Hill: Molecular analysis of avian retrovirus *env* genes.

Park, H., Kaji, A., Dept. of Microbiology, University of Pennsylvania, Philadelphia: Microinjection of RSV-DNA produces infectious RSV particles from the terminally differentiated myotubes which have withdrawn from cell cycle.

Perez, L.G., Hunter, E., Dept. of Microbiology, University of Alabama, Birmingham: Role of cellular proteases in the biosynthesis and function of the RSV *env* gene product.

Pinter, A., Honnen, W., Public Health Research Institute, New York, New York: *O*-Linked glycosylation of retroviral glycoproteins.



R. Swanstrom, S. Rasheed

Tsai, W.P., Copeland, T.D., Oroszlan, S., NCI-Frederick Cancer Research Facility, Frederick, Maryland: REV-A *env* gene products – Structure, biosynthesis, gene organization, and interviral relationship.

Ikuta, K.,[1,2] Luftig, R.B.,[1] [1]Dept. of Microbiology, Immunology, and Parasitology, Louisiana State University Medical Center, New Orleans; [2]Dept. of Pathology, Research Institute for Microbial Diseases, Osaka, Japan: The utility of cerulenin as a probe for MLV morphogenesis.

Olsen, J., Osheroff, W., Swanstrom, R., Dept. of Biochemistry and Cancer Research Center, University of North Carolina, Chapel Hill: Characterization of a replication-competent avian retrovirus shuttle vector.

Paolicchi, J.M.,[1] Snyder, S.H.,[2] Mulligan, R.C.,[2] [1]Dept. of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, Maryland; [2]Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: Generation of helper-free recombinant retrovirus designed for specific targeting.

Jørgensen, P., Mikkelsen, T., Pedersen, F.S., Kjeldgaard, N.O., Dept. of Molecular Biology and Plant Physiology, University of Aarhus, Denmark: A MLV transmission vector system designed to permit recovery in *E. coli* of proviral and cellular flanking sequences.

Gunzberg, W.H.,[1,2] Groner, B.,[2] Salmons, B.,[1,2] [1]Dept. of Cell and Molecular Biology, Medical College of Georgia, Augusta; [2]Ludwig Institute for Cancer Research, Bern, Switzerland: Infection of cultured cells by virus from cells productively transfected with cloned MMTV proviral DNA.

Frankel, W.,[1] Potter, T.A.,[2] Rajan, T.V.,[2] Depts. of [1]Genetics, [2]Pathology, Albert Einstein College of Medicine, Bronx, New York: Retroviral insertional mutagenesis of genes for cell-surface antigens.

Chang, S.M.W., Belmont, J., Henkel-Tigges, J., Wager-Smith, K., Caskey, C.T., Baylor College of Medicine, Houston, Texas: Retroviral gene transfer of human HPRT and human ADA in murine fibroblast and hematopoietic cells.

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Arrigo, S., Yun, M., Carlberg, K., Beemon, K., Dept. of Biology, Johns Hopkins University, Baltimore, Maryland: *Cis*-acting regulatory elements within the *gag* genes of avian retroviruses.

Chang, L.-J., Stoltzfus, C.M., Dept. of Microbiology, University of Iowa, Iowa City: Mutagenesis of 5′-leader of RSV – Effects on expression of *env* and *src* gene mRNAs.

Spodick, D.A.,[1] Lillehaug, J.,[2] Landolph, J.R.,[2,3] Roy-Burman, P.,[1,3] Depts. of [1]Biochemistry, [2]Microbiology, [3]Pathology, University of Southern California School of Medicine, Los Angeles: Transcriptional activity of feline RD-114 endogenous LTRs.

Jones, R.F., Schwartz, M., Maloney, T., McGrath, C.M., Dept. of Tumor Biology, Michigan Cancer Foundation, Detroit: Differentiation- and hyperplasia-specific patterns of mammary tumor proviral demethylation in mouse mammary tissues.

Buchhagen, D.L., Dept. of Microbiology and Immunology, State University of New York Downstate Medical Center, Brooklyn: *Trans*-suppression of transcription from retroviral LTRs.

Yoshinaka, Y.,[1] Sagata, N.,[2] Katoh, I.,[1] Adachi, S.,[1]

Ikawa, Y.,[2] [1]Japan Immuno-research Laboratories, Gunma; [2]Institute of Physical and Chemical Research, Wako, Japan: Identification and structural characterization of the X proteins of BLV.

Katoh, I.,[1] Sagata, N.,[2] Yoshinaka, Y.,[1] Adachi, S.,[1] Ikawa, Y.,[2] [1]Japan Immunoresearch Laboratories, Gunma; [2]Institute of Physical and Chemical Research, Wako, Japan: Biological activities of BLV *X* gene products.

Seiki, M., Kiyokawa, T., Fujisawa, J., Inoue, J., Nagashima, K., Yoshida, M., Dept. of Viral Oncology, Cancer Institute, Tokyo, Japan: New pX proteins of HTLV-I and identification of a product responsible for *trans* activation of gene expression

Williams, J L , Wachsman, W Gaynor R Chen I S Y University of California School of Medicine Los Angeles Differential *trans*-activation of an adenovirus E III promoter by the HTLV-II *x* and adenovirus E1A proteins

Wright, C.M.,[1] Felber, B K ,[1] Paskalis, H ,[1] Wong Staal F.,[2] Pavlakis, G N ,[1] [1]NCI-Frederick Cancer Research Facility, Frederick, [2]NCI, National Institutes of Health Bethesda, Maryland Expression and characterization of HTLV-III/LAV *trans*-activator protein in stable mouse cell lines.

SESSION 4 TRANSCRIPTIONAL/TRANSLATIONAL CONTROL

Chairmen: **H. Fan,** University of California, Irvine
M. Yoshida, Cancer Institute, Tokyo

Nowock, J.,[1] Miksicek, R.,[2] [1]Heinrich-Pette-Institut, Hamberg, [2]Deutsches Krebsforschungszentrum, Heidelberg, Federal Republic of Germany: TGGCA protein–DNA interaction as control element for MMTV transcription.

Derse, D., Casey, J.W., Laboratory of Viral Carcinogenesis, NCI, Frederick, Maryland: Promoter control elements in the BLV LTR responsive to pX gene expression.

Yoshida, M., Fujisawa, J., Inoue, J., Seiki, M., Dept. of Viral Oncology, Cancer Institute, Tokyo, Japan: Transcriptional enhancer of HTLV-I is responsible for activation of gene expression mediated by the viral product.

Cann, A.J., Wachsman, W., Shah, N.P., Chen, I.S.Y., Division of Hematology-Oncology, University of California School of Medicine, Los Angeles: Identification of a functional domain of the HTLV-I and -II *x* proteins.

Pavlakis, G.N.,[1] Felber, B.K.,[1] Paskalis, H.,[1] Kaplin, G.L.,[1] Kashmiri, S.V.S.,[2] Ferrer, G ,[2] Wright, C M ,[1] [1]NCI-Frederick Cancer Research Facility, Frederick, Maryland, [2]New Bolton Center, Kennett Square, Pennsylvania Regulation of expression of HTLV-I, HTLV-II, and BLV

Kitado, H.,[1] Chen, I.S.,[2] Shimotohno, K ,[3] Fan, H ,[1] [1]Dept. of Molecular Biology and Biochemistry, University of California, Irvine, [2]University of California School of Medicine, Los Angeles, [2]National Cancer Center Research Institute, Tokyo, Japan Generation of transactivatable Mo-MLV LTRs by insertion of sequences from HTLV-I and HTLV-II LTRs

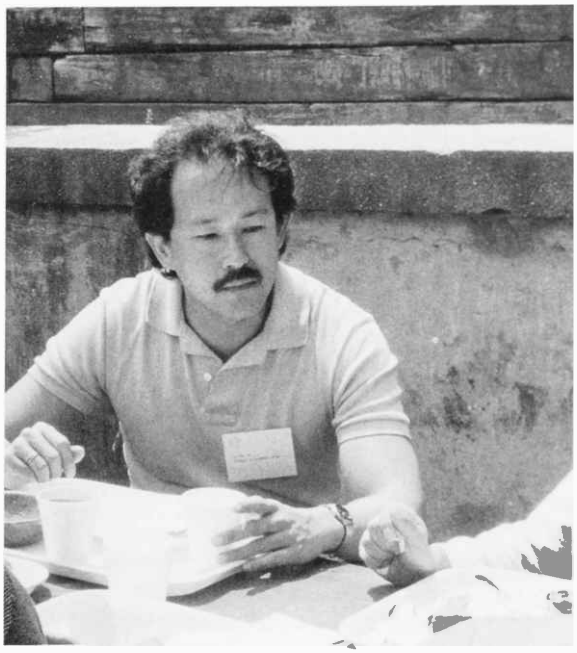
Rosen, C.,[1] Sodroski, J ,[1] Goh, W C ,[1] Haseltine, W ,[1] Wano, Y.,[2] Suetiik, P.,[2] Peffer, N ,[2] Leonard W ,[2] Greene, W , [1]Dana-Farber Cancer Institute, Harvard Medical School and School of Public Health, Boston Massachusetts; [2]NCI, National Institutes of Health Bethesda, Maryland The *trans*-activator gene of HTLV-II induces interleukin-2 receptor and interleukin-2 gene expression

Okamoto, T, Josephs, S F, Taguchi, Y, Sadaie, M R Wong-Staal, F, NCI, National Institutes of Health, Bethesda, Maryland Detection of a *trans*-acting transcriptional activator specific for HTLV-III in virus-infected cells

Cullen, B R , Dept of Molecular Genetics, Hoffmann-La Roche Inc , Nutley, New Jersey HTLV-III-encoded *trans*-activation is mediated by an increase in the steady-state level of target gene mRNAs

Josephs, S F,[1] Sadaie, M R [1] Heisig, V ,[1] Franza, B R [2] Renan, M J ,[3] Seigel L J ,[4] Wong-Staal, F.[1] [1]NCI, National Institutes of Health, Bethesda, Maryland [2]Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, [3]National Accelerator Center, Faure, South Africa, [4]Frederick Cancer Research Center, Frederick, Maryland Synergism of enhancer and Sp1 binding site elements for HTLV-III LTR linked gene expression

Sodroski, J , Rosen, C , Goh, W C , Dayton, A , Terwilliger, E., Campbell, K , Haseltine, W , Dana-Farber Cancer Institute and Harvard Medical and School of Public Health, Boston, Massachusetts HTLV-III determinants of latency and cytopathicity



H. Fan

SESSION 5 HTLVIII/PATHOGENESIS AND IMMUNITY

Chairmen: **J. Mullins,** Harvard School of Public Health
S. Rasheed, University of Southern California School of Medicine

Dayton, A.I., Sodroski, J., Rosen, C.A., Goh, W.C., Haseltine, W.A., Dana-Farber Cancer Institute, Harvard Medical School and Harvard School of Public Health, Boston, Massachusetts: Mutational analysis of the HTLV-III genome—The effects of mutations in the *trans*-activator gene and in other areas.

Ratner, L.,[1] Fisher, A.,[2] Jagodzinski, L.,[3] Gallo, R.C.,[2] Wong-Staal, F.,[2] [1]Washington University, St. Louis, Missouri; [2]NCI, National Institutes of Health, Bethesda; [3]Biotech Research Laboratories, Rockville, Maryland: Sequence and mutagenesis of a functional DNA clone of HTLV-III/LAV.

Franchini, G.,[1] Robert-Guroff, M.,[1] Ghrayeb, J.,[2] Chang, N.,[2] Wong-Staal, F.,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Centocor, Pennsylvania: The 3′ *orf* of HTLV-III is a gene expressed in vivo, the product of which is immunoreactive with human sera.

Fisher, A.G., Ratner, L., Mitsuya, H., Gallo, R.C., Wong-Staal, F., NCI, National Institutes of Health, Bethesda, Maryland: Characterization of noncytopathic variants of HTLV-III.

Garry, R.F.,[1] Rasheed, S.,[2] [1]Dept. of Microbiology and Immunology, Tulane University School of Medicine, New Orleans, Louisiana; [2]Dept. of Pathology, University of Southern California School of Medicine, Los Angeles: Cell killing by UV-inactivated HTLV-III.

Fenyö, E.M., Chiodi, F., Åsjö, B., Dept. of Virology, Karolinska Institutet, Stockholm, Sweden: Infection of brain-derived cells with LAV/HTLV-III.

Rasheed, S., Gowda, S., Gill, P.S., Meyer, P., Levine, A.M., University of Southern California School of Medicine, Los Angeles: Expression of HTLV-III genes in B-cell lymphoma tissues from patients with AIDS.

Barré-Sinoussi, F., Hazan, U., Nugeyre, M.T., Chermann, J.C., Viral Oncology Unit, Institut Pasteur, Paris, France: An approach to identify LAV target cells.

Kanki, P.,[1] Barin, F.,[2] M'Boup, S.,[3] Allan, J.,[1] Essex, M.,[1] [1]Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts; [2]CHRU Bretonneau and UER Pharmaceutical Sciences, Tours, France; [3]Dakar University, Senegal: Identification of a new HTLV related to STLV-III_{AGM}.

Hirsch, V., Kornfeld, H., Kanki, P., Riedel, N., Essex, M., Mullins, J.I., Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts: Cross-reactivity to HTLV-III/LAV and molecular cloning of the STLV-III_{AGM}.

SESSION 6 POSTER SESSION

Terwilliger, E., Dept. of Cancer Pharmacology, Dana-Farber Cancer Institute, Boston, Massachusetts: Mutational analysis of the 3′ end of the genome of HTLV III.

Spire, B., Barré-Sinoussi, F., Rey, F., Chermann, J.C., Institut Pasteur, Paris, France: Comparison of restriction fragment patterns of viral isolates from AIDS patients of different geographical and high-risk groups.

Desai, S.M.,[1] Kalyanaraman, V.S.,[2] Casey, J.M.,[1] Srinivasan, A.,[2] Andersen, P.R.,[1] Devare, S.G.,[1] [1]Dept. of Molecular Biology, Abbott Laboratories, North Chicago, Illinois; [2]AIDS Branch, Centers for Disease Control, Atlanta, Georgia: Genomic diversity in AIDS retroviruses—Primary nucleotide sequence analysis of molecularly cloned HTLV-III/LAV-CDC 451 reveals significant divergence in its genomic sequences.

Srinivasan, A.,[1] Anand, R.,[1] York, D.,[1] Curran, J.,[1] Sanchez-Pescador, R.,[2] Luciw, P.,[2] [1]Centers for Disease Control, Atlanta, Georgia; [2]Chiron Research Laboratories, Emeryville, California: Comparing molecular characteristics—HTLV-III/LAV from a Zairian versus other AIDS viral isolates.

Parker, D.,[1] Roberts, C.,[1] Comerford, S.,[1] Highfield, P.,[1] Weiss, R.,[2] [1]Wellcome Diagnostics, Beckenham, Kent; [2]Institute of Cancer Research, London, England: The cloning and expression of a British AIDS virus isolate.

Krust, B., Laurent, A.G., Hovanessian, A.G.H., Pasteur Institute, Paris, France: Precursor of the AIDS virus glycoproteins—Purification and immunogenecity.

Winkel, I.,[1] Bos, M.,[1] Tersmette, M.,[1] Miedema, F.,[1] Goudsmit, J.,[2] Huisman, H.,[1] [1]Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, [2]Dept. of Virology, University of Amsterdam, The Netherlands: Monoclonal antibodies to $p24^{gag}$ of LAV/HTLV-III.

Huisman, H.G.,[1] Winkel, I.,[1] Lelie, N.,[2] Tersmette, M.,[1] Goudsmit, J.,[2] Lange, J.,[2] Miedema, F.,[1] [1]Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, [2]Dept. of Virology, University Amsterdam, The Netherlands: Comparison of three different immunoassays in their ability to establish in human sera the onset of IgG antibody response to LAV/HTLV-III infection.

Pedersen, N.C.,[1] Higgins, J.R.,[2] Carlson, J.R.,[2] Depts. of [1]Medicine, School of Veterinary Medicine, [2]Medical Pathology, School of Medicine, University of California, Davis: Development of a double sandwich ELISA using monoclonal and polyclonal antibodies to HTLV-III-p24 and its usefulness in differentiating HTLV-III/LAV from ARV-like clinical isolates and for virus neutralizing antibody assays.

Beltz, G.A., Hung, C.-H., Thorn, R.M., Fallis, B., Storey, J.R., Winkle, S., Chen, K.L., Marciani, D.J., Cambridge BioScience Corp., Hopkinton, Massachusetts: A recombinant protein-based ELISA for antibodies against HTLV-III/LAV.

Allan, J.S.,[1] Lee, T.-H.,[1] Coligan, J.E.,[2] Groopman, J.E.,[3] Essex, M.,[1] [1]Harvard School of Public Health, Boston, Massachusetts; [2]NIAID, National Institutes of Health, Bethesda, Maryland; [3]New England Deaconess Hospital, Boston, Massachusetts: Identification of HTLV-III *pol* gene products and their relative immunogenicity.

Lange, J.M.A.,[1] de Wolf, F.,[1] Clapham, P.,[2] Ferns, B.,[3] Krone, W.J.,[1] Coutinho, R.A.,[4] Tedder, R.,[3] Goudsmit, J.,[1] [1]Dept. of Virology, Academic Medical Center, University of Amsterdam, The Netherlands; [2]Chester Beatty

Laboratories, London, England; [3]Virology Section, Middlesex Hospital Medical School, London, England; [4]Municipal Health Service, Amsterdam, The Netherlands: Antibody response to core and envelope antigen in primary HTLV-III/LAV infection.

Anand, R., Moore, J., Feorino, P., Schable, C., Jaffee, H., Srinivasan, A., Centers for Disease Control, Atlanta, Georgia: Heterogeneity of HTLV-III/LAV isolated serially from a blood donor who progressed from asymptomatic to frank AIDS stage.

Åsjö, B.,[1] Fenyö, E.M.,[1] Morfeldt-Månson, L.,[2] Biberfeld, G.,[3] [1]Dept. of Virology, Karolinska Institutet, [2]Dept. of Infectious Diseases, Karolinska Institutet, Roslagstulls Hospital, [3]Dept. of Immunology, National Bacteriology Laboratory, Stockholm, Sweden: A possible correlation between in vitro properties of LAV/HTLV-III and clinical progression in AIDS.

Barban, V., Quérat, G., Vigne, R., Laboratoire de Virologie, Faculté de Médecine Nord, Marseille, France: Analysis of RNA transcripts of visna virus.

Portis, J., McAtee, F., National Institutes of Health, NIAID, Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, Hamilton, Montana: Are murine retroviruses transmitted venereally?

Bilello, J.A., Cimino, E., Benjers, B., Hoffman, P.M., Veterans Administration Medical Center, Research Service, Baltimore, Maryland: Murine retrovirus replication in the central nervous system – Infection and transformation of brain capillary endothelial cells in vivo.

Hoover, E.A.,[1] Mullins, J.I.,[2] Donahue, P.R.,[2] Quackenbush, S.L.,[1] Gasper, P.W.,[1] [1]Dept. of Pathology, Colorado State University, Fort Collins; [2]Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts: Consistent patterns of disease progression in feline AIDS.

Carpenter, S., Chesebro, B., NIAID, National Institutes of Health, Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, Hamilton, Montana: Antigenic variation of equine infectious anemia virus can occur in the absence of neutralizing antibody.

Lagarias, D.,[1] Kramme, P.,[2] Grossman, D.,[1] Radke, K.,[1] Depts. of [1]Avian Sciences, [2]Veterinary Pathology, University of California, Davis: Expression of BLV in blood mononuclear cells from infected sheep.

Rebeyrotte, N., Mamoun, R.Z., Astier-Gin, T., Guillemain, B., INSERM, Bordeaux, France: Cellular oncogenes in bovine lymphosarcomas.

Hunter, E.,[1] Barker, C.,[1] Engler, J.,[1] Sonigo, P.,[2] Wain-Hobson, S.,[2] [1]Dept. of Microbiology, University of Alabama, Birmingham; [2]Recombinasion et Expression Génétique, Institut Pasteur, Paris, France: Genomic sequence analysis of a prototype D-type retrovirus. MPMV – Evidence for a role of the envelope glycoproteins in disease.

Ball, J.K., Dept. of Biochemistry, University of Western Ontario, Canada: Thymotropic and leukemogenic properties of a type-B retrovirus.

Carr, J.K., Cohen, J.C., Dept. of Biochemistry, Louisiana State University Medical Center, New Orleans: Lipopolysaccharide increases MMTV transcript level in the spleen of lactating C3HfB/HeN mice.

Sinclair, A., Dickson, C., Imperial Cancer Research Fund Laboratories, London, England: A structural and functional analysis of the protein encoded within the LTR of MMTV

Schwartz, M., Kittrell, F., Medina, D., Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas: Expression of endogenous MMTV in BALB/C hyperplasias and tumors

Geib, R.W., Seaward, M.S., Dept. of Microbiology and Immunology, Terre Haute Center for Medical Education, Indiana University School of Medicine: Formation of *Fv-2*^{r}-adapted strains of Friend virus

Jones, K.S., Lilly, F., Dept. of Genetics, Albert Einstein College of Medicine, Bronx, New York: Different pathogenic properties of SFFV rescued by Fr-MLV or by AKV

Robinson, H.L.,[1] Reinsch, S.S.,[2] Shank, P.R.,[2] [1]Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts; [2]Brown University, Division of Biology and Medicine, Providence, Rhode Island: Sequences near the 5′ LTR of ALVs determine the ability to induce osteopetrosis.

Fan, H.,[1] Mittal, S.,[1] Chute, H.,[1] Chao, E.,[1] Pattengale, P.K.,[2] [1]Dept. of Molecular Biology and Biochemistry, University of California, Irvine, [2]Dept. of Pathology, University of Southern California School of Medicine, Los Angeles: Effects of LTR sequence rearrangements and insertions on the biological activity of Mo-MLV

Davis, B.,[1] Chandy, G.K.,[2] Brightman, B.K.,[1] Gupta, S.,[2] Fan, H.,[1] Depts. of [1]Molecular Biology and Biochemistry [2]Medicine, University of California, Irvine: Preleukemic changes induced by wild-type and nonpathogenic Mo-MLV

McCaffrey, M., Teich, N., Imperial Cancer Research Fund Laboratories, London, England: Studies on MyLV – A murine myeloid leukemia virus

Rassart, E., Nelbach, L., Jolicoeur, P.: Clinical Research Institute of Montreal and Universite de Montreal, Canada: Molecular analysis of the 3′ end region harboring the determinant of paralysis of neurotropic Cas-Br-E MLV

Thomas, C.Y., Coppola, M.A., Depts. of Medicine and Microbiology, University of Virginia, Charlottesville: Host regulation of the *env* gene structure of tumor-associated recombinant MLVs

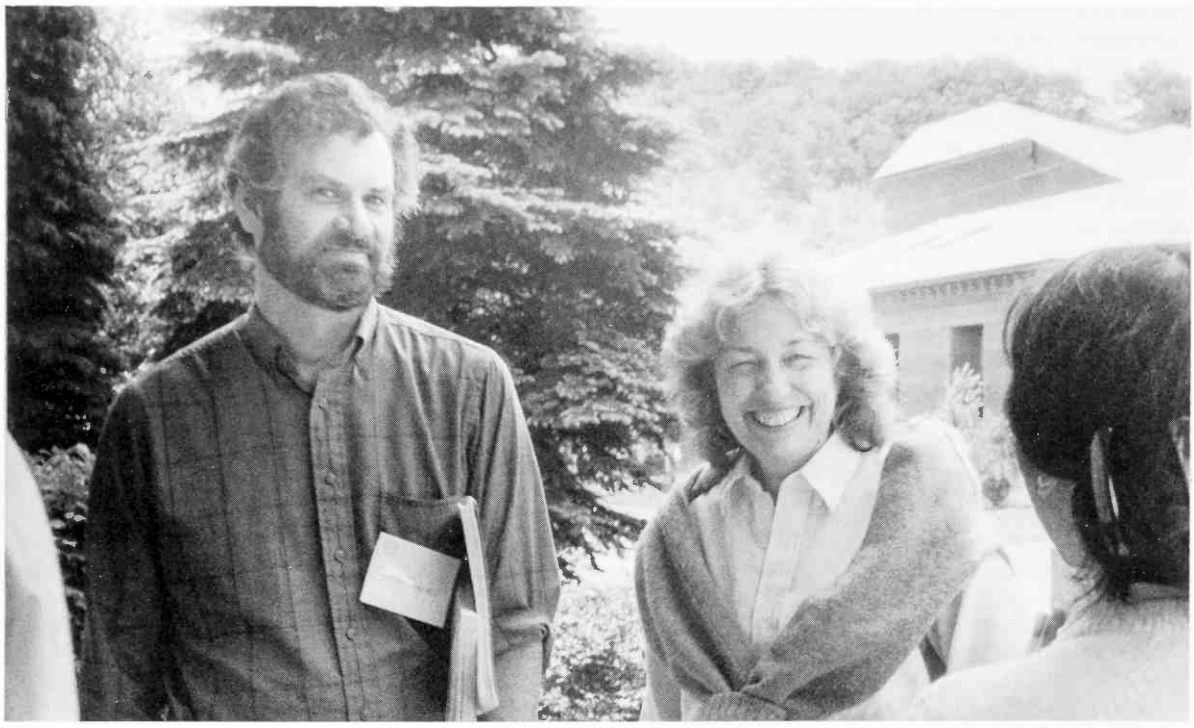
Lieberman, M., Scott, M.L., Hansteen, G.A., White, J.H.: Dept. of Radiology, Stanford University School of Medicine, California: Target cells for radiation lymphomagenesis

Evans, L., Malik, F.: NIAID, National Institutes of Health, Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, Hamilton, Montana: Type-II polytropic MLVs from young AKR/J mice; epShort, M., Okenquist, S., Lenz, J.: Dept. of Genetics, Albert Einstein College of Medicine, Bronx, New York: Leukemogenic potential of murine retroviruses correlates with transcriptional tissue preference of the viral LTRs

Lenz, J., Okenquist, S., Lopez, L.: Dept. of Genetics, Albert Einstein College of Medicine, Bronx, New York: Absence of recombinant provirus formation in SL3-3 virus-induced leukemias of DBA/2 mice

Hollon, T., Yoshimura, F.: Dept. of Biological Structure, University of Washington, Seattle: Characterization of T-cell-specific activity of the MCF MLV enhancer element

Buller, R., Portis, J.: NIAID, National Institutes of Health, Laboratory of Persistent Viral Diseases, Rocky Mountain



R. Eisenman, A. Skalka

Laboratories, Hamilton, Montana: Tissue-specific expression of two allelic forms of an endogenous gp70 linked to the Rmcf locus.

Policastro, P.F., McKinnon, R., Wilson, M.C., Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California: Analysis of Gv-1-regulated endogenous retrovirus loci in 129 mouse strains.

O'Neill, R.R.,[1] Hartley, J.W.,[1] Repaske, R.,[1] Friedrich, R.,[2] Kozak, C.A.,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Institute of Immunobiology, University of Freiburg, Federal Republic of Germany: Amphotropic MLV *env*-related segments are not endogenous in most inbred laboratory and wild mouse DNAs.

King, S.R., Horowitz, J.M., Holland, G.D., Risser, R., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Determinants of expression of endogenous ectotropic MLV.

Huang, L.-H.,[1] Henry, J.-M.,[1] Silberman, J.D.,[2] Rothschild, H.,[1] Cohen, C.,[1,2] Depts. of [1]Medicine, [2]Biochemistry, Louisiana State University Medical Center, New Orleans: Replication of BaEV DNA in human cells.

Kato, N.,[1] Kato, M.,[1] Pheifer-Ohlsson, S.,[2] Ohlsson, R.,[2] Larsson, E.,[2] Cohen, M.,[1] [1]NCI-Frederick Cancer Research Facility, Frederick, Maryland; [2]Uppsala University, Sweden: Expression of a human endogenous provirus, ERV3.

Gattoni-Celli, S., Kirsch, K., Kalled, S., Isselbacher, K.J., Massachusetts General Hospital and Harvard Medical School, Boston: Expression of type C-related endogenous retroviral sequences in human colon tumors.

Boyce-Jacino, M., Dunwiddie, C., Resnick, R., Alegre, J., Faras, A., Institute of Human Genetics and Dept. of Microbiology, University of Minnesota, Minneapolis: Characterization of a novel RSV-related family of endogenous retroviruses of chickens.

Brack, R.,[1] Leib-Mösch, C.,[2] Hehlmann, R.,[2] Erfle, V.,[2] [1]Abteilung für Pathologie, Gesellschaft für Strahlen-und Umweltforschung mbH Munchen, Neuherberg; [2]Med. Poliklinik LMU München, Federal Republic of Germany: Isolation and characterization of the SSAV-related molecular clone S71 – Representative of a new family of human endogenous retroviral sequences.

Obata, M., Khan, A.S., NIAID, National Institutes of Health, Bethesda, Maryland: Characterization of a new class of endogenous MLV-related DNAs containing a mosaic genomic structure.

Stoye, J.P., Coffin, J.M., Tufts University School of Medicine, Boston, Massachusetts: A new class of endogenous C-type retrovirus.

Chang, L.-Y.,[1] Myer, F.E.,[2] Yang, W.K.,[2] [1]Dept. of Microbiology, University of Tennessee, Knoxville; [2]Biology Division, Oak Ridge National Laboratories, Tennessee: On the defective MLV-related sequences – The involvement of short direct repeat in the generation of deletions.

Kuemmerle, N.B.,[1] Ch'ang, L.-Y.,[1] Koh, C.K.,[2] Boone, L.R.,[3] Yang, W.K.,[1,2] [1]University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences, [2]Biology Division, Oak Ridge National Laboratory, Tennessee; [3]NIEHS, Research Triangle Park, North Carolina: Solitary LTRs of the MLV type in chromosomal DNA of laboratory mouse strains.

Martinelli, S.C., Goff, S.P., Dept. of Biochemistry and Molecular Biophysics, Columbia University of Physicians and Surgeons, New York, New York: Cloning and charac-

terization of an infectious MLV formed by homologous recombination between a mutant Mo-MLV carrying a deletion in the *pol* gene and viral sequences in the genome of NIH/3T3 cells.

Mullins, J.I.,[1] Riedel, N.,[1] Elder, J.H.,[2] Beltz, G.A.,[3] Hoover, E.A.,[4] Binari, R.C., Jr.,[1] Hirsch, V.,[1] [1]Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts; [2]Scripps Clinic and Research Foundation, La Jolla, California; [3]Cambridge BioScience Corp., Hopkinton, Massachusetts; [4]Dept. of Pathology, Colorado State University, Fort Collins: Evolution of FeLVs.

Wolfe, J.H., Hardy, W.D., Jr., Hayward, W.S., Memorial Sloan-Kettering Cancer Center, New York, New York: A replication-defective transforming virus isolated from cultured cells from a cat with an FeLV-negative lymphosarcoma.

Morrey, J.D., Evans, L.H., NIAID, National Institutes of Health, Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, Hamilton, Montana: Polytropic viruses induced in SWR/J mice by a recombinant virus between Friend and Moloney MLVs.

Hofmann, B.,[1] Langhoff, E.,[1] Lindhardt, B.Ø.,[2] Platz, P.,[1] Ryder, L.P.,[1] Hyldig-Neilsen, J.J.,[1] Ødum, N.,[1] Morling, N.,[1] Svejgaard, A.,[1] Ulrich, K.,[2] [1]Tissue typing Laboratory, University Hospital, Copenhagen; [2]Laboratory of Tumor Virology, The Fibiger Institute, Copenhagen, Denmark: A factor with potent suppressive properties associated with HTLV-III.

SESSION 7 PATHOGENESIS, TROPISMS, AND HOST-CELL INTERACTIONS

Chairmen: **E. Fleissner,** Memorial Sloan-Kettering Cancer Center
P. Neiman, Fred Hutchinson Cancer Research Center

Overbaugh, J.,[1] Hoover, E.A.,[2] Mullins, J.I.,[1] [1]Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts; [2]Dept. of Pathology, Colorado State University, Fort Collins: Variability in feline AIDS virus genomes.

Elder, J.H.,[1] McGee, J.,[1] Grant, C.,[2] [1]Scripps Clinic and Research Foundation, La Jolla, California; [2]Pacific Northwest Research Foundation, Seattle, Washington: Differential neutralization of FeLV subtypes by antibodies which recognize homologous epitopes.

Davis, J.L., Clements, J.E., Johns Hopkins Medical Institutions, Baltimore, Maryland: Transcription of small ORFs of visna virus during productive infection.

Hess, J.L., Pyper, J.M., Clements, J.E., Depts. of Neurology and Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, Maryland: Transcriptional activity of the visna virus and caprine arthritis-encephalitis virus LTRs.

Gliniak, B., Spiro, C., Li, J.-P., Bestwick, R.K., Kabat, D., Dept. of Biochemistry, Oregon Health Sciences University, Portland: Progressive leukemogenesis by Friend erythroleukemia virus.

Amanuma, H., Nishi, M., Ikawa, Y., Tsukuba Life Science Center, Riken, Japan: Crucial role of the unique structure at the C-terminal end of the *env*-related glycoprotein for the leukemogenic potential of the Fr-SFFV.

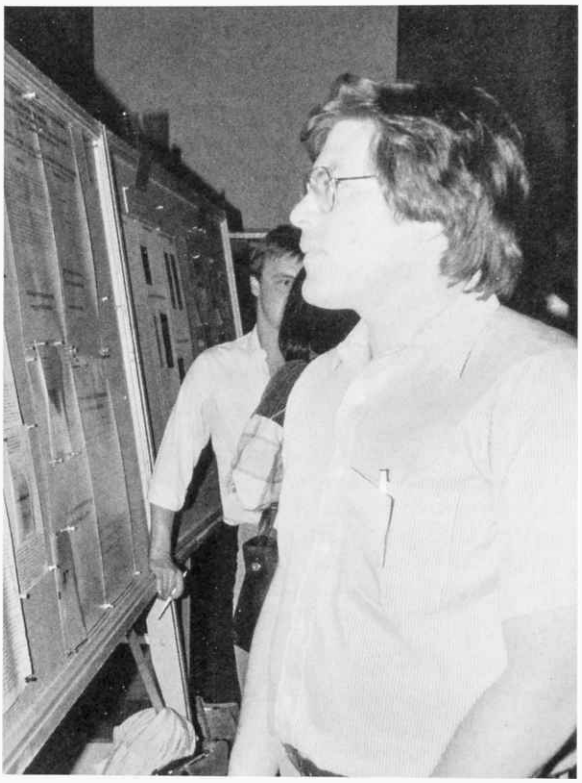
Chung, S.W., Wolf, L., Ruscetti, S., Laboratory of Genetics, NCI, National Institutes of Health, Bethesda, Maryland: 678-bp region at the 3′ end of the *env* gene of $SFFV_p$ is responsible for the altered Epo responsiveness of infected cells.

Sitbon, M.,[1] Sola, B.,[2] Nathanson, K.,[1] Nishio, J.,[1] Evans, L.,[1] Garon, C.,[1] Chesebro, B.,[1] [1]National Institutes of Health, NIAID, Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, Hamilton, Montana; [2]INSERM, Hôpital Cochin, Paris, France: Induction of early hemolytic anemia in newborn mice inoculated with Fr-MLV maps in the *pol-env* fragment of the viral genome.

Li, Y.,[1] Golemis, E.,[1] Mbangkollo, D.,[1] Izawa, M.,[1] Manley, N.,[1] Sharp, P.,[1] Hartley, J.,[2] Hopkins, N.,[1] [1]Massachusetts Institute of Technology, Cambridge; [2]NCI, National Institutes of Health, Bethesda, Maryland: Disease specificity of MLVs—Genetic analysis of enhancer regions.

Gisselbrecht, W.F., Martial, M.A., Varlet, P., Tambourin, P., INSERM, Hôpital Cochin, Paris, France: A novel "immortalizing" defective retrovirus inducing an acute myeloproliferative disorder in adult mice.

Vaidya, A.B., Connors, R.W., Anne, P.R., Long, C.A., Hahnemann University, Philadelphia, Pennsylvania: A molecular genetic explanation for the resistance of C57BL mice to tumorigenesis by MMTV(C3H).



C. Sheer

SESSION 8 INTEGRATION-ACTIVATION/*MYC*

Chairmen: **T.-J. Kung,** Case Western Reserve University
P. Tsichlis, Fox Chase Cancer Center

Strand, D.,[1] Lambert, M.,[2] Franza, B.R.,[2] McDonald, J.,[1] [1]Dept. of Genetics, University of Georgia, Athens; [2]Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: Molecular analysis of the transcriptional effects of insertion of a co*pia* element into the *Adh* locus of *D. melanogaster*.

Conklin, K.F., Groudine, M., Fred Hutchinson Cancer Research Center, Seattle, Washington: Varied interactions between integrated proviruses and adjacent host sequences.

Dudley, J., Dept. of Microbiology, University of Texas, Austin: The retrotransposon L1 may be a determinant of MMTV integration in T-cell lymphomas.

Witkowski, J., Moore, R., Dickson, C., Peters, G., Imperial Cancer Research Fund Laboratories, London, England: Multiple transcripts and potential for alternate exon usage in the mouse *int-2* gene.

Shackleford, G.,[1] Jakobovits, A.,[2] Joyner, A.,[2] Martin, G.,[2] Varmus, H.,[1] Depts. of [1]Microbiology, [2]Anatomy, University of California, San Francisco: *int*-1 and *int*-2 are independently regulated during development.

Tsichlis, P.N., Schafer, B., Fox Chase Cancer Center, Philadelphia, Pennsylvania: Recombination between two integrated proviruses, one of which was inserted near c-*myc* in Mo-MLV-induced rat thymomas.

Isfort, R.[1] Witter, R.,[2] Kung, H.-J.,[1] [1]Case Western Reserve University, Cleveland, Ohio; [2]USDA Regional Poultry Research Laboratory, East Lansing, Michigan: Activation of c-*myc* by REV insertion in a novel nonbursal lymphoma resembling Marek's disease herpesvirus-induced T-cell lymphoma.

Schubach, W.H., Dept of Medicine, State University of New York, Stony Brook: Structure and transcriptional analysis of the c-*myc* region in ALV-induced bursal lymphoma cell lines.

Nepveu, A., Marcu, K.B., Dept. of Biochemistry, State University of New York, Stony Brook: Transcriptional analysis of the c-*myc* oncogene—Antisense transcription and premature termination of sense transcription.

Rapp, U.R.,[1] Cleveland, J.L.,[2] Huleihel, M.,[2] Siebenlist, U.,[2] Eisenman, R.,[3] NCI, National Institutes of Health, [1]Frederick Cancer Research Facility, Frederick, [2]Bethesda, Maryland; [3]Fred Hutchinson Cancer Research Facility, Seattle, Washington: Regulation of c-*myc* expression.

SESSION 9 POSTER SESSION

Goodenow, M.M., Hayward, W.S., Sloan-Kettering Institute for Cancer Research, New York, New York: Nature of proviral deletions generated in vivo.

Steffen, D.L., Vijaya, S., Robinson, H.L., Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Identification and characterization of a selected proviral integration site in rat lymphomas induced by Mo-MLV.

Tsichlis, P.N., Prasad, V.R., Guzman, P., Fox Chase Cancer Center, Philadelphia, Pennsylvania: Analysis of LMVI-2, a common region for provirus insertion in Mo-MLV-induced rat thymomas.

Villeneuve, L.,[1] Villemur, R.,[1] Monczak, Y.,[1] Rassart, E.,[1] Kozak, C.,[2] Jolicoeur, P.,[1] [1]Clinical Research Institute of Montreal and Universite de Montreal, Canada; [2]NIAID, National Institutes of Health, Bethesda, Maryland: Further characterization of *Mis-1* and *Gin-1,* two putative new oncogenes representing frequent provirus integration in MLV-induced thymomas.

Nagarajan, L., Louie, E., Tsujimoto, Y., ar-Rushdi, A., Huebner, K., Croce, C.M., Wistar Institute, Philadelphia, Pennsylvania: Molecular cloning and chromosomal mapping of hPim-1 putative oncogene.

Nusse, R.,[1] Sonnenberg, A.,[2] Schuuring, E.,[1] Mester, J.,[1] Sluyser, M.,[2] Rijsewijk, F.,[1] Divisions of [1]Molecular Biology, [2]Tumor Biology, Netherlands Cancer Institute, Amsterdam, The Netherlands: Expression of *int-1* and *int-2* during progression of mouse mammary tumors.

Silver, J., Buckler, C., NIAID, National Institutes of Health, Bethesda, Maryland: *fis*-1 is very closely linked to *int*-2—Is this a superlocus for proviral insertion/chromosomal rearrangement in tumors?

Ali, I.U.,[1] Marianicostantini, R.,[1] Escot, C.,[1] Theillet, C.,[1] Lidereau, R.,[2] Callahan, R.,[1] [1]Laboratory of Tumor Immunology and Biology, NCI, Bethesda, Maryland; [2]Centre Rene Huguenin, St. Cloud, France: Analysis of the *ras* and *myc* proto-oncogenes in human breast tumors.

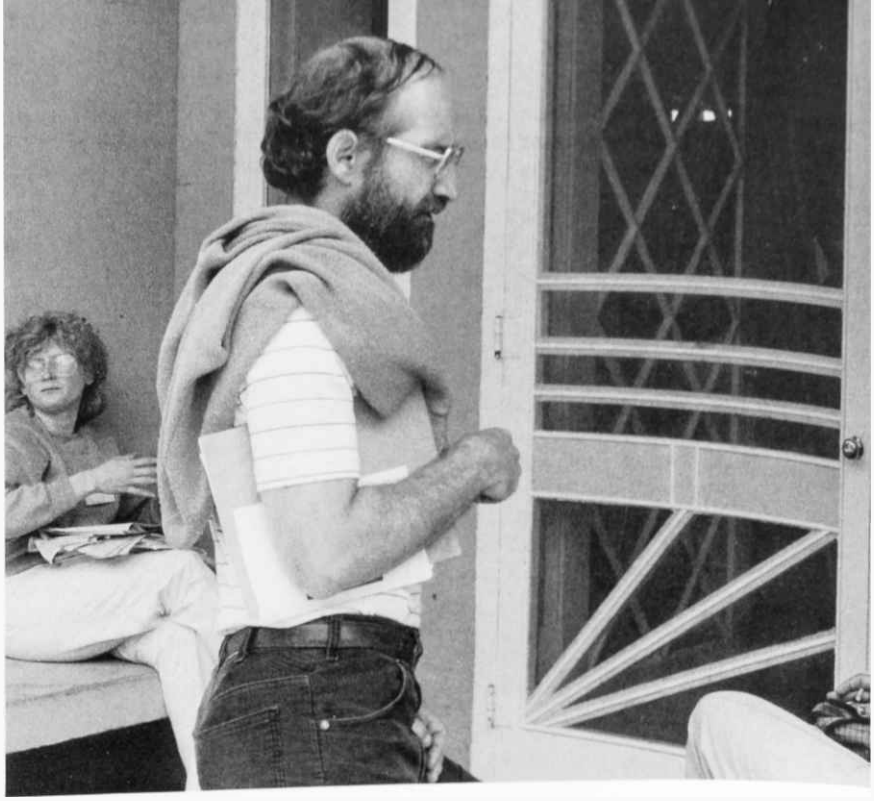
Palmieri, S., Vogel, M., National Institutes of Health, NIAID, Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, Hamilton, Montana: Fibroblast transformation parameters induced by the avian v-*mil* oncogene.

Friis, R.R.,[1] Eigenbrodt, E.,[2] [1]Ludwig Institute for Cancer Research, Bern, Switzerland; [2]Institute for Biochemistry, Veterinary Medical School, Justus-Liebig University, Giessen, Federal Republic of Germany: A different fibroblast transformation phenotype for myc.

O'Donnell, P.V., Woller, R., Koehne, C., Fotheringham, S., Jahnwar, S., Memorial Sloan-Kettering Cancer Center, New York, New York: Trisomy of chromosome 15 and insertional mutagenesis of c-*myc* appear to be unrelated events in murine T-cell leukemogenesis.

Wolff, L.,[1] Mushinski, J.F.,[1] Gilboa, E.,[2] Morse, H.C. III,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Molecular Biology, Princeton University, New Jersey: Neoplasms induced by a retrovirus vector containing c-*myc* cDNA from normal mouse spleen.

Bonham, L., Lobelle-Rich, P., Levy, L.S., Dept. of Microbiology and Immunology, Tulane Medical School, New Orleans, Louisiana: Isolation and biological activity of an in-



P. Jolicoeur

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Chenevix-Trench, G., Brown, J.A., Westin, E.H., Depts. of Human Genetics and Medicine, Medical College of Virginia, Richmond: Somatic rearrangement of the c-*myc* oncogene in primary diffuse large cell lymphoma.

Ely, C., Leftwich, J., Hall, R., Westin, R., Medical College of Virginia, Richmond: Sustained suppression of c-*myc* gene expression is required for terminal macrophage-like differentiation of HL-60 cells.

Lipsick, H.,[1,2] Ibanez, C.E.,[2] [1]Veteran's Administration Medical Center, San Diego; [2]Dept. of Pathology, University of California School of Medicine, San Diego, La Jolla: Expression of wild-type and mutant v-*myb* gene products in avian and mammalian cells.

Askew, D.,[1] May, S.,[2] Ihle, J.N.,[1] [1]NCI-Frederick Research Facility, Frederick; [2]Johns Hopkins Oncology Center, Baltimore, Maryland: Phenotypic properties of murine myeloid cell lines having retroviral insertions in the c-*myb* locus.

Kotler, M., Burstein, H., Roguel, N., Dept. of Molecular Genetics, Hebrew University-Hadassah Medical School, Jerusalem, Israel: A field strain of *myb* containing avian retrovirus responsible for hemangiosarcomas in chickens.

McDonough, M., Boettiger, D., Dept. of Microbiology, University of Pennsylvania, Philadelphia: Stage- and tissue-specific regulation of c-*mos* and c-*src* during chicken limb bud development.

Seth, A.,[1] Vande Woude, G.F.,[1] Black, R.,[2] Fisher, R.J.,[1] Pavlakis, G.,[1] Blair, D.,[1] [1]NCI-Frederick Cancer Research Facility, Frederick; [2]Uniformed Services University of Health Sciences, Bethesda, Maryland: Inducible expression of human growth hormone *mos* fusion protein in C127 cells using a BPV shuttle vector.

Iyer, A., Propst, F., Kaul, K., Oskarsson, M., Vande Woude, G.F., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Expression of the c-*mos* proto-oncogene during mouse spermatogenesis

Stein, R.B., Tai, J.Y., Scolnick, E.M.: Dept. of Virus and Cell Biology Research, Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania: Cloning of the temperature-sensitive 371 Ki-MSV and characterization of the mutant p21 *ras* protein

Connors, R.W., Sweet, R., Dept. of Molecular Genetics, Smith Kline and French Laboratories, Swedeland, Pennsylvania: Characterization of normal (gly-12) and mutant (val-12) *ras*-transformed cells

Newcomb, E.W., Pellicer, A.: Dept. of Pathology and Kaplan Cancer Center, New York University Medical Center, New York, New York: Murine transforming genes detected by tumorigenicity assay in nude mice

Leon, J., Steinberg, J.J., Pellicer, A.: Dept. of Pathology, New York University Medical Center, New York, New York: Ha-*ras* in benign skin tumors induced in rabbits

Chang, H.-Y.,[1] Guerrero, I.,[2] Lake, R.,[2] Pellicer, A.,[2] D'Eustachio, P.[1]: Depts. of [1]Biochemistry, [2]Pathology and Kaplan Cancer Center, New York University Medical Center, New York, New York: A novel *Nras* cDNA-like pseudogene in the mouse

Salmons, B.,[1,2] Jaggi, R.,[2] Friis, R.[2] Muellener, D.[2] Groner, B.,[2] [1]Dept. of Cell and Molecular Biology, Medical College of Georgia, Augusta, [2]Ludwig Institute for Cancer Research, Bern, Switzerland: Expression from the MMTV-LTR is repressed by oncogenes

Green, P.L., Risser, R., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Evidence that Ab-MLV tumors arise by a selective outgrowth of a few infected cells

Rees-Jones, R.W.,[1,2] Goff, S.P.[2] Depts. of [1]Medicine,

[2]Biochemistry, Columbia College of Physicians & Surgeons, New York, New York: Saturation insertional mutagenesis of Ab-MLV proviral DNA—Mutants with altered kinase activity and phenotype.

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Ramakrishnan, L., Rosenberg, N., Cancer Research Center and Immunology Graduate Program, Tufts Medical School, Boston, Massachusetts: "Fetal" target cells for Ab-MLV are present in adult bone marrow.

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Chung, S.-W.,[1] Wong, P.M.C.,[2] Shen-Ong, G.,[1] Ruscetti, S.,[1] Ishizaka, T.,[3] Eaves, C.J.,[4] [1]Laboratory of Genetics, NCI, [2]Clinical Hematology Laboratory, National Institutes of Health, Bethesda, Maryland; [3]Subdepartment of Immunology, Johns Hopkins University School of Medicine, Baltimore, Maryland; [4]Terry Fox Laboratory, British Columbia Cancer Research Center, Canada: Production of GM-CSF by Ab-MLV-induced tumorigenic mast cell lines.

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Wang, L.-C., Gao, C., Vass, W., Chang, K.S.S., NCI, National Institutes of Health, Bethesda, Maryland: Rearrangements of *abl* and endogenous ecotropic provirus loci in reticulum cell neoplasms of SJL/J mice.

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Schalken, J.A.,[1] Roebroek, A.J.M.,[2] Leunissen, J.A.M.,[2] Debruyne, F.M.J.,[1] Bloemers, H.P.J.,[2] Van de Ven, W.J.M.,[2] Depts. of [1]Biochemistry, [2]Urology, University of Nijmegen, The Netherlands: The c-*fes/fps* proto-oncogene is closely linked to a gene encoding a receptor-like protein.

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Piwnica-Worms, H., Roberts, T.M., Dana-Farber Cancer Institute, Boston, Massachusetts: Overproduction of kinase active $pp60^{c\text{-}src}$ using bacterial and baculovirus expression vectors.

Tanaka, A.,[1] Gibbs, C.P.,[2] Arthur, R.R.,[1] Anderson, S.K.,[1] Kung, H.-J.,[3] Fujita, D.J.,[1] [1]Cancer Research Laboratory, University of Western Ontario, London, Canada; [2]Rockefeller University, New York, New York; [3]Dept. of Molecular Biology and Microbiology, Case Western Reserve University, Cleveland, Ohio: The NH_2-terminal region of the human c-*src* protein—Its DNA coding sequence and a possible recognition domain for a *src*-specific target protein(s).

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Reynolds, A., Potts, W., Payne, M., Lansing, T., Weber, M., Parsons, J.T., Dept. of Microbiology, University of Virginia, Charlottesville: Activation of the cellular *src* gene—The role of somatic mutations.

Parsons, S.J., McCarley, D.J., Dept. of Microbiology, University of Virginia School of Medicine, Charlottesville: Reduced tyrosine kinase specific activity is associated with hypophosphorylation of $pp60^{c\text{-}src}$ in AEV-transformed avian and rodent fibroblasts.

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Laugier, D.,[1] Calothy, G.,[1] Jove, R.,[2] Cross, F.R.,[2] Iba, H.,[2] Hanafusa, H.,[2] [1]Institut Curie-Biologie, Orsay, France; [2]Rockefeller University, New York, New York: Myristylation of $p60^{src}$ is not required for the induction of chicken neuroretina cell proliferation by RSV.

Feuerman, M.H.,[1] Lee, W.K.,[1] Pattengale, P.K.,[2] Fan, H.,[1] [1]Dept. of Molecular Biology and Biochemistry, University of California, Irvine; [2]Dept. of Pathology, University of Southern California School of Medicine, Los Angeles: Biological comparison of different recombinant MLVs carrying the *v-src* gene of ASV.

Gee, C.E., Griffin, J., Sastre, L., Miller, L.J., Springer, T.A., Piwnica-Worms, H., Roberts, T.M., Dana-Farber Cancer Institute, Boston, Massachusetts: Differentiation of myeloid cells is accompanied by increased levels of $pp60^{c\text{-}src}$ protein and kinase activity.

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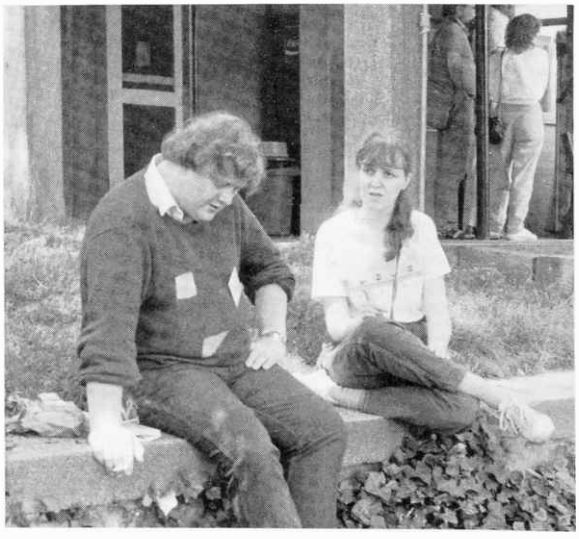
Burr, J.G.,[1] Linder, M.E.,[1] Lui, J.C.,[1] Ferracini, R.,[2] [1]Dept. of Biology, University of Texas, Dallas; [2]Instituto di Istologia, Facolta di Medicina, Torino, Italy: Comparison of tyrosine phosphoproteins detected by anti-ABP antibodies in RSV-infected cells with proteins phosphorylated in situ in Triton X-100 cytoskeletons.



J. Stoye

Rovigatti, U.,[1] Carroll, M.A.,[1] Sacher, R.A.,[2] Jacobson, R.J.,[2] Gallie, B.L.,[3] Yunis, J.J.,[4] [1]A. Ochsner Medical Foundation, New Orleans, Louisiana; [2]Georgetown University Hospital, Washington, D.C.; [3]Ontario Cancer Institute, Toronto, Canada; [4]University of Minnesota Medical School, Minneapolis: Alteration of c-*ets* oncogenes in human neoplasia.

Niman, H.L., Dept. of Molecular Biology, Scripps Clinic and Research Foundation, La Jolla, California: The use of site-directed monoclonal antibodies probes for the detection of oncogene-encoded and oncogene-related proteins.

Tamura, T., Hadwiger, A., Boschek, C.B., Niemann, H., Bauer, H., Institut für Medical Virologie, Justus-Liebig-Universität Giessen, Federal Republic of Germany Characterization of ts-td cell lines transformed by mutants of SM-FeSV.

Bassiri, M., Ng, M., Privalsky, M.L., Dept. of Bacteriology, University of California, Davis: Genetic dissection of erythroleukemia induction by AEV.

Karn, J., Lowe, T., Green, S., MRC Laboratory of Molecular Biology, Cambridge, England: Expression of human c-*myc* in murine fibroblasts and hemopoietic stem cells after retroviral infection.

Singh, B.,[1] Hannink, M.,[2] Donoghue, D.J.,[2] Arlinghaus, R.B.,[1] [1]Research Institute of Scripps Clinic, La Jolla; [2]University of California, San Diego: p37^{mos}-associated protein kinase activity is essential for cellular transformation by Mo-MSV.

SESSION 10 ONCOGENES. I. *myc/myb/mil/raf/mos*

Chairmen: **T. Parsons,** University of Virginia
U. Rapp, National Cancer Institute

Fleissner, E., Sorrentino, V., Droxdoff, V., Zeitz, L., Hume, C., Lee, J., Memorial Sloan-Kettering Cancer Center, New York, New York: Potentiation of growth factor effects by exogenous c-*myc* expression.

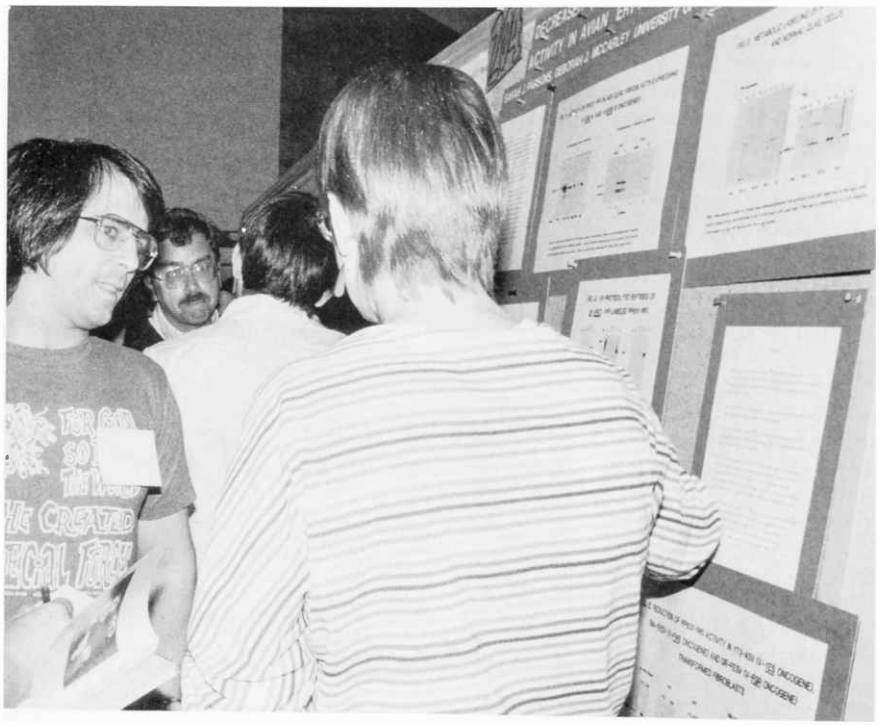
Schuetz, J.D., Westin, E., Depts. of Medicine and Microbiology, Medical College of Virginia, Richmond: c-*myc* amplification as a correlate in the development of drug resistance.

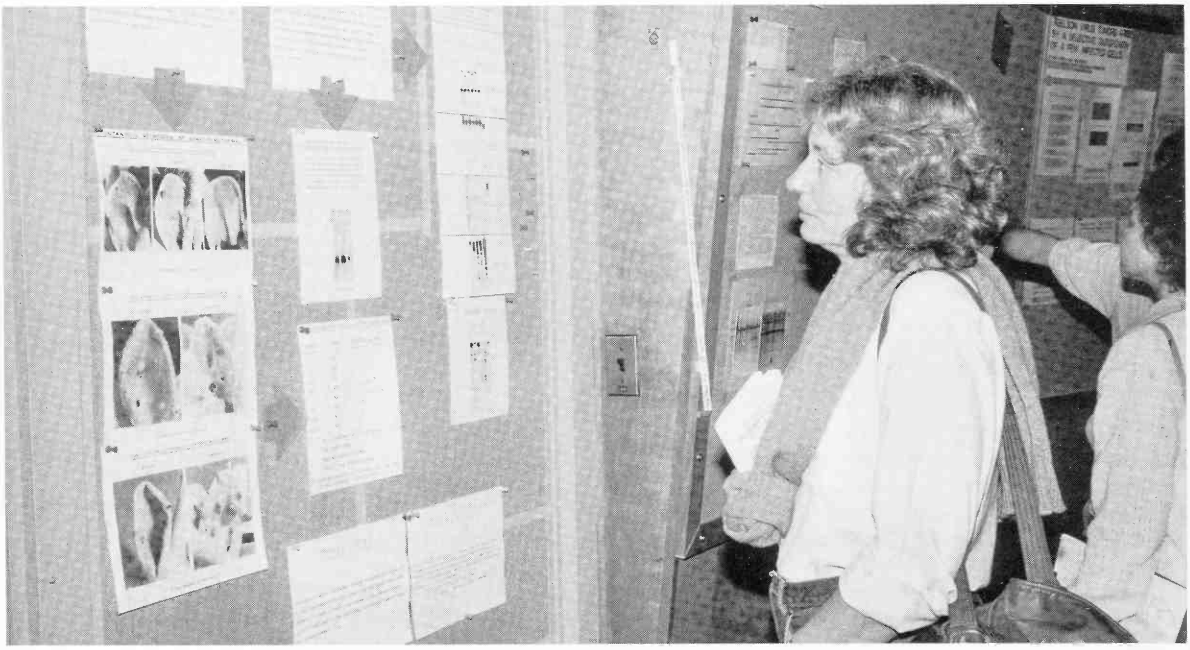
Hann, S.R., Eisenman, R.N., Fred Hutchinson Cancer Research Center, Seattle, Washington: Structural analysis of c-*myc* proteins.

de Lange, T.,[1] Stone, J.,[4] Ramsay, G.,[3] Bishop, J.M.,[1,3] Varmus, H.E.,[1] Lee, W.M.F.,[2] Depts. of [1]Microbiology, [2]Medicine, [3]G.W. Hooper Foundation, University of California, San Francisco; [4]Jackson Laboratory, Bar Harbor, Maine: Mapping the functional domains of normal human c-*myc* by mutagenesis of the normal gene.

Farina, S.F.,[1] Heaney, M.L.,[1] Pierce, J.H.,[2] Parsons, J.T.,[1] [1]Dept. of Microbiology, University of Virginia School of Medicine, Charlottesville; [2]Laboratory of Cell and Molecular Biology, NCI, Bethesda, Maryland: Linker insertion mutagenesis of the *gag-myc* gene of MC29.

Patschinsky, T., Jansen, H.W., Walther, N., Schroeer, B., Trachmann, C., Bister, K., Otto-Warburg-Laboratorium,





A. Skalka

Max-Planck-Institut für molekulare Genetik, Berlin, Federal Republic of Germany: Structure and transforming function of transduced mutant alleles of the chicken c-*myc* gene.

Klempnauer, K.-H., Bonifer, C., Sippel, A., ZMBL, University of Heidelberg, Federal Republic of Germany: Antibodies against conserved epitopes of avian *myb* proteins recognize the human c-*myb* protein and inhibit DNA binding.

Marx, M.,[1] Béchade, C.,[1] Laugier, D.,[1] Crisanti, P.,[2] Pessac, B.,[2] Calothy, G.,[1] [1]Institut Curie-Biologie, Orsay, [2]INSERM, Hôpital Paul Brousse, Villejuif, France: Induction of neuroretina cell proliferation by an avian lymphomatosis virus—A possible effect of c-*mil* activation.

Seth, A.,[1] Priel, E.,[2] Blair, D.,[1] Fisher, R.J.,[1] Vande Woude, G.F.,[1] [1]NCI-Frederick Cancer Research Facility, Frederick, Maryland; [2]Ben Gurion University of Negev, Beer Sheva, Israel: The *mos* protein has ATP-dependent nucleic acid binding properties.

Donoghue, D.J., Bold, R.J., Hannink, M., Dept. of Chemistry, University of California, San Diego, La Jolla: The v-*mos* gene product is transforming when its cytological localization is altered to the membrane.

Arlinghaus, R.B., Singh, B., Dept. of Molecular Biology, Scripps Clinic and Research Foundation, La Jolla, California: Expression of the v-*mos* gene reduces the cellular level of the intermediate filament protein, vimentin.

SESSION 11 ONCOGENES. II. *ras/abl/fps/met*

Chairmen: **D. Lowy**, National Cancer Institute
T. Pawson, Mt. Sinai Hospital Research Institute

Stacey, D.W., Smith, M.R., Roche Institute of Molecular Biology, Nutley, New Jersey: Cellular *ras* proteins and proliferative signal transduction in normal and tumor cells.

Kamata, T.,[1] Sullivan, N.R.,[1] Wooten, M.W.,[1] Bassin, R.H.,[2] Feramisco, J.R.,[1] [1]Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; [2]NCI, National Institutes of Health, Bethesda, Maryland: Altered levels of both C-kinase activity and phosphoinositide metabolites in a flat revertant cell line derived from Ki-MSV transformed cells.

Willumsen, B.V.,[1] Papageorge, A.G.,[2] Kung, H.-F.,[3] Velu, T.,[2] Johnsen, M.,[1] Lowy, D.R.,[2] [1]University Microbiology Institute, Copenhagen, Denmark; [2]NCI, National Institutes of Health, Bethesda, Maryland; [3]Hoffmann-La Roche Inc., Nutley, New Jersey: Mutational analysis of a *ras* catalytic domain.

Gibbs, J.B., Marshall, M.S., DeFeo-Jones, D., D'Alonzo, J.S., Ahern, J., Sigal, I.S., Scolnick, E.M., Virus and Cell Biology Research, Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania: Structural requirements of *ras* proteins for regulation of *Saccharomyces cerevisiae* adenylate cyclase activity.

Rein, A.,[1] McClure, M.,[1] Rice, N.,[1] Luftig, R.,[2] Schultz, A.,[1] [1]NCI-Frederick Cancer Research Facility, Frederick, Maryland; [2]Louisiana State University Medical Center, New Orleans: The myristylation site is required for particle formation by Mo-MLV pr65^{gag} and for transformation by the *gag-abl* transforming protein of Ab-MLV.

Wang, J.Y.J., Morla, A.O., Kipreos, E.T., King, C.Y., Lee, G.J., Dept. of Biology, University of California, San Diego, La Jolla: Tyrosine kinase activity of the c-*abl*- and v-*abl*-encoded proteins.

Daley, G.Q., Ben-Neriah, Y., Bernards, A., Baltimore, D., Whitehead Institute and Dept. of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts: K562 cells contain a breakpoint within the c-*abl* gene and produce a 210-kD *bcr/abl* hybrid protein.

Tidmarsh, G., Muller-Sieburg, C., Whitlock, C., Adkins, B., Sherwood, P., Spangrude, G., Weissman, I., Stanford University School of Medicine, California: A Thy-1^{lo}, B220^{hi} Ab-MLV(Mo) target cell.

Kaul, K., Dean, M., Park, M., Gonzatti-Haces, M., Vande Woude, G.F., NCI-Frederick Cancer Research Facility, Frederick, Maryland: The *met* oncogene in MNNG-HOS cells is a rearranged gene with homology to tyrosine kinases.

Sadowski, I.,[1] Stone, J.C.,[2] Pawson, T.,[1] [1]Mount Sinai Hospital Research Institute, Toronto, Canada; [2]Jackson Laboratory, Bar Harbor, Maine: Contiguous catalytic and extracatalytic domains required for transformation by FSV p130$^{gag\text{-}fps}$.

SESSION 12 ONCOGENES. III. *src/yes*

Chairmen: **H. Hanafusa**, Rockefeller University
J. Brugge, New York State University, Stony Brook

Anderson, S.K., Fujita, D.J., Cancer Research Laboratory and Dept. of Biochemistry, University of Western Ontario, Canada: Splicing of a cryptic intron may be involved in the generation of a class of fusiform (morph^f) mutants.

Mayer, B., Jove, R., Hanafusa, H., Rockefeller University, New York, New York: Stimulation of anchorage-independent growth by p60$^{c\text{-}src}$ at elevated temperature.

Levy, J.,[1] Iba, H.,[2] Hanafusa, H.,[1] [1]Rockefeller University, New York, New York; [2]University of Tokyo, Japan: Activation of the transforming potential of p60$^{c\text{-}src}$ by different structural alterations.

Cooper, J.A., Fred Hutchinson Cancer Research Center, Seattle, Washington: The carboxyterminus of p60$^{c\text{-}src}$ regulates its kinase activity.

Kmiecik, T.E., Shalloway, D., Dept. of Molecular and Cell Biology, Pennsylvania State University, University Park: Activation of pp60$^{c\text{-}src}$ transforming activity by an amino acid substitution at its major site of in vivo tyrosine phosphorylation.

Filson, A.,[1] Yonemoto, W.,[1] Lustig, A.,[1] Wang, J.,[2] Brugge, J.,[1] [1]Dept. of Microbiology, State University of New York, Stony Brook; [2]Dept. of Biology, University of California, San Diego, La Jolla: Detection of phosphotyrosine-containing proteins in polyoma-transformed cells.

Ballmer-Hofer, K., Burger, M.M., Biocenter of the University, Basel, Switzerland: Complex formation between pp60^{src} and vinculin in the plasma membrane of RSV-transformed cells.

Simon, M.,[1] Arrigo, A.-P.,[2] Spahr, P.-F.,[1] [1]Dept. of Molecular Biology, University of Geneva, Switzerland; [2]Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: Purification of a particle containing p34, the major target of the tyrosine kinase activity of pp60^{src}.

Carter, C.,[1] Howlett, A.R.,[2] Bissell, M.J.,[2] Martin G.S.,[1] [1]Dept of Zoology, [2]Lawrence Berkeley Laboratory, University of California, Berkeley: The tyrosine phosphorylation substrate p36 – Developmental regulation in avian limb, induction in cell culture, and phospholipid association.

Sukegawa, J., Semba, K., Yamamoto, T., Toyoshima, K., Institute of Medical Science, University of Tokyo, Japan: Nucleotide sequences of cellular homologs of the *yes* oncogene of Y73 sarcoma virus.

SESSION 13 ONCOGENES. IV. *sis/erbA-B/fms*

Chairmen: **C. Sherr,** St. Jude Children's Research Hospital
S. Martin, University of California, Berkeley

Hannink, M., Donoghue, D.J., Dept. of Chemistry, University of California, San Diego, La Jolla: Mutants of the v-*sis* gene encoding membrane-anchored and biologically active gene products.

Escobedo, J.A.,[1] Yarden, Y.,[2] Daniel, T.O.,[1] Fried, V.A.,[3] Ullrich, A.,[2] Williams, L.T.,[1] [1]Howard Hughes Medical Institute, University of California, San Francisco; [2]Genentech, Inc., San Bruno, California; [3]Dept. of Biochemistry, St. Jude Children's Hospital, Memphis, Tennessee: Structure of the PDGF receptor.

Ellis, L.,[1] Morgan, D.O.,[2] Clauser, E.,[1] Edery, M.,[1] Roth, R.A.,[2] Rutter, W.J.,[1] [1]Hormone Research Institute, University of California, San Francisco; [2]Dept. of Pharmacology, Stanford University, California: Functional analysis of genetically engineered insulin receptor mutants.

Rettenmier, C.W., Jackowski, S., Rock, C.O., Sherr, C.J., Depts. of Tumor Cell Biology and Biochemistry, St. Jude Children's Research Hospital, Memphis, Tennessee: Transformation by the v-*fms* oncogene product/ CSF-1 receptor.

Wheeler, E.F., Roussel, M.F., Rettenmier, C.W., Sherr, C.J., Dept. of Tumor Cell Biology, St. Jude Children's Research Hospital, Memphis, Tennessee: The v-*fms* gene product contains a functional leader peptide enabling transformation in the absence of FeLV *gag* sequences.

Van Beveren, C.,[1] Coussens, L.,[2] Wamsley, P.,[1] Dull, T.,[2] Ullrich, A.,[2] Verma, I.M.,[1] [1]Molecular Biology and Virology Laboratory, Salk Institute, San Diego; [2]Genentech, Inc., South San Francisco, California: The human c-*fms* gene – Structure and transforming potential.

Gamett, D.C.,[1] Tracy, S.E.,[2] Robinson, H.L.,[1] [1]Worcestor Foundation for Experimental Biology, Shrewsbury; [2]Dept. of Molecular Genetics and Microbiology, University of Massachusetts, Worcester: Sequences encoding the C-terminal domain of the EGF receptor determine the disease specificity of v-*erb*B genes.

Damm, K., Beug, H., Graf, T., Vennström, B., European Molecular Biology Laboratory, Differentiation Programme, Heidelberg, Federal Republic of Germany: The cooperative active of v-*erb*A with a defection v-*erb B* can transform a cell type not transformed by either oncogene alone.

Gandrillon, O., Jurdic, P., Xiao, J.-H., Samarut, J., CNRS, Biologie generale et appliquée, Université C. Bernard, France: Analysis of some functions of v-*erb*A oncogene.

Johnson, A.C.,[1] Ishii, S.,[1] Brady, J.,[1] Kadonaga, J.,[2] Tjian, R.,[2] Merlino, G.T.,[1] Pastan, I.,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Biochemistry, University of California, Berkeley: Analysis of the promoter region of the human EGF receptor protooncogene—Detection of binding factors.

SV40, Polyoma, and Adenoviruses

August 13–August 17

ARRANGED BY

Terri Grodzicker, Cold Spring Harbor Laboratory
Michael Botchan, University of California, Berkeley

390 participants

The Tumor Virus meeting on SV40, polyoma, and adenoviruses continues to be the major forum for bringing together a group of over 400 scientists, who presented their latest findings in a variety of areas. The small DNA tumor viruses continue to serve as model systems for dissecting the mechanisms of eukaryotic transcription, RNA processing, replication, and transformation.

There was a great deal of emphasis on the use of biochemical approaches and in vitro systems to analyze important control pathways. Many papers were presented in extremely active areas of research such as SV40, polyomavirus, and adenovirus in vitro DNA replication; the purification of transcription factors and enhancer-binding proteins and their interaction with target DNA sequences; regulation of promoters and enhancers by adenovirus E1A proteins and SV40 T antigen; the role of different domains and protein modifications on the function of E1A and SV40 T antigen proteins; the splicing, termination, and polyadenylation of viral mRNAs; control of translation by VA RNA; the role of different domains of virus-transforming proteins on immortalization and transformation; and interaction of transforming proteins with cellular proteins such as p53 and tyrosine kinases.

The many talks that were presented, as well as well-attended poster sessions and hours of informal discussion, attest to the continuing productivity of tumor virus research and the new findings that continue to arise in the field.

This meeting was supported in part by the Cancer Center Grant to Cold Spring Harbor Laboratory from the National Cancer Institute, National Institutes of Health.

SESSION 1 REPLICATION

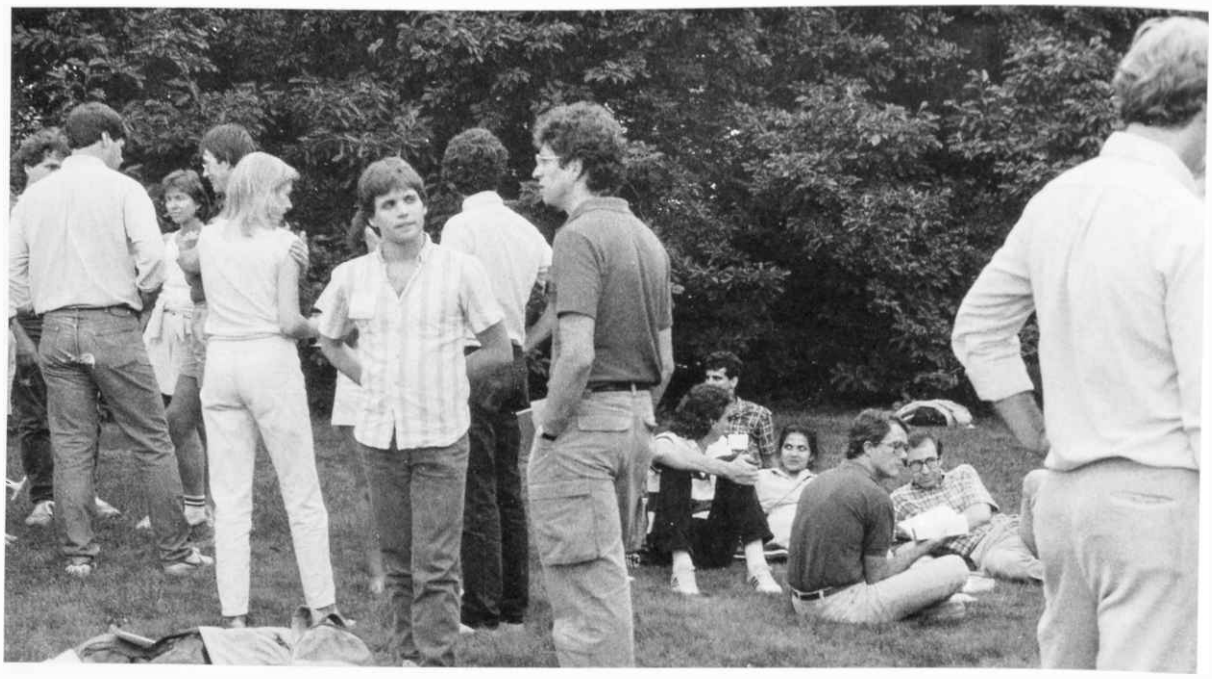
Chairman: Bruce Stillman, Cold Spring Harbor Laboratory

Wobbe, R., Weissbach, L., Dean, F., Murakami, Y., Hurwitz, J., Graduate Program in Molecular Biology and Virology, Sloan-Kettering Institute for Cancer Research, New York, New York: In vitro replication of SV40 DNA.

Prelich, G., Fairman, M., Stillman, B., Cold Spring Harbor Laboratory, New York: Cell-free replicaton of SV40 DNA.

Mohr, I., Gerard, R., Strauss, M., Argani, P., Gluzman, Y., Cold Spring Harbor Laboratory, New York: Properties of SV40 T antigen produced in HeLa cells or in *E. coli.*

Bradley, M.K.,[1] Weiner, B.,[2] Possenti, R.,[2] Decker, S.,[2] DePamphilis, M.L.,[2] [1]Dept. of Pathology, Harvard Medical School and Dana-Farber Cancer Institute, [2]Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: SV40 large T antigen and replication of



SV40 DNA in vitro.

Kenny, M.K., Balogh, L.A., Shneidman, P.S., Lindenbaum, J., Hurwitz, J., Graduate Program in Molecular Biology, Memorial Sloan-Kettering Cancer Research Center, New York, New York: Studies on the mechanism of initiation of adenovirus DNA replication.

Bennett, E.R., Naujokas, M., Collins, C., Hassell, J.A., Dept. of Microbiology and Immunology, McGill University, Montreal, Canada: The host range for replication of SV40-polyomavirus hybrid origins for DNA replication.

Campbell, B.A., Villarreal, L.P., Dept. of Molecular Biology and Biochemistry, University of California, Irvine: The polyomavirus B enhancer can activate or repress DNA replication in mouse lymphoid cell lines.

Bradley, M.K., Webster, T., Smith, T., Livingston, D.M., Dana-Farber Cancer Institute, Harvard Medical School and School of Public Health, Boston, Massachusetts: Further analyses of the SV40 large T antigen nucleotide-binding site and adenylation reaction.

Deb, S., DeLucia, A., Koff, A., Tsui, S., Tegtmeyer, P., Dept. of Microbiology, State University of New York, Stony Brook: The AT domain of the SV40 core origin regulates DNA replication and DNA bending.

Fanning, E., Vogt, B., Arthur, A., Vakalopoulou, E., Institute for Biochemistry, Munich, Federal Republic of Germany: Allosteric control of SV40 T antigen binding to viral origin DNA.

Roberts, J., Weintraub, H., Dept. of Genetics, Fred Hutchinson Cancer Center, Seattle, Washington: Negative control of DNA replication in SV40-BPV composite plasmids.

SESSION 2 TRANSCRIPTION. 1. ADENOVIRUSES

Chairman: A. Berk, University of California, Los Angeles

Postel, E.H., Marton, M., Flint, S.J., Dept. of Molecular Biology, Princeton University, New Jersey: A HeLa cell factor required for in vitro transcription of the Ad2 IVa2 gene.

Buckbinder, L., Flores, O., Reinberg, D., Dept. of Biochemistry, State University of New York, Stony Brook: Reconstitution of transcription at the adenovirus major late promoter.

Goding, C., Temperley, S., Fisher, F., Marie Curie Memorial Foundation, Research Institute, Surrey, England: Interactions between HeLa cell factors and the Ad2 E2-late promoter.

Garcia, J., Teitel, M., Wu, F., Gaynor, R., Dept. of Medicine, University of California School of Medicine, Los Angeles: Identification of regulatory sequences and cellular factors involved in adenovirus early region 3 transcription regulation.

Wu, L., Schmidt, M., Berk, A., Molecular Biology Institute, University of California, Los Angeles: Regulation of human Ad2 E1B transcription.

Parks, C.L., Spector, D.J., Dept. of Microbiology, Pennsylvania State University College of Medicine, Hershey: Genetic analysis of the E1B promoter of Ad5.

White, E., Denton, A., Stillman, B., Cold Spring Harbor Laboratory, New York: Role of the adenovirus E1B 19K tumor antigen in negatively regulating E1A-dependent early gene expression.

Senear, A.W.,[1] Anderson, C.W.,[2] Lewis, J.B.,[1] [1]Fred Hutchinson Cancer Research Center, Seattle, Washington; [2]Brookhaven National Laboratories, New York: Ad2 region E1B—New proteins and effects on expression of E1A.

Donath, C., Gander, R.I., Mack, A., Marschall, A., Meister-

ernst, M., Müller, U., Thalmeier, K., Winnacker, E.L., Institut für Biochemie, Universität München, Federal Republic of Germany: Purification and properties of nuclear factor I.

Weyer, U., Langner, K.-D., Klimkait, T., Knebel, D., Doerfler, W., Institute of Genetics, University of Cologne, Federal Republic of Germany: Complex controls in the activity of viral promoters.

SESSION 3 POSTER SESSION

Albin, R.L., Flint, S.J., Dept. of Molecular Biology, Princeton University, New Jersey: Stimulation of promoter element–protein interactions in adenovirus-infected HeLa cell extracts.

Cousin, C., Leite, J.P.G., D'Halluin, J.C., Laboratoire de Virologie Moléculaire, INSERM, Lille, France: Autoregulation of adenovirus DNA-binding protein preferentially occurred at the E2A early promoter level.

Brunet, L.J.,[1] Babiss, L.E.,[3] Mills, D.R.,[1] Young, C.S.H.,[2] Depts. of [1]Human Genetics and Development, [2]Microbiology, Columbia University, New York; [3]Rockefeller University, New York, New York: Role during infection of sequences flanking the TATA box of the Ad5 major late promoter.

Leza, A., Hearing, P., Dept. of Microbiology, State University of New York, Stony Brook: Genetic analysis of the Ad5 E4 transcriptional control region.

Hough, P.,[1] Mastrangelo, I.,[1] Sawadogo, M.,[2] Roeder, R.,[2] [1]Dept. of Biology, Brookhaven National Laboratory, Upton, [2]Rockefeller University, New York, New York: The Ad2 gene-specific transcription factor, USF–Mass and 15-Å structure by STEM.

SivaRaman, L., Bhat, G., Murthy, S., Domer, P., Thimmappaya, B., Dept. of Micro-Immunology, Northwestern University Medical School, Chicago, Illinois: Adenovirus E2A-late promoter–Transcriptional control regions and effect of the two E1A gene products on its expression.

Clark, L., Barrett, P., Hay, R.T., Dept. of Biochemistry and Microbiology, University of St. Andrews, Scotland: Interactions of cellular proteins with DNA sequences at the left terminus of Ad2.

Vasudevachari, M.B., Natarajan, V., Salzman, N.P., NIAID, National Institutes of Health, Bethesda, Maryland: Cotransfection with adenovirus DNA enhances transcription from linear plasmids containing eukaryotic promoters.

Takemori, N., Kumai, H., Ishi, M., Hashimoto, S., Division of Molecular Biology, Meiji Institute of Health Science, Odawara, Japan: Expression of E1B gene functions of human adenoviruses in *gpt*$^+$ KB cell lines.

Barker, D.D., Berk, A., Molecular Biology Institute, University of California, Los Angeles: The role of the Ad2 early region 1B gene products during infection and transformation.

Schaller, M.D., Caussy, D., Mak, S., Dept. of Biology, McMaster University, Hamilton, Canada: Regulation of gene expression by cytocidal mutants of Ad12.

McGlade, C.J., Tremblay, M.L., Branton, P.E., Dept. of Pathology, McMaster University, Ontario, Canada: Posttranslational modifications of the Ad5-176R E1B protein.

Chroboczek, J.,[1] Viard, F.,[1] D'Halluin, J.-C.,[2] [1]European Molecular Biology Laboratory, Grenoble, [2]Laboratoire de Virologie Moléculaire, Lille, France: Human Ad2 *ts* mutant 112 contains two mutations in the protein IIIa gene.

Lavery, D.,[1] Fu, S.M.,[2] Chen-Kiang, S.,[1] [1]Immunobiology Center, Mount Sinai Medical School, New York, New York; [2]Oklahoma Medical Research Foundation and Dept. of Medicine, University of Oklahoma, Oklahoma City: Molecular interaction of adenovirus with human lymphocytes.

Hong, J.S., Shu, L., Wei, Y.-F., Engler, J.A., Dept. of Biochemistry, University of Alabama, Birmingham: The nucleotide sequence of early region 2B of Ad12.

Wu, G.-J., Dept. of Microbiology and Immunology, Emory University School of Medicine, Atlanta, Georgia: Transcription of the canine Ad2-specific VA RNA gene.

Abmayr, S.M., Roeder, R.G., Rockefeller University, New York, New York: Further studies on the effect of the PRV IE protein on in vitro transcription.

Van Dyke, M., Roeder, R.G., Rockefeller University, New York, New York: Proteins that interact with the VA RNA genes of adenovirus.

Yoshinaga, S.K., Dean, N., Boulanger, P., Berk, A., Molecular Biology Institute, University of California, Los Angeles: Adenovirus E1A functions stimulate transcription of class III genes by increasing the amount of active transcription factor TFIIIC.

Mirza, A., Institut für Molekularbiologie, Essen, Federal Republic of Germany: Cellular proteins mediated binding of Ad12 E1A–encoded tumor antigens to viral transcriptional complexes.

Reichel, R., Kovesdi, I., Nevins, J., Rockefeller University, New York, New York: A developmentally regulated transcription factor is also regulated by the E1A gene product.

Nishigaki, T., Hanaka, S., Maeyanagi, H., Watanabe, H., Handa, H., Institute of Medical Science, University of Tokyo, Japan: Transactivation of transcription of adenovirus early region 4 by E1A gene products.

Webster, L.C., Friedman, D.J., Glenn, G.M., Ricciardi, R.P., Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania: Conserved cysteines in the 289R E1A protein are essential for E1A-induced transcription.

Stein, R.,[1] Ziff, E.,[2] [1]Dept. of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, Tennessee; [2]Dept. of Biochemistry, New York University Medical Center, New York, New York: Repression of insulin gene expression by Ad5 E1A proteins.

Zerler, B., Roberts, R.J., Mathews, M.B., Moran, E., Cold Spring Harbor Laboratory, New York: Different domains of the adenovirus E1A gene are involved in the regulation of host-cell-cycle-dependent genes.

Zullo, J., Stiles, C.D., Garcea, R.L., Dana-Farber Cancer Institute, Boston, Massachusetts: Induction of c-*myc* and c-*fos* by polyomavirus.

Lipp, M., Kausel, G., Institut für Biochemie der Universität, München, Federal Republic of Germany: *Trans*-activation of the human c-*myc* promoter by E1A of adenoviruses.

Pannuti, A., La Mantia, G., Pascucci, A., Lania, L., Dept. of

Genetics, University of Naples, Italy: Regulation of mouse *H-2* class-I gene expression by DNA tumor viruses.

Morimoto, R.I., Milarski-Brown, K., Williams, G., Wu, B.J., Dept. of Biochemistry, Molecular and Cellular Biology, Northwestern University, Evanston, Illinois: Trancription of a growth-regulated and adenovirus-inducible human heat-shock gene.

Solomon, W.,[1,2] Gwinn, K.,[1,2] Cowie, A.,[3] Kingston, R.,[1,2] [1]Dept. of Molecular Biology, Massachusetts General Hospital, Boston, Massachusetts; [2]Dept. of Genetics, Harvard Medical School, Cambridge, [3]Genetics Institute, Cambridge, Massachusetts: Regulation of the human hsp70 promoter by papovavirus T antigens.

Tsuzuki, H., Dept. of Microbiology, Immunology and Parasitology, Louisiana State University Medical Center, New Orleans: Effect of adenovirus E1A on protein synthesis in normal human cells.

Opalka, B., Roy, A., Reith, M., Koppe, J., Schulte-Holthausen, H., Institut für Molekularbiologie, Universitätsklinikum Essen, Federal Republic of Germany: Regulation of cellular genes by E1A deletion mutants of Ad12.

Picardi, J., Alwine, J.C., Dept. of Microbiology, University of Pennsylvania, Philadelphia: Evidence for repression of the SV40 late promoter during the early phase of infection.

Thompson, M.A., Brady, J., NCI, National Institutes of Health, Bethesda, Maryland: *Trans*-acting functions in SV40 late gene expresson.

Ghosh, P.K., Garcia-Blanco, M.A., Ivory, S., Payne, M., Lebowitz, P., Depts. of Internal Medicine and Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Connecticut: Viral late transcription and upstream shift of viral early start sites in the absence of DNA replication in HeLa cells transfected with SV40 DNA.

Ghosh, P.K., Garcia-Blanco, M.A., Rao, V.N., Payne, M., Bloodgood, R., Weissman, S.M., Lebowitz, P., Depts. of Internal Medicine, Human Genetics and Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Connecticut: SV40 late transcription in cycloheximide and cytosine arabinoside-treated cells – Two classes of late transcription initiation sites and negative regulation.

Kupelian, A., Acheson, N.H., Dept. of Microbiology and Immunology, McGill University, Montreal, Canada: Ribonucleoside triphosphate-dependent pausing of RNA polymerase II in vitro nearby the SV40 late promoter.

Das, G.C., Saikh, K.U., School of Basic Life Sciences, University of Missouri, Kansas: Organization and regulation of overlapping early and late promoters of SV40.

Chalifour, L.E.,[1] Wirak, D.O.,[3] Hansen, U.,[2] Wassarman, P.M.,[4] DePamphilis, M.L.,[3] [1]National Research Council on Biotechnology, Montreal, Canada; [2]Dana-Farber Cancer Institute [3]Harvard Medical School, Boston, Massachusetts; [4]Roche Institute, Nutley, New Jersey: SV40 promoter analysis in microinjected mouse oocytes.

Ondek, B., Sturm, R., Herr, W., Cold Spring Harbor Laboratory, New York: Multiple copies of separate SV40 enhancer "modules" confer transcriptional activation.

Green, J.C., Khoury, G., NCI, National Institutes of Health, Bethesda, Maryland: The 72-bp element contains a critical control region for SV40 late expression in *X. laevis* oocytes.

Firak, T.A., Chandrasekharappa, S.C., Gong, S.-S., Subramanian, K.N., Dept. of Microbiology and Immunology, University of Illinois College of Medicine, Chicago: Multiple functions of the SV40 72-bp repeat enhancer.

Gong, S.-S., Chandrasekharappa, S.C., Hartzell, S.W., Subramanian, K.N., Dept. of Microbiology and Immunology, University of Illinois College of Medicine, Chicago: The 72-bp repeat enhancer, large T antigen, and Sp1-binding sites and activation of the SV40 late promoter.

Lutter, L.C., Petryniak, B., Hadlock, K.G., Quasney, M.W., Dept. of Biological Chemistry, University of Michigan, Ann Arbor: T-antigen content and topological characterization of SV40 transcription complexes.

Brown, M., Livingston, D., Hansen, U., Roberts, T., Dana-Farber Cancer Institute and Harvard Medical School, Boston, Massachusetts: The *E. coli* lactose operon repressor and operator can function to regulate the activity of the SV40 early promoter in animal cells.

Pater, A., Pater, M., Faculty of Medicine, Memorial University of Newfoundland, St. John's, Canada: Differential expression of BK virus and SV40 early promoter sequences in nontransformed and transformed human embryonic kidney cells.

Min, H.Y., Auborn, K., Manley, J., Prives, C., Dept. of Biological Sciences, Columbia University, New York, New York: SV40 large T antigen binds specifically to human U-1 and U-2 genes.

Carey, M., Karambir, S., Cozzarelli, N.R., Botchan, M., Dept. of Molecular Biology, University of California, Berkeley: Induction of specific transcription by RNA polymerase III in SV40-transformed cells – An in vitro analysis.

Piette, J., Kryszke, M.-H., Yaniv, M., Institut Pasteur, Paris, France: Specific interaction of mouse cellular factors with the functional domains of the polyomavirus enhancer.

Perona, R.,[1] Wirak, D.,[1] Chalifour, L.,[2] Cupo, D.,[1] Hassell, J.,[3] Wassarman, P.,[4] DePamphilis, D.,[1] [1]Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts; [2]NRC Biotechnology Research Institute, [3]Dept. of Microbiology and Immunology, McGill University, Montreal, Canada; [4]Roche Institute of Molecular Biology, Nutley, New Jersey: Positive and negative regulation of transcriptional elements during mouse development and their role in DNA replication.

Bonnell, K., Carmichael, G., Dept. of Microbiology, University of Connecticut Health Center, Farmington: Sequences required for the function of the polyoma virus late promoter.

Grinnell, B., Berg, D., Walls, J., Dept. of Molecular Biology, Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, Indiana: The BK virus enhancer potentiates the adenovirus type-2 late promoter in the presence of *trans*-acting viral proteins.

Loeber, G., Dörries, K., Institut für Virologie, Universität Würzburg, Federal Republic of Germany: Structural rearrangements in the control region of the human polyomavirus JC, strain GS.

Abraham, G., Manor, H., Dept. of Biology, Technion-Israel-Institute of Technology, Haifa, Israel: Transcription map of the African green monkey lymphotropic papovavirus.

SESSION 4 TRANSCRIPTION. 2. TRANSACTIVATION

Chairman: **J. Alwine**, University of Pennsylvania, Philadelphia

Kovesdi, I., Reichel, R., Nevins, J., Rockefeller University, New York, New York: Role of the transcription factor E2F in E1A-mediated coordinate gene control.

Loeken, M.R., Duvall, J., Brady, J., NCI, National Institutes of Health, Bethesda, Maryland: *Trans*-activation of the adenovirus E2 early promoter by SV40 T antigen and adenovirus E1A may involve interaction between transcription factors bound to inducible promoter elements.

Subramanian, S., SivaRaman, L., Thimmappaya, B., Dept. of Microbiology and Immunology, Northwestern University Medical School, Chicago, Illinois: Identification of a factor in HeLa cells specific for an upstream transcriptional control sequence of an E1A- inducible adenovirus promoter and its relative abundance in infected and uninfected cells.

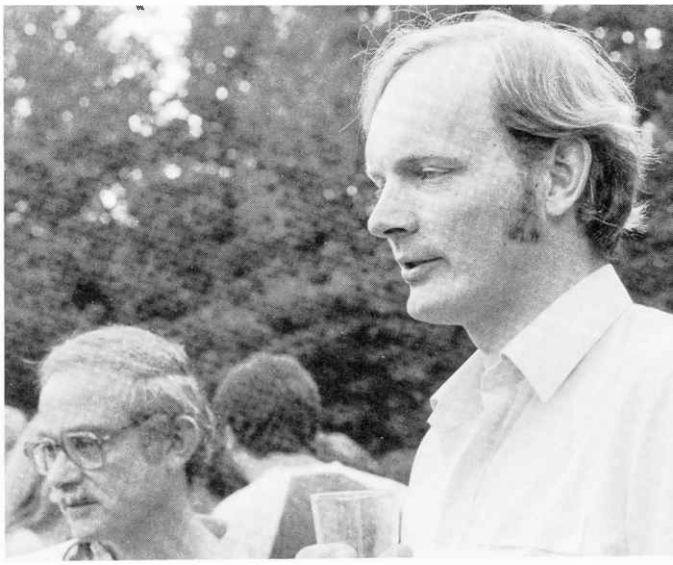
Leong, K., Brunet, L., Berk, A.J., Molecular Biology Institute and Dept. of Microbiology, University of California, Los Angeles: Adenovirus E1A protein increases the number of template molecules transcribed in cell-free extracts.

Glenn, G.M., Ricciardi, R.P., Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania: The protein product of an E1A missense mutant gene of adenovirus blocks wild-type E1A *trans*-activation by sequestering a positive transcription factor.

Velcich, A., Kern, F., Kegler, D., Basilico, C., Ziff, E., Depts. of Biochemistry and Pathology and Kaplan Cancer Center, New York University Medical Center, New York: Adenovirus proteins repress expression from the polyomavirus early and late promoters.

Sassone-Corsi, P., Fromental, C., Chambon, P., CNRS, INSERM, University of Strasbourg, France: A *trans*-acting repressor is responsible for the nonfunction of the polyomavirus enhancer in undifferentiated embryonal carcinoma cells.

La Thangue, N.B., Rigby, P.W.J., Laboratory of Eukaryotic Molecular Genetics, National Institute for Medical Research, London, England: Regulated expression of SV40 and adenovirus promoters in murine embryonal carcinoma stem cell extracts.



M. Horwitz, D. Lane

Lee, K.A.W., Lin, Y.S., Green, M.R., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: In vivo and in vitro analysis of an adenovirus E1A-dependent transcriptional enhancer.

Natarajan, V., Salzman, N.P., NIAID, National Institutes of Health, Bethesda, Maryland: Ad2 E1A and E1B gene products regulate enhancer-mediated transcription.

Bruder, J., Hearing, P., Dept. of Microbiology, State University of New York, Stony Brook: Genetic and biochemical analyses of the Ad5 E1A enhancer region.

SESSION 5 TRANSCRIPTION 3: SV40, POLYOMA

Chairman: **R. Tjian**, University of California, Berkeley

Tjian, R., Briggs, M., Mitchell, P., Lee, W., Kadonaga, J., Jones, K., Dept. of Biochemistry, University of California, Berkeley: Interplay of multiple sequence-specific transcription factors within the SV40 promoter and enhancer elements.

Gallo, G.J., Picardi, J., Alwine, J.C., Dept. of Microbiology, University of Pennsylvania School of Medicine, Philadelphia: Activation of the SV40 late promoter – Evidence that T antigen affects a cellular *trans*-acting factor.

Beard, P., Bruggmann, H., Swiss Institute for Experimental Cancer Research, Epalinges, Switzerland: A transcription factor activating the SV40 late promoter.

Kim, C.H., Heath, C., Bertuch, A., Hansen, U., Dana-Farber Cancer Institute, and Dept. of Pathology, Harvard Medical School, Boston, Massachusetts: Stimulation of the postreplicative (late) mode of SV40 transcription by a cellular factor that specifically binds the SV40 21-bp repeats.

Kern, F.G.,[1] Pellegrini, S.,[1] Cowie, A.,[2] Basilico, C.,[1] [1]Dept. of Pathology, New York University School of Medicine, New York; [2]Genetics Institute, Cambridge, Massachusetts: Effect of the polyomavirus early proteins on late promoter function.

Shepard, A., Clarke, J., Ondek, B., Herr, W., Cold Spring Harbor Laboratory, New York: Revertants of SV40 enhancer mutants – Structure and cell preference.

Ostapchuk, M.,[1] Diffley, J.,[2] Bruder, J.,[1] Stillman, B.,[2] Levine, A.,[3] Hearing, P.,[2] [1]Dept. of Microbiology, State University of New York, Stony Brook; [2]Cold Spring Harbor Laboratory, New York; [3]Dept. of Molecular Biology,

Princeton University, New Jersey: Interaction of a nuclear factor with the polyomavirus enhancer region.

Kryszke, M.-H., Piette, J., Yaniv, M., Dept. de Biologie Moléculaire, Institut Pasteur, Paris, France: Differentiation-stage-specific interaction of nuclear factors with the enhancer region of polyomavirus.

Stenlund, A., Bream, G., Botchan, M., Dept. of Molecular Biology, University of California, Berkeley: A cellular transcription factor–binding site is a *cis* regulatory element for two promoters and a replication origin in the bovine papilloma virus.

Passananti, C., Fried, M., Imperial Cancer Research Fund, London, England: Characterization of a mouse DNA sequence with enhancer activity.

Khalili, K., Khoury, G., NCI, National Institutes of Health, Bethesda, Maryland: The potential role of protein-protein interactions in the regulation of early RNA synthesis and gene expression.

C. Cole, D. Livingston, W. Eckhart, T. Grodzicker

SESSION 6 POSTER SESSION

Murakami, Y.,[1] Prives, C.,[2] Eki, T.,[3] Yamada, M.,[3] Hurwitz, J.,[1] [1]Graduate Progam in Molecular Biology, Sloan-Kettering Institute for Cancer Research, [2]Dept. of Biology, University of Columbia, New York, New York; [3]Faculty of Pharmaceutical Science, University of Tokyo, Japan: In vitro synthesis of DNA containing the polyomavirus replication origin.

Kumar, R., Yoon, K.P., Subramanian, K.N., Dept. of Microbiology and Immunology, University of Illinois College of Medicine, Chicago: Replication from the SV40 origin is inhibited by reiteration of the 72-bp repeat enhancer.

Gerard, R.D., Gluzman, Y., Cold Spring Harbor Laboratory, New York: A functional analysis of the AT-rich region and upstream flanking sequences in SV40 DNA replication.

Hendrickson, E.A.,[1] Heine, U.,[1] Fritze, C.E.,[1] Folk, W.R.,[2] DePamphilis, M.L.,[1] [1]Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts; [2]Dept. of Microbiology, University of Texas, Austin: Elements of papovavirus origins of replication that determine initiation sites for DNA synthesis.

Simmons, D., Chou, W., Rodgers, K., School of Life and Health Sciences, University of Delaware, Newark: Does T antigen have two SV40 DNA origin-binding regions?

Arthur, A., Vogt, B., Dehde, S., Fanning, E., Institute for Biochemistry, Munich, Federal Republic of Germany: Expression of the origin-DNA-binding domain of SV40 large T antigen in bacteria.

Vakalopoulou, E., Runzler, R., Fanning, E., Institute for Biochemistry, Munich, Federal Republic of Germany: Toward a mechanism of SV40 large T antigen DNA binding—The active form of T antigen and the sequence elements that determine binding affinity.

Graesser F.A., Walker, G., University of California, San Diego, La Jolla: State of phosphorylation of SV40 large T antigen has no effect on ATPase activity.

Muller, W.J., Hassell, J.A., Dept. of Microbiology and Immunology, McGill University, Montreal, Canada: Multiple sequence elements within the polyomavirus enhancer are required in *cis* for DNA replication.

Berger, H., Wintersberger, E., Institute of Molecular Biology, University of Vienna, Austria: Polyomavirus small T antigen enhances replication of viral genomes in 3T6 cells.

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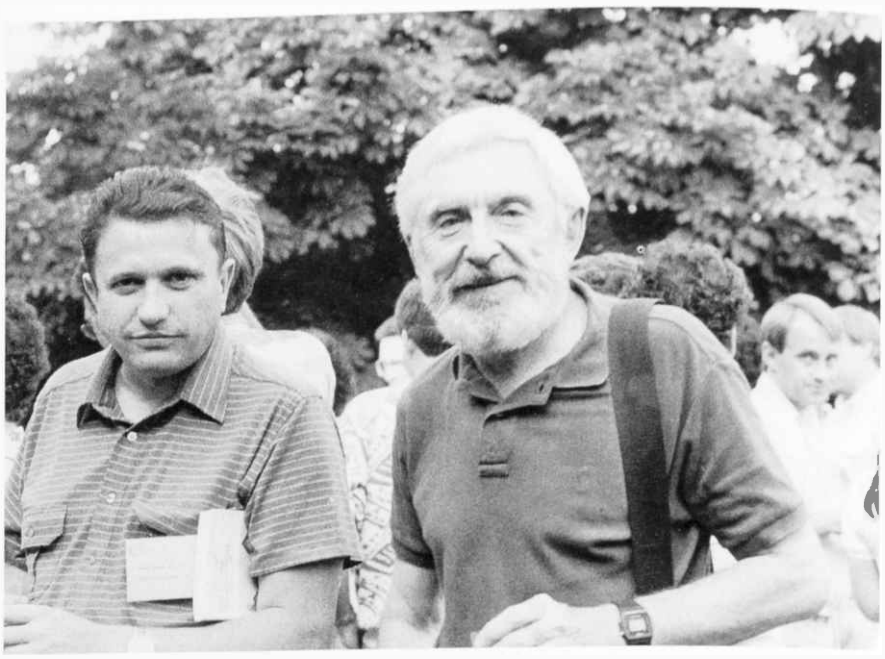

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Ulfendahl, P.J., Pettersson, U., Akusj Dept. of Medical Genetics, Uppsala University, Sweden: RNA sequence requirement for E1A pre-mRNA splicing.

SESSION 7 POSTER SESSION

Wilson, V.G., Dept. of Medical Microbiology and Immunology, Texas A&M University, College Station: Methylation of specific cytosines enhances binding of SV40 T antigen to origin DNA.

Mulgaonkar, P., Rundell, K., Dept. of Microbiology, Northwestern University, Chicago, Illinois: Anti-peptide antibodies to an internal unique determinant of SV40 small T antigen.

Mole, S.E., Lane, D.P., Imperial Cancer Research Fund, Clare Hall Laboratories, England: A function and immunochemical analysis of SV40 large T antigen using fusion proteins.

Rutila, J.E., Wilson-Gunn, S.I., Imperiale, M.J., Dept. of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor: Biological and biochemical characteristics of SV40 large T antigen mutants.

Lathe, R.,[1] Kieny, M.P.,[2] Gerlinger, P.,[1] Guizani, I.,[3] Clertant, P.,[3] [1]LGME-CNRS, INSERM, [2]Transgène S.A., Strasbourg, [3]INSERM, Nice, France: Vaccinia recombinants expressing polyomavirus T antigens – Biochemical and immunological studies.

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Lane, D.P., Gannon, J., Imperial Cancer Research Fund, Clare Hall Laboratories, England: Analysis of the large T antigen–p53 complex in vitro and in vivo.

Gamble, J., Milner, J., Dept. of Pathology, Cambridge University, England: Immunological variants of p53 in SV40-transformed cells.

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Kohrman, D.C.,[1] Christensen, J.B.,[1] Silverstein, G.H.,[2] Imperiale, M.J.,[1,2] [1]Dept. of Microbiology and Immunology, [2]Graduate Program in Cellular and Molecular Biology, University of Michigan, Ann Arbor: Identification of cellular genes involved in SV40 transformation.

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Cook, D.N., Hassell, J.A., Dept. of Microbiology and Immunology, McGill University, Montreal, Canada: The amino terminus of polyomavirus middle T antigen is required for transformation.

McBroom, C.A.K., Sheinin, R., Dept. of Microbiology, University of Toronto, Canada: Transformation of temperature-sensitive mouse cells by SV40.

Sagawa, K., Yamaguchi, N., Dept. of Virology, Institute of Medical Science, University of Tokyo, Japan: Intracellular changes induced in parallel with the increasing amount of SV40 large T antigen.

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Bethesda, Maryland: Adenovirus E1A gene control of susceptibility of infected rodent cells to lysis by host killer cells.

Sircar, S., Palkonyay, L., Horvath, J., Rodrigues, M., Weber, J., Dept. of Microbiology, Université de Sherbrooke, Canada: Correlation of drug-resistance and de-transformation in adenovirus-transformed cells.

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Barber, L.A., Gralla, J.D., Molecular Biology Institute, University of California, Los Angeles: Analysis of SV40 point mutants with inactivated initiation codons for VP2 and VP3.

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Choder, M., Aloni, Y., Dept. of Genetics, Weizmann Institute of Science, Rehovot, Israel: The actively transcribed, bulk SV40 minichromosomes have a low negative supercoiling, which can be enhanced in vivo by novobiocin.

Babé, L.M., Scott, W.A., Dept. of Biochemistry, University of Miami School of Medicine, Florida: Monoclonal antibodies to VP1 in SV40 chromatin.

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Hsu, M.-T., Wong, M.L., Gupta, S., Dept. of Microbiology, Mount Sinai Medical Center, New York, New York: Change in the structure of Ad5 and SV40 nucleoprotein complexes during lytic infection.

Milavetz, B.I., Mackinnon, J., DeMoor, J., Cancer Reseach Laboratory and Dept. of Biochemistry, University of Western Ontario, London, Canada: Restriction endonuclease digestion as a probe of higher-order chromatin structure in SV40 chromosomes.

Iacono, L.C., Kowalski, D., Dept. of Molecular and Cellular Biology, Roswell Park Memorial Institute, Buffalo, New York: Cruciforms in the enhancer region of torsionally stressed SV40 DNA.

O'Neill, F., Stevens, R., Miller, T., Dept. of Cellular, Viral, and Molecular Biology, University of Utah, Salt Lake City: Late SV40 sequences required for defective genome competition with wt SV40.

Ostrander, E., Kondoleon, S., Kurkinen, N., Karty, R., MacDonald, N., Hallick, L., Dept. of Microbiology and Immunology, Oregon Health Sciences University, Portland: Mapping the preferential psoralen-binding sites in the SV40 origin.

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Ludlow, J.W., Fattaey, A., Consigli, R.A., Dept. of Biology, Kansas State University, Manhattan: Comparison of biological activity and VP1 phosphorylation in polyomavirus propagated in primary mouse kidney and primary mouse embryo cells.

Marriott, S.J., Griffith, G.R., Consigli, R.A., Dept. of Biology, Kansas State University, Manhattan: Anti-idiotypic antibodies bind to the surface of mouse kidney cells and inhibit polyomavirus infection.

Cousin, C., Leite, J.P.G., D'Halluin, J.C., Laboratoire de Virologie Moléculaire, INSERM, Lille, France: Autoregulation of adenovirus DNA-binding protein preferentially occurred at the E2A promoter level.

SESSION 8 POST-TRANSCRIPTIONAL REGULATION

Chairman: J. Mertz, University of Wisconsin

Fu, X.-Y., Noble, J.C., Prives, C., Manley, J.L., Dept. of Biological Sciences, Columbia University, New York, New York: *Cis*- and *trans*-acting factor(s) control alternative splicing of SV40 early pre-mRNA.

Good, P.J., Mertz, J.E., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: In vitro splicing of the late RNAs of SV40.

Adami, G.R., Marlor, C.W., Carmichael, G.G., Dept. of Microbiology, University of Connecticut Health Center, Farmington: The length (but not the sequence) of the polyomavirus late leader is important for viability, late RNA splicing, and RNA stability.

Harper, J., Roberts, R., Herr, W., Cold Spring Harbor Laboratory, New York: Adenovirus E1A splicing—Modulation of splice-site selection in vitro.

Bhat, B., Wold, W., Institute for Molecular Virology, St. Louis University Medical School, Missouri: Differential pre-mRNA processing in region E3 of adenovirus at early and late stages of infection.

DeZazzo, J.D.,[1] Hales, K.H.,[2] Imperiale, M.J.,[1,2] [1]Graduate Program in Cellular and Molecular Biology, [2]Dept. of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor: Delineation of sequences required for cleavage and regulation of the adenovirus L1 and L2 poly(A) sites.

Resnekov, O., Pruzan, R., Bratosin, S., Aloni, Y., Dept. of Genetics, Weizmann Institute of Science, Rehovot, Israel: Analysis of in vitro termination at the L1 site of Ad2.

Yang, U.-C., Flint, S.J., Dept. of Molecular Biology, Princeton University, New Jersey: Transport of transcripts of inducible genes in adenovirus-infected cells.

O'Malley, R.P.,[1] Mellits, K.H.,[1] Rice, A.P.,[1] Kostura, M.,[1] Maran, A.,[1] Duncan, R.,[2] Hershey, J.W.B.,[2] Mathews, M.B.,[1] [1]Cold Spring Harbor Laboratory, New York;

[2]University of California, Davis: VA RNA regulates protein synthesis in adenovirus-infected cells.

Svensson, C.,[1] Kreivi, J.-P.,[1] Nygård, O.,[2] Akusjärvi, G.,[1] [1]Dept. of Medical Genetics, Uppsala University; [2]Dept. of Zoological Cell Biology, University of Stockholm, Sweden: Aberrant RNA splicing and reduced late mRNA accumulation in the absence of adenovirus VA RNA

Dabrowski, C., Alwine, J.C., Dept. of Microbiology, University of Pennsylvania School of Medicine, Philadelphia: Analysis of SV40 late gene expression—Effects of intragenic sequences on transcription and translation.

Soloway, P., Shenk, T., Dept. of Molecular Biology, Princeton University, New Jersey: An Ad5 mutant that fails to synthesize the i leader protein.

SESSION 9 TRANSFORMATION. 1. ADENOVIRUSES

Chairman: M. Green, St. Louis University Medical Center

Lillie, J.,[1] Green, M.,[2] Green, M.R.,[1] [1]Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts; [2]Institute for Molecular Virology, St. Louis University Medical Center, Missouri: An adenovirus E1A protein domain required for transformation and transcriptional repression.

Schneider, J.F.,[1] Richter, J.,[3] Fisher, F.,[2] Goding, C.,[2] Jones, N.C.,[1] [1]Gene Regulation Group, Imperial Cancer Research Fund, London, [2]Marie Curie Memorial Foundation, Research Institute, Oxted, England; [3]Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Genetic analysis of the E1A gene—Interrelationship of the different E1A functions.

Quinlan, M.P., Whyte, P., Grodzicker, T., Cold Spring Harbor Laboratory, New York: Analyses of induction of epithelial cell proliferation, growth factor production and transformation by the E1A 12S gene product.

Timmers, H.T.M.,[1] Offringa, R.,[1] Vaessen, R.T.M.J.,[1] Jochemsen, A.G.,[1] van Zoelen, E.E.J.,[2] Bos, J.L.,[1] van der Eb, A.J.,[1] [1]Sylvius Laboratories, Leiden, [2]Hubrecht Laboratory, Utrecht, The Netherlands: Altered expression of cellular genes in adenovirus-transformed cells.

Jelsma, A.N., Evelegh, C.M., Denman, J.E., Bayley, S.T., Dept. of Biology, McMaster University, Ontario, Canada: Site-specific mutations in the coding sequences of the E1A region of human Ad5.

Montano, X., Lane, D., Dept. of Biochemistry, Imperial College of Science and Technology, London, England: The adenovirus E1A gene induces differentiation of F9 teratocarcinoma cells.

Egan, C.,[1] Yee, S.-P.,[2] Ferguson, B.,[3] Bayley, S.T.,[1] Branton, P.E.,[1,2] Depts. of [1]Biology, [2]Pathology, McMaster University, Hamilton, Canada; [3]Experimental Station, E.I. DuPont de Nemours, Wilmington, Delaware: Analysis of cellular proteins associated with Ad5 E1A products.

Whyte, P., Harlow, E., Cold Spring Harbor Laboratory, New York: Association of adenovirus E1A proteins with cellular polypeptides.

Akagi, K., Murai, K., Haddada, H., Levine, A.S., Patch, C.T., NICHD, National Institutes of Health, Bethesda, Maryland: Mitogenic and antimitogenic transforming growth factors secreted by Ad2- and SV40-transformed hamster embryo cells—Possible role in promoting tumorigenesis.

Kenyon, D.J., Raska, K., Jr., Dept. of Pathology, UMDNJ-Rutgers Medical School, Piscataway, New Jersey: Region E1A of highly oncogenic Ad12 in transformed cells protects against NK but not LAK cytolysis.

Haddada, H.,[1] Lewis, A.M., Jr.,[1] Sogn, J.A.,[1] Coligan, J.E.,[1] Cook, J.L.,[2] Walker, T.A.,[2] Levine, A.S.,[1] [1]National Institutes of Health, Bethesda, Maryland; [2]National Jewish Hospital, Denver, Colorado: Tumorigenicity of BALB/c mouse cells transformed by Ad5 and Ad12 is not influenced by the level of class-I histocompatibility antigens expressed on the cells.

SESSION 10 T ANTIGENS

Chairman: W. Eckhart, Salk Institute

Cartwright, C.A., Kaplan, P.L., Simon, S., Hunter, T., Eckhart, W., Salk Institute, San Diego, California: Altered tyrosine phosphorylation and enhanced protein kinase activity of middle-T-antigen-associated $pp60^{c\text{-}src}$—Implications for cell transformation.

Kornbluth, S., Sudol, M., Hanafusa, H., Rockefeller University, New York, New York: Association of the polyomavirus middle T antigen with c-yes protein in middle-T-antigen-transformed chicken embryo fibroblasts.

Piwnica-Worms, H.,[1] Cheng, S.H.,[2] Saunders, K.B.,[1] Smith, A.E.,[2] Roberts, T.M.,[1] [1]Dana-Farber Cancer Institute, Boston, [1]Integrated Genetics, Framingham, Massachusetts: Interactions between polyomavirus middle T antigen and avian $pp60^{c\text{-}src}$.

Walter, G.,[1] Carbone, A.,[1] Welch, W.J.,[2] [1]Dept. of Pathology, University of California, San Diego, La Jolla: [2]Cold Spring Harbor Laboratory, New York: Medium T antigen of polyomavirus transformation-defective mutant NG59 is associated with the 73-kD heat-shock protein.

Jarvis, D.L., Chan, W.-K., Estes, M.K., Butel, J.S., Dept. of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas: The role of the secretory pathway in the biosynthesis, modification, and intracellular transport of SV40 large tumor antigen.

Lanford, R.E., Kennedy, R.C., White, R.G., Kanda, P., Dept. of Virology and Immunology, Southwest Foundation for Biomedical Research, San Antonio, Texas: Synthetic peptides homologous to SV40 T antigen induce nuclear transport of microinjected proteins.

Montenarh, M.,[1] Vesco, C.,[2] Kemmerling, G.,[1] Müller, D.,[1] Henning, R.,[1] [1]Dept. of Biochemistry, University of Ulm, Federal Republic of Germany; [2]Institute for Cell Biology, Rome, Italy: Mapping of regions of SV40 large T antigen for oligomerization and T-p53 complex formation using

various SV40 mutants.

Deppert, W., Haug, M., Dept. of Biochemistry, University of Ulm, Federal Republic of Germany: Metabolic stabilization of the cellular protein p53 in SV40-transformed cells is a transformation-specific event that is not mediated by complex formation with large T antigen.

Tack, L.C.,[1] Wright, J.H.,[1] Gurney, E.C.,[2] [1]Molecular Biology and Virology Laboratory, Salk Institute, San Diego, California; [2]Dept. of Biology, University of Utah, Salt Lake City: SV40 T antigen–p53 complexes are preferentially ATPase-active.

Farber, J., Peden, K., Dept. of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, Maryland: *Trans*-dominant defective mutants of SV40 T antigen.

SESSION 11 TRANSFORMATION. 2. SV40, POLYOMA

Chairman: H. L. Ozer, Hunter College

Small, J.A.,[1] Scangos, G.A.,[2] Cork, L.,[3] Trapp, B.,[4] Khoury, G.,[1] [1]National Institutes of Health, Bethesda, [2]Dept. of Biology, Johns Hopkins University, Depts. of [3]Pathology, [4]Neurology, Johns Hopkins Medical Institute, Baltimore, Maryland: Human papovavirus JC induces distinct phenotypes in transgenic mice.

Markland, W., Smith, A.E., Integrated Genetics, Framingham, Massachusetts: Transformation-associated domains contained within the first exon-encoded portion of polyomavirus middle T antigen.

Bouchard, L., Roberge, C., Vass-Marengo, J., Mathieu, F., Bastin, M., Dept. of Microbiology, University of Sherbrooke, Quebec, Canada: *Trans*-activation of the polyoma middle T gene stably integrated into the genome of rat cell lines.

Ozer, H.L.,[1] Caton, Y.,[1] Neufeld, D.,[1] Radna, R.,[1] Resnick, L.,[1] Ripley, S.,[1] Small, M.S.,[2] Zainul, B.,[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: Transformation and immortalization of human fibroblasts by SV40.

Cherington, V.,[1] Brown, M.,[2] Paucha, E.,[2] Spiegelman, B.,[2] Roberts, T.,[2] [1]Tufts University School of Medicine, [2]Dana-Farber Cancer Institute and Harvard Medical School, Boston, Massachusetts: Genetic analysis of the SV40 T antigen block of adipocyte differentiation.

Srinivasan, A.,[1] Spence, S.,[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: Genetic analysis of the SV40 large T antigen transforming function.

Cole, C.N.,[1] Haley, K.,[1] Tevethia, M.J.,[2] [1]Molecular Genetics Center and Dept. of Biochemistry, Dartmouth Medical School, Hanover, New Hampshire; [2]Dept. of Microbiology, Pennsylvania State University, Hershey: Immortalization, transformation and biochemical properties of SV40 deletion and linker insertion mutants.

Chen, S., Levesque, P., Pomert, E., Pollack, R.E., Dept. of Biological Sciences, Columbia University, New York, New York: Transformation of precrisis human cells by the cytoplasmic-localization SV40 mutant pSVCT3.

Vesco, C., Fischer-Fantuzzi, L., Istituto di Biologia Cellulare del CNR, Roma, Italy: Transforming cooperation between the polyomavirus large T antigen and the SV40 nonkaryotipic large T antigen, a mutant large T localized in the cytoplasm.

Phillips, B., Rundell, K., Dept. of Microbiology, Northwestern University, Chicago, Illinois: 10T1/2 cells expressing SV40 small T antigen.

Noda, T., Satake, M., Ito, Y., Institute for Virus Research, Kyoto University, Japan: Function of small T antigen of polyomavirus is not shared by middle T antigen despite their structural similarity.

Molecular Genetics of Bacteria and Phages

August 19–August 24

ARRANGED BY

Sankar Adhya, National Cancer Institute
Gary Gussin, University of Iowa
Gisela Mosig, Vanderbilt University
John Roth, University of Utah

264 participants

The 1986 meeting on bacteria and phages demonstrated again that work on phage and bacterial systems is making fundamental contributions to our understanding of basic molecular biology. This year's meeting was divided into ten sessions of oral presentations and two poster sessions, reflecting active areas of prokaryotic molecular biological research, including DNA replication, recombina-

tion and transposition, and regulation of gene expression. As a result of the expansion of the meetings a year ago to include bacterial molecular biology, several areas of interest were represented to a much greater extent than was true in the past. These included bacterial development (sporulation), physiological regulation (nitrogen metabolism and response to heat shock), and the synthesis of membrane proteins. There were 40–50 talks on detailed investigations of DNA-protein interactions and transcriptional regulation and exciting evidence was presented for the existence of enhancer-like elements essential for regulation of prokaryotic transcription.

SESSION 1 DNA REPLICATION

Novick, R.P., Gruss, A., Ross, H.F., Dept. of Plasmid Biology, Public Health Research Institute of the City of New York, Inc., New York: A palindromic sequence involved in the initiation of plasmid lagging-strand synthesis.

Gennaro, M.L., Novick, R.P., Dept. of Plasmid Biology, Public Health Research Institute, New York, New York: A *cis*-acting plasmid locus alters DNA supercoiling and the interaction between replication origin and initiator protein.

Gilchrist, C.A., Denhardt, D.T., Dept. of Biochemistry, University of Western Ontario, Canada: *E. coli rep* gene—Sequence of the gene, the encoded helicase, and its homology with *uvrD*.

Keller, J.A., Simon, L.D., Waksman Institute of Microbiology, Rutgers University, Piscataway, New Jersey: Phenotypic suppression of a bacteriophage λ *O*ts allele by *E. coli* mutants.

Greenstein, D., Fulford, W., Horiuchi, K., Rockefeller University, New York, New York: Multistep binding of the initiator protein to the replication region of bacteriophage f1.

Flensburg, J., Klevolin, M., Calendar, R., Christian, R., Dept. of Molecular Biology, University of California, Berkeley: P4 phage DNA replication.

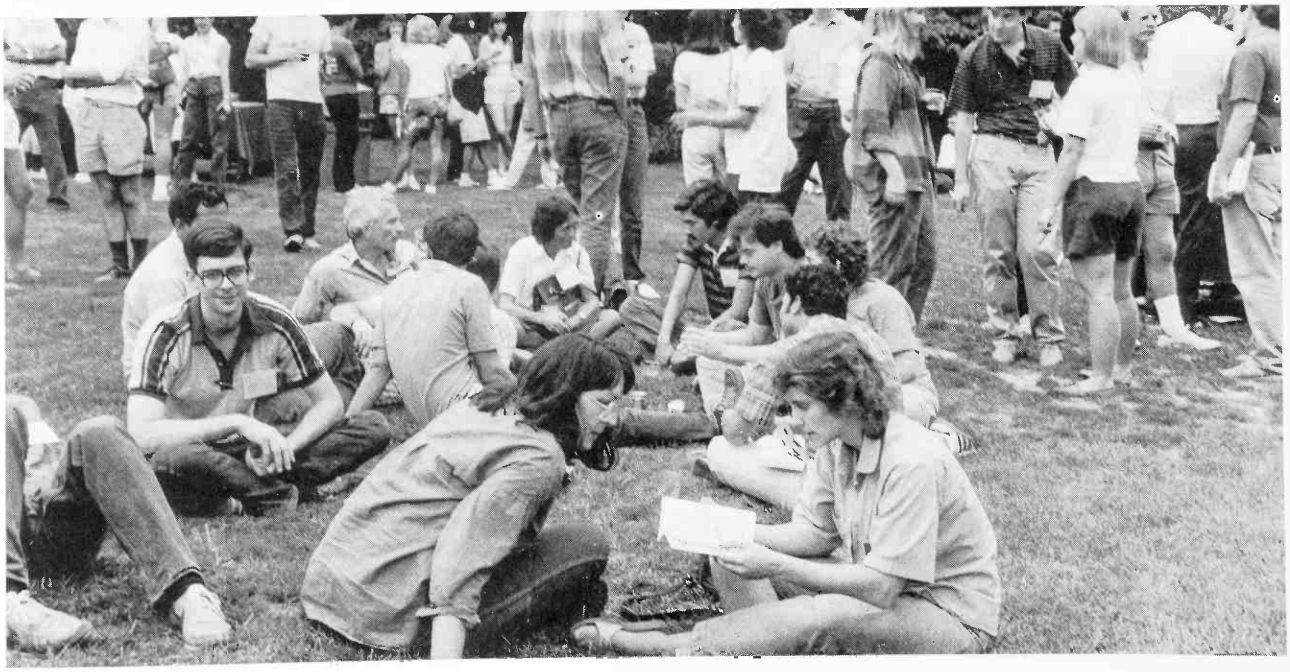
Wickner, S., Chattoraj, D., NCI, National Institutes of Health, Bethesda, Maryland: In vitro replication of a bacteriophage P1 miniplasmid.

Chattoraj, D.K., NCI, National Institutes of Health, Bethesda, Maryland: An autoregulation protein can be made rate-limiting by sequestration.

Powell, D.,[1] Franklin, J.,[1] Arisaka, F.,[2] Mosig, G.,[1] [1]Vanderbilt University, Nashville, Tennessee; [2]Hokkaido University, Sapporo, Japan: DNA sequence and transcription of the phage T4 DNA packaging genes 16 and 17—Partial homology with the replication/recombination gene 32.

Hill, T., Kuempel, P., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: The terminus region of the chromosome of *E. coli* contains two separate sites that exhibit polar inhibition of DNA replication.

Monod, M., Projan, S.J., Dubnau, D., Depts. of Microbiology and Plasmid Biology, Public Health Research Institute of the City of New York, Inc., New York: Interspecific plasmid mobility—pIM13, a naturally occurring *B. subtilis* plasmid, probably arose from pE5, a plasmid native to *S. aureus*.



SESSION 2 RECOMBINATION AND REPAIR

Thaler, D.S.,[1] Sampson, E.,[1] Rosenberg, S.M.,[1] Stahl, F.W.,[1] Stahl, M.M.,[1] Higgens, N.P.,[2] [1]Institute of Molecular Biology, University of Oregon, Eugene; [2]Dept. of Biochemistry, University of Alabama, Birmingham: Roles of double-chain ends in recombination of bacteriophage λ.

Rosenberg, S.M., Stahl, M.M., Stahl, F.W., Institute of Molecular Biology, University of Orgeon, Eugene: Asymmetry of Chi-stimulated patch recombinants.

Basu, S.K., Margolin, P., Dept. of Biology, City College of New York, New York: Transduction-mediated recombination between segments of a transducing DNA fragment.

Poteete, A.R., Fenton, A.C., Murphy, K., Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester: Activities of the *E. coli*, P22, and λ homologous recombination systems in a direct physical assay.

Sassanfar, M., Roberts, J., Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: DNA replication is required for UV induction of the SOS pathway

Ennis, D G , Ossanna, N Mount D W Dept of Molecular and Cellular Biology, University of Arizona Tucson Genetic analysis of the *recA* mutagenesis function

Tessman, E S , Tessman, I Peterson P K Forestal J D Dept. of Biological Sciences, Purdue University West Lafayette, Indiana Second role of the RecA protein in mutagenesis and repair

Wang, W.-B., Tessman, E S , Dept of Biological Sciences Purdue University, West Lafayette, Indiana Functional regions of the RecA protein of *E coli*

Heitman, J., Model, P, Rockefeller University, New York New York· A new locus in *E coli*, *mar*, mediates SOS induction in response to site-specific adenine methylation

Bhagwat, A.S., Roberts, R J , Cold Spring Harbor Laboratory, New York Possible repair of DNA-protein covalent complexes in *E coli* – 5-azaC sensitivity of DNA repair mutants.

SESSION 3 POSTER SESSION

Ang, D., Johnson, C., Chandresekhar, G.N., Zylicz, M., Georgopoulos, C., Dept. of Cellular, Viral, and Molecular Biology, University of Utah, Salt Lake City: The *E. coli grpE* gene product is required for λ replication.

Ball, T., Saurugger, P., Benedik, M., Dept. of Biology, Texas A&M University, College Station: Extracellular secretion of *Serratia marcescens* nuclease in *E. coli*.

Barth, K., Mosig, G., Trupin, M., Powell, D., Dept. of Molecular Biology, Vanderbilt University, Nashville, Tennessee: Phage T4 gene 49 (endonuclease VII) is transcribed early and late.

Bear, S.E.,[1] Clemens, J.B.,[1] Court, D.L.,[2] [1]E.I. du Pont de Nemours and Company, Wilmington, Delaware; [2]NCI-Frederick Cancer Research Facility, Frederick, Maryland· Mutational analysis of the λ *int* gene.

Bej, A., Jacob, R., Merrell, E., Perlin, M., University of Louisville and University of Kentucky Medical Center, Lexington: Restriction enzyme analysis and electron microscopic study of plasmid bacterial origin after reisolation from transformed fungal species.

Benson, K.H., Kreuzer, K.N., Dept. of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina: Selection of T4 mutants deficient in tertiary replication initiation.

Boyd, D., Manoil, C., Beckwith, J., Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Topology of an inner membrane protein of *E. coli*.

Calendar, R.,[1] Grossman, A.,[2] Yhou, Y.-N.,[2] Erickson, J.W.,[2] Gross, C.,[2] [1]Dept. of Molecular Biology, University of California, Berkeley; [2]Dept. of Bacteriology, University of Wisconsin, Madison: Mutations of the *htpR* (*rpoH*) gene that suppress the temperature sensitivity of *rpoD285(800)* and are incompatible with *rhoD*·.

Castaño, I.,[1] Bastarrachea, F.,[2] Covarrubias, A.A.,[1] [1]Centro de Investigación sobre Fijación de Nitrógeno, UNAM; [2]Instituto de Fisiología Celular, UNAM, Mexico: Control of the glutamate synthase synthesis of *E coli* K-12.

Catron, K.M , Schnaitman, C.A , Dept of Microbiology, University of Virginia, Charlottesville Analysis of an *ompC* mutant that affects protein transport in *E coli* K-12

Chang, B.,[1 2] Wanner, B L [1] [1]Purdue University, West Lafayette, Indiana; [2]Emory University, Atlanta Georgia Control of bacterial alkaline phosphatase synthesis in *E coli*.

Chung, Y-B , Hinkle, D C , Dept of Biology, University of Rochester, New York Recombinant plasmids containing the concatemer junction of bacteriophage T7 are packaged into transducing particles during phage infection

Clyman, J , Cunningham, R P Dept of Biological Science State University of New York Albany Characterization and cloning of the *rdgB* gene of *E coli*

Cowan, J , Drivdahl, R , D'Acci, K , Lindsay, P Williams S Loring, G , McKinney, J , Erickson, D Kutter E Evergreen State College, Olympia, Washington Detailed two-dimensional analysis of T4 proteins

Daniels, D L , Blattner, F R , Laboratory of Genetics University of Wisconsin, Madison Dissection of the *E coli* genome into bacteriophage λ clones and construction of detailed restriction maps

Daub, E , Walsh, C Botstein, D Depts of Chemistry and Biology, Massachusetts Institute of Technology, Cambridge Isolation and characterization of cell-wall biosynthesis genes in *S typhimurium*

de Boer, P A J , Rothfield, L I , Dept of Microbiology, University of Connecticut Health Center Farmington Isolation and properties of the *E coli minB* locus

Donovan, W P , Zheng L , Sandman, K , Losick, R., Dept of Cellular and Developmental Biology, Harvard University, Cambridge. Massachusetts Genes for spore coat proteins from *B subtilis*

French, S , Miller, O L , Jr , Dept of Biology, University of Virginia, Charlottesville Electron microscopic visualization of chromosome replication in *E coli* – A comparison of gene activity on sister strands

Gloor, G., Chaconas, G., Dept. of Biochemistry, University of Western Ontario, Canada: Cloning and identification of the gene coding for the 64-kD Mu virion protein, which is injected and circularizes infecting Mu DNA.

Guijarro, J.A., Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: Molecular cloning of *S. coelicolor* spore-associated proteins.

Gussin, G.N., Matz, K., Brown, S., Dept. of Biology, University of Iowa, Iowa City: New mutations causing defects in repressor synthesis directed by the λ P_{RM} promoter.

Hausmann, R., Lützelschwab, M., Messerschmid, M., Institut für Biologie, Universität Freiburg, Federal Republic of Germany: Genetic comparison of phages following the T7-specific strategy of infection.

Hayes, S., Hayes, C., Dept. of Microbiology, University of Saskatchewan, Saskatoon, Canada: Exclusion phenotype of selected replicative-killing-defective λ lysogens.

Heck, D., Hatfield, G.W., Dept. of Microbiology and Molecular Genetics, University of California College of Medicine, Irvine: In vivo and in vitro transcriptional analysis and complete nucleotide sequence of the *valS* gene of *E. coli* K-12.

Hsu, T., Wei, R., Dawson, M., Karam, J., Dept. of Biochemistry, Medical University of South Carolina, Charleston: Expression of an operon for T4-induced RNA polymerase-binding proteins.

Huff, A.C., Leatherwood, J.K., Kreuzer, K.N., Dept. of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina: Characterization of phage T4 DNA topoisomerase from mAMSA-resistant phage mutants.

Hwang, J.-J., Gussin, G.N., Dept. of Biology, University of Iowa, Iowa City: Kinetic analysis of transcription initiation at the λ P_{RM} promoter—Independent effects of *prm*E37 and *prmup-1* on promoter function.

Iordanescu, S., Dept. of Plasmid Biology, Public Health Research Institute, New York, New York: Change in the specificity of the interactions between the Rep proteins and replication origins of three staphylococcal plasmids in a host mutant.

Keeler, C.L., Jr., University of Maryland, Baltimore County, Catonsville: Overproducing the terminase proteins of λ.

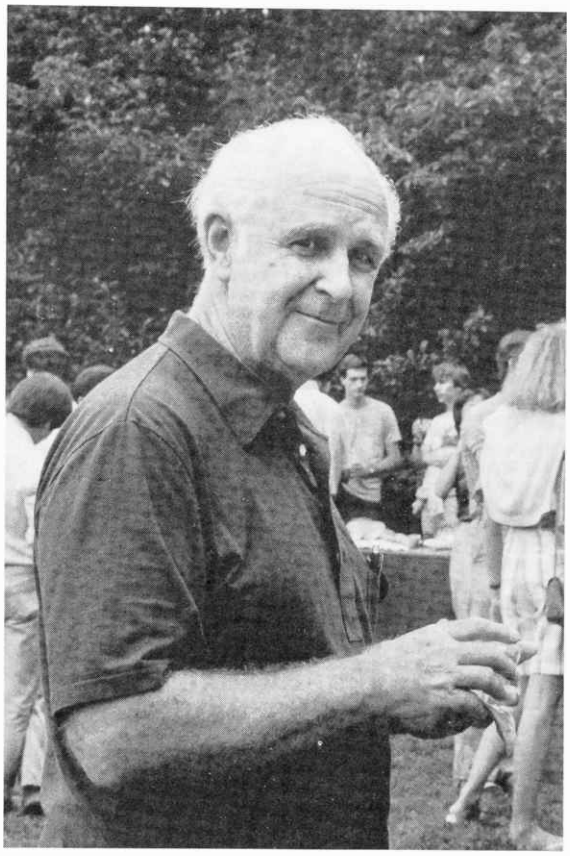
Kiino, D.R., Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois: *PrlfF1*, a mutation that relieves the lethality associated with export of β-galactosidase hybrid proteins in *E. coli*—Cloning and sequencing of the gene.

Kirby, T.W., Hindenach, B.R., Greene, R.C., Basic Science Laboratory, U.S. Veterans Administration Medical Center and Dept. of Biochemistry, Duke University Medical Center, Durham, North Carolina: Studies on the binding of the *E. coli metJ* regulatory protein to a DNA region between the *metJ* and *metB* genes.

Kleina, L.G.,[1] Masson, J.-M.,[1] Miller, J.H.,[1] Normanly, J.,[2] Abelson, J.N.,[2] [1]University of California, Los Angeles; [2]California Institute of Technology, Pasadena: Construction and use of an *E. coli* amber suppressor tRNA bank.

Kokjohn, T.A., Miller, R.V., Loyola University of Chicago, Maywood, Illinois: Control of expression of the *recA* gene of *P. aeruginosa* PAO.

Kwoh, T.J.,[1] Kwoh, D.Y.,[2] McCue, A.,[2] Davis, B.,[2] Patrick, D.,[2] Gingeras, T.R.,[2] [1]La Jolla Biological Laboratories,



W. Szybalski

San Diego; [2]Salk Institute Biotechnology/Industrial Associates, Inc., San Diego, California: Expression of a bacterial DNA methylase gene in mammalian cells.

Lazinski, D., Das, A., Program in Molecular Biology and Biochemistry, University of Connecticut Health Center, Farmington: Construction of hybrid proteins to determine the domain of N responsible for *nut*-site recognition.

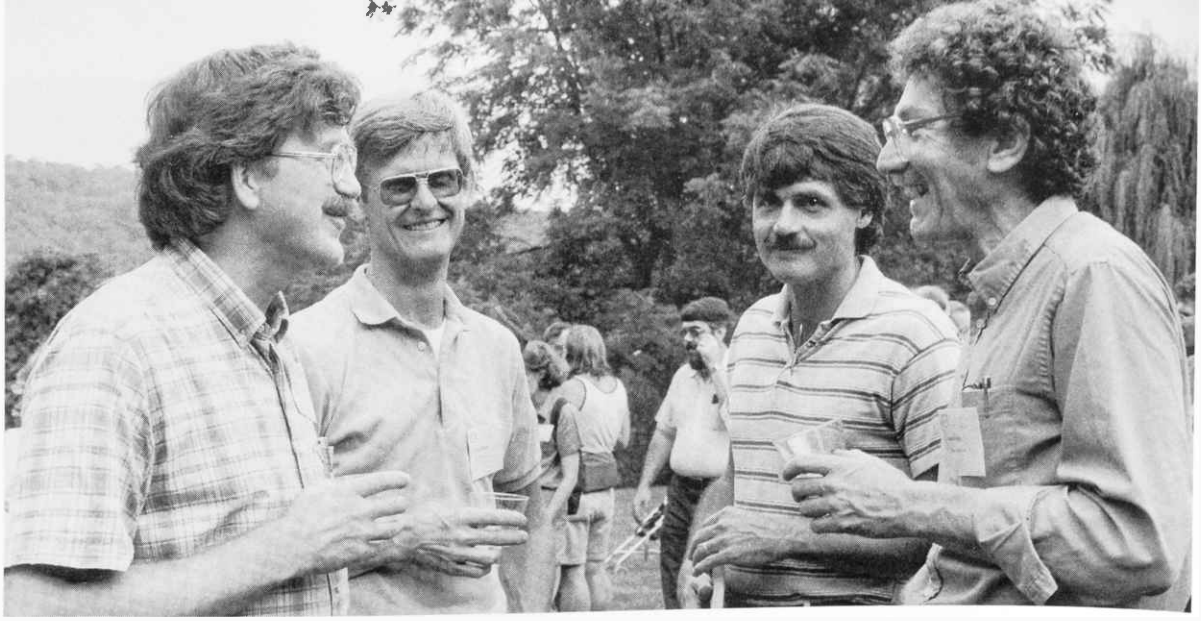
Lin, E., Wilson, D.B., Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Regulation of cellulase genes in *Thermomonospora fusca*.

Lin-Chao, S., Bremer, H., Dept. of Biology, University of Texas, Dallas: Physiological control of replication of plasmid pBR322.

Macaluso, A.,[1] Best, E.,[2] Bender, R.A.,[1] Depts. of [1]Biology, [2]Microbiology, and Immunology, University of Michigan, Ann Arbor: The nitrogen assimilation control (*nac*) gene is an essential element of the Ntr system of *K. aerogenes*.

Mandecki, W., Corporate Molecular Biology, Abbott Laboratories, Abbott Park, Illinois: Mutagenesis by oligonucleotide-directed repair of a double-strand break in plasmids of *E. coli*.

Marinus, M.G., Carraway, M., Wu, T., Dept. of Pharmacology, University of Massachusetts Medical School, Worcester: Mutation spectrum in a *dam* mutant of *E. coli* K-12.



J. Roth, P. Kuempel, L. Snyder, M. Pato

SESSION 4 TRANSPOSITION AND SITE-SPECIFIC RECOMBINATION

Ahmed, A., Dept. of Genetics, University of Alberta, Canada: Evidence for replicative transposition of Tn*5* and Tn*9*.

Lichens, A., Syvanen, M., Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Integration of bacteriophage λ by IS*50R* as a model system for studying IS*50*-mediated cointegration.

Rak, B., Brombacher, F., Eibel, H., Fuchs, K., von Reutern, M., Schnetz, K., Institut fur Biologie, Universität Freiburg, Federal Republic of Germany: Studies on DNA insertion element IS*5*.

Waddell, C., Arciszewska, L., Kubo, K., Orle, K., Craig, N., Dept. of Microbiology and Immunology and Hooper Research Foundation, University of California, San Francisco: Tn*7* transposition—DNA sites and proteins.

Nakayama, C.,[1] Teplow, D.B.,[2] Harshey, R.M.,[2] [1]Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, [2]Division of Biology, California Institute of Technology, Pasadena: Structural domains in the phage Mu transposase—Identification of the site-specific DNA-binding domain.

Surette, M., Buch, S., Chaconas, G., Dept. of Biochemistry, University of Western Ontario, Canada: Transpososomes—Stable protein-DNA complexes involved in the transposition of phage Mu DNA.

Stirling, C.J., Stewart, G., Sherratt, D.J., Institute of Genetics, University of Glasgow, Scotland: Site-specific recombination at the ColE1 c*er* locus is blocked by mutations in two different genes on the *E. coli* K-12 chromosome.

Miller, H.I., Kuang, W.J., Chen, E., Depts. of Cell Genetics and Molecular Biology, Genentech, Inc., South San Francisco, California: The *E. coli himA* gene is cotranscribed with the genes for phenylalanyl tRNA synthetase.

Granston, A.E., McGrath, D.M., Friedman, D.I., Dept. of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor: cos cleavage—Studies of host factors.

Ou, J.T., Baron, L.S., Rubin, F.A., Kopecko, D.J., Dept. of Bacteriology and Immunology, Walter Reed Army Institute of Research, Washington, D.C.: Reversible expression in *E. coli* of *Citrobacter freundii* Vi capsular antigen genes is controlled by the insertion and deletion of an IS1-like DNA element.

Hill, C.W., Pencek, M.S., Davidson, A.E., Kirschman, J.A., Vlazny, D.A., Gray, J.A., Dept. of Biological Chemistry, Hershey Medical Center, Pennsylvania State University, Hershey: A novel family of large repeated genetic elements of *E. coli*.

Youderian, P., Brewer, K.L., Sugiono, P., Dept. of Biological Sciences, University of Southern California, Los Angeles: Tn*10* transposition events in *Salmonella* that generate locked-in P22 prophages.

SESSION 5 DNA-PROTEIN INTERACTIONS

Bushman, F.D., Wharton, R.P., Ptashne, M., Harvard University, Cambridge, Massachusetts: The repressor of bacteriophage 434 activates transcription by two separate mechanisms.

Sarai, A., Jernigan, R.L., Takeda, Y., NCI, National Institutes of Health, Bethesda, and NCI-Frederick Cancer Research Facility, Frederick, Maryland: Quantitative analysis of Cro repressor-operator interaction.

Eliason, J.L., Sternberg, N.L., Central Research and Development Dept., E.I. du Pont de Nemours and Co., Wilmington, Delaware: The C1 repressor of phage P1 recognizes an asymmetric operator.

Benson, N., Sugiono, P., Youderian, P., Dept. of Biological Sciences, University of Southern California, Los Angeles:

DNA determinants of λ repressor binding.

Bass, S., Youderian, P., Dept. of Biological Sciences, University of Southern California, Los Angeles: Genetic analysis of *E. coli* tryptophan repressor binding.

Gardella, T., Susskind, M.M., Dept. of Biological Sciences, University of Southern California, Los Angeles: Mutations of the σ^{70} subunit of *E. coli* that suppress mutations of the P22 *ant* promoter.

Garges, S., Kim, J., Adhya, S., NCI, National Institutes of Health, Bethesda, Maryland: Sites of allosteric shift within the structure of the cAMP receptor protein of *E. coli*.

Ebright, R.,[1,2] Kolb, A.,[2] Buc, H.,[2] Cossart, P.,[2] Gicquel-Sanzey, B.,[2] Kunkel, T.,[3] Krakow, J.,[4] Beckwith, J.,[1] [1]Harvard Medical School, Boston, Massachusetts; [2]Institut Pasteur, Paris, France; [3]NIEHS, Research Triangle Park, North Carolina; [4]Hunter College, New York, New York: Role of Glu-181 and Lys-188 in DNA-sequence recognition by the catabolite gene activator protein (CAP).

Harman, J.G., McKenney, K., Peterkofsky, A., National Institutes of Health, Bethesda, Maryland: Structure-function analysis of three cAMP-independent forms of the cAMP receptor protein.

Kim, J.G.,[1] Takeda, Y.,[2] [1] NCI, National Institutes of Health, Bethesda, Maryland; [2]NCI-Frederick Cancer Research Facility, Frederick, Maryland: Kinetic studies of Cro repressor-operator DNA interaction.

SESSION 6 TRANSCRIPTION INITIATION AND REGULATION

Hammer, K., Dandanell, G., Institute of Biological Chemistry, University of Copenhagen, Denmark: DeoR repression in *E. coli* can be caused by a site located downstream from the regulated genes.

Miller, R.V., Kokjohn, T.A., Loyola University of Chicago, Maywood, Illinois: Role of the bacteriophage D3 repressor in lysogeny establishment in Rec$^+$ and Rec$^-$ *P. aeruginosa*.

Gourse, R.L., Dept. of Genetics, University of Georgia, Athens: Upstream activation of rRNA synthesis in *E. coli*.

Lampe, M., Binnie, C., Paoletti, R., Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: Cloned genes encoding the *B. subtilis* σ^{37} and δ RNA polymerase transcription factors.

Igo, M., Schafer, R., Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: Genetic studies on the regulation of a promoter that is utilized by minor forms of RNA polymerase in *B. subtilis*.

Lin, L.-L., Little, J.W., Dept. of Biochemistry, University of Arizona, Tucson: Isolation and characterization of noncleavable (Ind$^-$) mutants of LexA repressor.

Smith, C.M.,[1] Orrego, C.,[2] Eisenstadt, E.,[1,2] [1]Laboratory of Toxicology, [2]Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts: *TopA* and the SOS response in *S. typhimurium LT2*.

Salles, B., Weinstock, G.W., Dept. of Biochemistry and Molecular Biology, University of Texas Medical School, Houston: Regulation of *cea* gene expression.

Lamont, I.L., Egan, J.B., Dept. of Biochemistry, University of Adelaide, Australia: UV induction of coliphage 186—A novel control circuit involving LexA.

Schwartz, I., Wertheimer, S., Klotsky, R.A., Dept. of Biochemistry, New York Medical College, Valhalla: Transcriptional events in the *thrS-infC-rplT* region of the *E. coli* genome.

SESSION 7 DEVELOPMENTAL REGULATION

Grossman, A., Losick, R., Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: Extracellular control of sporulation in *B. subtilis*.

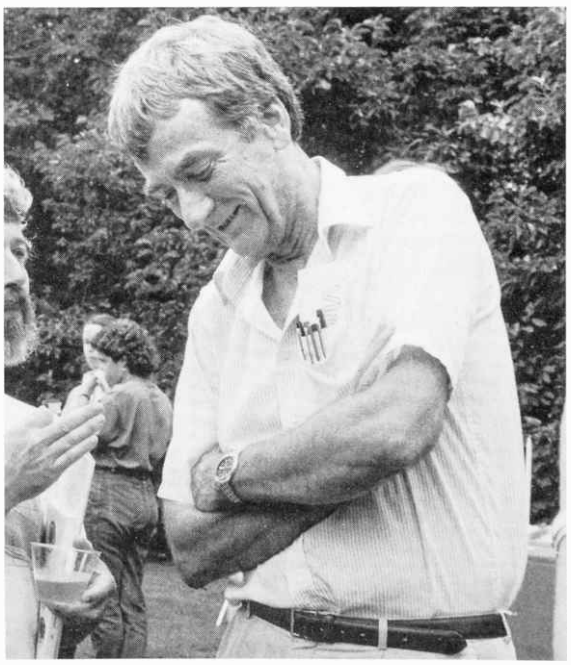
Zuber, P., Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: Transcription of a developmentally regulated gene of *B. subtilis* is under positive and negative control.

Carter, H.L. III, Moran C.P., Jr., Dept. of Microbiology and Immunology, Emory University School of Medicine, Atlanta, Georgia: New RNA polymerase σ factor under *spo0* control in *B. subtilis*.

Schauer, A., Losick, R., Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: Measuring *Streptomyces* gene expression with light.

Adams, T., Chelm, B., DOE-Plant Research Laboratory and Dept. of Microbiology, Michigan State University, East Lansing: A gene in *Bradyrhizobium japonicum* that is necessary for anaerobic gene expression and soybean symbiotic differentiation.

Mullin, D.A., Munnich, S.A., Newton, A., Dept. of Molecular Biology, Princeton University, New Jersey: Nucleotide consensus sequences in developmentally regulated flagellar promoters in C. *crescentus*.



F. Stahl

Minnich, S.A., Mullin, D.A., Chen, L.-S., Newton, A., Dept. of Molecular Biology, Princeton University, New Jersey: Evidence for a regulatory cascade in the temporal expression of *fla* genes in *C. crescentus*.

Pato, M., Toussaint, A., National Jewish Center, Denver, Colorado, and Universite Libre de Bruxelles, Belgium: Host functions required for late transcription of phage Mu.

Birkeland, N.K.,[1,2] Christie, G.E.,[1] Lindqvist, B.H.,[2] [1]Dept. of Microbiology and Immunology, Virginia Commonwealth Univeristy, Richmond; [2]Institute of Medical Biology, University of Tromsø, Norway: Analysis of P2 *ogr* gene expression.

Dehò, G., Zangrossi, S., Ghisotti, D., Sironi, G., Dipartimento di Genetica e di Biologia dei Microrganismi, Università di Milano, Italy: Alternative promoters for the development of the genetic element P4.

Dale, E.C.,[1] Halling, C.,[1] Calendar, R.,[1] Morrison, T.,[1] Christie, G.E.,[1] Zylbert, B.,[1] Keener, J.,[2] Kustu, S.G.,[2] Lane, K.,[3] Sunshine, G.,[3] Six, E.W.,[3] [1]Dept. of Molecular Biology, University of California, Berkeley; [2]Dept. of Bacteriology, University of California, Davis; [3]Dept. of Microbiology, University of Iowa, Iowa City: Expression of the P4 phage late promoter.

Downs, D., Roth, J., Dept. of Biology, University of Utah, Salt Lake City: *S. typhimurium* contains an archived P22-like prophage.

SESSION 8 POSTER SESSION

Makris, J.C., Reznikoff, W.S., Biochemistry Dept., University of Wisconsin, Madison: Effects of single base changes of the outer-ends of IS50 on Tn*5* transposition.

Moline, K.W., Barbieri, S., Musso, R.E., Dept. of Biology, University of South Carolina, Columbia: Genetic determination of transposition factors encoded by the DNA transposable element IS*2*.

Murphy, K.C., Fenton, A.C., Poteete, A.R., Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical Center, Worcester: Recombination system of bacteriophage P22.

Musso, R.E., Black, T., Dept. of Biology, University of South Carolina, Columbia: Transcription of the insertion sequence IS*2* is regulated by an IS*2*-encoded protein.

Nicosia, A., Rappuoli, R., Sclavo Research Center, Siena, Italy: Structure and expression of the operon coding for pertussis toxin.

Ossanna, N., Mount, D., Dept. of Molecular and Cellular Biology, University of Arizona, Tucson: Isolation and characterization of SOS constitutive mutants in *E. coli*.

Palchaudhuri, S.,[1] Patel, V.,[1] McFall, E.,[2] [1]Dept. of Microbiology, Wayne State University School of Medicine, Detroit, Michigan; [2]Dept. of Microbiology, New York University School of Medicine, New York: Sequence of the D-serine deaminase activator gene *dsdC*.

Plumbridge, J.A., Söll, D., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Mutations affecting glutamine-tRNA synthetase expression.

Popham, D.L., Wong, P.-K., Keener, J.X., Kustu, S.G., Dept. of Bacteriology, University of California, Davis: DNA sequence of the *S. typhimurium ntrA* gene.

Raleigh, E.A., Trimarchi, R., Latimer, E., New England Biolabs, Beverly, Massachusetts: Genetic mapping and molecular cloning of the *E. coli mcrA* and *mcrB* loci.

Ranes, M., Schauer, A., Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: In vivo and in vitro studies of the utilization of a promoter from *Streptomyces plicatus*.

Ray, J., Hess, J., Hong, S.-C., Bauerle, R., Dept. of Biology, University of Virginia, Charlottesville: Domain mapping of two tryptophan-regulated enzymes of aromatic biosynthesis.

Reitzer, L.J., Ninfa, A.J., Magasanik, B., Massachusetts Institute of Technology, Cambridge: Effects of relocation and deletion of activator NR_I binding sites on transcription from *glnAp2* in vitro and in vivo.

Rennell, D., Knight, J., Poteete, A.R., Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester: Genetic analysis of phage lysozyme structure.

Rockenbach, S.,[1,2] Lutkenhaus, J.,[1] [1]Dept. of Microbiology, University of Kansas Medical Center, Kansas City; [2]Upjohn Company, Kalamazoo, Michigan: Construction of a single copy, promoter assay vector that can be used in parallel with the pKO series of promoter detection vectors.

Rojiani, M., Goldman, E., Dept. of Microbiology, New Jersey Medical School, Newark: Dependence of MS2 and T4 phage growth on host amino acid biosynthesis during infections of *E. coli*.

Schlagman, S.L., Hattman, S., Dept. of Biology, University of Rochester, New York: Analysis of the translation initiation site of the T4 Dam DNA methyltransferase protein.

Schmellik-Sandage, C., Wanner, B., Purdue University, West Lafayette, Indiana: Control of bacterial alkaline phosphatase (BAP) clonal variation in a *E. coli* K-12 "phase mutant."

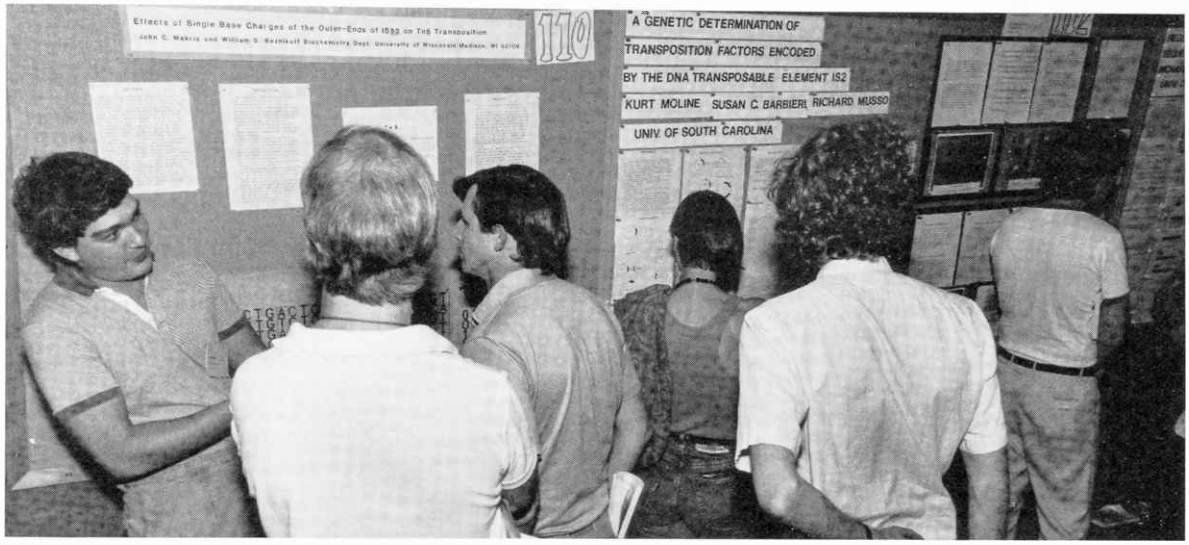
Semerjian, A.V., Poteete, A.R., Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester: Phage P22 genes between *erf* and *immC*.

Sengupta, D., Basu, S., Maitra, U., Division of Biology, Albert Einstein College of Medicine, Bronx, New York: Specific binding of bacteriophage T3 RNA polymerase to its cognate promoter requires the initiating nucleoside triphosphate (GTP).

Shapiro, J.A., Dept. of Biochemistry and Molecular Biology, University of Chicago, Illinois: Embryology of *E. coli* colonies viewed by SEM.

Snyder, L., Jorissen, L., Dept. of Microbiology, Michigan State University, East Lansing: *E. coli* mutations that delay the bacteriophage-T4-induced unfolding of the nucleoid.

Sparkowski, J., Das, A., Program in Molecular Biology and Biochemistry, University of Connecticut Health Center, Farmington: Temperature-sensitive alleles of RNA polymerase β-subunit that compensate for the antitermination defect of altered NusA protein.



Thomas, M.S., Nomura, M., Dept. of Biological Chemistry, University of California, Irvine: A novel form of translational coupling in *E. coli*.

Traxler, B., Minkley, E., Jr., Dept. of Biological Sciences and Mellon Institute, Carnegie-Mellon University, Pittsburgh, Pennsylvania: Study of the *traI* gene of the F sex factor.

Verkamp, E., Chelm, B., DOE-Plant Research Laboratory and Dept. of Microbiology, Michigan State University, East Lansing: Genetic analysis of heme biosynthesis in *E. coli*.

Vogel, J.L.,[1] Hawkins, M.D.,[1] Howe, M.M.,[2] Higgins, N.P.,[1] [1]Dept. of Biochemistry, University of Alabama, Birmingham; [2]Dept. of Bacteriology, University of Wisconsin, Madison: Characterization of mutations in bacteriophage Mu that cause thermoinducible prophage activation through different pathways.

Wang, P.-Z., Novick, R.P., Dept. of Plasmid Biology, Public Health Research Institute of the City of New York, New York: Construction of a series of gene fusion *S. aurerus–B. subtilis–E. coli* shuttle vectors using the staphylococcal β-lactamase as an index enzyme.

Wek, R.C., Hatfield, G.W., Dept. of Microbiology and Molecular Genetics, California College of Medicine, University of California, Irvine: Nucleotide sequence and in vivo expression of the *ilvY* and *ilvC* genes in *E. coli* K-12—Transcription from divergent overlapping promoters.

Wierzbicki, A., Abremski, K., Hoess, R., Central Research and Developmental Dept., E.I. Du Pont de Nemours and Co., Wilmington, Delaware: A mutational and biochemical analysis of the bacteriophage P1 recombinase, Cre.

Wu, J.H., Ippen-Ihler, K., Dept. of Medical Microbiology and Immunology, Texas A&M University, College Station: Identification of *traQ* and other new genes in the transfer operon of the *E. coli* K-12 F factor.

Yap, W.Y., Kreuzer, K.N., Dept. of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina: Tertiary replication initiation of phage T4 DNA.

Zagotta, M.T., Wilson, D.B., Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Time of appearance and localization of the λ S protein.

Zhu, L., Cudny, H., Deutscher, M.P., Dept. of Biochemistry, University of Connecticut Health Center, Farmington: A mutation of the cca gene that affects AMP incorporation by *E. coli* tRNA nucleotidyltransferase.

Kearney, C., Molineux, I.J., Dept. of Microbiology, University of Texas, Austin: "Incompatability" between gene products of phage T7 and the F plasmid.

SESSION 9 PHYSIOLOGICAL AND METABOLIC CONTROL

Straus, D., Cowing, D., Erickson, J., Zhou, Y.-N., Gross, C., Dept. of Bacteriology, University of Wisconsin, Madison: Negative regulation of the *E. coli* heat-shock response.

Johnson, C., Chandrasekhar, G.N., Ang, D., Zylicz, M., Georgopoulos, C., CVM Biology, University of Utah, Salt Lake City: *E. coli* heat-shock proteins dnaK and grpE—Isolation of *dnaK* suppressors for *grpE* 280.

Bahl, H.,[1] Straus, D.,[2] Crowl, R.,[3] Echols, H.,[1] [1]Dept. of Molecular Biology, University of California; [2]Dept. of Bacteriology, University of Wisconsin, Madison; [3]Dept. of Molecular Genetics, Hoffmann-La Roche, Inc., Nutley, New Jersey: Induction of the heat-shock response through stabilization of σ^{32} of *E. coli* by the bacteriophage λ cIII protein.

Bardwell, J.C.A., Craig, E.A., Dept. of Physiological Chemistry, University of Wisconsin, Madison: The *E. coli* heat-inducible gene homologous to the *Drosophila* 83,000-M_r heat-shock protein gene is dispensable.

Ninfa, A.J., Reitzer, L.J., Hunt, T.P., Magasanik, B., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Activation of transcription initiation from the nitrogen-regulated promoter *glnAp2* of *E. coli*.

Keener, J., Wong, P.-K., Wallis, J., Popham, D., Kustu, S., Dept. of Bacteriology, University of California, Davis: Metabolic regulation of *glnA* transcription.

Ludwig, R.A., Donald, R.G.K., Loroch, A.I., Nees, D.W.,

Dept. of Biology, Thimann Laboratories, University of California, Santa Cruz: Regulation of *Rhizobium* sp. ORS571 N_2 fixation genes.

Wanner, B.L., Purdue University, West Lafayette, Indiana: Bacterial alkaline phosphatase (BAP) clonal variation in phase and state mutants.

Storz, G., Christman, M.F., Jacobson, F.S., Ames, B.N., Dept. of Biochemistry, University of California, Berkeley: Characterization of the bacterial response to oxidative stress.

Jones, H.M., Kalman, L.V., Gunsalus, R.P., Dept. of Microbiology and Molecular Biology Institute, University of California, Los Angeles: Regulation of fumarate reductase gene expression (*frdABCD*) in *E. coli* by oxygen, nitrate, and fumarate.

Connell, N., Kolter, R., Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Genetic studies of a stationary-phase-induced promoter.

SESSION 10 TRANSCRIPTION TERMINATION AND ANTITERMINATION

Granston, A.E., Carver, D.L., Eades, L.J., Craven, M.G., Friedman, D.I., Dept. of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor: Studies on *nusA* and second-site supressors of *nus* mutations.

Ghosh, B., Zhu, L., DeVitto, J., Barik, S., Das, A., Program in Molecular Biology and Biochemistry, University of Connecticut Health Center, Farmington: Cellular components that participate in transcription antitermination.

Barik, S., Ghosh, B., Whalen, W., Lazinski, D., Das, A., Program in Molecular Biology and Biochemistry, University of Connecticut Health Center, Farmington: Site-specific entry and assembly of a regulatory component into the elongating transcription apparatus detected by its immunoprint.

Oberto, J.,[1] Sloan, S.,[1] Harbrecht, D.,[2] Gottesman, M.E.,[3] Weisberg, R.A.,[1] [1]NICHD, National Institutes of Health, Bethesda, Maryland; [2]Molecular Genetics, Inc., Minnetonka, Minnesota; [3]Institute of Cancer Research, Columbia University, New York, New York: Further studies on *nun*, a phage-encoded transcription termination factor.

Almond, N.M., Lupski, J.R., Godson, G.N., Dept. of Biochemistry, New York University Medical Center, New York: Regulation of transcription termination in the *rpsU-dnaG-rpoD* macromolecular synthesis operon.

Morgan, E., Chang, T., Sigmund, C., Ettayebi, M., Roswell Park Memorial Institute, Buffalo, New York: Effect of BoxA mutations on ribosomal RNA transcription in vivo.

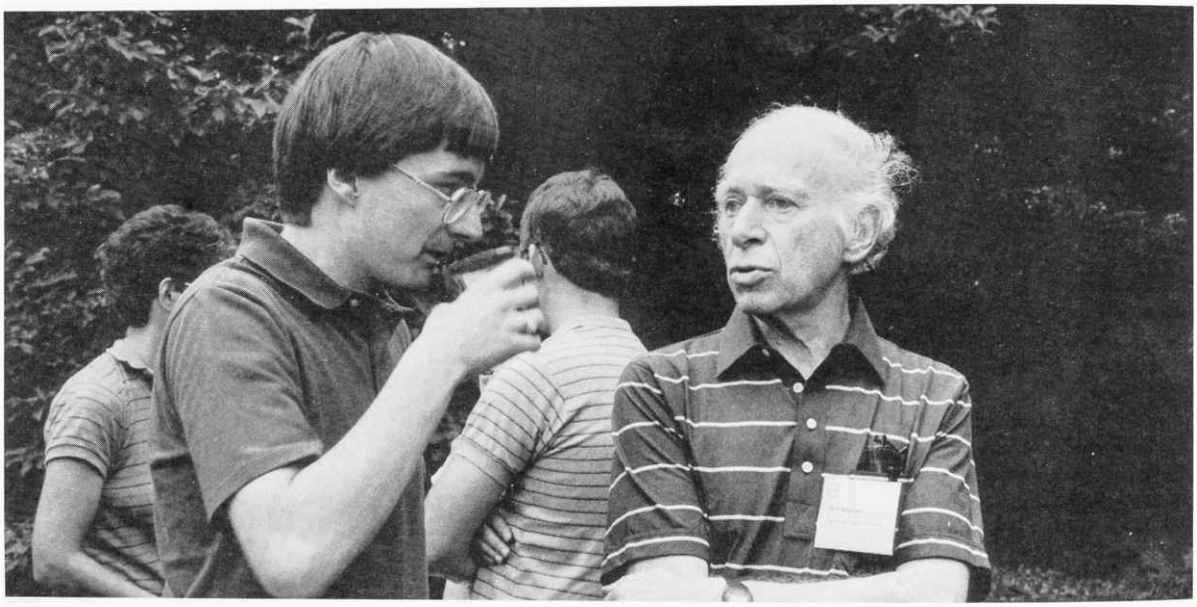
Whalen, W., Ghosh, B., Das, A., Program in Molecular Biology and Chemistry, University of Connecticut Health Center, Farmington: The *nutL* region of bacteriophage λ required for transcription antitermination encodes a signal for NusA-dependent termination.

Hasan, N., Szybalski, W., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Transcriptional activity of the promoter-less N^+ fragment of bacteriophage λ and its enhancement by the *nutL* module.

Somasekhar, G., Szybalski, W., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Q-utilization (*qut*) site required for antitermination of late transcription in bacteriophage λ – The functional boundaries.

Ferstandig Arnold, F., Fassler, J.S., Tessman, I., Dept. of Biological Sciences, Purdue University, West Lafayette, Indiana: Involvement of the *rho* protein of *E. coli* in the regulation of DNA supercoiling.

Drivdahl, R.H., Kutter, E., Evergreen State College, Olympia, Washington: Inhibition of transcription of dC-DNA by the *alc* gene product of phage T4.



R. Ebright, B. Magazanik

SESSION 11 RNA PROCESSING AND TRANSLATIONAL CONTROL

Shub, D.A.,[1] Gott, J.M.,[1] Xu, M.-Q.,[1] Belfort, M.,[2] [1]Dept. of Biology, State University of New York, Albany; [2]Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany: Multiple self-spliced introns in bacteriophage T4.

Chu, F.K., Maley, F., Maley, G.F., Wadsworth Center for Laboratories and Research, New York State Dept. of Health, Albany: Genomic localization of the *nrdA* gene encoding T4 phage ribonucleotide reductase A subunit and evidence for multiple introns in the T-even phages.

Hall, D.H.,[1] Povinelli, C.M.,[1] Ehrenman, K.,[2,3] Pedersen-Lane, J.,[2] Belfort, M.,[2] [1]School of Applied Biology, Georgia Tech, Atlanta; [2]Wadsworth Laboratories, N.Y. State Dept. of Health; [3]Dept. of Microbiology and Immunology, Albany Medical College, New York: Two splicing domains in the intron of the phage T4 *td* gene established by non-directed mutagenesis.

Takiff, H., Patterson, T., Court, D., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Cloning, genetic mapping, overexpression, and essential nature of the RNase III and *era* genes in *E. coli*.

Cole, J.R., Yamagishi, M., Nomura, M., Dept. of Biological Chemistry, University of California, Irvine: Overproduction of rRNA enhances the half-lives of specific mRNAs in *E. coli*.

Krinke, L., Cascio, M., Mahoney, M.E., Wulff, D.L., Dept. of Biological Sciences, State University of New York, Albany: Control of cII gene expression in bacteriophage λ.

Matthews, L.C., Nomura, M., Dept. of Biological Chemistry, University of California, Irvine: Protein S8 regulates L13 and L24 in the *spc* operon of *E. coli*.

Ivey, M.R., Steege, D.A., Dept. of Biochemistry, Duke University Medical Center, Durham, North Carolina: Translational regulation of bacteriophage f1 gene expression.

Ewen, M.E., Revel, H.R., Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois: Organization and expression of bacteriophage ϕ6 L-segment genes.

Engelberg-Kulka, H., Kopelowitz, J., Dept. of Molecular Biology, Hebrew University-Hadassah Medical School, Jerusalem, Israel: Influence of codon context on UGA read-through.

Whoriskey, S.K., Nghiem, V.-H., Leong, P.M., Masson, J.-M., Miller, J.H., Molecular Biology Institute, University of California, Los Angeles: Genetic rearrangements and gene amplification in *E. coli*.

Bergsland, K., Kao, C., Snyder, L., Dept. of Microbiology, Michigan State University, East Lansing: Interaction between the bacteriophage T4 *gol* site and the *lit* gene product of *E. coli*.

SESSION 12 MEMBRANE PROTEINS/DNA MANIPULATIONS

Manoil, C., Beckwith, J., Harvard Medical School, Boston, Massachusetts: Use of Tn*phoA* in determining the structure of integral membrane proteins.

Ito, K., Akiyama, Y., Shiba, K., Institute for Virus Research, Kyoto University, Japan: Characterization of the *secY* (*prlA*) gene-encoded membrane protein involved in protein export in *E. coli*.

Overdier, D.G., Druger-Liotta, J., Dunlap, V.J., Csonka, L.N., Dept. of Biological Sciences, Purdue University, West Lafayette, Indiana: Selection of osmoregulatory mutants of *S. typhimurium*.

Kadner, R.J., Friedrich, M.J., Weston, L.A., Dept. of Microbiology, University of Virginia, Charlottesville: Identification of the *uhp* polypeptides and their role in exogenous induction of the sugar phosphate transport system.

Friedman, S., Austin, S., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Regulation of P1 partition operon.

Taylor, R.,[1] Miller, V.,[2] Mekalanos, J.,[2] [1]University of Tennessee Center for the Health Sciences, Memphis; [2]Harvard Medical School, Boston, Massachusetts: Coordinate activation of genes encoding virulence determinants of *V. cholerae* by a transmembrane protein.

Smith, C.L., Dept. of Genetics and Development, Columbia University College of Physicians & Surgeons, New York, New York: Structure and dynamics of entire bacterial genomes.

Trun, N.J., Silhavy, T.J., Dept. of Molecular Biology, Princeton University, New Jersey: A general method for in vivo cloning in *E. coli*.

Ludwig, R.A., Dept. of Biology, Thimann Laboratories, University of California, Santa Cruz: Infection of diverse bacteria with *E. coli* bacteriophage λ.

Howard, K.A., Card, C., Benner, J.S., Callahan, H.L., Maunus, R., Silber, K., Wilson, G., Brooks, J.E., New England Biolabs, Beverly, Massachusetts: Cloning the *Dde*I restriction-modification system using a two-step method.

Moyle, H., Youderian, P., Dept. of Biological Sciences, University of Southern California, Los Angeles: Cloning vectors derived from phage P22.

Finkel, S., Halling, C., Calendar, R., Dept. of Molecular Biology, University of California, Berkeley: Selection of λ transducing phages using the P2 *old* gene cloned onto a plasmid.

G. Mosig

Molecular Biology of Hepatitis B Viruses

August 28–August 31

ARRANGED BY

Jesse Summers, Institute for Cancer Research, Philadelphia
Pierre Toillais, Institut Pasteur, Paris

198 participants

The second meeting on the Molecular Biology of Hepatitis B Viruses was attended by 212 registered participants. Although last year's meeting served to define the basic replication pathway, viral RNA transcripts, and viral gene products, more emphasis was apparent this year on factors that control gene expression and the function of viral gene products. Viral regulatory elements appear to be utilized with enhanced efficiencies after transfection into cells that already contain integrated viral DNA, suggesting a virus-encoded *trans*-acting control mechanism. Regions of the viral genome responsible for *cis*-acting transcriptional activation were identified, and evidence that these regions are acted on by tissue-specific factors was presented. Considerable evidence that virus-encoded proteins regulate intracellular and extracellular movement of viral gene products was also presented, and these minor proteins may have crucial regulatory roles in viral morphogenesis.

Hepatocellular carcinomas associated with hepadnaviral infections commonly contain integrated viral DNA. The general structures of these integrated DNAs now suggest that the replicative form of viral DNA is the precursor to the integrated form. A number of genetic changes in virus-induced hepatocellular carcinomas were described, some of which are associated with viral DNA integration. The role of these genetic changes in the etiology of hepatocellular carcinoma continues to be investigated.

Finally, the molecular biology of hepatitis B viruses will become more accessible to a genetic approach due to the availability of tissue-culture systems for HBV production from transfected cloned viral DNA. This long sought-after development was reported by a number of laboratories at this year's meeting and should add new and definitive data to next year's hepatitis B meeting, to be held at the end of September.

This meeting was supported in part by the following divisions of the National Institutes of Health: National Institute of Allergy and Infectious Diseases, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases; and National Science Foundation.

SESSION 1 REPLICATION AND THE CONTROL OF VIRAL GENE EXPRESSION

Chairman: J. Summers, Institute for Cancer Research, Philadelphia

Lien, J.-M., Petcu, D.J., Aldrich, C.E., Goldberg, J., Taylor, J.M., Mason, W.S., Institute for Cancer Research, Philadelphia, Pennsylvania: Initiation and termination of DHBV DNA synthesis.

Enders, G.,[1] Ganem, D.,[2,3] Varmus, H.,[1,2] Depts. of [1]Biochemistry and Biophysics, [2]Microbiology and Immunology, [3]Medicine, University of California, San Francisco: As few as 21 additional 5′-terminal nucleotides distinguish unpackaged from packaged GSHV RNAs.

Laub, O.,[1] Faktor, O.,[2] Shaul, Y.,[2] Depts. of [1]Genetics, [2]Virology, Weizmann Institute of Science, Rehovot, Israel: Efficient expression of the HBV enhancer element in hepatoma cell lines associated with HBV–Possible involvement of virus-coded transacting factor(s).

Spandau, D.F., Lee, C.H., Dept. of Pathology, Indiana University School of Medicine, Indianapolis: Evidence for a negative regulator that suppresses HBV enhancer activity.

Zahm, P., Hoschneider, P.H., Institute of Biochemistry, Martinsried, Federal Republic of Germany: *Trans*-activation by DNA sequences of HBV.

Farza, H.,[1] Hadchouel, M.,[1] Salmon, A.M.,[2] Babinet, C.,[2] Tiollais, P.,[1] Pourcel, C.,[1] [1]Unité de Recombinaison et Expression Génétique des Mammifères, Institut Pasteur, Paris, France: Developmental and hormonal regulation of HBsAg gene expression in transgenic mice.

Tur-Kaspa, R., Moore, D., Burk, R.D., Shafritz, D.A., Albert Einstein College of Medicine, Bronx, New York, and Massachusetts General Hospital, Boston: HBV DNA contains a binding site for the glucocorticoid receptor.

Bulla, G.A., Siddiqui, A., Dept. of Microbiology and Immunology, University of Colorado School of Medicine, Denver: Role of the HBV enhancer on HBsAg gene expression.

Burk, R.D.,[1] DeLoia, J.,[2] El Awady, M.K.,[1] Gearhart, J.,[2] [1]Albert Einstein College of Medicine, Bronx, New York; [2]Johns Hopkins University School of Medicine, Baltimore, Maryland: Putative HBsAg promoters not required for preferential liver gene expression of HBV DNA in transgenic mice.

Lever, A.M.L.,[1] Saito, I.,[2] Stark, G.,[2] Thomas, H.C.,[1] [1]Royal Free Hospital, [2]Imperial Cancer Research Fund, London, England: Interferon sensitivity in the HBV vivo and in vitro studies.

SESSION 2 BEHAVIOR AND FUNCTION OF VIRAL GENE PRODUCTS

Chairman: D. Ganem, University of California, San Francisco

Mack, D., Sninsky, J.J., Dept. of Diagnostics, Cetus Corporation, Emeryville, California: Two-dimensional protein analysis of hepadnaviral particles using antisera generated to ORFs expressed in *E. coli*.

McLachlan, A., Milich, D.R., Raney, A.K., Riggs, M.G., Hughes, J.L., Sorge, J., Chisari, F.V., Dept. of Basic and Clinical Research, Scripps Clinic and Research Foundation, La Jolla, California: A hepatitis-B amphotropic retroviral expression system.

Kay, A.,[1] d'Auriol, L.,[1] Mandart, E.,[1] Bouchier, C.,[1] Shamoon, B.,[1] Galibert, F.,[1] Trépo, C.,[2] [1]Laboratoire d'Hématologie Expérmentale, Centre Hayme, Hôpital St. Louis, Paris, [2]INSERM, Lyon, France: Site-directed mutagenesis of the pre-C region of WHV.

Brub, V., Gerlich, W.H., Dept. of Medical Microbiology, Göttingen, Federal Republic of Germany: The pre-core sequence of HBV functions as signal for translocation to the ER membrane.

Ou, J.,[1,2] Garcia, P.,[1] Walter, P.,[1] Standring, D.,[1,2] Rutter, W.J.,[1,2] [1]Depts. of Biochemistry and Biophysics, [2]Hormone Research Institute, University of California, San Francisco: Processing of the HBV core gene products.

Roossinck, M., Siddiqui, A., Dept. of Microbiology and Immunology, University of Colorado School of Medicine, Denver: Expression and biochemical analysis of HBV core protein.

Simon, K.,[1] Eble, B.,[2] Lingappa, V.,[1] Ganem, D.,[2] Depts. of [1]Physiology, [2]Microbiology, University of California, San Francisco: HBsAg is initially synthesized as a transmembrane polypeptide.

Eble, B.,[1] Lingappa, V.,[2] Ganem, D.,[1] Depts. of [1]Microbiology, [2]Physiology, University of California, San Francisco: Membrane insertion of the transmembrane form of HBsAg is directed by multiple uncleaved signal sequences.

Persing, D.H., Varmus, H.E., Ganem, D., Dept. of Microbiology, University of California, San Francisco: Inhibition of secretion of the hepatitis-B S-gene product by the pre-S1 protein.

Standring, D.N., Ou, J., Rutter, W.J., Hormone Research Institute, University of California, San Francisco: Pre-S proteins can inhibit the secretion of 22-nm S particles from *Xenopus* oocytes.

Chisari, F.V.,[1] Filippi, P.,[1] McLachlan, A.,[1] Milich, D.R.,[1] Riggs, M.,[1] Lee, S.,[1] Palmiter, R.,[2] Pinkert, C.,[3] Brinster, R.,[3] [1]Research Institute of Scripps Clinic, La Jolla, California; [2]University of Washington, Seattle; [3]University of Pennsylvania, Philadelphia: Expression of the HBV large envelope polypeptide inhibits HBsAg secretion in transgenic mice.

SESSION 3 POSTER SESSION

Siddiqui, A., Jameel, S., Dept. of Microbiology and Immunology, University of Colorado School of Medicine, Denver: Expression of the X region of HBV.

Marquardt, O.,[1] Heermann, K.H.,[2] Seifer, M.,[2] Gerlich, W.H.,[2] [1]Max Planck Institute of Biochemistry, Munich, [2]Dept. of Medical Microbiology, Göttingen, Federal Republic of Germany: Cell-type-dependent expressions and intracellular distribution of HBV pre-S1 surface antigen.

Wong, D.T.,[1] Sninsky. J.J.,[2] Shields, D.,[3] Depts. of [1]Microbiology and Immunology, [2]Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, New York; [3]Cetus Corporation, Emeryville, California: Synthesis and membrane insertion of pre-S and HBsAg in vitro.

Ma, X.K., Ding, G.Z., Li, L., Institute of Basic Medical Sciences, Beijing, People's Republic of China: Effect of pre-core and portion of core sequence on the expression of HBcAg in *E. coli*.

Lo, S.J.,[1] Chein, M.-L.,[1] Cheng, M.-L.,[1] Lee, Y.-H.W.,[2] Institutes of [1]Microbiology and Immunology, [2]Biochemistry, National Yang-Ming Medical College, Taipei, Taiwan, Republic of China: Analysis of X gene of HBV.

Kodama, K., Yoshikawa, H., Murakami, S., Biophysics Division, Cancer Research Institute, Kanazawa University, Japan: Expression of the product of X ORF of the WHV genome in *E. coli*.

Su, T.,[1,2] Lin, L.,[1] Lui, W.,[2] Chang, C.,[1,3] Chou, C.,[1,3] Ting, L.,[3] Hu, C.,[1,3] Han, S.,[1,3] P'eng, F.,[2] Depts. of [1]Medical Research, [2]General Surgery, [3]Veterans General Hospital and Graduate Institute of Microbiolgy and Immunology, National Yang-Ming Medical College, Taipei, Taiwan, Republic of China: Transcripts and the putative

RNA pregenome of HBV.

Bowyer, S., Dusheiko, G., Kew, M.C., University of the Witwatersrand Medical School, Johannesburg, South Africa: Expression of the HBV genome in chronic hepatitis-B carriers and patients with hepatocellular carcinoma.

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K. Koike, F. Imazeki

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Taiwan University College of Medicine, Taipei, Republic of China: Site-specific recombination of human HBV in hepatoma near a putative "triple-strand" structure.

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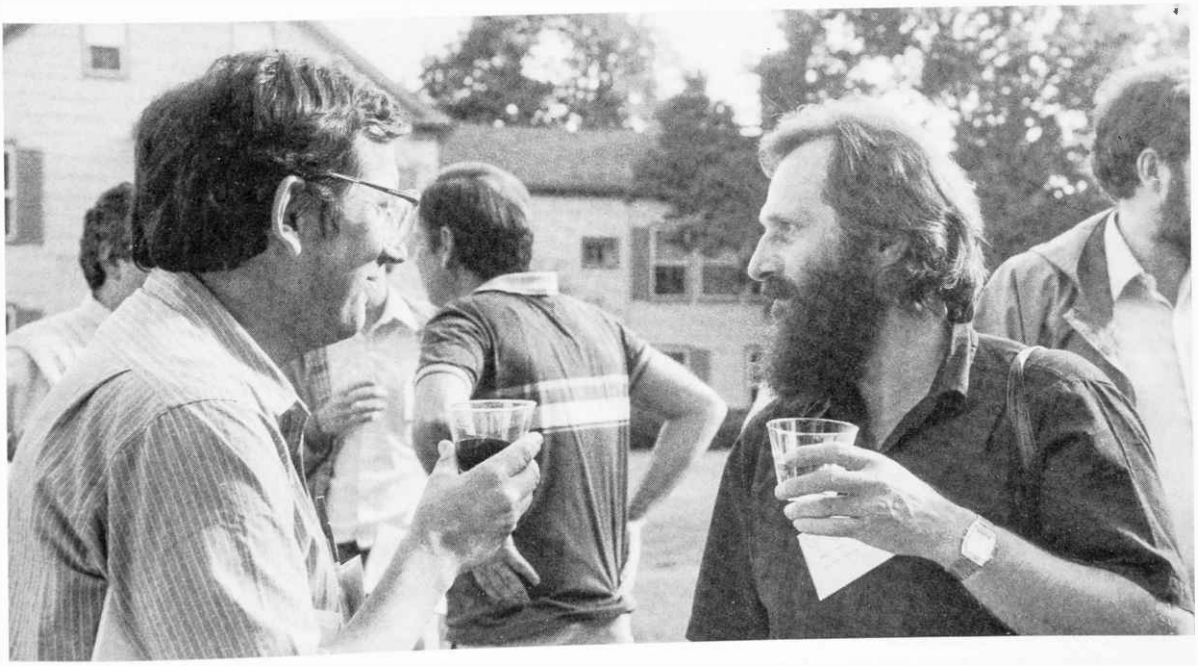

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Howard, C.,[1] Sundquist, B.,[2] Allan, J.,[1] Brown, S.,[1] Morein, B.,[2] [1]London School of Hygiene and Tropical Medicine, England; [2]National Veterinary Institute, Uppsala, Sweden: Immune-stimulating complexes (ISCOMs) containing HBsAg.

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Yu, W., Ping, C., Shaocai, D., Qimin, T., Institute of Hepatology, Beijing Medical College, People's Republic of China: Molecular cloning of the genome, gene *S*, and gene C of HBV, subtype *adr*.

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Roggendorf, M., Böhm, B., Rasshofer, R., Pahlke, C., Max von Pettenkofer Institute, University of Munich, Federal Republic of Germany: Characterization of proteins associated with the hepatitis delta virus (HDV).

SESSION 4 EXPRESSION OF VIRAL GENES IN INFECTED TISSUES

Chairman: W. Mason, Institute for Cancer Research, Philadelphia

Vitvitski, L.,[1] Meyers, M.L.,[2] Sninski, J.J.,[2] Chevallier, P.,[1] Acs, G.,[3] [1]INSERM, Lyon, France; [2]Dept. of Molecular Biology and Microbiology, Albert Einstein College of Medicine, Bronx, [3]Mount Sinai School of Medicine, New York, New York: Immunofluorescent studies of Hepadna X-gene products expression in liver of man, woodchuck, and duck—Evidence for cross-reactivity and correlation with replication.

Weber, C.,[1] Pugh, J.,[2] Murray, K.,[3] [1]Dept. of Internal Medicine, University of Mainz, Federal Republic of Germany; [2]Fox Chase Cancer Center, Philadelphia, Pennsylvania; [3]Dept. of Molecular Biology, University of Edinburgh, Scotland: Expression of the X gene of HBV.

Pfaff, E.,[1] Theilmann, L.,[2] Salfeld, J.,[1] Schaller, H.,[1] [1]Microbiology and ZMBH, University of Heidelberg, [2]Medizinische Universitätsklinik Heidelberg, Federal Republic of Germany: X-sequence-related proteins and x-specific antibodies.

Salfeld, J., Pfaff, E., Theilmann, L., Schaller, H., Dept. of Microbiology, ZMBH, University of Heidelberg, Federal Republic of Germany: The c-*pol* expression unit—Analysis of gene products from multifunctional genes.

Thung, S.N., Gerber, M.A., Kasambalides, E.J., Gilja, B.K., Keh, W., Gerlich, W.H., Dept. of Pathology, Mount Sinai School of Medicine and City Hospital, Elmhurst, New York, and Dept. of Medical Microbiology, University of Göttingen, Federal Republic of Germany: Demonstration of pre-S polypeptides of HBV in infected livers.

Korba, B.,[1] Wells, F.,[1] Tennant, B.,[2] Gerin, J.,[1] [1]Georgetown University, Rockville, Maryland; [2]College of Veterinary Medicine, Cornell University, Ithaca, New York: Lymphoid cells of the spleen are a site of active WHV replication in infected woodchucks.

Freiman, J.,[1] Cossart, Y.,[1] Holmes, M.,[1] Wills, E.,[2] [1]Dept.

of Infectious Diseases, University of Sydney, [2]Royal Prince Alfred Hospital, Sydney, Australia: Pathogenesis of DHBV infection.

Jilbert, A.R.,[1] Freiman, J.,[2] Gowans, E.J.,[1] Cossart, Y.E.,[2] Burrell, C.J.,[1] [1]Institute of Medical and Veterinary Science, Adelaide, [2]Dept. of Infectious Diseases, University of Sydney, Australia: Distribution and nature of DHBV DNA in infected liver, spleen, and pancreas—Analysis by in situ and Southern blot hybridization.

Tagawa, M., Marion, P.L., Dept. of Medicine, Stanford University, California: Viral transcripts in the yolk sac of duck hepatitis-B-infected duck eggs.

Halpern, M.S.,[1] Mason, W.S.,[2] Coates, L.,[2] O'Connell, A.P.,[2] England, J.M.,[3] [1]Wistar Institute, [2]Institute for Cancer Research, [3]Dept. of Pathology, University of Pennsylvania Hospital, Philadelphia: Humoral immune responsiveness in Pekin ducks experimentally infected with DHBV.

R. Miller, D. Govern, L. Johnson

SESSION 5 PATHOGENESIS AND IMMUNE RESPONSE

Chairman: C. Trepo, INSERM, Lyons

Di Bisceglie, A.M., Waggoner, J.G., Hoofnagle, J.H., NIDDK, NCI, Bethesda, Maryland: Changes in liver HBV DNA associated with seroconversion from HBeAg to antibody in chronic hepatitis B.

Carloni, G.,[1] Clementi, M.,[2] Colloce, S.,[3] Manzin, A.,[2] Galibert, K.,[4] Delfini, C.,[3] [1]Institute of Experimental Medicine, CNR, Rome, [2]Institute of Microbiology, University of Ancona, [3]Laboratory of Cell Biology, Institute Experiere Sanità, Rome, Italy; [4]Laboratory of Experimental Haematology, Centre Hayem, Paris, France: Detection of HBV replication in HBAg-negative carriers: HBV DNA in sera from symptomatic and asymptomatic subjects.

Raimondo, G., Burk, R.D., Lieberman, H.M., Muschel, J., Kew, M.C., Shafritz, D.A., Albert Einstein College of Medicine, Bronx, New York, and University of Witwatersrand, Johannesburg, South Africa: Replication state of HBV DNA in liver and tumor tissue of HBsAg carriers with hepatocellular carcinoma.

Lai, M.Y.,[1] Chen, D.S.,[1] Lee, S.C.,[1] Yang, P.M.,[2] Su, I.J.,[3] Sheu, J.C.,[2] Huang, G.T.,[2] Hsu, H.C.,[3] Wei, T.C.,[4] Sung, J.L.,[2] [1]Graduate Institute of Clinical Medicine, Depts. of [2]Internal Medicine, [3]Pathology, [4]Surgery, National Taiwan University College of Medicine, Taipei, People's Republic of China: Intrahepatic HBV DNA in HBsAg-positive chronic liver diseases.

Weimer, T., Fernholz, D., Wan, D.F., Will, H., Max-Planck-Institut für Biochemie, Martinsried, München, Federal Republic of Germany: Gene expression in hepadnavirus-infected livers and primary liver carcinoma.

Hadchouel, M.,[1] Pasquinelli, C.,[2] Hugon, R.N.,[1] Brechot, C.,[2] Bernard, O.,[1] [1]INSERM, Hôpital de Bicetre, [2]UREG, INSERM, Institut Pasteur, Paris, France: Detection of lymphocytes expressing HBV in peripheral blood from HBsAg-positive and -negative patients by in situ hybridization.

Knudsen, P.J., Zeldis, J.B., Dept. of Medicine, Beth Israel Hospital, Harvard Medical School, Boston, Massachusetts: HBV infection of monocytes in vitro blocks products of interleukin-1 activity.

Ferrari, C.,[1] Penna, A.,[2] Fiaccadori, F.,[2] Chisari, F.V.,[1] [1]Dept. of Basic and Clinical Research, Scripps Clinic and Research Foundation, La Jolla, California; [2]Cattedra Malattie Infettive Universita'di Parma, Italy: Intrahepatic, nucleocapsid antigen-specific T cells in chronic active hepatitis B.

Milich, D.R.,[1] McLachlan, A.,[1] Thornton, G.B.,[2] [1]Dept. of Basic and Clinical Research, Scripps Clinic and Research Foundation, [2]Johnson and Johnson, Biotechnol-

ogy Center, La Jolla, California: Immune response to the pre-S1 region of HBsAg.

Zeldis, J.B., Steinberg, H.N., Beth Israel Hospital, Harvard Medical School, Boston, Massachusetts: HBV inhibition of bone-marrow stem cells.

SESSION 6 MECHANISMS OF ONCOGENESIS

Chairman: K. Koike, Cancer Institute, Tokyo

Möröy, T.,[1] Marchio, A.,[1] Etiemble, J.,[1] Trepo, C.,[2] Tiollais, P.,[1] Buendia, M.A.,[1] [1]INSERM, CNRS, Institut Pasteur, Paris, [2]UER Alexis Carrel, Lyon, France: Rearrangement and activation of c-*myc* in hepatocellular carcinoma of woodchucks chronically infected with WHV.

Pasquinelli, C., Thiers, V., Garreau, F., Hadchouel, M., Dejean, A., Tiollais, P., Bréchot, C., Unité de Recombinaison et Expression Génétique, INSERM, Institut Pasteur, Paris, France: Search of preferential cellular DNA domains for HBV integration using unique flanking DNA sequences.

Wang, H.-P., Rogler, C.E., Dept. of Medicine, Albert Einstein College of Medicine, Bronx, New York: Deletion of the distal region of chromosome 11p in hepatocellular carcinomas from HBV carriers.

Simon, D., Knowles, B.B., Wistar Institute, Philadelphia, Pennsylvania: Karyotype abnormalities and chromosome instability in hepatocellular carcinoma.

Koshy, R., Max-Planck-Institut für Biochemie, Martinsried, Federal Republic of Germany: Structural alteration of specific cellular DNA sequences in some human hepatocellular carcinomas.

Ozturk, M.,[1] Carlson, R.,[1] Schouval, D.,[2] Wands, J.R.,[1] [1]Gastrointestinal Unit, Massachusetts General Hospital and Dept. of Medicine, Harvard Medical School, Boston; [2]Liver Unit, Dept. of Medicine, Hadassah University Hospital, Jersalem, Israel: Isolation and partial characterization of a human hepatoma-associated cell-surface antigen.

Kuroki, K.,[1] Kaneko, K.,[2] Kodama, K.,[1] Kobayashi, K.,[2] Hattori, N.,[2] Murakami, S.,[1] [1]Biophysics Division, Cancer Research Institute, [2]Internal Medicine, Kanazawa University Medical School, Japan: Transcriptional activation of a chromosome region where the enhancer of WHV DNA harbors in a cell line of woodchuck hepatoma.

Dejean, A.,[1] Bougueleret, L.,[2] Tiollais, P.,[1] [1]INSERM, CNRS, [2]Institut Pasteur, Paris, France: HBV DNA integration in a sequence homologous to v-*erb*-A and steroid receptor genes in a hepatocellular carcinoma.

Ochiya, T., Fujiyama, A., Fukushige, S., Hatada, I., Matsubara, K., Institute for Molecular and Cellular Biology, Osaka University, Japan: Molecular cloning of a novel oncogene from an HBV-related human hepatocellular carcinoma.

Zhou, Y.-Z., Butel, J.S., Dept. of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas: Integrated state of subgenomic fragments of HBV DNA in hepatocellular carcinoma from mainland China.

SESSION 7 NEW EXPERIMENTAL SYSTEMS

Chairman: H. Will, Max-Planck Institute, Munich

Marion, P.L.,[1] Azcarraga, R.,[1] Van Davelaar, M.J.,[1] Garcia, G.,[1] Popper, H.,[2] Robinson, W.S.,[1] [1]Dept. of Medicine, Stanford University, California; [2]Mount Sinai School of Medicine, New York, New York: Hepatocellular carcinoma in ground squirrels persistently infected with GSHV.

Paul, D.,[1] Farza, H.,[2] Pourcel, C.,[2] [1]Dept. of Cell Biologie, Fraunhoffer Institute of Toxicologie, Hannover, Federal Republic of Germany; [2]Recombinaison et Expression Génétique, INSERM, Institut Pasteur, Paris, France: Production of HBsAg in normal and immortalized transgenic mouse hepatocytes.

Pugh, J.,[1] Schaeffer, E.,[2] Summers, J.,[1] Sninsky, J.,[3] [1]Institute for Cancer Research, Philadelphia, Pennsylvania; [2]Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York; [3]Cetus Corporation, Emeryville, California: Antisera against the DHBV pre-S domain recognize a 36-kD viral envelope polypeptide and abolish viral infectivity in vitro.

Feitelson, M.A., DeTolla, L., Zhou, X.D., Fox Chase Cancer Center, Philadelphia, Pennsylvania: Mice infected intrahepatically with HBV DNA become chronic carriers and develop hepatitis.

Marion, P.L.,[1] Cullen, J.M.,[2] Robinson, W.S.,[1] Azcarraga, R.,[1] Van Davelaar, M.J.,[1] [1]Dept. of Medicine, Stanford University, California; [2]Dept. of Microbiology, Pathology, and Parasitology, North Carolina State University School of Veterinary Medicine, Raleigh: Experimental transmission of DHBV to domestic geese.

Tsurimoto, T., Matsubara, K., Institute for Molecular and Cellular Biology, Osaka University, Japan: Stable expression and replication of the HBV genome in an integrated state in a human hepatoma cell line transfected with the cloned viral DNA.

Yaginuma, K., Shirakata, Y., Kobayashi, M., Koike, K., Dept. of Gene Research, Cancer Institute, Tokyo, Japan: Formation of HBV-like particles in cell culture with a transient expression system.

Sureau, C., Romet-Lemonne, J.-L., Essex, M., Dept. of Cancer Biology, Harvard University School of Public Health, Boston, Massachusetts: Production of HBV-like particles in Hep G2 after transfection with cloned, circular HBV DNA.

Sureau, C., Romet-Lemonne, J.-L., Essex, M., Dept. of Cancer Biology, Harvard University School of Public Health, Boston, Massachusetts: Replication of HBV DNA in human lymphoblastoid cells after transfection with cloned, circular HBV DNA.

Sells, M.A., Acs, G., Dept. of Biochemistry, Mount Sinai School of Medicine, New York, New York: In vitro HBV DNA expression in transfected human liver cells.

Sells, M.A., Zelent, A., Shvartsman, M., Price, P.M., Acs, G., Dept. of Biochemistry, Mount Sinai School of Medicine, New York, New York: Expression of HBV core protein, pregenomic RNA, and episomal DNA by transfected murine cells.

Colucci, G.,[1] Lyons, P.,[1] Waksal, S.D.,[2] [1]Dept. of Medicine, Cornell University Medical College, [2]Institute for Cellular and Molecular Oncology, New York, New York: Transcription and translation of the HBV genome in a human-human hybridoma.

International Workshop on Papillomaviruses

September 3–September 7

ARRANGED BY

Bettie M. Steinberg, Long Island Jewish Medical Center
Janet L. Brandsma, Long Island Jewish Medical Center
Lorne B. Taichman, State University of New York, Stony Brook

286 participants

This year, the International Workshop on Papillomaviruses attracted the largest group ever, comprising more than 200 conferees from around the globe. An enormous interest currently centers on papillomavirus research because it represents an area of direct clinical and epidemiologic relevance, as well as an area where many basic molecular findings are now coming into focus. Because of the unique blend of basic scientists and clinicians who join together in efforts to understand this group of viruses, two short workshops were offered: Molecular Biology for the Clinician and Pathology for the Molecular Biologist.

The meeting was dedicated to Richard Shope and Yokei Ito for their pioneering work in this field using animal models. Interestingly, some of the more significant developments this year related again to animal models, including a method to grow human tissue explants infected with HPV in vitro in the nude mouse and the development of transgenic mice carrying the complete BPV genome.

Abstracts on the involvement of HPVs in human disease accounted for well over one third of the presentations and revealed the complexity of the association of various types of HPV with both benign and malignant disease. Prevalence rates of subclinical infection were also reported in many cases to be unexpectedly high.

The meeting also gave considerable attention to the molecular biology of papillomaviruses. BPV was shown to express an E2 gene product that *trans*-activates two transcriptional enhancer regions of BPV. In addition, evidence for a negative transcriptional regulatory factor was presented. Some studies addressed the mapping of particular viral transcripts in vivo, whereas other studies looked at the replication and transformation of papillomaviruses in vivo and in vitro. A number of groups presented the generation of papilloma-specific proteins and analysis of their distribution in various cells and tissues.

This meeting was supported in part by the following divisions of the National Institutes of Health: the National Cancer Institute and the National Institute of Allergy and Infectious Diseases; the National Science Foundation; the Burroughs Wellcome Company; the Council for Tobacco Research-USA, Inc.; the Otolaryngology Foundation, Inc.; and the Sanford C. Bernstein & Company Foundation, Inc.

SESSION 1 ANIMAL PAPILLOMAVIRUSES

Chairperson: M.S. Campo, The Beatson Institute for Cancer Research

Dedicatory Address: **C. Olson,** Dept. of Veterinary Science, University of Wisconsin, Madison: Richard Shope and Yokei Ito and the Origins of Papillomavirus Research.

Seto, A.,[1] Miyoshi, I.,[2] Ito, Y.,[1] [1]Dept. of Microbiology, Kyoto University, [2]Dept. of Medicine, Kochi Medical School, Japan: Introduction of inbred rabbits into tumor virus research.

Amtmann, E.,[1] Wayss, K.,[2] Institute for [1]Virus Research, [2]Pathology, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Interaction of MnPV and carcinogenic agents.epSundberg, J.P.,[1] O'Banion, M.K.,[2] Reichmann, M.E.,[2] [1]Dept. of Pathobiology, College of Veterinary Medicine, [2]Dept. of Microbiology, School of Life Sciences, University of Illinois, Urbana: Mouse papillomavirus—Pathology and viral characterization.

Schneider-Maunaury, S., Georges, E., Orth, G., Institut Pasteur, Paris, France: Structure and expression of CRPV genome and of the *myc* and Ha-*ras* genes during progression of domestic rabbit skin tumors.

M.S. Campo

SESSION 2 HUMAN DISEASES

Chairperson: C. Crum, Columbia University

Gissmann, L., German Cancer Research Center, Heidelberg: Association of papillomaviruses with human malignancies.

Syrjänen, K.,[1] Mäntyjärvi, R.,[2] Väyrynen, M.,[3] Syrjänen, S.,[4] Parkkinen, S.,[2] Yliskoski, M.,[3] Saarikoski, S.,[3] Sarkkinen, H.,[2] Nurmi, T.,[2] Castrén, O.,[3] [1]Laboraroty of Pathology and Cancer Research, Finnish Cancer Society, Depts. of [2]Clinical Microbiology, [3]Gynecology and Obstetrics, [4]Oral Pathology, University of Kuopio, Finland: Assessing the biological potential of HPV infection in cervical carcinogenesis.

Shimoda, K., Lancaster, W.D., Dept. of Obstetrics and Gynecology, Georgetown University, Washington, D.C.: Integration of HPV-16—related sequences in mild cervical dysplasia.

Ostrow, R.,[1] Manias, D.,[1] Clark, B.,[2] Okagaki, T.,[2,3] Twiggs, L.,[2] Faras, A.,[1] [1]Dept. of Microbiology and Institute of Human Genetics, [2]Dept. of Obstetrics and Gynecology, [3]Laboratory Medicine and Pathology, University of Minnesota, Minneapolis: The analysis of malignant tumors of the vulva, vagina, and cervix for HPV DNA.

Bistoletti, P.,[1] Zellbi, A.,[1] Hjerpe, A.,[2] [1]Dept. of Obstetrics and Gynecology, [2]Dept. of Pathology, Huddinge Hospital, Karolinska Institutet, Stockholm, Sweden: Genital papillomavirus infection in women with adenomatous endometrial hyperplasia.

Jablonska, S., Dept. of Dermatology, Warsaw School of Medicine, Poland: Natural killer cell activity in patients with skin lesions induced by HPV.

Fey, S.J., Institute for Medical Microbiology and Institute for Human Genetics, Arhus University, Denmark: New technique for viral diagnosis.

Syrjänen, S.,[1] Partanen, P.,[2] Syrjänen, K.,[3] [1]Dept. of Oral Pathology, University of Kuopio, [2]Labsystems Oy, Helsinki, [3]Finnish Cancer Society, Kuopio, Finland: Comparison of the in situ DNA hybridization protocols using ^{35}S-labeled probes and biotin-labeled probes in detection of HPV DNA sequences.

Fuchs, P.G.,[1] Girardi, F.,[2] Engelhardt, A.,[1] Pfister, H.,[1] [1]Institut für Klinische Virologie der Universität Erlangen-Nürnberg, Federal Republic of Germany; [2]Geburtschilflich-Gynä-kologische Universitätsklinik, Graz, Austria: Papillomaviruses in genital tumors of Austrian patients.

SESSION 3 WORKSHOPS

Lowy, R., National Cancer Institue, NIH, Bethesda, Maryland: Workshop on Molecular Biology for the Clinician.

Kurman, R., Dept. of Pathology, Georgetown University, Washington, D.C.: Workshop on Pathology for the Molecular Biologist.

SESSION 4 THERAPIES

Chairperson: A.L. Abramson, Long Island Jewish Medical Center

Weck, P.K., Whisnant, J.K., Dept. of Immunology and Oncology, Burroughs Wellcome Co., Research Triangle Park, North Carolina: Therapeutic approaches to the treatment of human papillomavirus diseases.

Steinberg, B.,[1] Gallagher, T.,[1] Trobridge, L.,[2] Healy, G.,[2] Abramson, A.,[1] [1]Dept. of Otolarynogology, Long Island Jewish Medical Center, New Hyde Park, New York; [2]Dept. of Otolarynogology, Childrens Hospital Medical Center, Boston, Massachusetts: Interferon and laryngeal papillomatosis, effect of HPV type, and possible IFN mechanism.

Shikowitz, M.,[1] Steinberg, B.,[1] Galli, R.L.,[1] Wettstein, F.O.,[2] [1]Dept. of Otolaryngology, Long Island Jewish Medical Center, New Hyde Park, New York; [2]Dept. of Microbiology and Immunology, University of California, Los Angeles: Molecular analysis of CRPV-induced papillomas treated with hematoporphyrin photodynamic therapy.

SESSION 5 POSTER SESSION: ANIMAL PAPILLOMAVIRUS; HUMAN DISEASES. I.

O'Banion, M.K.,[1] Reichmann, M.E.,[1] Sundberg, J.P.,[2] [1]Dept. of Microbiology, School of Life Sciences, [2]Dept. of Pathobiology, College of Veterinary Medicine, University of Illinois, Urbana: Homologous DNA and protein sequences of the rabbit oral papillomavirus with CRPV, HPV-1, and HPV-16.

Trenfield, K.,[1] Spradbrow, P.B.,[2] Vanselow, B.A.,[3] [1]Dept. of Medicine, Princess Alexandra Hospital, [2]Dept. of Veterinary Pathology and Public Health, University of Queensland, Brisbane; [3]Braund Street, Armidale, New South Wales,. Australia: Papillomavirus DNA resembling BPV-2 DNA detected in an ovine cutaneous horn of the ear.

Bunney,. M.H., Dept. of Dermatology, Royal Infirmary, Edinburgh, Scotland: Experience of five allograft patients with extensive HPV infections treated with human lymphoblastoid interferon α.

Friedman-Kien, A.E.,[1] Eron, L.,[2] Conant, M.,[2] Badiak, H.,[2] Bradstreet, P.,[2] Geffen, J.,[1] Fedorczyk, D.,[2] Trout, J.R.,[2] Plasse, T.,[2] [1]New York University Medical Center, New York; [2]Interferon Sciences, Inc., New Brunswick, New Jersey: Intralesional α-interferon treatment of condylomata acuminata – A multicenter trial.

Gross, G.,[1] Roussaki, A.,[1] Drees, N.,[3] Ikenberg, H.,[2] Dept. of [1]Dermatology, [2]Gynecology and Obstetrics, University of Freiberg, Federal Republic of Germany; [3]Hoffmann-La Roche Inc., Grenzach-Wyhlen: Systemic treatment of genital warts with recombinant interferon-2A – HPV DNA persistence in epithelia after regression of the lesions?

Bernstein, A.,[1] Lanier, J.[1] Buddin, D.A., [2] Weck, P.K.,[2] [1]Georgia Baptist Hospital, Atlanta; [2]Burroughs Wellcome Co., Research Triangle Park, North Carolina: Interferon alfa-n1 as adjuvant to laser therapy for severe or recurrent condyloma acuminata.

Mills, J.,[1] Gottleib, A.,[1] Britt, P.H.,[2] Weck, P.K.,[2] [1]University of California, San Francisco; [2]Burroughs Wellcome Co., Research Triangle Park, North Carolina: Interferon alfa-n1 for the treatment of refractory perianal and rectal condylomata acuminata.

Smotkin, D.,[1] Fu, Y.S.,[2] Wettstein, F.O.,[3] [1]Division of Gynecologic Oncology, Depts. of [2]Pathology, [3]Microbiology and Immunology, University of California School of Medicine, Los Angeles: Distribution of HPV-16 and HPV-18 in lesions of the female lower genital tract.

Villa, L.L.,[1] Tamashiro, M.S.,[2] Pereyra, E.,[2] [1]Ludwig Institute for Cancer Research, [2]Dept. of Gynecology, Universidade de São Paulo, Brasil: HPV DNA in cervical and urethral swabs from São Paulo.

von Krogh, G.,[1] Syrjänen, S.M.,[2] Syrjänen, K.J.,[3] [1]Dept. of Dermatology, Karolinska Institutet, Stockholm, Sweden; [2]Dept. of Oral Pathology and Radiology, University of Kuopio, [3]Laboratory of Pathology and Cancer Research, Finland Cancer Society, Kuopio: Detection of HPV DNA in genitoanal warts of the male using in situ DNA hybridization applied on paraffin sections.

Malcolm, A.D.B.,[1] Wickenden, C.,[1] Coleman, D.V.,[2] [1]Dept. of Biochemistry, Charing Cross and Westminster Medical School, [2]Dept. of Pathology, St. Mary's Hospital Medical School, London, England: Papillomaviruses in cervical epithelial scrapes.

Webb, D.H., Rogers, R., Fife, K., Indiana University Medical School, Indianapolis: Detection of HPV genomes in cervical scrapes using reverse-blot DNA hybridization.

Stoler, M.,[1] Wolinsky, S.,[2] Chow, L.,[3] Broker, T.,[3] Depts. of [1]Pathology, [2]Medicine, [3]Biochemistry, University of Rochester Medical Center, New York: In situ hybridization using HPV type-specific subgenomic RNA probes.

Wilczynski, S.,[1] Bergen, S.,[2] Sheets, E.,[2] Depts. of [1]Pathology, [2]Obstetrics and Gynecology, University of California, Irvine: Analysis of HPV DNA in cervical carcinomas.

Giorgi, C.,[1] Leopardi, O.,[2] Goisis, F.,[1] Bolis, P.F.,[3] de Virgiliis, G.,[4] Broich, G.,[5] Divisions of [1]Obstetrics and Gynecology, [2]Pathology, Ospedale di Lodi; [3]Dept. of Obstetrics and Gynecology, University of Pavia; [4]Section of Obstetrical and Gynecological Pathology, University of Milan, Italy; [5]Dept. of Oral Biology and Pathology, State University of New York, Stony Brook: Immunohistochemical demonstration of HPV and carcino-embryogenic antigens in the lower female genitary tract.Franceschi, S.,[1] Zaninetti, P.,[2] Baccolo, M.,[2] Bonazzi, B.,[2] Gottardi, G., Serraino, D.,[1] [1]Centro di Riferimento Oncologico, Aviano, [2]Istitute of Obstetrical and Gynecological Pathology, University of Milan, Italy: Papillomavirus infection and cervical intraepithelial neoplasia in 2,040 women under 20.

de Villiers, E.-M.,[1] Wagner, D.,[2] Schneider, A.,[3] Miklaw, H.,[4] Grussendorf-Conen, W.,[5] zur Hausen, H.,[1] [1]Referenzzuntrum für humanpathogene Papillomviren, Deutsches Krebsforschungszentru,, Heidelberg; [2]University of Freiburg; [3]Universität Frauenklinik, Ulm; [4]Salem-Krankenhaus, Heidelberg, Federal Republic of Germany: A survey of the infection rate in a normal popu-

lation with genital papillomaviruses.

Pater, M.M., Dunne, J., Hogan, G., Ghatage, P., Pater, A., Dept. of Obstetrics and Gynecology, Memorial University of Newfoundland, St. John's, Canada: Association of HPVs with precancerous and cancerous lesions of the cervix.

Reeves, W.C.,[1] Caussy, H.,[3] Cuevas, M.,[1] Arosemena, J.,[1] Torrazza, I.,[2] Rawls, W.E.,[3] [1]Gorgas Memorial Laboratory, [2]Oncology Institute, Panama, Republic of Panama; [3]McMaster University, Hamilton, Canada: HPV infection in Panamanian prostitutes.

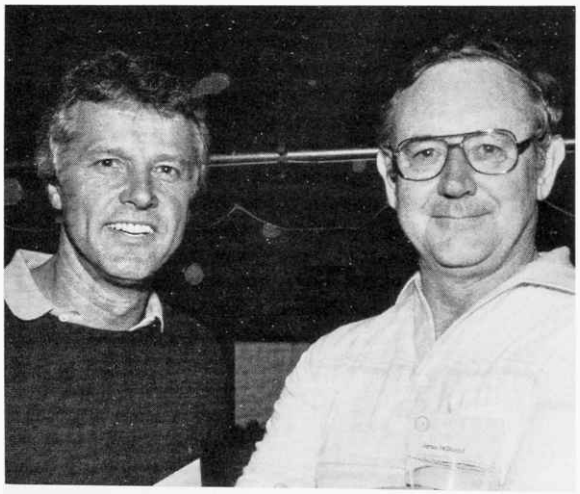
Reeves, W.,[1] Caussy, H.,[3] Brinton, L.,[4] Gomez, B.,[1] Brenes, M.M.,[1] Torrazza, I.,[2] Rawls, W.E.,[3] [1]Gorgas Memorial Laboratory, [2]Oncology Institute, Panama, Republic of Panama: [3]McMaster University, Hamilton, Canada; [4]NCI, National Institutes of Health, Bethesda, Maryland: Case-control study of HPV and cervical cancer in Latin America.

Barraso, O., Debrux, J., Croissant, O., Orth, G., Unité des Papillomavirus, Institut Pasteur, Paris, France: HPV-associated lesions of male genitalia.

Reid, R.,[1] Greenberg, M.,[1] Jenson, A.B.,[2] Husain, M.,[1] Willett, J.,[2] Daoud, Y.,[1] Temple, G.,[3] Stanhope, C.R.,[4] Sherman, A.,[1] Phibbs, G.,[5] Lorincz, A.,[3] [1]Sinai Hospital, Detroit, Michigan; [2]Georgetown University School of Medicine, Washington, D.C.; [3]Bethesda Research Laboratories, Gaithersburg, Maryland; [4]Mayo Clinic, Rochester, Minnesota; [5]Medical College of Ohio, Toledo: Sexually transmitted papillomaviral infections. I. The anatomic distribution and pathologic grade of neoplastic lesions associated with different viral types.

Bodén, E.,[1] Evander, M.,[2] Rylander, E.,[1] Wadell, G.,[2] Depts. of [1]Obstetrics and Gynecology, [2]Virology, Umeå, Sweden: HPV present in benign cervical epithelium several years after surgical removal of precancerous lesions.

ter Schegget, J.,[1] Walboomers, J.M.M.,[2] Fokke, H.E.,[1] Briët, M.A.,[3] Struyk, A.P.H.B.,[3] Wouters, E.,[1] Lammes, F.B.,[3] van der Noordaa, J.,[1] Depts. of [1]Virology, [2]Pathology, [3]Obstetrics and Gynecology, Academic Medical Centre, University of Amsterdam, The Netherlands: Detection of HPV DNA in cervical neoplasias.



A. Faras, J. McDougall

Schneider, A.,[1] Hotz, M.,[2] Gissmann, L.,[2] [1]Dept. of Obstetrics and Gynecology, Univeristy of Ulm, [2]German Cancer Research Center, Heidelberg, Federal Republic of Germany: Prevalence of genital HPV infections in pregnant women.

Toon, P.G.,[1.2] Wilson, L.P.,[1] Sharp, D.S.,[2] Arrand, J.R.,[1] [1]Paterson Laboratories, [2]North Manchester General Hospital, Manchester, England: HPV infection of the uterine cervix of women without any cytological signs of neoplasia.

Bergeron, C.,[1] Naghashfar, Z.,[2] Shah, K.,[2] Fu, Y.,[3] Ferenczy, A.,[1] [1]Dept. of Pathology, Sir Mortimer B. Davis Jewish General Hospital, Montreal, Canada; [2]Dept. of Immunology and Infectious Diseases, Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland; [3]Dept. of Pathology, University of California, Los Angeles: HPV-16 in intraepithelial neoplasia (Bowenoid papulosis) and coexistent invasive carcinoma of the vulva.

Bistoletti, P.,[1] Zellbi, A.,[1] Hjerpe, A.,[2] Depts. of [1]Obstetrics and Gynecology, [2]Pathology, Huddinge Hospital, Karolinska Institutet, Stockholm, Sweden: Genital papillomavirus infection after conization for cervical intraepithelial neoplasia.

Carson, L.F., Twiggs, L.B., Okagaki, T., Manias, D., Clark, B., Faras, A.J., Depts. of Obstetrics and Gynecology, Pathology and Microbiology, University of Minnesota, Minneapolis: Incidence of HPV DNA in specific histologic types of invasive vulvar carcinoma.

Chen, R.-H.,[1] Lee, S.-C.,[2] Hsieh, C.-Y.,[3] Huang, S.-C.,[3] [1]Dept. of Clinical Research, National Taiwan University Hospital, [2]Graduate Institute of Clinical Medicine, School of Medicine, National Taiwan University, and Institute of Biological Chemistry, Academia Sinica, Tapei, Taiwan, [3]Dept. of Obstetrics and Gynecology, School of Medicine, National Taiwan University, People's Republic of China: Presence and expression of HPV-16 and HPV-18 DNA in cervical carcinomas.

Del Mistro, A.,[1] Vallejos, H.,[1] Kleinhaus, S.,[2] Braunstein, J.D.,[1] Halwer, M.,[1] Koss, L.G.,[1] Depts. of [1]Pathology, [2]Surgery, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, New York: HPV types in condyloma acuminata in prepubertal children.

Fife, K.H., Boggs, D., Zwickl, B., Rogers, R., Indiana University School of Medicine, Indianapolis: Asymptomatic cervical infection with HPV during pregnancy.

Fu, Y.S.,[1] Huang, I.,[1] Beaudenon, S.,[2] Ionesco, M.,[3] Barrasso, R.,[3] de Brux, J.,[3] [1]Dept. of Pathology, University of California School of Medicine, Los Angeles; [2]Institut Pasteur, [3]IPECA, Paris, France: Correlative study of HPV DNA, histopathology, and morphometry in cervical condyloma and intraepithelial neoplasia.

Ikenberg, H., Kleine, W., Pfleiderer, A., Dept. of Gynecology and Obstetrics, University of Freiburg, Federal Republic of Germany: HPV DNA in primary tumors and lymph node metastases of genital carcinomas—Correlation of HPV prevalence with clinical stage and histology.

Murdoch, J.B., Cassidy, L., Cordiner, J.W., Macnab, J.C.M., MRC Institute of Virology, University of Glasgow. Scotland: The HPV genome and its association with colposcopically normal and colposcopically abnormal tissue of the uterine cervix.

J. Brandsma, L. Taichman, B. Steinberg

Caussy, D.,[1] Roth, P.,[1] Reeves, W.,[2] Rawls, W.,[1] [1]McMaster University, Hamilton, Canada; [2]Gorgas Memorial Institute, Panama, Republic of Panama: Evaluation of methods for detecting HPV DNA in clinical specimens.

Parkkinen, S.,[1] Mäntyjärvi, R.,[1] Syrjänen, K.,[2] Ranki, M.,[3] [1]Dept. of Clinical Microbiology, University of Kuopio, [2]Laboratory of Pathology, Finnish Cancer Society Kuopio, [3]Orion Genetic Engineering Laboratory Helsinki, Finland: Sandwich hybridization for detecting papillomavirus DNA in cervical scrapings.

Malcolm, A.D.B.,[1] Nicholls, P.J.,[1] Wickenden, C.,[1] Coleman, D.V.,[2] [1]Dept. of Biochemistry, Charing Cross and Westminster Medical School, [2]Dept. of Pathology, St. Mary's Hospital Medical School, London, England: Sandwich hybridizations in the detection of HPV strains.

Syrjänen, S.,[1] Happonen, R.-P.,[2] Virolainen, E.,[3] Siivonen, L.,[3] Syrjänen, K.,[4] [1]Dept. of Oral Pathology and Radiology, University of Kuopio, Depts. of [2]Oral Pathology, [3]Otorhinolaryngology, University of Turku, [4]Finnish Cancer Society, Kuopio, Finland: Demonstrations of HPV-11 and HPV-16 DNA in nasal inverted papillomas and squamous cell carcinomas by in situ hybridization.

Fey, S.J., Mose Larsen, P., Institute for Medical Microbiology and Institute for Human Genetics, Århus University, Denmark: Identification of putative HPV coat proteins (L1) in cervical biopsies by 2D gel electrophoresis.

Mose Larsen, P.,[1] Hansen, K.,[2] Frandsen, K.,[2] Vetner, M.,[3] Gissmann, L.,[4] [1]Institute for Medical Microbiology and Institute for Human Genetics, Århus University, [2]Herning Central Hospital, [3]Holstebro Central Hospital, Denmark; [4]German Cancer Research Center, Heidelberg, Federal Republic of Germany: A clinical study of cervical HPV infections using the putative coat proteins to determine the type of HPV present.

Ritter, D.B., Kadish, A.S., Vermund, S.H., Romney, S.L., Saed, S.O., Burk, R.D., Albert Einstein College of Medicine, Bronx, New York: Detection of HPV infection of the cervix by cervicovaginal lavage and molecular hybridization.

Goldsborough, M.D., DiSilvestre, D.A., Temple, G.F., Lörincz, A.T., Bethesda Research Laboratories, Life Technologies, Inc., Gaithersburg, Maryland: Nucleotide sequence analysis of HPV-31.

Lörincz, A.T., Quinn, A.P., Temple, G.F., Bethesda Research Laboratories, Life Technologies, Inc., Gaithersburg, Maryland: Cloning and characterization of three new HPV predominantly associated with mild cervical lesions.

Naghashfar, Z.,[1] Rosenshein, N.,[1] Lorincz, A.,[2] Shah, K.V.,[1] [1]Johns Hopkins Medical Institutions, Baltimore, [2]Bethesda Research Laboratory, Maryland: A new HPV-18–related genital tract papillomavirus from a patient with mild cervical pathology.

Beaudenon, S., Kremodor, D., Croissant, O., Orth, G., Unité des Papillomaviruses, Institut Pasteur, Paris, France: Two novel types of genital human papillomaviruses.

Lazo, P.A., Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania: Structure of papillomavirus (HPV-18) integrated in the genome of HeLa cells.

El Awady, M.K.,[1] Kaplan, J.,[1] O'Brien, S.,[2] Burk, R.D.,[1] [1]Albert Einstein College of Medicine, Bronx, New York; [2]NCI-Frederick Cancer Research Facility, Frederick, Maryland: Molecular analysis of integrated HPV-16 in cervical cancer.

Campion, M.J.,[1] McCance, D.J.,[2] Cuzick, J.,[3] Singer, A.,[1] [1]Dept. of Gynecology, Whittington Hospital, [2]Dept. of Microbiology, Guy's Medical School, [3]Imperial Cancer Research Fund, London, England: The progressive potential of mild cervical atypia–A prospective cytological, colposcopic, and virological study.

SESSION 6 TRANSCRIPTION

Chairperson: J. Brandsma, Long Island Jewish Medical Center

Spalholz, B.A., Lambert, P.F., Howley, P.M., NCI, National Institutes of Health, Bethesda, Maryland: Mapping of the BPV-1 LCR enhancer and promoters responsive to E2 transcriptional *trans*-activation.

Lambert, P.F., Spalholz, B.A., Howley, P.M., NCI, National Institutes of Health, Bethesda, Maryland: Evidence that papillomaviruses may encode a transcriptional repressor.

Thierry, F., Carranca, A.G., Heard, J.-M., Cereghini, S., Yaniv, M., Institut Pasteur, Paris, France: Characterization of transcription control elements in the noncoding region of HPV-18.

Seeberger, R.,[1] Haugen, T.,[2] Turek, L.,[2] Pfister, H.,[1] [1]Institut für Klinische Virologie, Erlangen, Federal Republic of Germany; [2]Dept. of Pathology, Veternas Administration Medical Center, Iowa City, Iowa: An enhancer of HPV-8 is *trans*-activated by the BPV-1 E2 function.

Schwarz, E., Boukamp, P., Fusenig, N.E., zur Hausen, H., Deutsches Krebsforschungszentrum, Heidelberg, Federal Republic of Germany: HPV-18 transcription in HeLa¼normal cell hybrids.

Chow, L.T.,[1] Nasseri, M.,[1] Wolinsky, S.M.,[2] Broker, T.R.,[1] Depts. of [1]Biochemistry, [2]Medicine, University of Roches-

ter School of Medicine, New York: Electron microscopic analysis of R-loops of HPV-6 and HPV-11 mRNAs from condyloma acumina.

Ward, P., Mounts, P., Dept. of Immunology and Infectious Diseases, Johns Hopkins University, Baltimore, Maryland: Transcriptional activity of HPV-6 subtypes in squamous papilloma of the respiratory tract.

Crum, C.,[1,3] Nuovo, G.,[1] Friedman, D.,[1] Silverstein, S.,[2,3] Depts. of [1]Pathology, [2] Microbiology, [3]Cancer Research Center, Columbia University, New York, New York: In situ hybridization analysis of HPV-16 RNA in genital precancers.

SESSION 7 WORKSHOP

T. Broker, Dept. of Biochemistry, University of Rochester, New York: Workshop on Computer-associated Bibliography and Word Processing for Papillomavirus Research.

SESSION 8 REPLICATION

Chairperson: K. Shah, Johns Hopkins University

Prives, C., Dept. of Biological Sciences, Columbia University, New York, New York: The replication of SV40 and polyomaviruses.

Botchan, M., Molecular Biology and Virus Laboratory, University of California, Berkeley: Papillomavirus replication.

Reilly, S. S., Taichman, L., Dept. of Oral Biology and Pathology, School of Dental Medicine, State University of New York, Stony Brook: Underreplication of HPV-1 DNA in cultured epidermal keratinocytes.

Christian, C.B.,[1] Reddel, R.R.,[2] Shah, K.V.,[1] [1]Dept. of Immunology and Infectious Diseases, Johns Hopkins University School of Hygiene and Public Health, Baltimore, [2]NCI, National Institutes of Health, Bethesda, Maryland: Papillomavirus DNA replication in cultured cells of respiratory tract origin.

B. Steinberg, E.-M. de Villiers

SESSION 9 POSTER SESSION: HUMAN DISEASES. II. PAPILLOMAVIRUS REPLICATION AND TRANSCRIPTION

Beiss, B.,[1] Sundberg, J.,[4] Douglas, J.,[5] Burk, R.,[2] Ritter, D.,[3] Kadish, A.,[1] Depts. of [1]Pathology, [2]Pediatrics, [3]Obstetrics and Gynecology, Albert Einstein College of Medicine, Bronx, New York; [4]Dept. of Veterinary Pathobiology, College of Veterinary Medicine, University of Illinois, Urbana; [5]Dept. of Health and Hospitals, Disease Control Service, Denver, Colorado: Host immune responses to genital or laryngeal HPV infections.

Rüdlinger, R.,[1] Bunney, M.H.,[2] Smith, I.W.,[3] Hunter, J.A.A.,[2] [1]Dermatologische Klinik, Universitatsspital Zurich, Switzerland; Depts. of [2]Dermatology, [3]Virology, University of Edinburgh, Scotland: Detection of HPV-5 DNA in a renal allograft patient from Scotland.

Zachow, K., Ostrow, R., Faras, A., Dept. of Microbiology and Institute of Human Genetics, University of Minnesota, Minneapolis: Nucleotide sequence of HPV-5.

Tanigaki, T.,[1] Kanda, R.,[1] Yoshikawa, K.,[1] Kitano, Y.,[1] Yutsudo, M.,[2] Hakura, A.,[2] [1]Dept. of Dermatology, Osaka University Medical School, [2]Research Institute for Microbial Diseases, Osaka University, Japan: Epidemiologic aspects of epidermodysplasia verruciformis (L-L 1922) in Japan—Clinical observations and virological studies in 11 patients with epidermodysplasia verruciformis.

Tanigaki, T.,[1] Kanda, R.,[1] Yoshikawa, K.,[1] Kitano, Y.,[1] Yutsudo, M.,[2] Hakura, A.,[2] [1]Dept. of Dermatology, Osaka University Medical School, [2]Research Institute for Microbial Diseases, Osaka University, Japan: Epidermodysplasia verruciformis in Japan—Characteristics of the lesions and risk of widespread conversion in relation to the types of HPV involved.

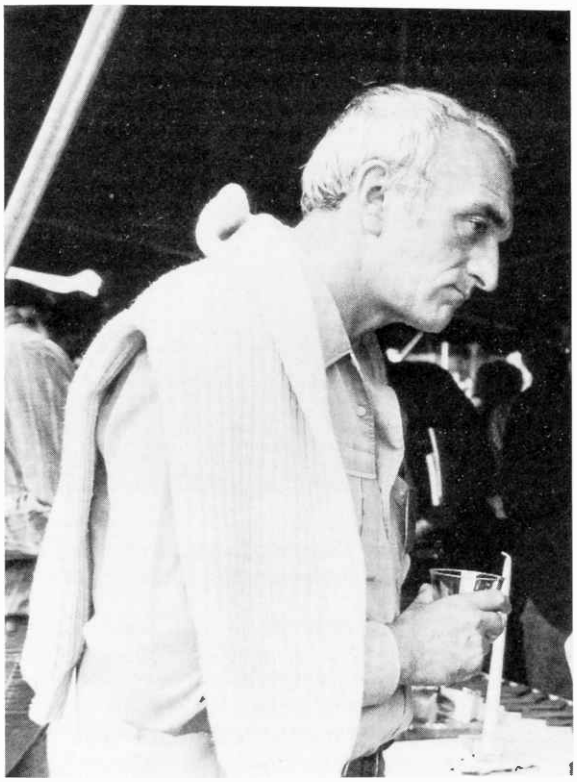
Brandsma, J.,[1] Barrezueta, N.,[1] Galli, R.,[1] Shah, K.,[2] [1]Long Island Jewish Medical Center, New Hyde Park, New York; [2]Johns Hopkins University, Baltimore, Maryland: HPV-11 in some cases of nasal inverted papilloma.

de Villiers, E.-M.,[1] Le, J.-Y.,[1] Weidauer, H.,[2] Greenspan, D.,[3] zur Hausen, H.,[1] [1]Referenzzentrum für humanpathogene Papillomviren, Deutsches Krebsforschungszentrum, Heidelberg, [2]University of Heidelberg, Federal Republic of Germany; [3]University of California, San Francisco: DNA related to HPV-32 in a fibrolipoma of the human parotid and HPV-7 DNA in some oral papillomas.

Takagi, M.,[1] Yamada, T.,[1] Yamamoto, H.,[1] Terada, M.,[2] [1]Dept. of Oral Pathology, Tokyo Medical and Dental University, [2]National Cancer Center Research Institute, Tokyo, Japan: HPV in oral lesions.

Adler-Storthz, K.,[1] Newland, J.R.,[2] Tessin, B.A.,[1] Yeudall, W.A.,[3] Shillitoe, E.J.,[1] Depts. of [1]Microbiology, [2]Pathology and Radiology, University of Texas Dental Branch, Houston; [3]Dept. of Oral Medicine and Pathology, Glasgow Dental Hospital, Scotland: HPV-2 DNA in oral verrucous carcinoma.

Hernandez-Jáuregui, P.,[1] Perez, R.T.,[1] Ericsson, A.,[2] Pet-



F. Cuzin

tersson, U.,[3] Moreno-L., J.,[2] [1]Unidad de Investigacion Médica Instituto Mexicano del Seguro Social, México; [2]Dept. of Veterinary Microbiology, Virology Biomedical Center, [3]Dept. of Medical Genetics and Microbiology, Biomedical Center, Uppsala, Sweden: HPV-13 DNA in focal epithelial hyperplasia among Mexicans.

Kashima, H.,[1] Mounts, P.,[3] Kuhajda, F.,[2] Loury M.,[1] Depts. of [1]Otolaryngology-Head and Neck Surgery, [2]Pathology, Johns Hopkins Hospital, [3]Dept. of Immunology and Infectious Diseases, Johns Hopkins University, Baltimore, Maryland: Demonstration of HPV capsid antigen in carcinoma-in-situ of the larynx.

Zaslav, A.,[1,2,3] Steinberg, B.,[2] Stamberg, J.,[1] Lin, Y.,[3] [1]Schneider Children's Hospital, [2]Dept. of Otolaryngology, Long Island Jewish Medical Center, New Hyde Park; [3]St. John's University, Jamaica, New York: Cytogenetic evaluation of laryngeal papillomas.

McDonnell, P.J., McDonnell, J.M., Kessis, T., Green, W.R., Shah, K.V., Johns Hopkins Medical Institutions, Baltimore, Maryland: Detection of HPV-6 DNA in conjunctival neoplasia by in situ hybridization with radioactive probes.

Nasseri, M., Hirochika, R., Broker, T.R., Chow, L.T., Dept. of Biochemistry, University of Rochester School of Medicine, New York: An HPV-11 transcript encoding an E1-E4 fusion protein.

Wolinsky, S.M.,[1] Stoler, M.H.,[2] Broker, T.R.,[3] Chow, L.T.,[3] Depts. of [1]Medicine, [2]Pathology, [3]Biochemistry, University of Rochester School of Medicine, New York: Messenger RNA exon-specific probes for HPV transcriptional analysis.

Mitrani-Rosenbaum, S., Kitron, N., Dept. of Virology, Hebrew University-Hadassah Medical School, Jerusalem, Israel: Expression of integrated HPV-6 recombinant DNA in mouse cells.

Baker, C.,[1] Phelps, W.,[1] Lingren, V.,[1] Braun, M.,[2] Gonda, M.,[2] Howley, P.,[1] [1]NCI, National Institutes of Health, Bethesda, [2]NCI-Frederick Cancer Research Facility, Frederick, Maryland: A structural and transcriptional analysis of integrated HPV-16 DNA in human cervical carcinoma cell lines.

Scott, C.L., Temple, G.F., Lörincz, A.T., Bethesda Research Laboratories, Life Technologies, Inc., Gaithersburg, Maryland: Construction and characterization of a partial cDNA library from a cervical intraepithelial neoplasia.

Hirochika, H., Chow, L.T., Broker, T.R., Dept. of Biochemistry, University of Rochester School of Medicine and Dentistry, New York: Enhancer structure and *trans*-acting E2 transcriptional factors of HPVs.

Hurley-Guis, D.,[1] Laimins, L., [1]Committee on Virology, [2]Howard Hughes Medical Institute, University of Chicago, Illinois: Characterization of an HPV-18 enhancer element.

Phelps, W.C., Howley, P.M., NCI, National Institutes of Health, Bethesda, Maryland: Transcriptional *trans*-activation of a conditional enhancer in HPV-16.

Marshall, T., Pater, A., Pater, M.M., Memorial University of Newfoundland, St. John's, Canada: Identification of regulatory elements in the genomes of oncogenic human papillomaviruses.

Haugen, T.H.,[1] Cripe, T.P.,[1,2] Karin, M.,[3] Turek, L.P.,[1] [1]Dept. of Pathology, [2]Dept. of Genetics, VAMC and University of Iowa, Iowa City; [3]Dept. of Medicine, University of California, San Diego: BPV E2 function *trans*-activates the major early viral promoter.

Cripe, T.P.,[1,2] Haugen, T.H.,[1] Karin, M.,[3] Turek, L.P.,[1] [1]Dept. of Pathology, [2]Dept. of Genetics, VAMC and University of Iowa, Iowa City; [3]Dept. of Medicine, University of California, San Diego: An HPV-16 E2 function *trans*-activates a cryptic *cis* enhancer upstream of the early viral genes.

Harrison, S.M., Midgley, C.A., Kim, S., King, L.A., Kingsman, A.J., Kingsman, S.M., Dept. of Biochemistry, University of Oxford, England: Analysis of the *cis*- and *trans*-acting factors involved in the regulation of transcription in BPV-1.

Baker, C.C., Howley, P.M., NCI, National Institutes of Health, Bethesda, Maryland: Control of late transcription in BPV.

Stamps, A., Campo, M.S., Beatson Institute for Cancer Research, Glasgow, Scotland: Analysis of the mRNA transcripts of BPV-4.

Lusky, M., Berg, L., Stenlund, A., Botchan, M., Dept. of Molecular Biology, University of California, Berkeley: A modulator function for BPV replication is required for the stable maintenance of the viral plasmid state.

Holmstedt, E., Nilsson, S., Pharmacia Biotechnology, Uppsala, Sweden: The E1 ORF is not sufficient to maintain pBPV as a stably replicating episome in transfected cells.

King, L.A., Harrison, S.M., Wilson, L.E., Kingsman, A.J., Kingsman, S.M., Dept. of Biochemistry, University of Oxford, England: Analysis of the *cis*- and *trans*-acting sequences involved in the replication of BPV.

L. Taichman, L. Chow

Dietrich, W., Pfister, H., Institut für klinische Virologie der Universität Erlangen-Nürnberg, Federal Republic of Germany: Mutation in BPV-1 ORF E4 affects persistence of viral DNA.

Romanczuk, H., Wormington, M., Brandeis University, Rosenstiel Center, Waltham, Massachusetts: Replication and persistence of BPV in *Xenopus* embryos.

Ahola, H.,[1] Ström, A.-C.,[2] Bergman, P.,[2] Eriksson, A.,[1] Moreno-Lopez, G.,[1] Pettersson, U.,[2] Depts. of [1]Veterinary Microbiology (Virology), [2]Medical Genetics, Uppsala University, Biomedial Center, Sweden: Structure and expression of the transforming region from the European elk papillomavirus.

Sarver, N., Link, J., Nathan, M., Meloy Laboratories, Molecular Biology Division, Springfield, Virginia: Modulation of cDNA expression in mammalian cells by 3′ flanking sequences.

Law, M.-F.,[1] Baker, C.,[2] Gardner, S.M.,[1] Register, R.B.,[1] [1]Salk Institute Biotechnology/Industrial Associates Inc., La Jolla, California; [2]NCI, National Institutes of Health, Bethesda, Maryland: A combination cDNA cloning and expression vector derived from BPV DNA.

Jensen, N.A., Pedersen, F.S., Dept. of Molecular Biology, University of Aarhus, Denmark: Expression of BPV-1 late genes in a retroviral expression and expression-transmission vector system.

Giordano, T.J., McAllister, W.T., Dept. of Microbiology and Immunology, State University of New York Science Center, Brooklyn: BPV-derived shuttle vectors containing the hygromycin B resistance gene.

Jablonska, S.,[1] Kawashima, M.,[2] Obalek, S.,[1] Orth, G.,[2] [1]Dept. of Dermatology, Warsaw School of Medicine, Poland; [2]Institut Pasteur, Paris, France: Human cutaneous papillomas.

Malejczyk, J.,[1] Majewski, S.,[2] Jablonska, S.,[2] Orth, G.,[3] [1]Dept. of Histology and Embryology, [2]Dept. of Dermatology, Warsaw Medical School, Poland; [3]Institut Pasteur, Paris, France: Natural cell-mediated cytotoxicity (NCMC) in patients with an O-genital lesions induced by potentially oncogenic HPVs.

Majewski, S.,[1] Malejczyk, J.,[2] Jablonska, S.,[1] Orth, G.,[3] [1]Dept. of Dermatology, [2]Dept. of Histology and Embryology, Warsaw Medical School, Poland; [3]Institut Pasteur, Paris, France: In vitro effects of interferons α and γ on SKV cells.

SESSION 10 TRANSFORMATION

Chairperson: J.K. McDougall, Fred Hutchinson Cancer Research Center

Lowy, D.R., Androphy, E.J., Vousden, K.H., Schiller, J.T., NCI, National Institutes of Health, Bethesda, Maryland: Cellular transformation by BPV.

DiMaio, D.,[1] Neary, K.,[1] Kaczmarek, L.,[2] Andrews, E.,[1] Horwitz, B.,[1] Guralski, D.,[1] [1]Dept. of Human Genetics, Yale University School of Medicine, New Haven, Connecticut; [2]Dept. of Pathology, Temple Medical School, Philadelphia, Pennsylvania: Mutational analysis of BPV transformation functions.

DiPaolo, J.A., Doniger, J., Popescu, N., Yasumoto, S., Laboratory of Biology, NCI, Bethesda, Maryland: Transformation and extended life span of human fibroblasts and epithelial cells.

Brandt, C.R., McDougall, J.K., Galloway, D.A., Fred Hutchinson Cancer Research Center, Seattle, Washington: Amplification of HPV DNA in cervical carcinoma cell lines occurs following HSV-2 infection or treatment with a chemical carcinogen.

Jones, K., Long, J., Laimins, L., Howard Hughes Medical Institute, University of Chicago, Illinois: Transformation of primary cells with HPV-16 and HPV-18 sequences.

Burnett, S., Pettersson, U., Dept. of Medical Genetics, Biomedical Center, Uppsala, Sweden: Transformation of C127 cells by BPV-1 with its noncoding region replaced by that of HPV-1.

Levenson, R.M., Brinckmann, U.G., Androphy, E. Schiller,

J., Broker, T.R., Chow, L.T., Young, D.A., Depts. of Medicine, Biochemistry, and Biophysics, University of Rochester, New York: Papillomavirus-specific inductions of cellular proteins in murine C127 cells—Role of the E5 and E6 transforming regions.

Nasseri, M., Wettstein, F.O., Dept. of Microbiology and Immunology, School of Medicine and Molecular Biology Institute, University of California, Los Angeles: Characterization of CRPV deletion maintained as a plasmid in both rabbit tissue and NIH-3T3 cells and its transforming activity.

SESSION 11 TISSUE-SPECIFIC EXPRESSION

Chairperson: F. Cuzin, Université de Nice

Kreider, J.W.,[1,2] Howett, M.K.,[2] Lill, N.L.,[2] Leure-Dupree, A.E.,[3] Zaino, R.J.,[1] Depts. of [1]Pathology, [2]Microbiology, [3]Anatomy, Hershey Medical Center, Pennsylvania: Tissue-specific expression of HPV-11.

Sippola-Thiele, M.,[1] Hanahan, D.,[2] Lindgren, V.,[1] Howley, P.M.,[1] [1]Laboratory of Tumor Virus, NCI, Bethesda, Maryland; [2]Cold Spring Harbor Laboratory, New York: Analysis of BPV-1 expression in transgenic mice.

Campione-Piccardo, J., Fanok, A., Garvie, P., Mandy, F., Health and Welfare Canada, LCDC, Ottawa: Cell differentiation independent expression of late HPV-1a genes in human and monkey cell lines.

Watts, S.L., Brewer, E.E., Walton, L.A., University of North Carolina, Chapel Hill: Culture of dysplastic uterine exocervical cells and analysis of HPV DNA and mRNA in cervical lesions.

SESSION 12 POSTER SESSION: TISSUE-SPECIFIC EXPRESSION, TRANSFORMATION, AND PAPILLOMAVIRUS-SPECIFIC PROTEINS

Dürst, M.,[1] Boukamp, P.,[2] Gissmann, L.,[1] Institut für [1]Virusforschung, [2]Biochemie, Duetsches Krebsforschungszentrum, Heidelberg, Federal Republic of Germany: Transfection of primary human keratinocytes using the complete HPV-16 genome—Analysis of a stable cell clone.

Watts, S.L., Brewer, E.E., University of North Carolina, Chapel Hill: Transformation of mouse C127 cells by HPVs associated with genital neoplasia.

Kaur, P., McDougall, J.K., Fred Hutchinson Cancer Research Center, Seattle, Washington: Transformation of cells with cloned HPV-6b and HPV-18 DNAs.

Matlashewski, G., Banks, L., Spence, P., Murray, A., Crawford, L., Imperial Cancer Research Fund, London, England: Transformation by HPV-16 and production of tumors in nude mice.

Sarkkinen, H.,[1] Syrjänen, S.,[2] Mäntyjärvi, R.,[1] Depts. of [1]Clinical Microbiology, [2]Oral Pathology and Radiology, University of Kuopia, Finland: Transformation of C127 cells by HPV-16 and HPV-31.

Yasumoto, S., Doniger, J., DiPaolo, J.A., NCI, National Institutes of Health, Bethesda, Maryland: Analysis of two stage-transformation of HPV-16—transfected NIH-3T3 cells.

Chesters, P.M., McCance, D.J., Dept. of Microbiology, Guy's Medical School, London, England: Transformation of NIH-3T3 cells with pSVNEO2 cloned HPV-6 and -16.

Green, M., Brackmann, K.H., Loewenstein, P.M., Institute for Molecular Virology, St. Louis University Medical Center, Missouri: Rat embryo fibroblast cells expressing HPV-1a genes exhibit altered growth properties and tumorigenicity.

Modjtahedi, N., Feunteun, J., Brison, O., Institut Gustave Roussy, Villejuif, France: *Cis*-activation of the *myc* gene in a BPV-1/human c-*myc* hybrid plasmid.

Groff, D.E., Dept. of Obstetrics and Gynecology, Georgetown University Medical Center, Washington, D.C.: HPV-16 DNA enhances stable biochemical transformation of mouse cells.

Binétruy, B.,[1] Meneguzzi, G.,[1] Cerni, C.,[2] Cuzin, F.,[1] [1]INSERM, Université de Nice, France; [2]Institut für Tumorbiologie der Universität, Vienna, Austria: Tumoral progression induced by BPV-1 oncogenes in rodent fibroblasts.

Levenson, R.M., Brinckmann, U.G., Broker, T.R., Chow, L.T., Young, D.A., Depts. of Medicine, Biochemistry, and Biophysics, University of Rochester, New York: Specific protein phosphorylations in murine C127 cells—Effects of transformation by BPV-1 and v-Ha-*ras* and treatment with the phorbol tumor promoter, TPA.

Brinckmann, U.G., Levenson, R.M., Broker, T.R., Chow, L.T., Young, D.A., Depts. of Medicine and Biochemistry, and Biophysics, University of Rochester, New York: Specific proteins secreted by murine C127 cells transformed by HPV, BPV, and CRPV, v-*fes*, v-*mos* and v-Ha-*ras*.

Bergman, P.,[1] Ustav, M.,[2] Vennström, B.,[3] Pettersson, U.,[1] [1]Dept. of Medical Genetics, Uppsala University, Sweden; [2]Dept. of Molecular Biology, University of Tartu, Estonia, USSR; [3]EMBL, Heidelberg, Federal Republic of Germany: Analysis of BPV-1 transforming function by the use of a retrovirus vector.

Viac, J., Bouvard, V., Chardonnet, Y., Thivolet, J., INSERM, CNRS, Hopital E. Herriot, Lyon, France: Incidence of HPV infection on epithelial antigen expression in papillomas.

Metcalfe, L., Mounts, P., Dept. of Immunology and Infectious Diseases, Johns Hopkins University, Baltimore, Maryland: Sequence homology among HPV-6 isolates from condyloma acuminata and juvenile-onset and adult-onset respiratory papillomata.

Farr, A., Roman, A., Dept. of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis: Transfection of human keratinocytes with pRSVcat and HPV-6 DNA.

Morgan, D.M., Pecoraro, G., Le, J., Defendi, V., Dept. of Pathology, New York University Medical Center, New York: Persistence, physical state, and transforming ability of papillomavirus DNA in fibroblast/epithelial cells.

DiLorenzo, T., Risdon, G., Pumo, D.E., Hofstra University, Hempstead, New York: Defined culture conditions for C127 and BPV-infected ID13 cells.

Thompson, C., Rose, B., Parker, B., Morris, B., Cossart, Y., Depts. of Infectious Diseases and Physiology, University of Sydney, Australia: Persistence of papillomavirus DNA in keratinocytes cultured from anogenital warts.

Wilbanks, G.,[1] Hawkins, J.,[1] Turyk, M.,[1] Golub, T.,[1] Mercer, L.,[2] McCance, D.,[3] [1]Dept. of Obstetrics and Gynecology, Rush University, [2]Dept. of Obstetrics and Gynecology, University of Chicago, Illinois; [3]Dept. of Microbiology, Guy's Hospital Medical School, London, England: Tissue culture of human cervical intraepithelial neoplasias.

Villari, D.,[1] Kress, Y.,[1] Burk, R.,[2] Kadish, A.,[1] Depts. of [1]Pathology, [2]Pediatrics, Albert Einstein College of Medicine, Bronx, New York: Ultrastructural identification of viral particles and filamentous forms in dysplastic cervical lesions associated with different HPV types.

Gallagher, T.,[1,2] Chardonnet, Y.,[1] Viac, J.,[1] Steinberg, B.,[2] [1]CNRS, Lyons, France; [2]Long Island Jewish Medical Center, New Hyde Park, New York: Comparative investigation of somatic hybrids, human carcinoma HeLa cells (containing HPV-18 DNA)x mouse fibroblasts 3T3.4E, prepared with PEG and electrofusion.

Schiller, J.T., Androphy, E.J., Lowy, D.R., NCI, National Institutes of Health, Bethesda, Maryland: Genetic and biochemical analysis of the BPV E1 ORF and its encoded polypeptide.

Seedorf, K., Bernard, H.U., Roewekamp, W., Institute of Cell and Tumor Biology, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Expression of HPV ORFs in *E. coli* and transfected mammalian cells.

Giri, I.,[1] Danos, O.,[1] Yaniv, M.,[1] Drigeon, G.,[2] Haenni, A.L.,[2] [1]Institut Pasteur, [2]Université Paris, France: Characterization and properties of polypeptides encoded by the CRPV early region.

Thompson, G.H., Roman, A., Dept. of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis: Expression of HPV-6b ORFs in *E. coli*.

Li, C.-C.H.,[1,2] Shah, K.,[1] Seth, A.,[3] Gilden, R.V.,[1,2] [1]Johns Hopkins University, Baltimore, [2]PRI, NCI-Frederick Cancer Research Facility, [3]Litton Bionetics, Inc., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Expression of L1 ORF of HPV-6b—Potential of expressed protein in serological surveys.

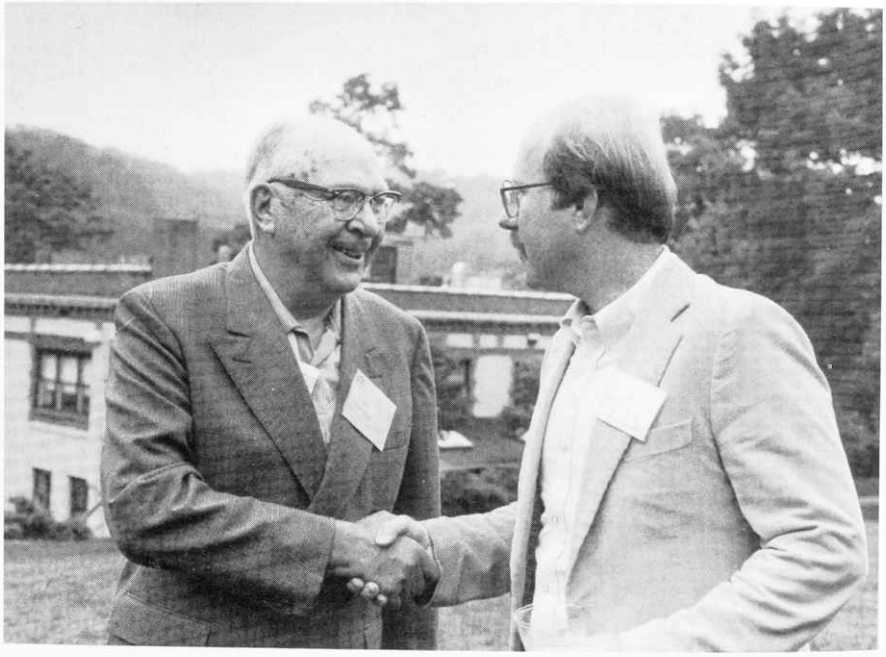
Browne, H.M., Minson, A.C., Smith, G.L., Stanley, M.A., Dept. of Pathology, University of Cambridge, England: Production of vaccinia virus recombinants containing ORFs of HPV-16.

Cowsert, L.M.,[1] Jenson, B.,[2] Depts. of [1]Microbiology, [2]Pathology, Georgetown University, Washington, D.C., Monoclonal antibodies define conformational and nonconformational epitopes on BPV-1.

Doorbar, J., Gallimore, P.H., Dept. of Cancer Studies, University of Birmingham, England: Analysis of proteins encoded by HPV-1 and HPV-2.

Schneider-Gädicke, A.,[1] Schwarz, E.,[1] Kaul, S.,[2] Ganseпohl, H.,[3] Frank, R.,[3] Röwekamp, W.,[1] Bastert, G.,[4] [1]Deutsches Krebsforschungszentrum, Heidelberg, [2]Institut für Biologische Chemie, Frankfurt, [3]EMBL, Heidelberg, [4]Universitäts-Frauenklinik, Homburg, Federal Republic of Germany: Expression of HPV-18 in human cervical carcinoma cell lines and production of monoclonal antibodies against the HPV-18 E6 polypeptide.

Danos, O.,[1,2] Yaniv, M., [1]Dept. de Biologie Moléculaire, Institut Pasteur, Paris, France; [2]Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: Evolution of the E6 and E7 gene products of papillomavirus from a 33 amino acid peptide with a potential nucleic acid-binding structure.



C. Olson, D. Owen

Patel, D., Chesters, P.M., McCance, D.J., Dept. of Microbiology, Guy's Medical School, London, England: Expression of HPV-16 ORFs E6, L1, and E4 and a spliced fragment of HPV-6 in bacteria and immunochemical analysis of the proteins.

Barbosa, M.S., Wettstein, F.O., Dept. of Microbiology and Immunology, University of California School of Medicine, Los Angeles: Identification of the protein encoded by the E6 ORF of the CRPV.

Nakai, Y., Lancaster, W.D., Jenson, A.B., Dept. of Pathology, Georgetown University, Washington, D.C.: Isolation of papillomavirus structural polypeptides by immunoaffinity columns using monoclonal antibodies.

Breitburd, F., Croissant, O., Orth, G., Unité des Papillomavirus, Institut Pasteur, Paris, France: Expression of E4 gene products in the HPV-1–induced warts.

Smith, K.T., Campo, M.S., Beatson Institute for Cancer Research, Glasgow, Scotland: BPV-4 – In vitro cell transformation and viral specific transcription.

SESSION 13 PAPILLOMA-SPECIFIC PROTEINS

Chairperson: F. Breitburd, Institut Pasteur

Smotkin, D.,[1] Wettstein, F.O.,[2] [1]Division of Gynecologic Oncology, [2]Dept. of Microbiology and Immunology, University of California School of Medicine, Los Angeles: E7, the product of the major HPV-16 transcript in cervical cancer, is a partially aggregated cytoplasmic phosphoprotein.

Firzlaff, J.M., Hsia, C.N., Halbert, C., Galloway, D.A., Fred Hutchinson Cancer Research Center, Seattle, Washington: Generation and use of HPV-6B and HPV-16 antigens and antibodies to detect viral gene expression and immune response.

Palefsky, J.,[1] Winkler, B.,[2] Nizet, V.,[1] Kidd, J.L.,[1] Schoolnik, G.K.,[1] [1]Stanford Medical School, Stanford, [2]University of California, San Franscisco Medical School: HPV-16 DNA–positive cervical intraepithelial neoplasia and cervical carcinoma demonstrate expression of an E6 ORF protein in the more differentiated cell layers.

Mallon, R., Wojciechowicz, D., Defendi, V., Dept. of Pathology, New York University Medical Center, New York: Expression and characterization of viral proteins from BPV-1, HPV-6b, and HPV-16.

Androphy, E.J., Schiller, J.T., Lowy, D.R., NCI, National Institutes of Health, Bethesda, Maryland: A peptide encoded by the BPV-1 E2 ORF specifically binds to the upstream regulatory regions of the BPV-1 and HPV-16 genomes.

Schlegel, R., Wade-Glass, M., Rabson, M., Yang, Y.-C., NCI, National Institutes of Health, Bethesda, Maryland: The E5 transforming gene of BPV directs synthesis of a small hydrophobic polypeptide.

Summary: P.M. Howley, National Cancer Institute

P. Howley

Modern Approaches to New Vaccines Including Prevention of AIDS

September 9–September 14

ARRANGED BY

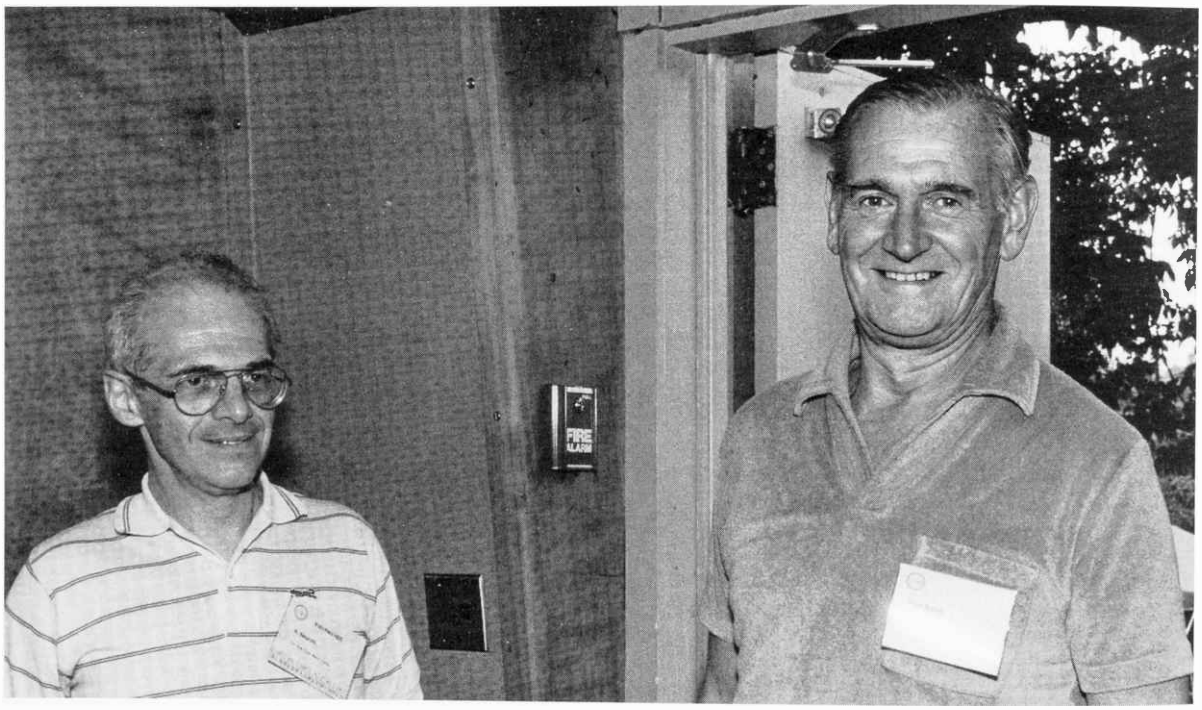
Robert Chanock, NIAID National Institutes of Health
Harold Ginsberg, Columbia University
Richard Lerner, Research Institute of Scripps Clinic
Fred Brown, Wellcome Biotechnology Ltd.

253 participants

The series of annual meetings on Modern Approaches to Vaccines, held at the Cold Spring Harbor Laboratory since 1983, has proved to be of considerable popular interest. Although the general pattern of the program in 1986 was similar to that of previous years, it was decided that the meeting should be extended by one day so that a full discussion of all aspects of AIDS could be included. The shadow of AIDS continues to spread over many parts of the world and its prevention presents a challenge not only to governments and their agencies, but also to those of us who believe that modern approaches to vaccination can offer some answers.

The program also included sessions on immunology; recombinant vectors; pathogenic bacteria and viral glycoproteins; and pathogenesis and attenuation; as well as a poster session.

This meeting was funded in part by The Rockefeller Foundation, the National Institute of Allergy and Infectious Diseases Intramural Research Program, and the Johnson & Johnson Biotechnology Center, Inc.



A. Neurath, F. Brown

SESSION 1 IMMUNOLOGY. I.

Chairman: R. Lerner, Research Institute of Scripps Clinic

Lerner, R.A., Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California: Catalytic antibodies.

Houghten, R.A., Dept. of Molecular Biology, Scripps Clinic and Research Foundation, La Jolla, California: Determination of the precise amino acids and their relative importance in peptide antigen/monoclonal antibody interactions.

Finberg, R., Ertl, H., Laboratory of Infectious Diseases, Dana-Farber Cancer Institute, Boston, Massachusetts: Use of anti-idiotype antibodies as immunizing antigens.

Newton, S.E.,[1] Clarke, B.E.,[1] Appleyard, G.,[1] Carroll, A.R.,[2] Francis, M.J.,[1] Rowlands, D.J.,[1] Brown, F.,[1] Skehel, J.J.,[2] [1]Wellcome Biotechnology Ltd., [2]National Institute for Medical Research, London, England: Novel approaches to antigen presentation using vaccinia virus.

O'Hanley, P.,[1] Weyand, C.,[2] Goronzy, J.,[2] Fathman, G.,[2] [1]Dept. of Medicine, Division of Infectious Diseases, [2]Division of Immunology, Stanford University, California: IL-2 administration in vivo enhances the anti-core glycolipid IgM response and prevents *E. coli* septic death in mice.

Anderson, G.,[1] Urban, O.,[1] Fedorka-Cray, P.,[1] Newell, A.,[2] Nunberg, J.,[2] Doyle, M.,[2] [1]Dept. of Veterinary Science, University of Nebraska, Lincoln; [2]Cetus Corporation, Emeryville, California: IL-2 and protective immunity in *H. pleuropneumoniae* – Preliminary studies.

Berzofsky, J.A., NCI, National Institutes of Health, Bethesda, Maryland: Antigen presentation and recognition by T cells.

Cease, K.B., Buckenmeyer, G., Berkower, I., York-Jolley, J., Berzofsky, J.A., NCI, National Institutes of Health, Bethesda, Maryland: Antigen seen by T cells is localized to the cell surface – Implications for vaccine design.

Neurath, A.R.,[1] Kent, S.B.H.,[2] Strick, N.,[1] Parker, K.,[2] [1]L.F. Kimball Research Institute of the New York Blood Center, New York, New York; [2]Division of Biology, California Institute of Technology, Pasadena, California: Approaches to prepare highly immunogenic synthetic peptide candidate vaccines against hepatitis B.

SESSION 2 IMMUNOLOGY. II.

Chairman: M. Oldstone, Research Institute of Scripps Clinic

Dryberg, T.,[1] Oldstone, M.,[2] [1]Hagedorn Research Laboratory, Gentofte, Denmark; [2]Research Institute of Scripps Clinic, La Jolla, California: Synthetic peptides as antigens – Importance of orientation.

Eisenlohr, L.C., Gerhard, W., Hackett, C.J., Wistar Institute, Philadelphia, Pennsylvania: Presentation of influenza virus antigens in helper T cells (T_H) – Viral HA activity in antigen focusing and identification of transitory stages in antigen presentation.

Milich, D.R.,[1] McLachlan, A.,[1] Thornton, G.B.,[2] [1]Scripps Clinic and Research Foundation, Dept. of Basic and Clinical Research, [2]Johnson and Johnson, Biotechnology Center, La Jolla, California: An immune response to the pre-S1 region of HBsAg can bypass nonresponse to the pre-S2 and S regions.

Allison, A.C., Byars, N., Institute of Biological Sciences, Syntex Research, Palo Alto, California: An adjuvant formulation eliciting cell-mediated and protective immunity with defined antigens and inactivated viruses.

Francis, M.J.,[1] Fry, C.M.,[1] Clarke, B.E.,[1] Rowlands, D.J., Brown, F.,[1] Bittle, J.L.,[2] Houghten, R.A.,[2] Lerner, R.A.,[2] [1]Wellcome Biotechnology Ltd., England;[2] Scripps Clinic and Research Foundation, La Jolla, California: FMDV synthetic peptide containing B- and T-cell determinants.

Zamvil, S.S.,[1] Mitchell, D.J.,[1] Steinman, L.,[1] Rothbard, J.B.,[2] [1]Stanford Medical School, California; [2]Imperial Cancer Research Fund, London, England: Encephalitogenic T-cell epitope of the autoantigen myelin basic protein.

Charbit, A.,[1] Boulain, J.C.,[3] Ryter, A.,[2] Hofnung, M.,[1] [1]UPMTG, [2]Unité de Microscopie Electronique, Institut Pasteur, Paris, [3]CEA, Saclay, France: Stable expression of a eukaryotic epitope at the surface of *E. coli* K-12 and the construction of "exposition vectors."

Andrew, M.,[1] Coupar, B.,[1] Both, G.,[2] Boyle, D.,[1] [1]Dept. of Microbiology, JCSMR, Australian National University, Canberra; [2]CSIRO Division of Molecular Biology, North Ryde, Australia: Temporal regulation of influenza HA expression in vaccinia virus recombinants and effects on the immune response.

SESSION 3 IMMUNOLOGY. II (continued) PARASITES

Chairman: A. Sher, NIAID, National Institutes of Health

Good, M.F.,[1] Berzofsky, J.A.,[2] Maloy, W.L.,[1] Hayashi, Y.,[3] Fujii, N.,[3] Hockmeyer, W.T.,[4] Miller, L.H.,[1] [1]NIAID, [2]NCI, National Institutes of Health, Bethesda, Maryland; [3]Faculty of Pharmaceutical Sciences, Kyoto University, Japan; [4]Walter Reed Army Institute of Research, Washington, D.C.: Widespread nonresponsiveness in mice to immunodominant repeat of *P. falciparum* sporozoite vaccine.

Perlmann, P.,[1] Berzins, K.,[1] Björkman, A.,[2] Kabilan, L.,[1] Högh-Pedersen, B.,[2] Patarroyo, E.,[3] Perlmann, H.,[1] Troye-Blomberg, M.,[1] Wåhlin, B.,[1] [1]Dept. of Immunology, University of Stockholm, [2]Dept. of Infectious Diseases, Karolinska Institute, Stockholm, Sweden; [3]Dept. of Immunology, National University of Colombia, Bogatá: Humoral and cellular immune responses to the carboxy-terminal amino acid repeats of Pf 155 – A potential vac-

cine candidate of the human malaria parasite, *P. falciparum*.

Edwards, S.J.,[1] Woodrow, G.C.,[2] Anders, R.F.,[3] Langford, C.J.,[3] [1]Commonwealth Serum Laboratories, Parkville, Victoria, [2]Biotechnology Australia, Roseville, [3]Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria: Immunization with a malaria blood-stage antigen by live recombinant vaccinia virus induces antibodies that cross-react with the circumsporozoite coat protein repeating epitope.

Coppel, R.L.,[1] Anders, R.F.,[1] Pappaioanou, M.,[2] Campbell, G.H.,[2] Brown, G.V.,[1] Kemp, D.J.,[1] Skinner, J.C.,[2] Andrysiak, P.M.,[2] Favaloro, J.M.,[1] Corcoran, L.M.,[1] Broderson, J.R.,[2] Mitchell, G.F.,[1] Campbell, C.C.,[2] Collins, W.E.,[2] [1]Walter and Eliza Hall Institute of Medical Research, Victoria, Australia; [2]Centers for Disease Control, Atlanta, Georgia: Protection of *Aotus* monkeys by immunization with recombinant proteins of the RESA of *P. falciparum*.

Barr, P.J.,[1] Gibson, H.L.,[1] Hollingdale, M.,[2] Arnot, D.E.,[3] Nussenzweig, V.,[3] [1]Chiron Corporation, Emeryville, California; [2]Biochemical Research Institute, Rockville, Maryland; [3]New York University Medical Center, New York, New York: Antigenicity and immunogenicity of *P. vivax* circumsporozoite proteins produced in yeast.

Scott, P., Pearce, E., Natovitz, P., Sher, A., NIAID, National Institutes of Health, Bethesda, Maryland: Soluble Leishmania antigens that elicit T-cell reactivity and protective immunity against Leishmania major in BALB/c mice.

Pearce, E.,[1] James, S.,[2] Lanar, D.,[1] Sher, A.,[1] [1]NIAID, National Institutes of Health, Bethesda, Maryland; [2]George Washington University Medical School, Washington, D.C.: Molecular characterization of a murine vaccine against *S. mansoni*.

Patarroyo, M., Dept. of Immunology, Hospital San Juan de Dios, Bogota, Columbia: Immunogenic peptides of malaria antigens.

Thornton, G.,[1] Milich, D.,[2] Chisari, F.,[2] Mitamura, K.,[3] Kent, S.,[4] Neurath, R.,[5] Purcell, R.,[6] Gerin, J.,[7] [1]Johnson and Johnson Biotechnology, San Diego, California; [2]Scripps Clinic and Research Foundation, La Jolla, California; [3]Tokyo Medical Institute, Tokyo, Japan; [4]California Institute of Technology, Pasadena, California; [5]New York Blood Center, New York; [6]NIH, Bethesda, [7]Georgetown University, Rockville, Maryland: Immune response to the pre-S2 region of HBV in human and nonhuman primates and its relevance to protection.

SESSION 4 AIDS. 1.

Chairman: W. Parks, University of Miami School of Medicine

Parks, W.,[1] Parks, E.,[1] Geffin, R.,[1] Hahn, B.,[2] Shaw, G.,[2] [1]Dept. of Pediatrics and Microbiology, University of Miami School of Medicine, Florida; [2]Dept. of Hematology and Oncology, University of Alabama, Birmingham: Group- and type-specific neutralization of AIDS virus isolates from longitudinal natural history studies.

Luciw, P.,[1] Cheng-Mayer, C.,[2] Levy, J.,[2] Barr, P.,[1] Walker, M.,[2] Peterlin, B.M.,[2] [1]Chiron Corp., Emeryville, [2]University of California, San Francisco: Replication of HIV—Genetic and molecular analyses.

Haseltine, W., Dept. of Pathology, Dana-Farber Cancer Center, Harvard Medical School, Boston, Massachusetts: Molecular characterization of genome of AIDS retrovirus.

Wong-Staal, F., Gallo, R.C., NCI, National Institutes of Health, Bethesda, Maryland: Defining the viral genes for HTLV-III replication and cytopathicity—Implications for prevention and treatment of AIDS.

Broder, S., NCI, National Institutes of Health, Bethesda, Maryland: Inhibition of the in vitro infectivity and cytopathic effect of HTLV-III/LAV by purines and pyrimidines with the ribose moiety in a 2′, 3′-dideoxy configuration.

Folks, T.M., Justement, J.S., Powell, D., Martin, M., Fauci, A.S., NIAID, National Institutes of Health, Bethesda, Maryland: Constitutive production of a reverse transcriptase negative AIDS retrovirus particle.

Oroszlan, S., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Chemical characterization of the protein components of HTLV-III.

Dubovi, E.J.,[1] Donis, R.,[1] White, R.T.,[2] Cordell, B., Dales, B.,[2] [1]Diagnostic Laboratory, New York State College of Veterinary Medicine at Cornell, Ithaca; [2]California Biotechnology, Inc., Mountain View: Expression of a glycoprotein antigen of BVD virus that elicits neutralizing antibody production in mice.

SESSION 5 AIDS. 2. PATHOGENESIS

Chairman: A. Fauci, NIAID, National Institutes of Health

Fauci, A.S., NIAID, National Institutes of Health, Bethesda, Maryland: Immunopathogenesis and immunologic reconstitution in AIDS.

Gallo, R.C., Popovic, M., Wong-Staal, F., NCI, National Institutes of Health, Bethesda, Maryland: HTLV-I and II—T-tropic human retroviruses that cause leukemia and transform T cells in vitro by a *trans*-activating mechanism.

Fultz, P.,[1] McClure, H.,[2] Switzer, W.,[1] McGrath, N.,[1] Swenson, B.,[2] Srinivasan, A.,[1] [1]AIDS Program, Centers for Disease Control, [2]Yerkes Regional Primate Research Center, Emory University, Atlanta, Georgia: Development of animal models to test potential vaccines for protection against infection by T-lymphotropic retroviruses.

Letvin, N.L.,[1] Daniel, M.D.,[1] King, N.W.,[1] Kiyotaki, M.,[1] Kannagi, M.,[1] Sehgal, P.K.,[1] Arthur, L.O.,[2] Allison, A.C.,[3] Hunt, R.D.,[1] Desrosiers, R.C.,[1] [1]Harvard Medical School, New England Regional Primate Research Center, Southborough, Massachusetts; [2]NCI-Frederick Cancer Re-

search Facility, Frederick, Maryland; [3]Syntex Research, Palo Alto, California: AIDS-like disease in macaque monkeys induced by STLV-III—Model for AIDS vaccine development.

Shaw, G.M.,[1] Hahn, B.H.,[1] Wong-Staal, F.,[2] Gallo, R.C.,[2] Parks, E.S.,[3] Parks, W.P.,[3] [1]University of Alabama, Birmingham; [2]National Institutes of Health, Bethesda, Maryland; [3]University of Miami School of Medicine, Florida: Genetic variation in HTLV-III/LAV.

Levy, J.A., Cancer Research Institute, University of California, San Francisco: Features of HIV infection that influence vaccine development.

Clapham, P.R.,[1] Dalgleish, A.G.,[1] Weiss, R.A.,[1] Beverly, P.C.L.,[2] Sattentau, Q.J.,[2] Lasky, L.A.,[3] Berman, P.W.,[3] Maddon, P.,[4] Axel, R.,[4] [1]Chester Beatty Laboratories, London, [2]University College, London, England; [3]Genentech, San Francisco, California; [4]Columbia University, New York, New York: HIV-I—Neutralizing antibodies and cellular receptors.

Montagnier, L., Institut Pasteur, Paris, France: IDS—Pathogenesis.

SESSION 6 PATHOGENIC BACTERIA AND VIRAL GLYCOPROTEINS

Chairman: J. Keith, NIAID, Rocky Mountain Laboratories

Locht, C., Keith, J.M., Laboratory of Pathobiology, NIAID, Rocky Mountain Laboratories, Hamilton, Montana: Expression of pertussis toxin subunit S1 in *E. coli*.

Dougan, G.,[1] Maskell, D.,[1] Sweeney, K.,[1] Hormaeche, C.,[2] [1]Wellcome Research Laboratories, Beckenham, Kent, [2]Dept. of Pathology, University of Cambridge, England: Attenuated *Salmonella* as carriers of heterologous antigens.

Kawaoka, Y.,[1] Bean, W.J.,[1] Fried, V.,[2] Chambers, T.M.,[1] Webster, R.G.,[1] [1]Dept. of Virology and Molecular Biology, [2]Dept. of Biochemistry, St. Jude Children's Hospital, Memphis, Tennessee: A single change in the HA of H5N2 influenza virus is associated with high virulence.

Murphy, B.R.,[1] Prince, G.,[1] Wagner, D.,[2] Walsh, E.E.,[3] Chanock, R.M.,[1] [1]NIAID; [2]NCI, National Institutes of Health, Bethesda, Maryland; [3]University of Rochester, New York: Immune response of humans and cotton rats to RS virus infection or formalin-inactivated vaccine.

Coelingh, K., Winter, C.C., Murphy, B.R., NIAID, National Institutes of Health, Bethesda, Maryland: Antigenic and functional structure of the human and bovine type-3 parainfluenza virus HN proteins—Sequence analysis of variants selected with monoclonal antibodies.

Southern, P., Buchmeier, M.J., Oldstone, M.B.A., Dept. of Immunology, Scripps Clinic and Research Foundation, La Jolla, California: Arenavirus glycoprotein expression in acute and persistent infections.

Schlesinger, J.J.,[1] Brandriss, M.W.,[1] Cropp, C.B.,[2] Monath, T.P.,[2] [1]University of Rochester and Rochester General Hospital, New York; [2]Centers for Disease Control, Fort Collins, Colorado: Protection against yellow fever in monkeys immunized with a purified 17D YF nonstructural glycoprotein, NS1.

Mach, M., Utz, U., Fleckenstein, B., Institut für Klinische Virologie der Universität Erlangen-Nürnberg, Federal Republic of Germany: Mapping and expression cloning of the major glycoprotein gene of HCMV.

Norrby, E., Karolinska Institute, Stockholm, Sweden: Characterization of paramyxovirus glycoproteins.

SESSION 7 AIDS. 3. EPIDEMIOLOGY AND ANIMAL MODELS

Chairman: M. Essex, Harvard School of Public Health

Essex, M., Kanki, P., Allan, J., Lee, T.H., Mullins, J., Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts: Relationship between HTLV-III/LAV and HTLV-IV/STLV-III retroviruses.

Plata, F., Langlade-Demoyen, P., Abastado, J.P., Berbar, R., Kourilsky, P., Unité de Biologie Moléculaire du Gène, Institut Pasteur, Paris, France: Molecular analysis of the T-lymphocyte response elicited by retrovirus-induced tumors in mice.

Alizon, M., Sonigo, P., Wain-Hobson, S., Institut Pasteur, Paris, France: Genetic variability of the human and animal lentiviruses.

Jaffe, H., Morgan, W.M., Darrow, W., Curran, J., AIDS Program, Centers for Disease Control, Atlanta, Georgia: Epidemiology of AIDS in the United States.

Quinn, T.C., NIAID, National Institutes of Health, Bethesda, Maryland: Epidemiologic features of AIDS in Africa—Implications for HIV vaccine development.

Rosen, J., Hom, Y.-L., Naso, R., Johnson and Johnson Biotechnology Center, Inc., La Jolla, California: Reactivity of HIV-related antibodies in AIDS patient sera to synthetic oligopeptides.

Cosand, W., Harris, L., Genetic Systems Corp., Seattle, Washington: Human antiviral antibodies recognize a chemically synthesized peptide from the transmembrane protein of the HIV.

Krohn, K.,[1] Ranki, A.,[1] Robey, W.G.,[2] Putney, S.,[3] [1]NCI, National Institutes of Health, Bethesda, [2]NCI-Frederick Cancer Research Facility, Frederick, Maryland; [3]RepliGen Corporation, Cambridge, Massachusetts: Characterization of the protective immune response in animals immunized with HTLV-III envelope proteins and in infected individuals.

Ranki, A., Krohn, K., NCI, National Institutes of Health, Bethesda, Maryland: Characterization of the neutralizing antibody in HTLV-III infected individuals.

SESSION 8 RECOMBINANT VECTORS. I.

Chairman: **B. Murphy**, NIAID, National Institutes of Health

Olmsted, R.A.,[1] Elango, N.,[2] Prince, G.A.,[1] Murphy, B.R.,[1] Johnson, P.R.,[1] Moss, B.,[3] Chanock, R.A.,[1] Collins, B.R.,[1] Laboratories of [1]Infectious Diseases, [2]Viral Diseases, NIAID, National Institutes of Health, Bethesda, Maryland; [3]National Institute of Virology, Pune, India: Expression of the F glycoprotein of RS virus by a recombinant vaccinia virus—Comparison of the individual contributions of the F and G glycoproteins to host immunity.

Fuerst, T.R., Moss, B., NIAID, National Institutes of Health, Bethesda, Maryland: Use of a hybrid vaccinia virus/T7 RNA polymerase system for high-level eukaryotic gene expression.

Young, K.K.Y.,[1] Stott, E.J.,[1,2] Ball, L.A.,[3] Wertz, G.W.,[1] [1]Dept. of Microbiology and Immunology, University of North Carolina, Chapel Hill; [2]Institute for Research on Animal Diseases, Compton, England; [3]Biophysics Laboratory and Dept. of Biochemistry, University of Wisconsin, Madison: Human RS virus proteins expressed from vaccinia virus vectors can confer protection against live virus challenge in mice.

Lowe, R.,[1] Keller, P.,[1] Davison, A.,[2] Kieff, E.,[3] Morgan, A.,[3] Ellis, R.,[1] [1]Dept. of Virus and Cell Biology, Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania; [2]MRC Virology Group, Institute of Virology, Glasgow, Scotland; [3]Dept. of Medicine, University of Chicago, Illinois: VZV as a live vector for the expression of foreign genes.

Meigner, B., Longnecker, R., Roizman, B., University of Chicago, Illinois: Construction and in vivo evaluation of two genetically engineered prototypes of live attenuated HSV vaccines.

Motz, M., Deby, G., Wolf, H., Max von Pettenkofer Institute, University of Munich, Federal Republic of Germany: Secretion of the two major membrane proteins of EBV from recombinant CHO cells into culture medium.

Flexner, C., Hugin, A., Moss, B., NIAID, National Institutes of Health, Bethesda, Maryland: Expression of human IL-2 by live recombinant vaccinia virus.

Cochran, M.A., Ericson, B.L., Knell, J., Smith, G.E., Dept. of Molecular Biology, MicrogeneSys, Inc., West Haven, Connecticut: Use of baculovirus recombinants as a general method for the production of subunit vaccines.

SESSION 9 POSTERS

Ambrosio, R.E.,[1] Potgieter, F.T.,[2] Depts. of [1]Molecular Biology, [2]Protozoology, Veterinary Research Institute, Onderstepoort, South Africa: Molecular hybridization of *Anaplasma marginale* and *Anaplasma centrale* DNAs.

Arora, D.J.S., Houde, M., Justewicz, D.M., Biochemicals Division and Virology Research Center, Institute Armand-Frappier, University of Quebec, Canada: In vivo stimulation of NK cell activity by purified influenza viral neuraminidase.

Azad, A., CSIRO, Parkville, Australia: Studies on the gene products involved in pathogenicity of adenovirus.

Azad, A.A., Jagadish, M., Hudson, P., CSIRO, Division of Protein Chemistry, Parkville, Australia: Strategies for a genetically engineered subunit vaccine against an avian immunosuppressive viral disease.

Bangham, C.R.M.,[1] Cannon, M.J.,[2] McMichael, A.J.,[1] Askonas, B.A.[2] [1]John Radcliffe Hospital, Oxford; [2]National Institute for Medical Research, London, England: Antigen specificity of human and murine MHC-restricted cytotoxic T cells specific to RS virus.

Beuvery, E.C.,[1] Hazendonk, T.G.,[1] Enger-Valk, B.,[2] Henneke, M.,[1] Kottenhage, M.,[2] van Wezel, T.L.,[1] [1]Rijksinstituut voor Volksgezondheid en Milieuhygiene, Bilthoven, [2]Medisch-Biologisch Laboratorium, Rijswijk, The Netherlands: Primary antibody responses to neutralization epitopes of poliovirus are enhanced by preimmunization with purified capsid proteins or with fusion proteins containing these capsid proteins.

Beuvery, E.C.,[1] Jiskoot, W.,[2] Teerlink, T.,[1] Kersten, G.F.A.,[1] van Dalen, F.,[2] Jeurissen, S.,[3] Crommelin, D.J.A.,[2] van Wezel, T.L.,[1] [1]Rijksinstituut voor Volksgezondheid en Milieuhygiene, Bilthoven, [2]State University of Utrecht, [3]Free University, Amsterdam, The Netherlands: Immunogenic activity of gonococcal P1 in mice delivered in liposomes, ISCOMs, and detergent complexes.

Chanh, T.C., Dreesman, G.R., Kennedy, R.C., Southwest Foundation for Biomedical Research, Dept. of Virology and Immunology, San Antonio, Texas: A monoclonal anti-idiotypic antibody mimics the CD_4 receptor and reacts with HTLV-III/LAV envelope glycoprotein(s).

Cote, P.J., Taylor, D.W., Gerin, J.L., Division of Molecular Virology and Immunology, Rockville, Maryland, and Dept. of Biology, Georgetown University, Washington, D.C.: Quantitative immunochemical analysis of pre-S epitopes in the quaternary structure of hepadnavirus surface antigen particles.

Delpeyroux, F.,[1] Blondel, B.,[2] Crainic, R.,[2] Streeck, R.,[1] [1]Unité des Applications du Génie Génétique, [2]Unité de Virologie Médicale, Institut Pasteur, Paris, France: Immune response in rabbits to a poliovirus epitope carried by HBsAg particles.

Donabedian, A.M., DeBorde, D.C., Smitka, C.W., Herlocher, M.L., Maassab, H.F., Dept. of Epidemiology, School of Public Health, University of Michigan, Ann Arbor: Molecular changes in the PA protein of the cold-adapted influenza B/Ann Arbor/1/66 vaccine donor strain associated with loss of the temperature-sensitive phenotype and reversion to virulence.

Dubeaux, C.,[1] Gras-Masse, H.,[1] Jolivet, M.,[2] Audibert, F.,[2] Tartar, A.,[1] Chedid, L.,[2] [1]Service de Chimie des Biomolécules, Institut Pasteur, Lille, France; [2]Dept. of Pharmacology and Therapeutics, University of South Florida Medical Center, Tampa: Selection of optimal sequences

to use as synthetic immunogens—Study of the reliability of currently used approaches to predict antigenic sites, with regard to type I (Mahoney) poliovirus.

Enger-Valk, B.E., Broekhuysen, M.P., Blom, A., Pouwels, P.H., Medical Biological Laboratory, Rijswijk, The Netherlands: Synthesis of protein complexes that contain multiple copies of the antigenic determinant of FMDV.

Giri, C.P., Klutch, M.J., Kaufman, J.D., Shepp, D.H., Quinnan, G.V., Jr., Wright, S.E., FDA, Bethesda, Maryland: Expression of the external portion of HTLV-III envelope–VSV-G transmembrane fusion protein by recombinant vaccinia viruses.

Israel, N., Streeck, R.E., Unité des Applications du Génie Génétique, Institut Pasteur, Paris, France: Construction of vectors for efficient expression of viral antigens in mammalian cell lines.

Jessup, J.M., University of Texas, M.D., Anderson Hospital, Houston: Active specific immunotherapy for human colorectal carcinoma.

Johnston, J.M., Harmon, S.A., Richards, O.C., Summers, D.F., Ehrenfeld, E., Dept. of Cellular, Viral, and Molecular Biology and Biochemistry, University of Utah School of Medicine, Salt Lake City: Expression of HAV VP1 in *E. coli* for use as a vaccine.

Jorgensen, E.D., Dept. of Cell Biology, New York University, New York, New York: Cloning, nucleotide sequence, and expression of NDV HN protein—Identification of a putative sialic acid binding site.

Keller, P.M.,[1] Davison, A.J.,[2] Lowe, R.S.,[1] Bennett, C.D.,[1] Rieman, M.,[1] Ellis, R.W.,[1] [1]Dept. of Virus and Cell Biology, Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania; [2]Dept. of Medicinal Chemistry, MRC Virology Group, Glasgow, Scotland: Genetic mapping and sequencing of the major glycoprotein gpIII of VZV.

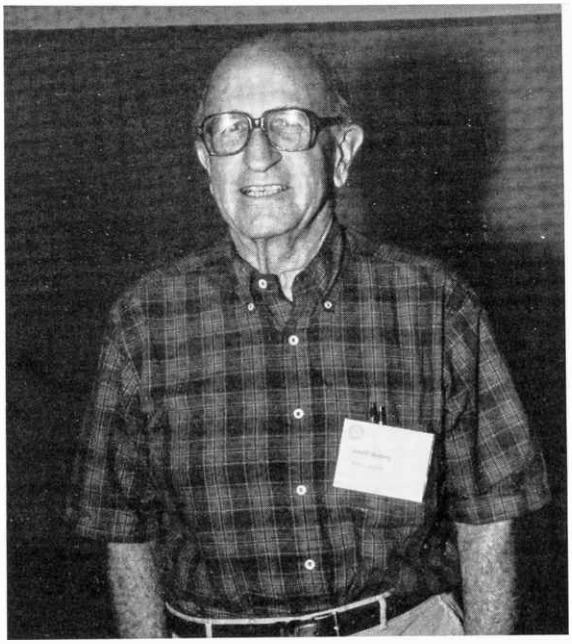
Kniskern, P.J., Hagopian, A., Miller, W.J., Yamazaki, S., Emini, E.A., Ellis, R.W., Dept. of Virus and Cell Biology, Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania: Expression of the complete surface antigen (pre-S1 + pre-S2 + S) of HBV in the yeast *S. cerevisiae*.

Kusters, J.G.,[1] Niesters, H.G.M.,[1] Spaan, W.J.M.,[1] Lenstra, J.A.,[2] Horzinek, M.C.,[1] van der Zeijst, B.A.M.,[2] [1]Institute of Virology, [2]Section of Bacteriology, Veterinary Faculty, State University, Utrecht, The Netherlands: Molecular epidemiology of infectious bronchitis virus in The Netherlands.

Ladant, D.,[1] Brezin, C.,[2] Alonso, J.-M.,[2] Crenon, I.,[1] Guiso, N.,[1] Unité de Biochimie des Régulations cellulaires, [2]Unité d'Ecologie bactérienne, Institut Pasteur, Paris, France: *B. pertussis* adenylate cyclase—Purification, characterization, and radioimmunoassay.

Lal, A.A.,[1] de la Cruz, V.,[1] Charoenuit, Y.,[2] Miller, L.H.,[1] McCutchan, T.F.,[1] [1]NIAID, National Institutes of Health, [2]Malaria Unit, Naval Medical Research Institute, Bethesda, Maryland: Gene structure of circumsporozoite protein of *P. yoeilli*—A rodent model for vaccine studies.

La Monica, N., Racaniello, V.R., Dept. of Microbiology, Columbia University College of Physicians & Surgeons, New York, New York: Reduced mouse neurovirulence of poliovirus antigenic variants selected with monoclonal antibodies.



H. Ginsberg

Lathe, R.,[1] Clertant, P.,[2] Kieny, M.P.,[3] [1]CNRS-LGME, INSERM, Strasbourg, [2]INSERM, Nice, [3]Transgene S.A., Strasbourg, France: Vaccinia/polyoma recombinant virus—A model for tumor immunity?

Mento, S., Kowal, K., Weeks-Levy, C., Dubpernell, S., Richey, M., Cano, F., Lederle Biologicals, American Cyanamid Company, Pearl River, New York: Cell substrate affects on poliovirus replication.

Mento, S., Weeks-Levy, C., Swanson, S., Ritchey, M., Cano, F., Lederle Biologicals, American Cyanamid Company, Pearl River, New York: Simian and human cell substrate retrovirus analyses.

Miller, T.J.,[1] Jones, E.,[2] Reed, A.P.,[1] [1]Norden Laboratories, Lincoln, Nebraska; [2]Dept. of Molecular Genetics, Swedeland, Pennsylvania: Alternate assembly of CPV capsids by expression of capsid protein genes using BPV vectors in hamster cells.

Norman, G.L., Weiss, J.N., Rasheed, S., and the Transfusion Safety Study Group, University of Southern California, Los Angeles, and other participating institutions: Variations in antibody reactivity to HIV detected by protein immunoblot and correlation with individual clinical history.

Nunberg, J.H.,[1] Gilbert, J.H.,[1] Pedersen, N.C.,[2] [1]Cetus Corporation, Emeryville, [2]University of California, Davis: FeLV envelope protein expression encoded by a recombinant vaccinia virus—Apparent lack of immunogenicity in vaccinated animals.

Paloheimo, M., Ölander, R.-M., Nyman, K., Dept. of Bacteriology, National Public Health Institute, Helsinki, Finland: Failure to convert *B. parapertussis* to *B. pertussis* with a *B. pertussis* phage.

Pluschke, G., Basel Institute for Immunology, Basel, Switzerland: Antibodies to the O antigen of lipopolysaccharide are protective against infection with K1 *E. coli*.

Pologe, L.G., Ravetch, J.V., Dept. of Biochemical Genetics, Sloan-Kettering Institute, New York, New York: Progress on the role of the knob-associated histidine-rich protein of *P. falciparum* in sequestration.

Quakyi, I.A., Carter, R., Rener, J., Kumar, N., Miller, L.H., NIAID, National Institutes of Health, Bethesda, Maryland: A 230-kD gamete surface protein of *P. falciparum* is a target of transmission-blocking antibodies.

Shaw, M.W.,[1] De, B.K.,[1] Esposito, J.J.,[1] Brownlee, G.G.,[2] Kendal, A.P.,[1] [1]Division of Viral Diseases, Centers for Disease Control, Atlanta, Georgia; [2]Sir William Dunn School of Pathology, University of Oxford, England: Protection against a virulent avian influenza virus after immunization with vaccinia-vectored influenza virus HA.

Stave, J.W., Morgan, D.O., Plum Island Animal Disease Center, Greenport, New York: Production, purification, and characterization of bovine anti-idiotype antibodies to FMDV neutralization epitopes.

Stevens, V.C., Dept. of Obstetrics and Gynecology, Ohio State University, Columbus: Definition of an epitope on hCG β-subunit using a monoclonal antibody and a series of synthetic peptides.

Tannock, G.A., Paul, J.A., Faculty of Medicine, University of Newcastle, New South Wales, Australia: Homotypic and heterotypic immunity to influenza-A viruses by recombinants of the cold-adapted master strain A/Ann Arbor/1/66-ca.

Vrijsen, R., Boeyé, A., Dept. of Microbiology and Hygiene, Universiteit Brussel, Brussels, Belgium: Poliovirus neutralization and aggregation by monoclonal antibodies—A comparison of wild-type virus and neutralization-resistant mutants.

Yilma, T.,[1] Ristow, S.S.,[1] Moss, B.,[2] Jones, L.,[1] [1]Dept. of Veterinary Microbiology and Immunology, University of California, Davis; [2]NIAID, National Institutes of Health, Bethesda, Maryland: A novel approach for the production of monoclonal antibodies using infectious vaccinia virus recombinants.

Yilma, T.,[1] Reeves, R.,[2] Buck, C.,[2] Moss, B.,[3] Dani, G.,[1] [1]Dept. of Veterinary Microbiology and Immunology, University of California, Davis; [2]Dept. of Biochemistry/Biophysics, Washington State University, Pullman; [3]Laboratory of Viral Diseases, NIAID, National Institutes of Health, Bethesda, Maryland: Infectious vaccinia virus recombinant expresses the bovine interleukin gene.

SESSION 10 AIDS. 4. VACCINES AND PAPILLOMA

Chairman: M. Martin, National Institutes of Health

Wiley, R., Gendelman, H., Daugherty, D., Adachi, A., Venkatesan, S., Folks, T., Rutledge, R., Rabson, A., Martin, M., NIAID-Frederick Cancer Research Center, Frederick, Maryland: Functional analysis of mutant AIDS retrovirus genomes.

Prince, A.M., Pascual, D., Lindsley F. Kimball Research Institute of the New York Blood Center, New York, New York: Variation of susceptibility of HIV to neutralizing antibodies.

Robey, W.G.,[1] Arthur, L.O.,[1] Matthews, T.J.,[2] Langlois, A.,[2] Copeland, T.D.,[1] Lerche, N.W.,[3] Oroszlan, S.,[1] Bolognesi, D.P.,[2] Gilden, R.V.,[1] Fischinger, P.J.,[1] [1]NCI-Frederick Cancer Research Facility, Frederick, Maryland; [2]Duke University Medical School, Durham, North Carolina; [3]California Primate Research Center, Davis: Prospect for prevention of HTLV-III infection—Purified 120,000-dalton envelope glycoprotein induces neutralizing antibody.

Bolognesi, D.P.,[1] Matthews, T.J.,[1] Robey, W.G.,[2] Arthur, L.,[2] Mara, P.,[2] Gallo, R.C.,[2] Fischinger, P.J.,[2] [1]Dept. of Surgery, Duke University Medical Center, Durham, North Carolina; [2]NCI, National Institutes of Health, Bethesda, Maryland: Immunobiological properties of the major envelope glycoprotein of HTLV-III_B.

Hu, S.-L.,[1] Morton, W.,[2] Moran, P.A.,[1] McClure, J.,[3] Kosowski, S.G.,[1] Zarling, J.M.,[1] [1]Oncogen, [2]Oncogen, [2]Regional Primate Center, University of Washington, [3]Genetic Systems Corp., Seattle, Washington: Immune response to HIV in macaques immunized with recombinant vaccinia virus expressing HIV envelope glycoproteins.

Steimer, K.S., Dina, D., Van Nest, G., Luciw, P.A., Barr, P.J., Miller, E.T., Chiron Research Laboratories, Chiron Corporation, Emeryville, California: Studies to evaluate recombinant envelope polypeptides of HIV as potential subunit vaccines.

Berman, P.W.,[1] Groopman, J.E.,[3] Fennie, C.,[1] Benz, P.,[2] Capon, D.,[1] Dowbenko, D.,[1] Nakamura, G.,[1] Nunes, W.,[1] Renz, M.,[1] Lasky, L.A.,[1] Depts. of [1]Molecular Biology, [2]Hybridoma, Genentech, Inc., S. San Francisco, California; [3]Division of Hematology-Oncology, New England Deaconess Hospital, Boston, Massachusetts: Expression of a secreted form of the AIDS retrovirus envelope antigen in mammalian cells—Vaccination studies.

DeLorbe, W.J., Pilacinski, W.P., Lum, M., Cooney, W., Marshall, R., Molecular Genetics, Inc., Minnetonka, Minnesota: A recombinant vaccine for the prevention of bovine papillomatosis.

SESSION 11 RECOMBINANT VECTORS. II. AND PARVOVIRUSES

Chairman: B. Cheseboro, NIAID, Rocky Mountain Laboratories

Rupprecht, C.E.,[1] Johnston, D.H.,[2] Dietzschold, B.,[1] Koprowski, H.,[1] [1]Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania; [2]Wildlife Research Section, Ontario Ministry of Natural Resources, Canada: Development of an oral wildlife rabies vaccine—Long-term protection of raccoons using a vaccinia-rabies glycoprotein recombinant virus.

Yilma, T.,[1] Anderson, K.,[2] Ristow, S.,[1] Brechling, K.,[3] Chakrabarti, S.,[3] Moss, B.,[3] [1]Dept. of Veterinary Microbiology and Immunology, University of California, Davis;

[2]Genetech, Inc., South San Francisco, California; [3]National Institutes of Health, Bethesda, Maryland: Infectious vaccinia virus recombinant expresses the bovine interferon-γ gene.

Lai, C.-J., Zhao, B., Mackow, E., Moss, B., Chanock, R.M., NIAID, National Institutes of Health, Bethesda, Maryland: Expression of structural proteins and nonstructural protein NS1 by vaccinia–dengue recombinant virus.

Panicali, D.,[1] Skarnes, W.,[1] McKenzie, S.,[1] Destree, A.,[1] Young, R.,[2] [1]Applied bioTechnology, Inc., [2]Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: Expression of the 65K antigen of *M. laprae* by a recombinant vaccinia virus.

Auperin, D., Esposito, J., Lange, J., McCormick, J., Division of Viral Diseases, Centers for Disease Control, Atlanta, Georgia: A vaccinia virus recombinant expressing the glycoprotein gene of Lassa virus—A potential vaccine against Lassa fever.

Andrew, M.,[1] Bellamy, A.,[2] Coupar, B.,[1] Both, G.,[3] Boyle, D.,[1] [1]Dept. of Microbiology, JCSMR, Australian National University, Canberra; [2]Dept. of Cell Biology, University of Auckland, New Zealand; [3]CSIRO Division of Molecular Biology, North Ryde, Australia: Immune recognition of rotavirus antigens expressed by recombinant vaccinia viruses.

Emini, E.A.,[1] Qualtiere, L.F.,[2] Pearson, G.R.,[3] Kieff, E.,[4] Silberklang, M.,[1] Schultz, L.D.,[1] Ellis, R.W.,[1] [1]Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania; [2]University of Saskatchewan, Canada; [3]University of Chicago, Illinois; [4]Georgetown University, Washington, D.C.: Antigenic analysis of the EBV major membrane protein (gp350/gp220) expressed in yeast and mammalian cells.

Mazzara, G.,[1] Parrish, C.,[2] Destree, A.,[1] Sue, J.,[1] Panicali, D.,[1] [1]Applied bioTechnology, Inc., Cambridge, Massachusetts; [2]James A. Baker Institute for Animal Health, New York State College of Veterinary Medicine, Cornell University, New York: Expression of empty CPV capsids by murine cells transformed with a BPV/CPV recombinant plasmid.

SESSION 12 AIDS. 5. VACCINES

Chairman: R. Chanock, NIAID, National Institutes of Health

Parker, D., Cheingsong-Popov, R., Roberts, C., Walker, J., Duncan, R.J.S., Highfield, P.E., Wellcome Research Laboratories, Kent, England: Characterization of the antigenic determinants of the British isolate of the AIDS virus (CBL-1) by cloning and expression of gene deletions in *E. coli*.

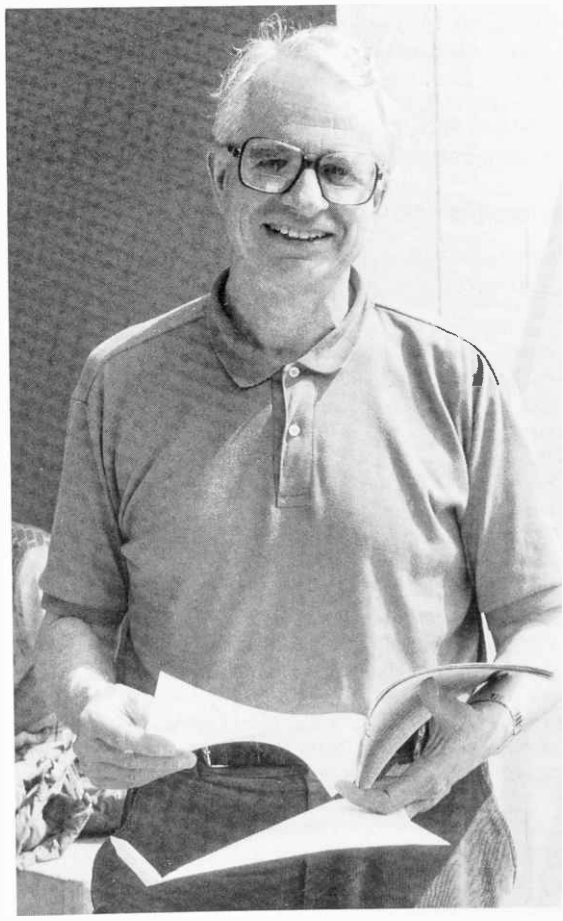
Kieny, M.P.,[1] Rautmann, G.,[1] Girard, M.,[2] Wain-Hobson, S.,[3] Montagnier, L.,[3] Lecocq, J.P.,[1] [1]Transgéne S.A., Strasbourg, [2]Pasteur-Vaccins, [3]Institut Pasteur, Paris, France: Vaccinia/AIDS virus recombinant primarily elicits anti-gp40 (*env*) antibodies; shedding of gp120/110 (*env*) from the cell surface.

Jameson, B.,[1] Guertler, L.,[1] Deinhardt, F.,[1] Gelderblom, H.,[2] [1]Max von Pettenkofer Institute, Munich, [2]Robert Koch Institute, Berlin, Federal Republic of Germany: Characterization of an HTLV-III (*env*)-derived synthetic peptide.

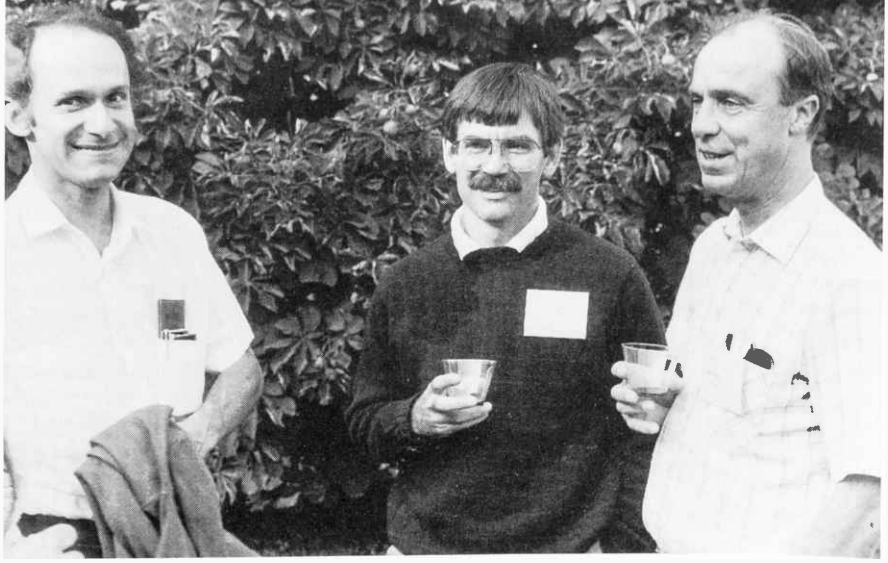
Gardner, M.,[1] Marx, P.,[2] Luciw, P.,[1] Lerche, N.,[2] Maul, D.,[2] Pedersen, N.,[3] [1]Dept. of Medical Pathology, [2]California Primate Center, [3]Dept. of Veterinary Medicine, University of California, Davis: Prevention of simian AIDS by an inactivated type-D whole-virus vaccine.

Elder, J.H.,[1] McGee, J.S.,[1] Munson, M.,[1] Houghten, R.,[1] Kloetzer, W.,[2] Bittle, J.,[1] Grant, C.,[3] [1]Scripps Clinic and Research Foundation, La Jolla, [2]Johnson and Johnson Biotechnology Center, San Diego, California; [3]Pacific Northwest Research Foundation, Seattle, Washington: Use of synthetic peptides in the development of a vaccine to FeLV.

Morrison, R.,[1] Chesebro, B.,[1] Earl, P.,[2] Nishio, J.,[1] Wehrly, K.,[1] Moss, B.,[2] [1]NIAID, National Institutes of Health, Rocky Mountain Laboratories, Hamilton, Montana; [2]National Institutes of Health, Bethesda, Maryland: Induction of primed T lymphocytes and protection against Friend murine leukemia by a recombinant vaccinia virus expressing the Friend retrovirus envelope gene.



R. Chanock



M. Patarroyo, R. Houghton, E. Norrby

Kennedy, R.C.,[1] Kanda, P.,[1] Dreesman, G.R.,[1] Allan, J.S.,[2] Lee, T.H.,[2] Essex, M.,[2] Chanh, T.C.,[1] [1]Dept. of Virology and Immunology, Southwest Foundation for Biomedical Research, San Antonio, Texas; [2]Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts: Synthetic peptides identifying neutralizing epitopes associated with HTLV-III/LAV envelope glycoprotein.

Dreesman, G.R., Chanh, T.C., Kanda, P., Eichberg, J.W., Kennedy, R.D., Southwest Foundation for Biomedical Research, Dept. of Virology and Immunology, San Antonio, Texas: A prototype synthetic peptide vaccine for HTLV-III/LAV chimpanzees.

Putney, S.,[1] Rusche, J.,[1] Lynn, D.,[1] Javaherian, K.,[1] Jackson, J.,[1] Mueller, T.,[1] Carson, H.,[1] Robey, G.,[2] Wong-Staal, F.,[3] Khron, K.,[3] Ranki, A.M.,[3] [1]RepliGen Corporation, Cambridge, Massachusetts; [2]NCI-Frederick Cancer Research Facility, Frederick, [3]NCI, National Institutes of Health, Bethesda, Maryland: Recombinant HTLV-III envelope proteins produced in *E. coli* and insect cells are AIDS vaccine candidates.

SESSION 13 PATHOGENESIS AND ATTENUATION

Chairman: **F. Brown**, Wellcome Biotechnology Ltd.

Hahn, C.S.,[1] Rice, C.M.,[1,2] Dalrymple, J.,[2] Strauss, J.H.,[1] [1]Division of Biology, California Institute of Technology, Pasadena; [2]Virology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland: Comparison of Asibi and 17D strains of yellow fever virus.

Ginsberg, H.S., Valdesuso, J., Horswood, R., Chanock, R.M., Prince, G., Laboratory of Infectious Diseases, NIAID, National Institutes of Health, Bethesda, Maryland: Studies on the gene products involved in pathogenicity of adenovirus.

Cohen, J., Rosenblum, B., Feinstone, S., Daemer, R., Ticehurst, J., Purcell, R., NIAID, National Institutes of Health, Bethesda, Maryland: Comparison of the nucleotide sequence of wild-type HAV with an attenuated candidate vaccine derivative.

Gorziglia, M., Flores, J., Hoshino, Y., Buckler-White, A., Blumentals, I., Glass, R., Kapikian, A.Z., Chanock, R.M., NIAID, National Institutes of Health, Bethesda, Maryland: Conservation of amino acid sequence of VP8 and cleavage region of 84K outer capsid protein among rotaviruses recovered from asymptomatic neonatal infection and its possible role in attenuation.

La Monica, N., Racaniello, V.R., Dept. of Microbiology, Columbia University College of Physicians & Surgeons, New York, New York: Reduced mouse neurovirulence of poliovirus antigenic variants selected with monoclonal antibodies.

Howley, P.M., Baker, C.C., Phelps, W.C., Spalholz, B.A., Laboratory of Tumor Virus Biology, National Cancer Institute, Bethesda, Maryland: Papillomavirus transformation and transcriptional *trans*-activation functions.

Kit, S.,[1] Kit, M.,[1] Bartkoski, M.J.,[2] Dees, C.,[2] [1]Division of Biochemical Virology, Baylor College College of Medicine, Houston, Texas; [2]Biologics Corporation/Division of TechAmerican Group, Inc., Omaha, Nebraska: Genetically engineered pseudorabies virus vaccine with deletions in thymidine kinase and glycoprotein genes.

Summary: **H. Ginsberg**, Columbia University College of Physicians & Surgeons and NIAID, National Institutes of Health

Biological Effects of DNA Topology

September 17–September 21

ARRANGED BY

Nicholas Cozzarelli, University of California, Berkeley
James Wang, Harvard University

176 participants

The Biological Effects of DNA Topology is the first meeting on this rapidly expanding subject held at Cold Spring Harbor Laboratory. This early fall meeting focused on the effects of DNA topology on biological processes including replication, transcription, and recombination, and the mechanistic and functional aspects of DNA topoisomerases.

The five-day program comprised two full poster sessions and 43 oral presentations divided into eight sessions covering forms of DNA supercoiling, mechanisms and structure of topoisomerases, genetic studies of topoisomerases, inhibitors and cellular localization of topoisomerases, DNA replication topology, topological approaches to site-specific recombination, and effects of DNA supercoiling and bending on transcription

This meeting was supported in part by the National Science Foundation and the National Institute of General Medical Sciences.

SESSION 1 FORMS OF DNA SUPERCOILING

Chairman: M. Gellert, National Institutes of Health

Opening Remarks: A. Klug, A.A. Travers, Medical Research Council

Pettijohn, D.E., Broyles, S., Dept. of Biochemistry, Biophysics, and Genetics, University of Colorado Health Sciences Center, Denver: Protein-DNA interactions stabilizing DNA packaging in prokaryotic cells.

White, J.,[1] Bauer, W.,[2] [1]Dept. of Mathematics, University of California, Los Angeles; [2]Dept. of Microbiology, State University of New York, Stony Brook: Analysis of superhelical DNA containing local substructures in terms of the intersection number and the ladder-like correspondence surface.

Kikuchi, A., Nakasu, S., Mitsubishi-Kasel Institute of Life Sciences, Machida, Tokyo, Japan: Reverse gyrase and type II DNA topoisomerase in *Sulfolobus*, an acidothermophilic Archaebacterium.

Forterre, P.,[1] Nadal, M.,[2] Mirambeau, G.,[2] Moldy, S.,[1] Jaxel, C.,[2] Reiter, W.D.,[3] Duguet, M.,[2] [1]Laboratoire de Biologie Moléculaire de la Réplication, Institut de Recherches Scientifiques sur le Cancer, Villejuif, [2]Laboratoire d'Enzymologie des Acides Nucleiques, Université Pierre et Marie Curie, Paris, France; [3]Max-Planck-Institut für Biochemie, Martinsried, Federal Republic of Germany: DNA topology in Archaebacteria.

SESSION 2 MECHANISM AND STRUCTURE OF TOPOISOMERASES

Chairman: J. Champoux, University of Washington

Bjornsti, M.-A., Abulafia, R., Giaever, G., Horowitz, D.S., Kim, R., Lynn, R., Swanberg, S., Wilkinson, A., Worland, S., Wang, J.C., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Recent studies of DNA topoisomerases.

Maxwell, A.,[1,2] Rau, D.C.,[1] Gellert, M.,[1] [1]NIDDK, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Biochemistry, University of Leicester, England: A structural transition in the gyrase-DNA complex.

Huang, W.M., Nicholson, G., Fang, M., Gibson, A., Dept. of Cellular, Viral, and Molecular Biology, University of Utah Medical Center, Salt Lake City: Structure and function of T4 DNA topoisomerase subunits.

Osheroff, N., Dept. of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee: Trapping the covalent topoisomerase-II-cleaved DNA complex in an active form.

Nolan, J., Wyckoff, E., Natalie, D., Lee, M., Hsieh, T., Dept.

of Biochemistry, Duke University Medical Center, Durham, North Carolina: Cytogenetic and structural analysis of topoisomerase II gene from *D. melanogaster*.

Heller, R.A., Fairman, R., Philip, M., Brutlag, D.L., Dept. of Biochemistry, Stanford University Medical Center, California: Cloning and characterization of the *Drosophilia*-DNA topoisomerase II gene and its expression during development.

SESSION 3 POSTERS

Uemura, T., Uzawa, S., Morino, K., Yanagida, M., Dept. of Biophysics, Faculty of Science, Kyoto University, Japan: Structure and function studies on fission yeast DNA topoisomerase I gene.

Gilmour, D.S., Elgin, S.C.R.,[1] Lis, J.T.,[2] [1]Washington University, St. Louis, Missouri; [2]Cornell University, Ithaca, New York: Topoisomerase I interacts with sites on each strand of the transcribed heat shock genes in *Drosophilia*.

D'Urso, G.F.,[1] Lewis, J.B.,[1,2] [1]Dept. of Pathology, University of Washington, [2]Fred Hutchinson Cancer Research Center, Seattle: Effects of adenovirus infection on the levels of DNA topoisomerase I.

Olivera, B.M., Ferro, A.M., Gandhi, R.T., Cordray, P., Dept. of Biology, University of Utah, Salt Lake City: Poly ADP-ribosylation of DNA topoisomerase I.

Andoh, T.,[1] Ishii, K.,[1] Suzuki, Y.,[1] Ikegami, Y.,[1] Iida, K.,[1] Okada, K.,[2] Kusunoki, Y.,[3] Takemoto, Y.,[4] Oguro, M.,[5] [1]Meiji College of Pharmacy, Tokyo, [2]Dept. of Blood Transfusion, Hiroshima University Hospital, [3]Dept. of Hematology, Research Institute for Nuclear Medicine and Biology, [4]Dept. of Physiology, School of Medicine, Hiroshima University, [5]Division of Hematology and Chemotherapy, Chiba Cancer Center Hospital, Japan: Isolation and characterization of camptothecin-resistant human T-ALL cell line possessing altered DNA topoisomerase I.

Patterson, E.B., Keene, J.D., Dept. of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina: Isolation and analysis of cDNA clones encoding topoisomerase I using anti-Scl 70 autoimmume antisera.

Schaack, J., Schedl, P., Shenk, T., Depts. of Molecular Biology and Biology, Princeton University, New Jersey: Topoisomerase interaction with adenovirus.

Uemura, T., Yanagida, M., Dept. of Biophysics, Faculty of Science, Kyoto University, Japan: Functional domains in DNA topoisomerase II.

Muller, M.T., Spitzner, J.R., Molecular, Cellular and Developmental Biology Program, Ohio State University, Columbus: Analysis of the role of eukaryotic topoisomerase II in DNA supercoiling.

Sahai, B.M., Kowalski, D., Dept. of Molecular and Cellular Biology, Roswell Park Memorial Institute, Buffalo, New York: Large changes in DNA topoisomerase II activity mark the transition between quiescent and proliferating cells.

Sahai, B.M., Kaplan, J.G., Dept. of Biochemistry, University of Alberta, Edmonton, Canada: On the possible role of type II topoisomerase in the transition of quiescent cells into cycling cells.

Squinto, S.P.,[1] Cross, S.M.,[2] Morin, M.J.,[2] [1]Dept. of Biochemistry and Molecular Biology, Louisiana State University Medical Center, New Orleans; [2]Dept. of Pharmacology and the Cancer Center, Northwestern University Medical School, Chicago, Illinois: Stimulation of topoisomerase II by activators of protein kinase C in HL-60 cells.

J. Wang

Stewart, A.F., Becker, P., Schütz, G., German Cancer Research Center, Heidelberg, Federal Republic of Germany: Characteristics of topoisomerase I and II cleavage sites mapped in vivo on the rat tyrosine aminotransferase gene.

Weinberg, R., Gallagher, M., Simpson, M.V., Dept. of Biochemistry, State University of New York, Stony Brook: The effect of oxolinic acid, novobiocin, and nalidixic acid on the transcription of the rat mitochondrial genome.

Bonven, B., Andersen, A., Christiansen, K., Thomsen, B., Nielsen, O.F., Westergaard, O., Institute of Molecular Biology, University of Aarhus, Denmark: Sequence-specific topoisomerase I and II action in rDNA spacer chromatin.

Strauss, P.R.,[1] Zhang, L.H.,[1] Pommier, Y.,[2] [1]Dept. of Biology, Northeastern University, Boston, Massachusetts; [2]Laboratory of Molecular Pharmacology, DCT-NCI, National Institutes of Health, Bethesda, Maryland: Detergent soluble DNA contains tightly bound protein.

Russo, P.,[1] Pedrini, A.M.,[2] Tornaletti, S.,[2] Peluso, M.,[1] Palitti, F.,[3] Kihlman, B.A.,[4] Parodi, S.,[1] [1]Istituto Nazionale per la Ricerca sul Cancro, Genova, [2]Istituto di Genetica Biochimica ed Evoluzionistica del CNR, Pavia, [3]Centro di Genetica Evoluzionistica del CNR, Rome, Italy; [4]Dept. of Genetics, University of Uppsala, Sweden: Is DNA topoisomerase II the target of caffeine and its derivatives?

Hickson, I.D., Hoban, P.R., Harris, A.L., Robson, C.N., Cancer Research Unit, University of Newcastle upon Tyne, England: A CHO cell hypersensitive to topoisomerase II inhibitors.

Pocklington, M., Orr, E., Dept. of Genetics, University of Leicester, England: Novobiocin resistance in yeast.

Cotten, M., Sealy, L., Chalkley, R., Dept. of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, Tennessee: Novobiocin precipitation of chromosomal histones.

Thompson, R., Mosig, G., Dept. of Molecular Biology, Vanderbilt University, Nashville, Tennessee: Control of a chloroplast promoter of *C. reinhardtii* by torsional stress.

Shlomai, J., Linial, M., Kuvin Centre for the Study of Infectious and Tropical Diseases, Hebrew University, Jerusalem, Israel: A nicking enzyme from trypanosomatids detects the bent structure in kinetoplast DNA.

Bressler, S.L., Cattolico, R.A., Dept. of Botany, University of Washington, Seattle: Topoisomerase activities in the marine alga *O. luteus*.

Shaffer, R., Traktman, P., Depts. of Microbiology and Cell Biology, Cornell University Medical College, New York, New York: Isolation and characterization of a topoisomerase activity from vaccinia virus.

Douc-Rasy, S., Riou, J.F., Riou, G., Laboratoire de Pharmacologie Clinique et Moléculaire, Institut Gustave Roussy, Villejuif, France: Characterization in trypanosomes of an ATP-independent type II topoisomerase.

Sioud, M.,[1] Possot, O.,[2] Elie, C.,[1] Sibold, L.,[2] Baldacci, J.,[1] Forterre, P.,[1] [1]CNRS, Biologie Moléculaire de la Réplication, Villejuif, [2]Unité de Physiologie Cellulaire, Institut Pasteur, Paris, France: Sensitivity of Archaebacteria to inhibitors of type II DNA topoisomerases.

SESSION 4 GENETIC STUDIES OF TOPOISOMERASES

Chairman: R. Sternglanz, State University of New York, Stony Brook

Brill, S., DiNardo, S., Voelkel-Meiman, K., Sternglanz, R., Dept. of Biochemistry, State University of New York, Stony Brook: Yeast DNA topoisomerases and their roles in DNA replication and transcription.

Holm, C.,[1] Stearns, T.,[2] Botstein, D.,[2] [1]Dept. of Cellular and Developmental Biology, Harvard University, [2]Dept. of Biology, Massachusetts Institute of Technology, Cambridge: The role of DNA topoisomerase II in the cell cycle of yeast.

Uemura, T., Ohkura, H., Uzawa, S., Yanagida, M., Dept. of Biophysics, Faculty of Science, Kyoto University, Japan: In vivo roles of DNA topoisomerase I and II in fission yeast.

Drlica, K.,[1] Pruss, G.,[2] Franco, R.,[1] Chevalier, S.,[1] Steck, T.,[3] [1]Public Health Research Institute, New York, [2]Dept. of Biology, University of Rochester, New York; [3]Dept. of Microbiology, University of Pittsburgh School of Medicine, Pennsylvania: Mutations and inhibitors affecting bacterial topoisomerases alter titratable supercoiling.

Depew, R.E., Dept. of Microbiology and Immunology, Northeastern Ohio Universities College of Medicine, Rootstown: Genetic studies of DNA topoisomerase I mutations and compensatory mutations in *E. coli*.

Higgins, P., Krause, H., Kilpatrick, M., Collier, D., Wells, R., Dept. of Biochemistry, University of Alabama, Birmingham: *E. coli* histone-like proteins modify supercoiled DNA structure and function in phage Mu.

Camilloni, G., Negri, R., Della Seta, F., Ficca, A.G., Di Mauro, E., Dipt. di Genetica e Biologia molecolare e Centro Acidi Nucleici CNR, Università La Sapienza, Rome, Italy: The intrinsic topological organization of *S. cerevisiae* GAL1-GAL10 and ADHII promoters.

Chen, J.H., Shen, D.-F., Dept. of Biochemistry, New York University Dental Center, New York: The involvement of DNA topology in gene expression of lens tissue.

Luchnik, A.N.,[1] Dubinina. E.N.,[2] Institute of [1]Developmental Biology, [2]Molecular Biology, USSR Academy of Science, Moscow: Switching off transcription in mammalian cells after violation of topological constraint in superhelical DNA loops by X-irradiation.

Amirhaeri, S.,[1] Vacante, D.,[1] Major, E.,[1] Wohlrab, F.,[2] Wells, R. D.,[2] [1]NINCDS, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Biochemistry, University of Alabama, Birmingham: Unusual DNA structures in the regulatory region of the human polyomavirus JC.

Wohlrab, F., McLean, M.J., Wells, R.D., Dept. of Biochemistry, Schools of Medicine and Dentistry, University of Alabama, Birmingham: Site of segment inversion in HSV-1 adopts a novel DNA structure.

van Mansfeld, A.D.M., van Teeffelen, H.A.A.M., Baas, P.D., Jansz, H.S., Institute of Molecular Biology and Medical Biotechnology, University of Utrecht, The Netherlands: Two neighboring tyrosyl residues are involved in cleavage and ligation of DNA by ϕX174 gene *A* protein.

Ghelardini, P.,[1] Liebart, J.C.,[2] Paolozzi, L.,[1] Pedrini, A.M.,[3] [1]Centro Acidi Nucleici del CNR, Rome, Italy; [2]Institut de Microbiologie, Faculté des Sciences d'Orsay, France; [3]Istituto di Genetica Biochimica ed Evoluzionistica del CNR, Pavia, Italy: The *lig* product of phage Mu affects the superhelicity of intracellular DNA.

Gennaro, M.L., Novick, R.P., Dept. of Plasmid Biology, Public Health Research Institute, New York, New York: A *cis*-acting plasmid locus alters DNA supercoiling and the interaction between replication origin and initiator protein.

Umek, R.M., Kowalski, D., Dept. of Molecular and Cellular Biology, Roswell Park Memorial Institute, Buffalo, New York: Torsional stress induces an altered DNA conformation in a yeast ARS and gene terminator region.

Lockshon, D., Galloway, D.A., Fred Hutchinson Cancer Research Center, Seattle, Washington: Palindromic replication origins of HSV.

Sumners, D.W.,[1] Cozzarelli, N.R.,[2] Spengler, S.J.,[3] [1]Dept.

of Mathematics, Florida State University, Tallahassee; [2]Dept. of Molecular Biology, University of California, Berkeley; [3]Biomed Division, Lawrence Berkeley Laboratory, California: A topological model for site-specific recombination.

Kim, B.D., Dept. of Plant Sciences, University of Rhode Island, Kingston: Four-stranded DNA and intercoiled DNA—Intermediates of recombination.

Weber, P.C., Dinh, Y.-C.T., Abremski, K., Beran, R.K., Brenner, S.L., Cox, M.J., Salemme, F.R., Zlotnick, A., Ohlendorf, D.H., E.I. du Pont de Nemours and Company, Dept. of Central Research and Development, Wilmington, Delaware: Structural studies of proteins involved in DNA recombination and strand topology.

Hoess, R., Abremski, K., Wierzbicki, A., E.I. du Pont de Nemours and Company, Dept. of Central Research and Development, Wilmington, Delaware: A mutational and biochemical analysis of the site-specific topoisomerase *Cre*.

Kanaar, R.,[1] van de Putte, P.,[1] Cozzarelli, N.R.,[2] [1]Dept. of Biochemistry, State University of Leiden, The Netherlands; [2]Dept. of Molecular Biology, University of California, Berkeley: Gin-mediated DNA inversion—Product structure and strand exchange.

Stasiak, A., Müller, B., Koller, T., Institute for Cell Biology, ETH-Hönggerberg, Zürich, Switzerland: DNA structure in recombination intermediates.

Blaho, J.A., Wells, R.D., Dept. of Biochemistry, Schools of Medicine and Dentistry, University of Alabama, Birmingham: Left-handed Z-DNA binding by the RecA protein of *E. coli*.

Leis, J.,[1] Cobrinik, D.,[1] Katz, R.A.,[2] Soltis, D.,[2] Terry, R.,[2] Skalka, A.M.,[2] [1]Dept. of Biochemistry, Case Western Reserve University, Cleveland, Ohio; [2]Dept. of Molecular Oncology, Roche Institute of Molecular Biology, Nutley, New Jersey: Biochemical studies of retroviral DNA integration.

SESSION 5 INHIBITORS AND CELLULAR LOCALIZATION OF TOPOISOMERASES

Chairman: L. Liu, Johns Hopkins University

Earnshaw, W.C., Heck, M.M.S., Dept. of Cell Biology and Anatomy, Johns Hopkins School of Medicine, Baltimore, Maryland: Topoisomerase II— A specific marker for cell proliferation.

Cockerill, P.N., Blasquez, V.C., Huang, S.-Y., Yuen, M.-H., Garrard, W.T., Dept. of Biochemistry, University of Texas Health Science Center, Dallas: Immunoglobulin gene chromosomal loops are anchored adjacent to enhancers by (A + T)-rich DNA elements containing topoisomerase II sites.

Laemmli, U.K., Gasser, S.M., Mirkovitch, J., Dept. of Molecular Biology, University of Geneva, Switzerland: Cohabitation of scaffold binding regions with upstream/enhancer elements of three developmentally regulated genes of *D. melanogaster*.

Darby, M.K.,[1] Herrera, R.E.,[2] Vosberg, H.-P.,[1] Nordheim, A.,[2] [1]Abteilung Molekulare Biologie, Max-Planck-Institut für medizinische Forschung, [2]Zentrum für Molekulare Biologie, Heidelberg, Federal Republic of Germany: DNA topoisomerase II cleaves at specific sites in the 5′-flanking region of c-*fos* proto-oncogenes in vitro.

Pommier, Y., Markovits, J., Kerrigan, D., Kohn, K.W., NCI, National Institutes of Health, Bethesda, Maryland: Alterations in topoisomerase activities associated with cell proliferation and drug resistance.

Shen, L.L., Abbott Laboratories, Abbott Park, Illinois: Quinolone antibacterials—Structure, activity, and mechanism of inhibition of DNA gyrase.

SESSION 6 POSTERS

Scholten, P.M., Runkel, L., Herrera, R.E., Nordheim, A., Zentrum für Molekulare Biologie, Heidelberg, Federal Republic of Germany: In vitro DNA supercoiling stabilizes conformations that exhibit increased chemical reactivities with diethylpyrocarbonate.

Walba, D.M.,[1] Armstrong, J.D. III,[1] Simon, J.K.,[2] [1]Dept. of Chemistry and Biochemistry, University of Colorado, Boulder; [2]Dept. of Mathematics, University of Iowa, Iowa City: Topological stereochemistry from an organic chemist's perspective.

Beran, R.K., Dinh, Y.-C.T., E.I. du Pont de Nemours and Company, Dept. of Central Research and Development, Wilmington, Delaware: Simplified procedure for the purification of *E. coli* DNA topoisomerase I from an overproducing strain.

Domanico, P.L., Beran, R.K., Doran, E., Dinh, Y.-C.T., E.I. du Pont de Nemours and Company, Dept. of Central Research and Development, Wilmington, Delaware: Physical and kinetic studies on the interaction between *E. coli* topoisomerase I and oligonucleotides.

Barot, H.A., Fisher, L.M., Dept. of Biochemistry, St. George's Hospital Medical School, University of London, England: Site-specific DNA breakage by *E. coli* DNA gyrase.

Hooper, D., Wolfson, J., Souza, K., Ng, E., McHugh, G., Swartz, M., Infectious Disease Unit, Massachusetts General Hospital, Boston: Quinolone antimicrobial agents—Inhibition of DNA gyrase and mechanisms of resistance in *E. coli*.

Tornaletti, S., Pedrini, A.M., Istituto di Genetica Biochimica ed Evoluzionistica del CNR, Pavia, Italy: Effect of nalidixic acid on DNA conformation.

Lin, G., Mosig, G., Dept. of Molecular Biology, Vanderbilt University, Nashville, Tennessee: Inactivation of both phage T4 DNA topoisomerase and *E. coli* DNA gyrase differentially affects T4 late transcripts.

Balke, V.L., Gralla, J.D., Molecular Biology Institute and Dept. of Chemistry and Biochemistry, University of California, Los Angeles: Changes in *E. coli* plasmid supercoiling in response to growth transitions.

Yamamoto, N.,[1] Yoshizawa, Y.,[1] Thomulka, K.W.,[2] Hoober, J.K.,[3] [1]Dept. of Microbiology and Immunology, Hahnemann University School of Medicine, [2]Dept. of Biological Sciences, Philadelphia College of Pharmacy and Sciences, [3]Dept. of Biochemistry, Temple University School of Medicine, Philadelphia, Pennsylvania: Relation between strict aerobic mutants of facultative anaerobes (*E. coli* and *S. typhimurium*) and the action of DNA gyrase inhibitors.

Jovanovich, S.B.,[1] Martinell, M.,[1] Lebowitz, J.,[2] Burgess, R.R.,[1] [1]McArdle Laboratory for Cancer Research, University of Wisconsin, Madison; [2]Dept. of Microbiology, University of Alabama, Birmingham: Use of operon fusions to examine the in vivo effects of supercoiling.

Yamamoto, N.,[1] Thomulka, K.W.,[2] Yoshizawa, Y.,[1] Hoober, J.K.,[3] [1]Dept. of Microbiology and Immunology, Hahnemann University School of Medicine, [2]Dept. of Biological Sciences, Philadelphia College of Pharmacy and Science, [3]Dept. of Biochemistry, Temple University School of Medicine, Philadelphia, Pennsylvania: Control of gene expression by aerobic or anaerobic conditions.

Hsieh, W.-T., Wells, R.D., Dept. of Biochemistry, University of Alabama, Birmingham: Influence of left-handed Z-DNA and supercoiling on the binding of the lactose repressor to its operator.

SESSION 7 DNA REPLICATION TOPOLOGY

Chairman: J. Hurwitz, Memorial Sloan-Kettering Cancer Center

Baker, T.A., Funnell, B.E., Sekimizu, K., Bramhill, D., Kornberg, A., Dept. of Biochemistry, Stanford University School of Medicine, California: Enzymatic studies on replication of *oriC* plasmids.

McMacken, R., Wold, M., Mensa-Wilmot, K., Seaby, R., Dept. of Biochemistry, Johns Hopkins University, Baltimore, Maryland: Roles of DNA gyrase in the replication of bacteriophage λ DNA.

Marians, K., Minden, J., Graduate Program in Molecular Biology, Memorial Sloan-Kettering Cancer Center, New York, New York: Segregation of replicating pBR322 DNA daughter molecules in vitro.

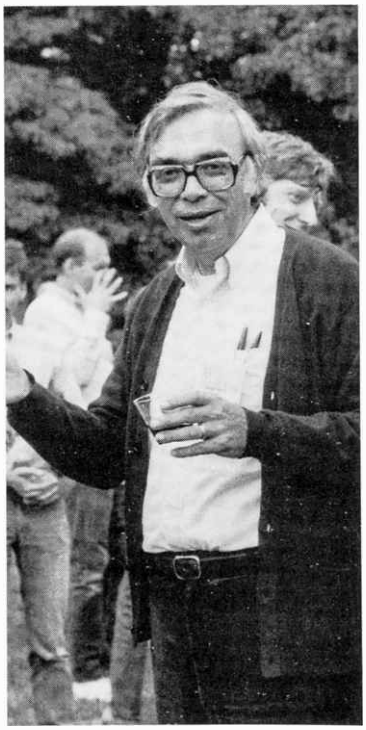
Dean, F., Bullock, P., Murakami, Y., Weissbach, L., Wobbe, R., Borowiec, J., Hurwitz, J., Graduate Program in Molecular Biology and Virology, Sloan-Kettering Institute for Cancer Research, New York, New York: SV40 T antigen is required for unwinding template plasmid DNA containing the SV40 origin of replication.

Yang, L.,[1] Wold, M.S.,[2] Li, J.J.,[2] Kelly, T.J.,[2] Liu, L.F.,[1] Depts. of [1]Biological Chemistry, [2]Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, Maryland: Multiple roles of DNA topoisomerases in SV40 DNA replication in vitro.

Fields-Berry, S., DePamphilis, M.L., Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Effect of yeast centromeric sequences on formation of catenated dimers during plasmid DNA replication in mammalian cells.

K. Mizuuchi

A. Klug



N. Cozarelli

SESSION 8 TOPOLOGICAL APPROACHES TO SITE-SPECIFIC RECOMBINATION

Chairman: H. Nash, National Institutes of Health

Nash, H., Abcarian, P., Kitts, P., Richet, E., Laboratory of Molecular Biology, National Institute of Mental Health, Bethesda, Maryland: Mechanism of λ integrative recombination—Topological studies and beyond.

Nunes-Düby, S., Pargellis, C., Moitoso de Vargas, L., Egner, C., Franz, B., Landy, A., Division of Biology and Medicine, Brown University, Providence, Rhode Island: Design and use of suicide substrates to study topoisomerase and recombination intermediates in the Int-dependent pathway.

Hatfull, G.F., Salvo, J.J., Grindley, N.D.F., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Site-specific recombination by the γδ resolvase—Formation of a kink at the crossover site?

Benjamin, H., Dungan, J., Haskins, B., Kanaar, R., Richardson, M., Wasserman, S., Weinberg, R., Cozzarelli, N., Dept. of Molecular Biology, University of California, Berkeley: Specificity in synapsis and exchange in site-specific recombination.

Boocock, M.R., Brown, J.L., Sherratt, D.J., Institute of Genetics, University of Glasgow, Scotland: Topological specificity of Tn*3* resolvase.

Abremski, K., Hoess, R.H., Frommer, B., Wierzbicki, A., E.I. Du Pont de Nemours and Co., Dept. of Central Research and Development, Wilmington, Delaware: Mechanisms of synapsis and strand exchange during Cre-*loxP* site-specific recombination.

SESSION 9 DNA TOPOLOGY AND GENERAL RECOMBINATION

Chairman: K. Mizuuchi, National Institutes of Health

Kahmann, R., Bräuer, B., Max-Planck-Institut für Molekulare Genetik, Berlin, Federal Republic of Germany: DNA topology changes associated with G inversion.

Craigie, R., Mizuuchi, K., NIADDKD, National Institutes of Health, Bethesda, Maryland: Role of DNA topology in Mu transposition—Mechanism of sensing the relative orientation of two DNA segments.

Morisato, D., Kleckner, N., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Tn*10* and IS*10* transposition in vitro.

Radding, C.M., Bainton, R.J., Chow, S.A., Flory, J., Gonda, D.K., Honigberg, S.M., Leahy, M., Muniyappa, K., Tsang, S.S., Depts. of Human Genetics and Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Connecticut: Topology and topography of homologous pairing and strand exchange.

Griffith, J.D.,[1] Christiansen, G.C.,[2] Register, J.,[1] Harris, L.D.,[1] [1]Lineberger Cancer Center, University of North Carolina, Chapel Hill; [2]Dept. of Medical Microbiology, University of Aarhus, Denmark: Electron microscopic visualization of the intermediates in strand exchange reactions.

Champoux, J., Bullock, P., McCoubrey, W., Dept. of Microbiology and Immunology, University of Washington, Seattle: Evidence consistent with a role for the eukaryotic type I topoisomerase in nonhomologous recombination.

SESSION 10 EFFECTS OF DNA SUPERCOILONG AND BENDING ON TRANSCRIPTION

Chairman: H. Weintraub, Fred Hutchinson Cancer Research Center

Worcel, A.,[1] Kmiec, E.,[1] Ruberti, I.,[2] [1]Dept. of Biology, University of Rochester, New York; [2]Istituto di Fisiologia Generale, Universita degli Studi di Roma, Italy: Chromatin assembly and gene expression in vitro.

Hochschild, A.,[1] Griffith, J.,[2] Ptashne, M.,[1] [1]Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts; [2]Lineberger Cancer Research Center, University of North Carolina Medical School, Chapel Hill: Cooperative binding of λ repressors to separated operator sites.

Gralla, J., Borowiec, J., Dept. of Chemistry and Biochemistry, University of California, Los Angeles: DNA supercoiling induces bent DNA during recognition of the *lac* operator and promoter.

Dinh, Y.-C.T., E.I. du Pont de Nemours and Company, Dept. of Central Research and Development, Wilmington, Delaware: Transcription of *E. coli* DNA topoisomerase I gene from multiple promoters and its regulation by DNA supercoiling.

Summary: M. Gellert, National Institutes of Health

Poxvirus/Iridovirus

September 24–September 26

ARRANGED BY

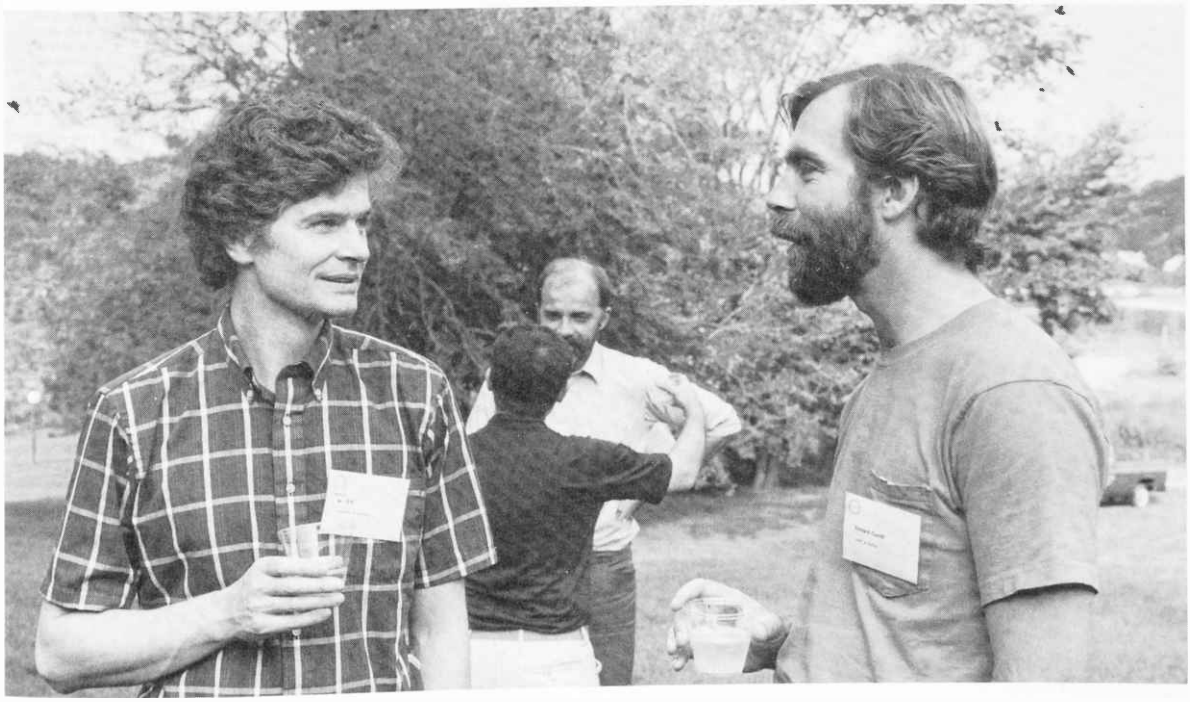
Richard Condit, State University of New York, Stony Brook
Dennis Hruby, Oregon State University

132 participants

The 1986 Poxvirus/Iridovirus Workshop was the sixth in a series that began in 1977. The purpose of the meetings is to discuss all aspects of the biology of DNA-containing viruses that undergo a significant portion of their replication cycle in the cytoplasm of infected cells. The viruses considered include a large variety of poxviruses and iridoviruses, with the current emphasis focusing on vaccinia virus, Shope fibroma virus, African swine fever virus, and frog virus 3. Sessions in this year's meeting covered virus structure, virus-cell interactions, genome structure and organization, transcription, DNA metabolism and recombination, virus-host interactions, including studies on the interferon-induced antivirus state, persistent infections, and transformation, and finally the use of vaccinia virus as an expression vector and as a live recombinant vaccine.

The meeting was the largest yet, with worldwide participation by over 100 investigators, over 90 oral presentations, and a poster session. As in the past, the group continues to draw strength from the diversity of virus systems represented, and from an extraordinary degree of cooperation and interaction among the participants.

This meeting was supported in part by the Bethesda Research Laboratories and Life Technologies, Inc.



A. Ball, R. Condit

SESSION 1 VIRION STRUCTURE AND VIRUS-CELL INTERACTIONS

Chairman: S. Dales, University of Western Ontario

Berthiaume, L.,[1] Alain, R.,[1] Robin, J.,[2] [1]Institut Armand-Frappier, [2]Dept. of Biology, University of Sherbrooke, Quebec, Canada: Electron microscopic study of lymphocystis disease virus (LDV) grown in cell cultures—Ultrastructure and morphogenesis.epDales, S., Dept. of Microbiology and Immunology, University of Western Ontario, London, Canada: Concerning definitions of "membrane" and "envelope" in the biology of poxviruses.

Schloer, G.M., Geering, G., Plum Island Animal Disease Center, Greenport, New York: Isolation of African swine fever virus polypeptides.

Carrascosa, A.L., Santarén, J.F., Viñuela, E., Centro de Biología Molecular (CSIC-UAM), Universidad Autónoma, Madrid, Spain: External proteins of African swine fever virus (ASFV) particles.

López-Otín, C., Rey, J., Simón, C., Méndez, E., Carrascosa, A.L., Viñuela, E., Centro de Biologia Molecular, Universidad Autónoma (CSIC-UAM), Madrid, Spain: African swine fever virus (ASFV) late proteins.

del Val, M., Viñuela, E., Centro de Biología Molecular (CSIC-UAM), Universidad Autónoma, Madrid, Spain: Glycosylated components of African swine fever virus (ASFV) in virus particles and virus-infected cells.

Cabirac, G.F., Strayer, D.S., Leibowitz, J.L., University of Texas Health Science Center, Houston: The surface polypeptides of three leporipoxviruses.

Rodriguez, J.F., Paez, E., Esteban, M., Depts. of Biochemistry, Microbiology and Immunology, Health Science Center, Brooklyn, New York: A 14K envelope protein of vaccinia virus is involved in virus-induced cell fusion.

Gordon, J., Dales, S., Dept. of Microbiology and Immunology, University of Western Ontario, London, Canada: Physical mapping of the gene encoding a prominent vaccinia virus antigen.

Alvarez, R.M., Schlemm, D.J., Keller, S.J., Dept. of Biological Sciences, University of Cincinnati, Ohio: Association of host cytoskeletal proteins with vaccinia virus.

SESSION 2 GENOME STRUCTURE

Chairman: L. Archard, Westminster Medical School

Robinson, A.J., Barnes, G., Carpenter, E., Mercer, A.A., MRC Virus Research Unit, Dunedin, New Zealand: Conservation and variation in parapoxvirus genomes.

Binns, M.M., Tomley, F.M., Boursnell, M.E.G., Mockett, A.P.A., Houghton Poultry Research Station, Huntingdon, England: Cloning and restriction mapping studies on fowlpox virus.

Gershon, P.D., Black, D.N., Animal Virus Research Institute, Pirbright, England: Location of genomic differences between capripoxvirus isolates.

Blasco, R., Almendral, J.M., Agúero, M., Almazán, F., Viñuela, E., Centro de Biología Molecular (CSIC-UAM), Universidad Autónoma, Madrid, Spain: Multigene families and genetic variation of African swine fever virus (ASFV).

Dixon, L.K., Wilkinson, P.J., Hutchings, G., Animal Virus Research Institute, Pirbright, England: Molecular analysis of African swine fever virus isolates from tick populations in Zambia and Malawi.

Porter, C.D., Archard, L.C., Dept. of Biochemistry, Charing Cross and Westminster Medical School, London, England: Structure of molluscum contagiosum virus DNA and mapping of a novel EGF-like gene.

Reisner, H., Scholz, J., Darai, G., Institut of Medical Virology of the University, Heidelberg, Federal Republic of Germany: Characterization of the genome of molluscum contagiosum virus type 2.

Rösen, A.,[1] Pilaski, J.,[2] Darai, G.,[1] [1]Institute of Medical Virology of the University, Heidelberg, [2]Medizinisches Institut für Umwelthygiene, Düsseldorf, Federal Republic of Germany: Genomic characterization of a poxvirus isolated from a child.

Soltau, J., Schnitzler, P., Reisner, H., Scholz, J., Darai, G., Institute of Medical Virology of the University, Heidelberg, Federal Republic of Germany: Analysis of the genome of insect iridescent virus type 6 by molecular cloning and physical mapping.

Schnitzler, P.,[1] Delius, H.,[2] Darai, G.,[1] [1]Institute of Medical Virology of the University, [2]European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: Analysis of the repetitive DNA sequences in the genome of fish lymphocystis disease virus.

SESSION 3 GENOME ORGANIZATION

Chairman: R. Wittek, Institut de Biologie Animale, Lausanne

Tomley, F.M., Binns, M.M., Boursnell, M.E.G., Mockett, A.P.A., Houghton Poultry Research Station, Cambridge, England: Molecular structure and organization of an 11.3-kb fragment of fowlpox virus.

Tham, T.N., Mesnard, J.M., Tondre, L., Aubertin, A.M., Groupe de Recherches INSERM, Laboratoire de Virologie, Strasbourg, France: RNA transcript organization within the *Sal* I F fragment of frog virus 3—Nucleotide sequences of two genes.

Beckman, W., Willis, D.B., Dept. of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, Tennessee: Cloning and sequencing of the immediate-early frog virus 3 gene that encodes ICR489.

Macaulay, C., Upton, C., Macen, J., McFadden, G., Dept.

of Biochemistry, University of Alberta, Canada: Characterization of the genes within the terminal inverted repeat of Shope fibroma virus.

Patel, D., Pickup, D.J., Dept. of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina: Identification and characterization of the gene encoding the major component of the A-type inclusions formed by cowpox virus.

Niles, E., Condit, R., Seto, J., Dept. of Biochemistry, State University of New York, Buffalo: Gene organization in the vaccinia virus *Hin*dIII D fragment.

Lee-Chen, J., Davidson, K., Bourgeois, N., Condit, R., Niles, E., Dept. of Biochemistry, State University of New York, Buffalo: Transcript mapping of the genes in the *Hin*dIII D fragment.

Weinrich, S.L., Hruby, D.E., Dept. of Microbiology, Oregon State University, Corvallis: Expression of a tandemly oriented late gene cluster of vaccinia virus.

Ryan, T.E., Ball, L.A., Biophysics Laboratory and Dept. of Biochemistry, University of Wisconsin, Madison: Deletion of the thymidine kinase gene of vaccinia virus and its reinsertion at a new locus in the viral genome.

SESSION 4 TRANSCRIPTION

Chairman: B. Moss, National Institutes of Health

Bertholet, C., Van Meir, E., Wittek, R., Institut de Biologie animale, Batiment de Biologie, Lausanne, Switzerland: Evidence for processing at the 5′ end of vaccinia virus late mRNAs.

Thompson, J.P., Willis, D.B., Granoff, A., Dept. of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, Tennessee: Transcription of methylated DNA in frog virus 3-infected cells.

Willis, D.B., Dept. of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, Tennessee: DNA sequences required for virus-induced activation of an immediate-early frog virus 3 promoter.

Ryan, T.W., Ball, L.A., Biophysics Laboratory and Dept. of Biochemistry, University of Wisconsin, Madison: Promoter activity for *E. coli* RNA polymerase associated with the vaccinia virus thymidine kinase gene – Analysis of sequence requirements.

Vassef, A., Institut Jacques Monod, Paris, France: Transcriptional characterization of vaccinia virus genomic fragments possessing the early promoter activity.

Davison, A.J., Weir, J.P., Rosel, J.L., Moss, B., NIAID, National Institutes of Health, Bethesda, Maryland: Structural and functional analysis of vaccinia virus late promoters.

Talavera, A.,[1] Beloso, A.,[1] Granoff, E.,[2] Willis, D.,[2] Moss, B.,[3] Viñuela, E.,[1] [1]Centro de Biologia Molecular (CSIC-UAM), Universidad Autonoma, Madrid, Spain; [2]Division of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, Tennessee; [3]NIAID, National Institutes of Health, Bethesda, Maryland: RNA polymerase-promoter recognition in African swine fever virus (ASFV). vaccinia virus (VV), and frog virus 3 (FV3).

D. Hruby, M. Ferguson, D. Brown, R. Pacha

Yuen, L., Moss, B., NIAID, National Institutes of Health, Bethesda, Maryland: Identification of vaccinia virus regulatory sequences responsible for termination of early transcription.

Broyles, S., Moss, B., NIAID, National Institutes of Health, Bethesda, Maryland: Sedimentation of an RNA polymerase complex from vaccinia virus that specifically initiates and terminates transcription.

Thompson, C., Condit, R., Dept. of Biochemistry, State University of New York, Buffalo: Characterization of two *ts* mutants affecting a 22-kD subunit of the vaccinia virus-encoded RNA polymerase.

SESSION 5 DNA METABOLISM

Chairman: M. Slabaugh, Oregon State University

Esteves, A., Costa, J.V., Gulbenkian Institute of Science, Oeiras, Portugal: DNA-binding proteins of African swine fever virus.

Barros, M.F., Cunha, C.V., Costa, J.V., Gulbenkian Institute of Science, Oeiras, Portugal: Single-stranded DNase of African swine fever virus.

Essani, K., Goorha, R., Granoff, A., Dept. of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, Tennessee: Mutation in a DNA-binding protein reveals an association between DNA methyltransferase activity and a 26,000-dalton polypeptide in frog virus 3-infected cells.

Upton, C., McFadden, G., Dept. of Biochemistry, University of Alberta, Canada: Identification and nucleotide sequence of the thymidine kinase gene of Shope fibroma virus.

Slabaugh, M., Davis, R., Mathews, C.K., Dept. of Biochemistry and Biophysics, Oregon State University, Corvallis: The ribonucleotide reductase genes of vaccinia virus—Isolation and analysis of HU^R variants.

Shaffer, R., Kunzi, M., Traktman, P., Depts. of Microbiology and Cell Biology and Anatomy, Cornell University Medical College, New York, New York: Isolation and characterization of enzymes associated with DNA replication from vaccinia virions.

Kahn, J.S., Rodriguez, J., Esteban, M., Depts. of Biochemistry, Microbiology and Immunology, Health Science Center, Brooklyn, New York: Molecular cloning, encoding sequence, and expression of vaccinia virus nucleic acid-dependent nucleoside triphosphatase gene.

Traktman, P., Crozel, V., Pacheco, S., Dept. of Cell Biology and Anatomy, Cornell University Medical College, New York, New York: Molecular genetic analysis of the vaccinia virus DNA polymerase.

Evans, E., Traktman, P., Dept. of Cell Biology and Anatomy, Cornell University Medical College, New York, New York: Molecular genetic analysis of vaccinia virus mutants deficient in DNA replication.

Roseman, N.A., Hruby, D.E., Dept. of Microbiology, Oregon State University, Corvallis: Analysis of a vaccinia virus gene required for DNA replication.

SESSION 6 POSTERS

Lichtenstein, D.,[1] Wertz, G.,[2] Ball, A.,[1] [1]Biophysics Laboratory and Dept. of Biochemistry, University of Wisconsin, Madison; [2]Dept. of Microbiology and Immunology, University of North Carolina, Chapel Hill: Site-specific mutagenesis of the putative membrane anchor region of an unusual viral envelope glycoprotein.

Orange, N.,[1] Cerutti, M.,[1] DeVauchelle, G.,[2] [1]Laboratoire de microbiologie de la faculté des sciences de Rouen, Mont Saint Aignan, [2]Laboratoire de pathologie comparée INRA CNRS, Saint Christol les Ales, France: Characterization and localization of Chilo iridescent virus (CIV) polypeptides.

Nii, S., Uno, F., Tsutsui, K., Yoshida, M., Dept. of Virology, Okayama University Medical School, Japan: The interaction between virions of poxviruses and the surface membrane of infected cells.

Tajbakhsh, S.,[1,2] Lee, P.E.,[2] Seligy, V.L.,[1,2] [1]Division of Biological Sciences, National Research Council, [2]Dept. of Biology, Carleton University, Ontario, Canada: Molecular studies on *Tipula* iridescent virus (TIV).

SESSION 7 DNA REPLICATION, RECOMBINATION, TRANSFORMATION

Chairman: G. McFadden, University of Alberta

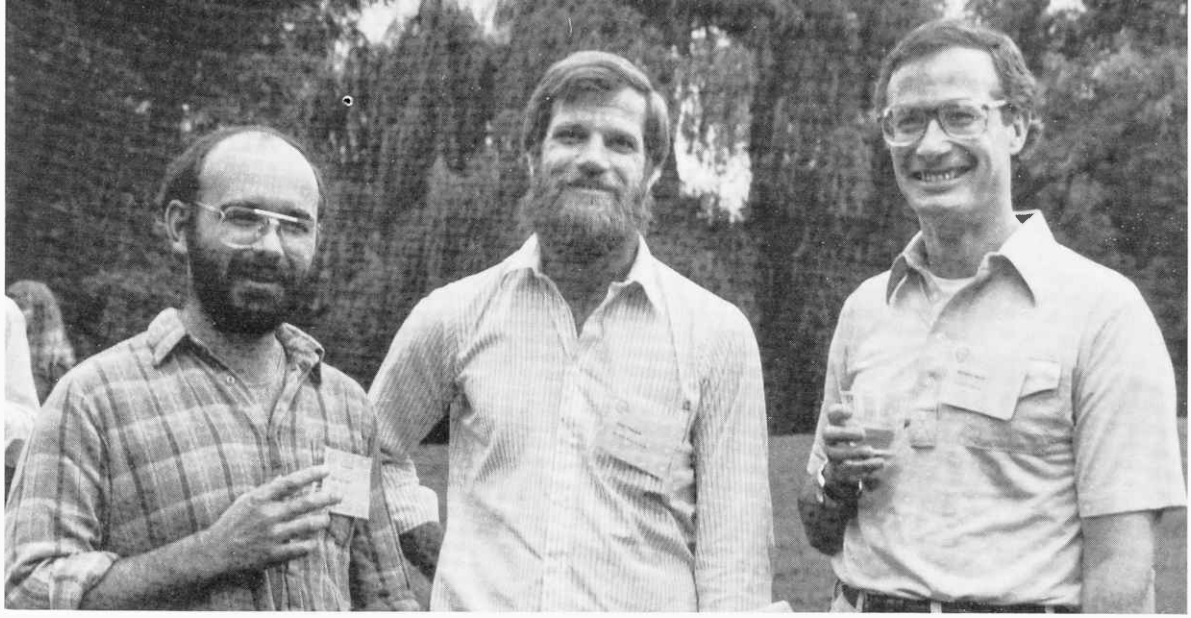
González, A., Calvo, V., Viñuela, E., Centro de Biologío Molecular (CSIC-UAM), Universidad Autónoma, Madrid, Spain: Structural features at the ends of the African swine fever virus (ASFV) genome.

Parsons, B.L., Pickup, D.J., Dept. of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina: Tandemly repeated sequences are present at the ends of the DNA of racoonpox virus.

Merchlinsky, M., Moss, B., NIAID, National Institutes of Health, Bethesda, Maryland: Resolution of recombinant plasmids containing the terminal sequences of vaccinia virus into replicating linear minichromosomes.

McFadden, G.,[1] Delange, A.M.,[2] Upton, C.,[1] Reddy, M.,[3] [1]Dept. of Biochemistry, University of Alberta, [2]Dept. of Human Genetics, University of Manitoba, Canada; [3]Dept. of Microbiology, State University of New York, Stony Brook: Mutational analysis of cloned poxvirus telomeres reveals conserved core sequences required for the replication and resolution of linear viral minichromosomes.

Reddy, M.,[1] McFadden, G.,[2] Bauer, W.,[1] [1]Dept. of Microbiology, State University of New York, Stony Brook; [2]Dept. of Biochemistry, University of Alberta, Canada: In vitro resolution of poxvirus telomeres.



G. McFadden, E. Paoletti, R. Moyer

Fathi, Z., Sridhar, P., Pacha, R., Condit, R., Dept. of Biochemistry, State University of New York, Buffalo: Efficient targeted insertion of an unselected marker into the vaccinia virus genome.

Spyropoulos, D.,[1] Panicali, D.,[2] Cohen, L.K.,[1,2] Roberts, B.E.,[1] [1]Dept. of Biological Chemistry, Harvard Medical School, Boston, [2]Applied bioTechnology, Inc., Cambridge, Massachusetts: In vivo recombination in vaccinia virus.

Archard, L.C.,[1] Kinchington, D.,[2] Rice, A.P.,[2] [1]Dept. of Biochemistry, Charing Cross and Westminster Medical School, [2]Imperial Cancer Research Fund Laboratories, London, England: Duplication of an orthopox virus gene by genomic rearrangement.

Ball, A., Biophysics Laboratory and Dept. of Biochemistry, University of Wisconsin, Madison: An approach to the isolation of recombination-deficient mutants of vaccinia virus.

Obom, K.M., Pogo, B.G.T., Center for Experimental Cell Biology and Dept. of Microbiology, Mount Sinai School of Medicine, New York, New York: Characterization of the transformation properties of Shope fibroma virus.

SESSION 8 VIRUS HOST INTERACTIONS. I.

Chairman: D. Hruby, Oregon State University

Wilton, S., Dales, S., Dept. of Microbiology and Immunology, University of Western Ontario, London, Canada: RNA polymerase II (Pol II) in vaccinia virus (VV) transcription and translation.

Hruby, D.E., Villarreal, E.C., Dept. of Microbiology, Oregon State University, Corvallis: Host cell nuclear involvement in vaccinia virus replication.

Chinchar, V.G.,[1] Caughman, G.B.,[2] [1]Dept. of Microbiology, University of Mississippi Medical Center, Jackson; [2]Dept. of Oral Biology and Microbiology, Medical College of Georgia, Augusta: Selective shutoff of equine herpesvirus type 1 protein synthesis by heat-inactivated frog virus 3.

Beaud, G.,[1] Person-Fernandez, A.,[1] Buendia, B.,[2] Madjar, J.J.,[2] [1]Institut J. Monod, Paris, [2]UFR de Médicine A. Carrel, Lyon, France: Shutoff of host protein synthesis and ribosomal protein phosphorylation by vaccinia virus.

Moussatché, N., Lobo, D.S., Damaso, C.R.A., Rebello, M.A., Instituto de Bio fisica Carlos Chagas Filho, Rio de Janeiro, Brazil: In vitro inhibition of protein synthesis induced by vaccinia virus is restored by ribonucleoside triphosphates.

Bablanian, R.,[1] Goswami, S.K.,[1] Esteban, M.,[1] Banerjee, A.K.,[2] [1]State University of New York Health Science Center, Brooklyn; [2]Roche Institute, Nutley, New Jersey: Mechanism of selective inhibition of protein synthesis by vaccinia virus—Role of poly A.

Paez, E., Esteban, M., Depts. of Biochemistry, Microbiology and Immunology, Health Science Center, Brooklyn, New York: Genetic and biological properties of spontaneous deletion mutants of vaccinia virus.

Paez, E., Esteban, M., Depts. of Biochemistry, Microbiology and Immunology, Health Science Center, Brookiyn, New York: Isolation and characterization of spontaneous mutants of vaccinia virus sensitive to inhibition by interferon.

Pacha, R., Condit, R., Dept. of Biochemistry, State University of New York, Buffalo: Precise genetic characterization of a vaccinia virus mutant (*ts*22) with an abortive late phenotype, and investigation of the relationship between *ts*22 and the effects of isatin-β-thiosemicarbazone (IBT).

Cohrs, R.J.,[1] Goswami, B.B.,[1] Condit, R.C.,[2] Pacha, R.F.,[2] Sharma, O.K.,[1] [1]Dept. of Molecular Biology, AMC Cancer Research Center, Denver, Colorado; [2]Dept. of Biochemistry, State University of New York, Buffalo: Synthesis of 2-5A during infection with a temperature-sensitive mutant of vaccinia virus (*ts*22) at nonpermissive temperature.

SESSION 9 VIRUS HOST INTERACTIONS. II.

Chairman: D. Moyer, Vanderbilt University

Novembre, F., Natuk, R., Holowczak, J.A., Dept. of Molecular Genetics and Microbiology, UMDNJ, Piscataway, New Jersey: The immune response to infection with vaccinia virus in mice.

Novembre, F., Holowczak, J.A., Dept. of Molecular Genetics and Microbiology, UMDNJ, Piscataway, New Jersey: The humoral response to infection with vaccinia virus in mice.

Alcamí, A., Carrascoca, A.L., Viñuela, E., Centro de Biología, Molecular (CSIC-UAM), Universidad Autónoma, Madrid, Spain: Role of receptor-mediated interaction in African swine fever virus (ASFV) infection of sensitive and resistant cells.

Spehner, D., Drillien, R., Transgène, Laboratoire de Virology, Strasbourg, France: Characterization of a cowpox gene required for multiplication in Chinese hamster ovary cells.

Chang, W.,[1] Upton, C.,[3] Hu, S.-L.,[2] Purchio, A.,[2] McFadden, G.,[3] [1]Dept. of Microbiology and Immunology, University of Washington, [2]Oncogen, Seattle, Washington; [3]Dept. of Biochemistry, University of Alberta, Canada: Cloning of an EGF-related growth factor from Shope fibroma virus.

Strayer, D.S., Leibowitz, J.L., University of Texas Health Science Center, Houston: Shope fibroma virus and malignant rabbit virus code for factors that inhibit epidermal growth factor-induced cellular proliferation.

Buller, R.M., Fredrickson, T.N., Chakrabarti, S., Moss, B., NIAID, National Institutes of Health, Bethesda, Maryland: Vaccinia virus growth factor is important for virus virulence.

Pogo, B.G.-T., Lai, A.C.-K., Holland, J.G., Friend, C., Center for Experimental Cell Biology and Dept. of Microbiology, Mount Sinai School of Medicine, New York, New York: Differences in the susceptibility of hematopoietic human cell lines to vaccinia virus.

Lai, A.C.-K., Obom, K.M., Holland, J.G., Pogo, B.G.-T., Friend, C., Center for Experimental Cell Biology and Dept. of Microbiology, Mount Sinai School of Medicine, New York, New York: Isolation and characterization of vaccinia virus mutants from persistently infected Friend erythroleukemia cells.

Dallo, S., Maa, J.-S., Esteban, M., Depts. of Biochemistry, Microbiology, and Immunology, Health Science Center, Brooklyn, New York: Isolation and characterization of attenuated mutants of vaccinia virus.

S. Dales, J. Holowczak, A. Granoff

R. Wittek, B. Moss

SESSION 10 VECTOROLOGY

Chairman: E. Paoletti, New York State Dept. of Health

Hruby, D.E., Wilson, E.M., Hodges, W.M., Franke, C.A., Dept. of Microbiology, Oregon State University, Corvallis: Development of alternative vaccinia virus expression vector systems.

Fuerst, T.R.,[1] Niles, E.G.,[2] Studier, F.W.,[3] Moss, B.,[1] [1]NIAID, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Biochemistry, State University of New York, Buffalo; [3]Dept. of Biology, Brookhaven National Laboratory, Upton, New York: Eukaryotic transient expression system based on recombinant vaccinia virus that synthesizes bacteriophage T7 RNA polymerase.

Shida, H.,[1] Hinuma, Y.I.,[1] Hatanaka, S.,[1] Ito, Y.,[1] Hayami, M.,[2] Sugimoto, M.,[3] Morita, M.,[4] [1]Institute for Virus Research, Kyoto University, [2]Institute of Medical Science, University of Tokyo, [3]Tao Nenryo Kogyo, K.K., [4]Chiba Serum Institute, Japan: Expression of the HTLV-1 envelope gene inserted in the hemagglutinin gene of vaccinia virus.

Ball, A.,[1,2] Ryan, T.,[1] King, A.,[3] Stott, J.,[3] Wertz, G.,[3] [1]Biophysics Laboratory, [2]Dept. of Biochemistry, University of Wisconsin, Madison; [3]Dept. of Microbiology and Immunology, University of North Carolina, Chapel Hill: Construction and analysis of a double vector—A recombinant vaccinia virus that expresses both the N and G genes of human respiratory syncytial virus.

Hruby, D.E.,[1] Thomas, G.,[2] Hodges, W.M.,[1] Herbert, E.,[2] [1]Dept. of Microbiology, Oregon State University, Corvallis; [2]Oregon Health Sciences Center, Portland: Use of vaccinia virus as a neuropeptide expression vector.

Edwards, S.J.,[1] Corcoran, L.M.,[2] Langford, C.J.,[2] [1]Commonwealth Serum Laboratories, [2]Walter and Eliza Hall Institute of Medical Research, Victoria, Australia: Expression of malaria antigens in recombinant vaccinia virus.

Belanger, L., Cormier, N., Destree, A., Dunn, J., Gritz, L., Hudson, G., Jensen, E., Katz, S., Mazzara, G., McKenzie, S., Panicali, D., Applied bioTechnology, Inc., Cambridge, Massachusetts: Expression of multiple pseudorabies glycoproteins in recombinant vaccinia virus.

Kost, T.A.,[1] Jones, E.V.,[2] Miller, T.J.,[1] Smith, K.M.,[1] Brown, A.L.,[1] [1]Norden Laboratories, Lincoln, Nebraska; [2]Smith Kline and French Molecular Genetics, Swedeland, Pennsylvania: Expression of pseudorabies gp50 from a recombinant vaccinia virus.

Esposito, J.,[1] Brechling, K.,[2] Baer, G.,[1] Auperin, D.,[1] McCormick, J.,[1] Kinney, R.,[1] Trent, D.,[1] Moss, B.,[2] [1]Centers for Disease Control, Atlanta, Georgia; [2]NIAID, National Institutes of Health, Bethesda, Maryland: Progress on vaccinia recombinant vaccines for rabies, Lassa fever, and Venezualan equine encephalitis.

Franke, C.A., Hruby, D.E., Dept. of Microbiology, Oregon State University, Corvallis: Vaccinia virus—The hitchhiker syndrome.

Molecular Neurobiology of *Drosophila*

October 6–October 9

ARRANGED BY

Corey Goodman, Stanford University
Gerald Rubin, University of California, Berkeley

134 participants

The neurobiology of *Drosophila*, initiated nearly two decades ago by Seymour Benzer (to whom our meeting was dedicated), has finally come of age. Kindled by the application of genetic approaches to neurobiology, and fueled by the infusion of modern recombinant DNA and moloclonal antibody approaches, the last few years have witnessed an enormous explosion of interest in the neurobiology of *Drosophila*. In this first meeting on the topic, the sessions were devoted to studies on neuronal development (neurogenesis, axon guidance, and synaptogenesis), to the visual system as a model system, to studies on receptors, channels, excitability, and synaptic transmission, and to studies on behavior, biological rhythms, and learning. The quality of the science presented was excellent, and the overall feeling of those that attended was that this is a very exciting field that is bursting with interesting mutations, genes, and molecules.

G. Rubin, C. Goodman, W. Quinn

SESSION 1 NEUROGENESIS

Chairman: S. Artavanis-Tsakonas, Yale University

Artavanis-Tsakonas, S., Grimwade, B., Hartley, D., Johansen, K.M., Preiss, A., Ramos, R., Wharton, K., Xu, T., Dept. of Biology, Yale University, New Haven, Connecticut: Molecular biology of neurogenesis.

Brand, M., Bremer, K., de la Concha, A., Dietrich, U., Jimenez, F., Knust, E., Technau, G.M., Tepass, U., Vässin, H., Weigel, D., Campos-Ortega, J.A., Institut für Entwicklungsphysiologie, Köln, Federal Republic of Germany: Genetics of early neurogenesis in *Drosophila*.

Muskavitch, M.A.T.,[1,2] Alton, A.K.,[1,2] Fechtel, K.,[2] Kopczynski, C.C.,[1,2] Shepard, S.B.,[2] Terry, A.L.,[2] Meikle, S.B.,[2] [1]Institute for Molecular and Cellular Biology, [2]Dept. of Biology, Indiana University, Bloomington: Molecular genetics of neurogenesis in *Drosophila*.

Mahowald, A., Furst, A., Perkins, L., Neumann, E., Huff, R., Perrimon, N., Dept. of Developmental Genetics and Anatomy, Case Western Reserve University, Cleveland, Ohio: Cellular, genetic, and molecular analyses of embryonic neurogenesis.

Thomas, J.B., Crews, S.T., Goodman, C.S., Dept. of Biological Sciences, Stanford University, California: Molecular genetics of the *S8* locus—A gene involved in the early events of neurogenesis in the *Drosophila* embryo.

Jan, Y.N.,[1] Bodmer, R.,[1] Jan, L.Y.,[1] Grell, E.,[1] Ghysen, A.,[2] Dambly-Chaudiere, C.,[2] [1]Howard Hughes Medical Institute and Dept. of Physiology, University of California, San Francisco; [2]Universite Libre de Bruxulles, Faculte des Sciences-Genetique, Belgium: Mutations affecting neurogenesis, neuronal identity, and path finding in *D. melanogaster*.

SESSION 2 AXON GUIDANCE AND SYNAPTOGENESIS

Chairman: C.S. Goodman, Stanford University

Patel, N.H., Snow, P.M., Goodman, C.S., Dept. of Biological Sciences, Stanford University, California: Cellular and molecular characterization of a cellsurface glycoprotein expressed on a subset of axons in the *Drosophila* embryo.

Schubiger, M., Blair, S.S., Palka, J., Dept. of Zoology, University of Washington, Seattle: Changing spatial patterns of DNA replication in developing wings—Are they related to axon guidance?

Murphey, R.K., Neurobiology Research Center, State University of New York, Albany: Mechanisms for the formation of selective synaptic connections in crickets and flies.

Meinertzhagen, I.A.,[1] Kral, K.,[2] [1]Dept. of Psychology, Dalhousie University, Halifax, Canada; [2]Institut für Zoologie, Universität Graz, Austria: Functional plasticity of the feedback synapses in the lamina ganglionaris of the fly, *M. domestica*.

Ghysen, A., Laboratory of Genetics, University Libre de Bruxelles, Belgium: Origin of peripheral nerves in *Drosophila*.

Steller, H., Rubin, G.M., Dept. of Biochemistry, University of California, Berkeley: disconnected—A locus required for neuronal pathway formation in the visual system of *Drosophila*.

SESSION 3 VISUAL SYSTEM

Chairman: S. Benzer, California Institute of Technology

Benzer, S., California Institute of Technology, Pasadena: Molecular genetics of visual system development.

Tomlinson, A., Ready, D.F., Dept. of Biology, Princeton University, New Jersey: *Drosophila* photoreceptors express neural antigens in a precise sequence.

Heisenberg, M., Wolf, R., Institut für Genetik und Mikrobiologie, Würzburg, Federal Republic of Germany: Synergistic effects of two mutant genes reducing cell number in the optic lobes.

Matsumoto, H., Ozaki, K., Isono, K., Randall, L., Floreani, M., Larrivee, D., Pye, Q., Pak, W.L., Dept. of Biological Sciences, Purdue University, West Lafayette, Indiana: *nina*—Class of mutations affecting photoreceptor-specific gene functions.

Montell, C., Zuker, C., Rubin, G., Dept. of Biochemistry, University of California, Berkeley: Molecular analysis of phototransduction—Identification and characterization of the genes encoding *trp*, *nina*C, and two R7-specific opsins.

Hotta, Y.,[1] Yoshioka, T.,[2] Inoue, H.,[2] [1]Dept. of Physics, Faculty of Science, University of Tokyo, [2]Dept. of Physiology, School of Medicine, Yokohama City University, Japan: Molecular and biochemical analyses of *Drosophila* visual behavior mutants.

SESSION 4 RECEPTORS, CHANNELS, EXCITABILITY. I.

Chairman: O. Siddiqi, Tata Institute

Siddiqi, O., Rodrigues, V., Ayyub, C., Molecular Biology Unit, Tata Institute of Fundamental Research, Bombay, India: Odor perception in *Drosophila*—A neurogenetic approach.

Wu, C.-F., Haugland, F., Sun, Y.-A., Komatsu, A., Dept. of Biology, University of Iowa, Iowa City: K^+ channels in *Drosophila*

Solc, C., O'Dowd, D., Zagotta, W., Germeraad, S., Aldrich,

R., Dept. of Neurobiology, Stanford University, California: Gating of A-type K^+ channels and Na^+ channels in wild-type and mutant *Drosophila* neurons and muscle cells.

Bowers, C., Branton, D., Lee, P., Phillips, H., Jan, Y.N., Jan, L.Y., Howard Hughes Medical Institute and Dept. of Physiology, University of California, San Francisco: Studies of neuronal excitability and ion channels in *Drosophila*.

Tanouye, M.A., Division of Biology, California Institute of Technology, Pasadena: The Shaker locus.

Ferrús, A., Llamazares, S., Canal, I., de la Pompa, J.L., Yuste, R., Centro de Biologia Molecular, Universidad Autónoma de Madrid, Spain: Biological functions of the Shaker complex.

SESSION 5 RECEPTORS, CHANNELS, EXCITABILITY. II.

Chairman: B. Ganetzky, University of Wisconsin

Ganetzky, B., Loughney, K., Kreber, R., Laboratory of Genetics, University of Wisconsin, Madison: Genetic and molecular analysis of the *para* locus.

Salkoff, L.,[1] Butler, A.,[1] Hiken, M.,[1] Wei, A.,[1] Giffen, K.,[1] Ifune, C.,[1] Goodman, R.,[2] Mandel, G.,[2] [1]Dept. of Anatomy and Neurobiology, Washington School of Medicine, St. Louis, Missouri; [2]Dept. of Medicine, Tufts New England Medical Center, Boston, Massachusetts: A *Drosophila* gene with homology to the vertebrate Na^+ channel.

Hall, L.M.,[1] Kasbekar, D.P.,[1] Gil, D.W.,[1] Keen, J.K.,[1] Urquhart, D.,[1] Nelson, J.C.,[2] Jackson, F.R.,[3] [1]Dept. of Genetics, Albert Einstein College of Medicine, Bronx, New York; [2]Dept. of Biology, Yale University, New Haven, Connecticut; [3]Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Molecular and genetic analysis of voltage-sensitive Na channels from *D. melanogaster*.

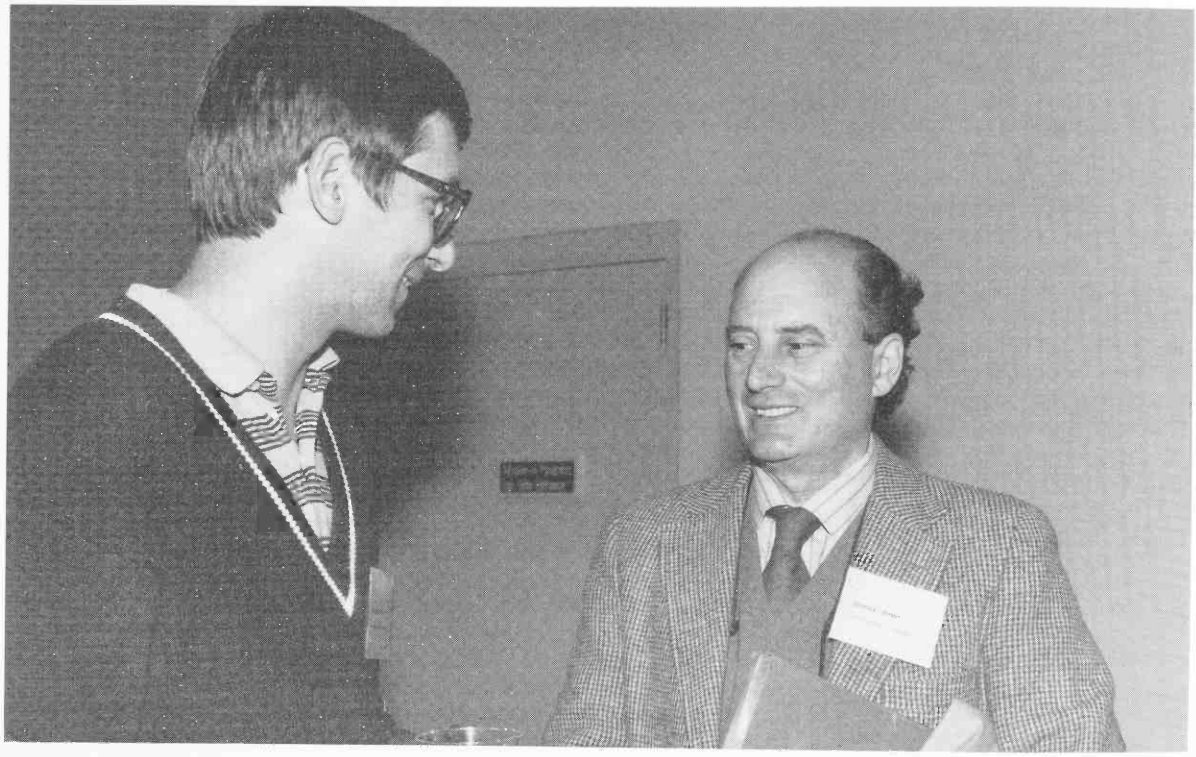
Nelson, J.C., Wyman, R.J., Dept. of Biology, Yale University, New Haven, Connecticut: *Drosophila* neurological mutations do not block the giant fiber pathway.

Hermans-Borgmeyer, I., Sawruk, E., Zopf, D., Gundelfinger, E., Betz, H., Zentrum für Molekulare Biologie, Heidelberg, Federal Republic of Germany: Characterization of the mRNA and the gene of a putative neuronal nicotinic acetylcholine receptor protein from *D. melanogaster*.

Bossy, B.,[1] Hall, L.M.C.,[1] Ballivet, M.,[2] Spierer, P.,[1] Depts. of [1]Molecular Biology, [2]Biochemistry, University of Geneva, Switzerland: Molecular genetics of the cholinergic synapse of *Drosophila*.

Salvaterra, P.M.,[1] Itoh, N.,[1] Maines, V.,[1] Slemmon, R.,[1] Mori, N.,[2] [1]Division of Neurosciences, [2]Dept. of Biology, Beckman Research Institute of the City of Hope, Duarte, California: *Drosophila* choline acetyltransferase—Molecular biology and evolution.

Gnagey, A.L., Forte, M., Rosenberry, T.L., Dept. of Pharmacology, Case Western Reserve University, Cleveland, Ohio: Isolation and characterization of acetylcholinesterase from *Drosophila*.



H. Stellar, S. Benzer

SESSION 6 BEHAVIOR, RHYTHMS, LEARNING

Chairman: J. Hall, Brandeis University

Yu, Q., Abovich, N., Citri, Y., Colot, H., Ewer, J., Hamblen, M., Lorenz, L., Petersen, G., Siwicki, K., Wheeler, D., Hall, J., Rosbash, M., Dept. of Biology, Brandeis University, Waltham, Massachusetts: Molecular studies on biological rhythms in *D. melanogaster.*

Young, M.W.,[1] Bargiello, T.,[1] Jackson, F.R.,[2] [1]Rockefeller University, New York, New York; [2]Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Abundance of *per* locus product sets the pace of the *Drosophila* clock.

Smith, R.F., Choi, K.-W., Mardon, G., Tully, T., Quinn, W.G., Dept. of Brain and Cognitive Science and Biology, Massachusetts Institute of Technology, Cambridge: Deficient protein-kinase-C activity in turnip, a *Drosophila* learning mutant.

Dudai, Y., Buxbaum, J., Corfas, G., Orgad, S., Segal, D., Sher, B., Shilo, B., Yovell, Y., Weizmann Institute of Science, Rehovot, Israel: Role of the *rut* gene in neuronal and behavioral plasticity.

Chen, C.-N., Takayasu, H., Davis, R.L., Dept. of Biochemistry, Michigan State University, East Lansing: Molecular characterization of the dunce locus.

Gabor Miklos, G.L.,[1] Kelly, L.,[2] Coombe, P.,[1] Davies, J.,[1] De Couet, G.,[1] Pirrotta, V.,[3] Yamamoto, M.,[1] Schalet, A.,[4] Lefevre, G.,[5] [1]Research School of Biological Sciences, Australian National University, Canberra; [2]Dept. of Genetics, University of Melbourne, Australia; [3]Baylor College of Medicine, Houston, Texas; [4]Dept. of Radiation Genetics, State University of Leiden, The Netherlands; [5]California State University, Northridge: Mapping, characterizing, and cloning behavioral genes in a small region at the base of the X chromosome.

Tompkins, L., Dept. of Biology, Temple University, Philadelphia, Pennsylvania: Regulation of female- and male-specific sexual behaviors by the sex-lethal gene.

POSTERS

Yedvobnick, B., Smoller, D., Dept. of Biology, Emory University, Atlanta, Georgia: Transposon tagging of the neurogenic locus mastermind.

Knust, E., Dietrich, U., Weigel, D., Tepass, U., Vässin, H., Bremer, K., Campos-Ortega, J.A., Entwicklungsphysiologie, Köln, Federal Republic of Germany: Molecular genetics of neurogenic genes in *Drosophila*.

Technau, G.M., Campos-Ortega, J.A., Institut für Entwicklungsphysiologie der Universität, Köln, Federal Republic of Germany: Lineage studies of singly transplanted ectodermal cells of the *Drosophila* embryo.

Johansen, K.M.,[1] Slaughter, C.,[2] Artavanis-Tsakonas, S.,[1] [1]Dept. of Biology, Yale University, New Haven, Connecticut; [2]Dept. of Biochemistry, University of Texas, Dallas: Characterization and localization of the embryonic Notch product in *D. melanogaster* using antibodies.

Hartley, D., Dept. of Biology, Yale University, New Haven, Connecticut: Spatial pattern of Notch gene expression during *D. melanogaster* embryogenesis.

Hoppe, P.E., Greenspan, R.J., Dept. of Biology, Princeton University, New Jersey: Localized requirement for Notch gene product in embryonic neural-epidermal determination.

Alton, A.K.,[1] Terry, A.L.,[2] Meikle, S.B.,[2] Muskavitch, M.A.T.,[1,2] [1]Institute of Molecular and Cellular Biology, [2]Dept. of Biology, Indiana University, Bloomington: Genetic analysis of a chromosomal interval including Delta, a neurogenic gene in *D. melanogaster.*

Smouse, D.,[1] Perrimon, N.,[2] Mahowald, A.,[2] Goodman, C.,[1] [1]Dept. of Biological Sciences, Stanford University, California; [2]Dept. of Developmental Genetics and Anatomy, Case Western Reserve University, Cleveland, Ohio: topless—A new class of zygotic mutations that causes hypertrophy of the nervous system in the *Drosophila* embryo.

Hayashi, I., Division of Cytogenetics, City of Hope National Medical Center, Duarte, California: Function of nerve growth factor in early nervous system development.

Beachy, P.A., Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: Regulatory targets of a protein encoded by the homeotic locus Ultrabithorax.

Helfand, S.L.,[1] Lipshitz, H.D.,[2] Hogness, D.S.,[2] [1]Dept. of Biology, Yale University, New Haven, Connecticut; [2]Dept. of Biochemistry, Stanford University, California: Regulation of *Ubx* expression in the CNS.

Schneiderman, A.M., Tao, M.L., Wyman, R.J., Dept. of Biology, Yale University, New Haven, Connecticut: Transformation of identified muscle and its motorneuron in bithorax *Drosophila.*

Schneuwly, S.,[1] Gehring, W.J.,[2] [1]Dept. of Biological Sciences, Purdue University, West Lafayette, Indiana; [2]Dept. of Cell Biology, Biozentrum der Universitaet Basel, Switzerland: Molecular and functional analysis of the homeotic gene Antennapedia in *D. melanogaster.*

Doe, C.Q.,[1] Hiromi, Y.,[2] Gehring, W.,[2] Goodman, C.S.,[1] [1]Dept. of Biological Sciences, Stanford University, California; [2]Biozentrum, University of Basel, Switzerland: Expression and function of fushi tarazu during neurogenesis.

Hiromi, Y., Gehring, W.J., Biocenter, University of Basel, Switzerland: Regulation of fushi tarazu gene expression during *Drosophila* embryogenesis.

Dambly-Chaudière, C.,[1] Ghysen, A.,[1] Jan, Y.N.,[2] Jan, L.Y.,[2] [1]Laboratory of Genetics, Libre de Bruxelles, Belgium; [2]Howard Hughes Medical Institute, University of California, San Francisco: Genetics of the determination of larval sense organs.

Campos, A.R., Fleming, R.J., Robinow, S., Rosen, D., White, K., Brandeis University, Waltham, Massachusetts: Molecular analysis of *ewg* and *elav* loci.

O. Siddiqi, E. Macagno

Taylor, B.J., Dept. of Biology, University of California, San Diego, La Jolla: Sexually dimorphic neurons in the genital disk of *D. melanogaster*.

Stocker, R.F., Gendre, N., Institute of Zoology, University of Fribourg, Switzerland: lozenge – A mutant in *Drosophila* devoid of basiconic antennal sensilla.

Katz, F.,[1] Moats, W.,[1] Jan, Y.N.,[2] Howard Hughes Medical Institute, [1]University of Texas Health Science Center, Dallas; [2]University of California, San Francisco: Mutations in the development of the imaginal nervous system of *D. melanogaster*.

Zinn, K., McAllister, L., Snow, P., Harrelson, A., Bastiani, M., Goodman, C., Dept. of Biological Sciences, Stanford University, California: Molecular characterization of fasciclin I, a cell-surface glycoprotein expressed on a subset of axon pathways in the insect embryo.

Baird, D.,[1] Aceves, E.,[1] Wyman, R.,[1] Davies, J.,[2] Miklos, G.,[2] [1]Dept. of Biology, Yale University, New Haven, Connecticut; [2]Pop. Biology, Australian National University, Canberra: Genetics and cloning of Passover, a mutant with altered neural connectivity.

Caudy, M., Jan, Y.N., Jan, L.Y., Howard Hughes Medical Institute, University of California, San Francisco: Pioneer growth cone guidance by segment boundary epithelium in *Drosophila* embryos.

Ready, D.F., Dept. of Biology, Princeton University, New Jersey: Segmental nerves, a connection between segments and parasegments?

Burg, M.G., Wu, C.-F., Dept. of Biology, University of Iowa, Iowa City: Effects of excitability on the development, projection, and arborization of mechanosensory cells in *Drosophila* mosaics.

Costello, W.J., Hummon, M.R., Dept. of Zoology and Biomedical Science/COM, Ohio University, Athens: Coated vesicles and formation of adult motor systems in *Drosophila*.

Anderson, H., Dept. of Zoology, University of California, Davis: Pioneer neurons use basal lamina as a substrate for axon outgrowth.

Fessler, L.I., Campbell, A.G., Stern, R., Fessler, J.H., Molecular Biology Institute and Biology Dept., University of California, Los Angeles: Basement membranes of embryonic nervous system as seen by immunofluorescence with antibodies made against *Drosophila* laminin and entactin.

Banerjee, U., Renfranz, P., Benzer, S., Division of Biology, Caltech, Pasadena, California: Molecular cloning of the sevenless gene.

Bernstein, L.B., Larison, K.D., Venkatesh, T.R., Institutes of Molecular Biology and Neuroscience, University of Oregon, Eugene: P-element-induced mutations that perturb the structure and development of the photoreceptor cluster of the *Drosophila* compound eye.

Ballinger, D., Benzer, S., Division of Biology, Caltech, Pasadena, California: Photophobe – A mutation that causes negative phototaxis in *Drosophila*.

O'Tousa, J.E.,[1] Leonard, D.S.,[2] Pak, W.L.,[2] Dept. of Biological Sciences, [1]University of Notre Dame, [2]Purdue University, West Lafayette, Indiana: Morphological defects in *ora*^{JK84} photoreceptors caused by mutation in R1-6 opsin gene of *Drosophila*.

Zuker, C.,[1] Ozaki, K.,[2] Rubin, G.M.,[1] Pak, W.L.,[2] [1]Dept. of Biochemistry, University of California, Berkeley; [2]Dept. of Biological Sciences, Purdue University, West Lafayette, Indiana: Physiological effects of deleting potential phosphorylation sites from *ninaE,* R1-6 opsin gene.

Carlson, J., Helfand, S., Sun, H., Dept. of Biology, Yale University, New Haven, Connecticut: Molecular and genetic analysis of olfaction.

Hasan, G., Ayyub, C., Molecular Biology Unit, Tata Institute of Fundamental Research, Bombay, India: P-M hybrid dysgenesis-induced olfactory mutants in *Drosophila*.

Mistri, R., Siddiqi, O., Molecular Biology Unit, Tata Institute

of Fundamental Research, Bombay, India: A sex-specific olfactory protein detected by a monoclonal antibody in *D. melanogaster*.

Jones, K., Steller, H., Rubin, G., Dept. of Biochemistry, University of California, Berkeley: Molecular characterization of two paralytic mutations.

Royden, C.S., Jan, L.Y., Dept. of Physiology, University of California, San Francisco: Molecular analysis of the *tko* locus in *D. melanogaster*.

Singh, S.,[1] Wu, C.-F.,[1] Ganetzky, B.,[2] [1]Dept. of Biology, University of Iowa, Iowa City; [2]Laboratory of Genetics, University of Wisconsin, Madison: Roles of different membrane currents in muscle excitability in normal and mutant *Drosophila* larvae.

Solc, C., Aldrich, R., Dept. of Neurobiology, Stanford University, California: Whole-cell and single-channel patch-clamp analysis of larval *Drosophila* CNS neurons.

Pongs, O.,[1] Baumann, A.,[1] Kecskemethy, N.,[1] Seidel, R.,[1] Krah, I.,[1] Wenzel, B.,[1] Müller, R.,[1] Weber, K.,[1] Ferrus, A.,[2] [1]Lehrstuhl für Biochemie, Ruhr-Universität Bochum, Federal Republic of Germany; [2]Instituto de Biologia Molecular, Madrid, Spain: Molecular genetic analysis of the Shaker complex in *Drosophila*.

Tempel, B., Papazian, D., Schwarz, T., Jan, Y.N., Jan, L.Y., Howard Hughes Medical Institute and Dept. of Physiology, University of California, San Francisco: Molecular analysis of Shaker, a genetic locus that affects A current.

Timpe, L.C., Jan, L.Y., Howard Hughes Medical Institute and Dept. of Physiology, University of California, San Francisco: Gene dosage and complementation analysis of the Shaker locus in *Drosophila*.

Wei, A., Salkoff, L., Dept. of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri: Twinning of K^+ channels and occult Ca^{++} channels in *Drosophila*.

Gil, D.W., Keen, J.K., Hall, L.M., Dept. of Genetics, Albert Einstein College of Medicine, Bronx, New York: Identification of four *Drosophila* sequences homologous to the voltage-sensitive Na channel gene from electric eel.

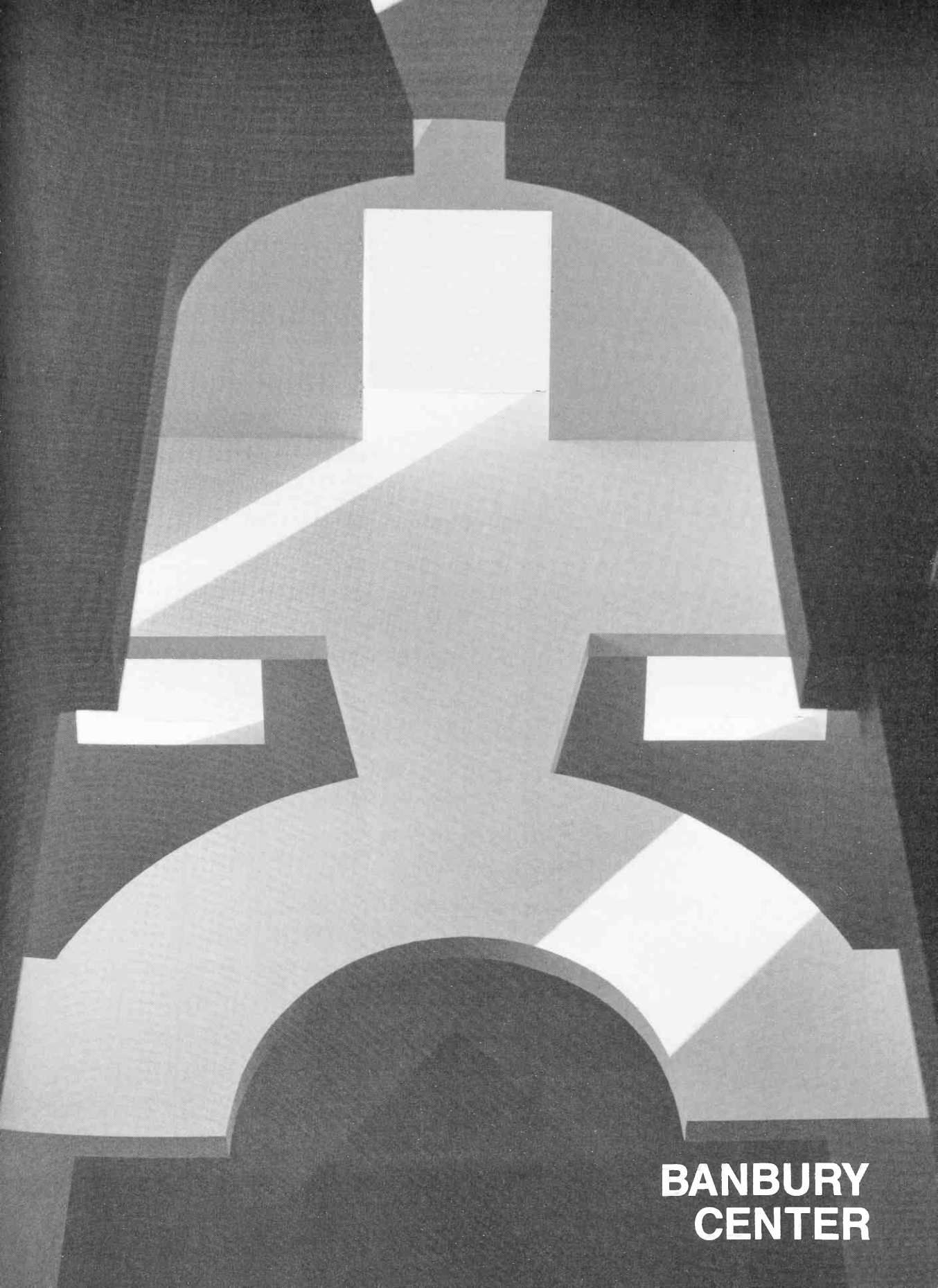
Scholnick, S.B., Morgan, B.A., Bray, S.J., Johnson, W.A., Beall, C.J., McCormick, C.A., Hirsh, J., Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Dual regulatory modes for expression of the *D. melanogaster Ddc* gene in the CNS.

Neckameyer, W.S., Quinn, W.G., Dept. of Brain and Cognitive Sciences and Biology, Massachusetts Institute of Technology, Cambridge: Molecular cloning of a tyrosine hydroxylase gene in *D. melanogaster*.

Healy, M.J., Davis, R.L., Dept. of Biochemistry, Michigan State University, East Lansing: Studies with antibodies directed against the cAMP phosphodiesterase of *D. melanogaster*.

Konopka, R., Dept. of Biology, Clarkson University, Potsdam, New York: Variegating clock mutants of *D. melanogaster*.

Cobb, M., Jallon, J.-M., Laboratoire de Biologie et Génétique Evolutives, CNRS, France: Pheromonal signals in *Drosophila* species recognition.


BANBURY
CENTER

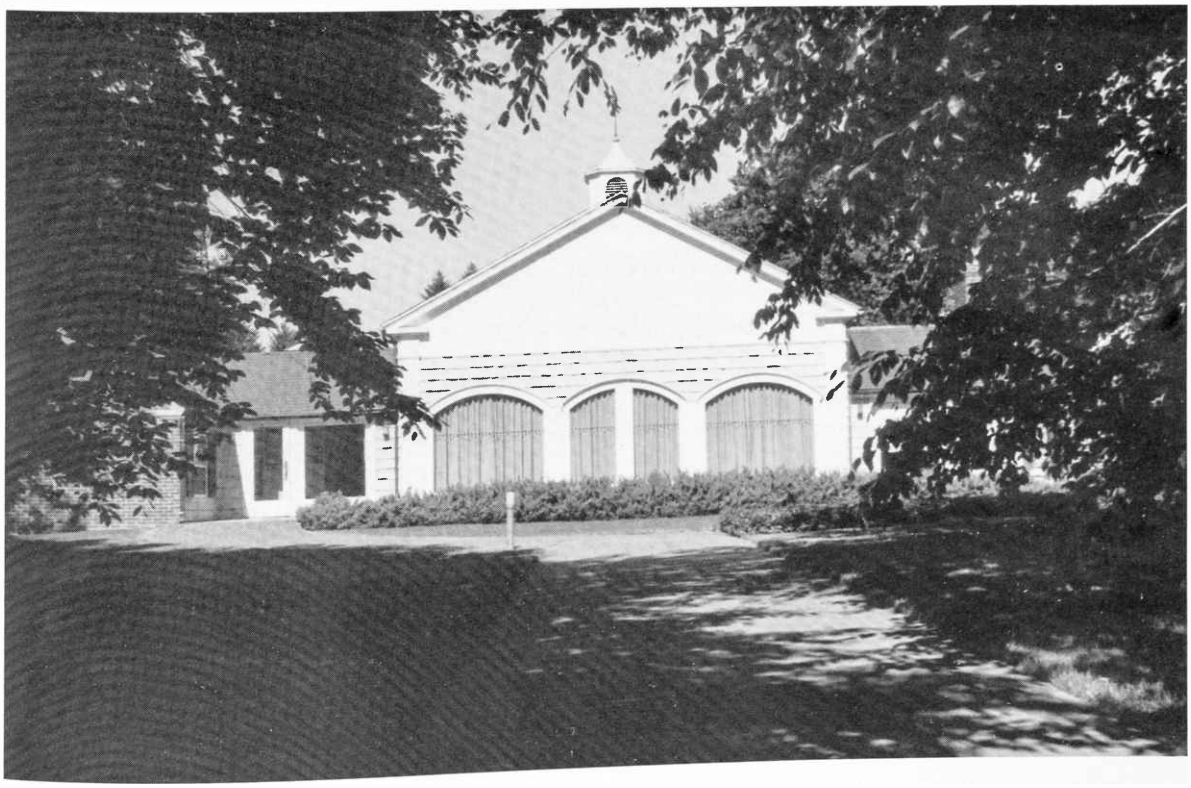
BANBURY CENTER DIRECTOR'S REPORT

The ninth year of the Banbury Center's operation saw the continuation of the Center's well-established programs, along with indications of novel directions for our meetings and publications in the next few years. Fourteen meetings were held and, as in previous years, the Center was the location for four advanced lecture courses.

A New Program on the Origins and Detection of Human Cancer

Among the newer aspects of the Center's activities is one that promises to be particularly important for the future—a collaboration with the Preventive Medicine Institute/Strang Clinic of New York City. PMI/Strang has agreed to fund a series of three meetings a year on the Origins and Detection of Human Cancer. These meetings will build on the Center's well-established reputation as a leading location for cancer-related meetings and as a prime source of publications on such topics.

In addition, we hope to bring information from certain Banbury meetings to a larger audience. Our established range of books (discussed below) has an excellent reputation for reporting the data and views of the small groups of about 35 leading scientists who attend each meeting. Such books will continue to play a vital role in disseminating research results to specialists who were not at the meeting. However, it is clear that much of the information discussed at our



Banbury Meeting House

meetings would be very useful and interesting to less-specialized readers, if it were presented in an appropriate form. Therefore, it has been decided that the Center will begin to publish booklets written for nonspecialist scientists and clinicians. Such booklets will be written in-house and will be based on those Banbury meetings that have especially broad implications.

This new source of funding from PMI/Strang Clinic came only a year after the $300,000 grant over three years from the James S. McDonnell Foundation. As expected, this grant has greatly facilitated the Center's ability to organize first-rate meetings at a time when grants from federal agencies have become both scarcer and smaller.

Risk Assessment Program

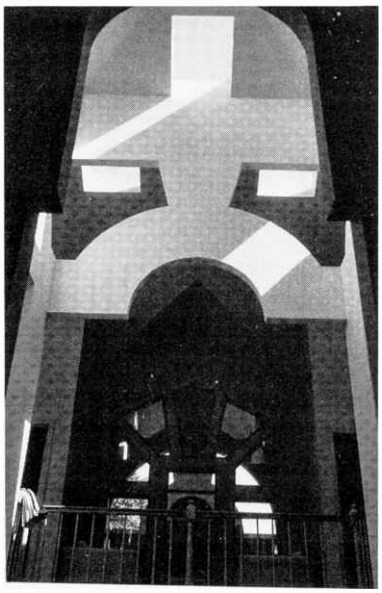
In 1986, four meetings were held within the Risk Assessment Program. This program forms the original core of the Center's activities and has lost none of its importance since the addition of various other programs.

The first meeting in the program concerned Antibiotic Resistance Genes, a topic of great concern, given the ever-increasing use of antibiotics in medicine and animal farming, which has already led to a worrying spread of antibiotic-resistant microbes. The next meeting, on Nongenotoxic Mechanisms in Carcinogenesis, examined the multitude of ways in which known carcinogens interact with cells, apart from their effects on DNA. The most complex problem is to distinguish between the primary and secondary effects of carcinogens. Clearly, this aspect of research will continue to require further intensive study before definitive answers can be obtained.

The first fall meeting was entitled Mechanistic Approaches to Developmental Toxicology. The molecular mechanisms by which certain chemicals interfere with normal development in mammals are now becoming understood in much greater detail. The experts at this meeting reviewed recent results and addressed the vitally important possibility of predicting deleterious effects of new chemicals in medicines or in the environment. The final meeting of the year, the Neurochemistry of Aging, was particularly felicitously timed. Here, several groups reported major advances in our knowledge of the genes and proteins implicated in Alzheimer's disease.

Finally, a workshop closely related to our Risk Assessment program, but organized independently by the National Institute of Environmental Health Sciences, was held in October on DNA Adducts.

Interior of Sammis Hall



Corporate Sponsor Program Continues Its Emphasis on the Applications of Science

The Corporate Sponsor program had another active year. These meetings, which address issues of particular interest to molecular biologists and biotechnologists, have proved to be increasingly popular. Like all other Banbury meetings, invitations to attend are almost invariably accepted—a fact that is very gratifying when one bears in mind that leaders in science receive a steady stream of invitations to meetings that take them away from their laboratories.

The three spring meetings in the Corporate Sponsor series focused on Microbial Energy Transduction, Mechanisms of Yeast Recombination, and DNA Probes. The first addressed fundamental questions about the methods that microorganisms use to convert the energy they receive from nutrients (and, in

some cases, from light) into forms that can be used to maintain their metabolism. The second meeting reviewed the latest knowledge about the molecular mechanisms by which genetic information is recombined and modified in yeasts. Meetings in the Corporate Sponsor series are intended to have a particularly strong emphasis on the application of molecular biology to human health. This aim was very well exemplified by the DNA Probes meeting, at which there were many reports on the ways that diseases can be diagnosed and better understood by examining certain parts of an individual's genetic constitution. Particular interest was also generated by reports on "genetic fingerprinting," a method of examining DNA that can precisely distinguish one individual from any other.

Both of the fall meetings also had very clear applied aspects. Participants at the Tumor Angiogenesis meeting examined newly discovered clues about the manner in which blood is supplied to cancers. Obviously, the hope is that some way will be found to starve tumors of the blood they need to survive. The meeting on Gene Transfer Vectors brought a valuable interchange of knowledge concerning new methods of introducing DNA into cells, one of the crucial steps in all forms of genetic engineering.

The third main facet of the Center's activities consists of the meetings funded by the Alfred P. Sloan Foundation grant, first received in 1980. The 1986 Workshop for Congressional Aides examined the problems caused by radon in homes. Exposure to radon gas, which collects in some homes as it seeps out of the ground, is now thought to be a very significant health hazard, perhaps responsible for as many as 10,000 cases of lung cancer a year in the United States. The experts at this meeting discussed the geographical distribution of radon-rich materials in the ground, the methods by which it can accumulate in houses, the biological effects of high concentrations of radon, and methods for making houses safer. An unusual combination of approaches, ranging from radiation biology through epidemiology to civil engineering, made for an especially stimulating and useful workshop. The aides, many of whom work for members of Congress who represent areas with a major radon problem, received a day and a half of "expert testimony" in an informal setting. Equally importantly, they were able to provide information on the political and economic aspects of the issue.

In keeping with Banbury's purpose of promoting the application of knowledge, chief executive officers of major corporations spent a weekend in October meeting eight of the country's leading scientists. During this meeting, which was organized in collaboration with Shearson Lehman Brothers Inc., the company representatives were able to learn more about the future impact of biological research on their businesses.

Finally, the staff of the Banbury Center was particularly pleased to welcome an assembly of people connected with the Esther A. and Joseph Klingenstein Fund, Inc. The Fund has provided generous support to many younger neuroscientists; this meeting brought them together with the trustees and scientific advisors of the Fund to exchange information on their research. The Banbury Center of Cold Spring Harbor Laboratory was an especially appropriate site for such a gathering, since the Klingenstein Fund has been very generous in its support for the Center and the Laboratory over many years.

Banbury Publications

As noted elsewhere in this Annual Report (see Publications, under Departmental Reports), the organization of Banbury's publications program has been modified. To ensure that the books and other types of publications arising from future

Banbury meetings are published as efficiently and rapidly as possible, the Banbury editorial activities are being more closely integrated into the larger publications department at Cold Spring Harbor Laboratory. This will eliminate some duplication of effort while retaining a full-time editor at the Center.

Departure of Michael Shodell

In March 1986, Michael Shodell left the Center after four years as its Director. Mike's enthusiasm and knowledge had been instrumental in expanding Banbury's activities and consolidating the Center's international reputation. Furthermore, his efforts in raising support have been very important in putting the Center's finances on a sound foundation. We wish him well in his return to a career of teaching at C.W. Post College of Long Island University and scientific writing.

Steve Prentis

Robertson House provides housing and dining accommodations at Banbury Center

MEETINGS

Microbial Energy Transduction: Genetics, Structure and Function

February 23–February 26

ARRANGED BY

D. C. Youvan, Cold Spring Harbor Laboratory, New York

SESSION 1 REACTION CENTER

Chairperson: S. G. Boxer, Stanford University, California

H. Michel, Max-Planck Institut für Biochemie, Munich, Federal Republic of Germany: Structure of the photosynthetic reaction center from *Rhodopseudomonas viridis*.

W. Parson, University of Washington, Seattle: Fast electron transfer steps in photosynthetic reaction centers.

W. Lubitz, Freie Universität Berlin, Federal Republic of Germany: Structural aspects of primary reactants in bacterial reaction centers studied by ENDOR spectroscopy.

B. Marrs, E. I. du Pont de Nemours & Company, Wilmington, Delaware: Molecular genetics in *Rhodopseudomonas capsulata*.

J. T. Beatty, University of British Columbia, Vancouver, Canada: Regulation of expression of the *rxcA* operon of *Rhodopseudomonas capsulata*.

C. Arntzen, E. I. du Pont de Nemours & Company, Wilmington, Delaware: Genetic analysis of PSII polypeptides.

SESSION 2 LIGHT HARVESTING ANTENNAE

Chairperson: S. Kaplan, University of Illinois, Urbana

A. N. Glazer, University of California, Berkeley: Phycobilisomes—Relationship of structure to energy flow dynamics.

R. Huber, Max-Planck Institut für Biochemie, Munich, Federal Republic of Germany: Crystal structural studies of cyanobacterial C-phycocyanins and functional aspects.

D. A. Bryant, Pennsylvania State University, University Park: Genetic analysis of the cyanobacterial phycobilisome.

R. J. Cogdell, University of Glasgow, Scotland: The structure and function of purple bacteria antenna complexes.

H. Zuber, Institut für Molekularbiologie und Biophysik, ETH, Zurich, Switzerland: Structural principles and variability of light-harvesting antennae.

SESSION 3 OXIDOREDUCTASE AND TERMINAL OXIDASE

Chairperson: P. L. Dutton, University of Pennsylvania Medical School, Philadelphia

G. von Jagow, University of Munich, Federal Republic of Germany: Structural and functional diversity of microbial and mitochondrial ubiquinol–Cytochrome *c* reductase.

B. L. Trumpower, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: Purification and properties of a three subunit cytochrome bc_1 complex from *Paracoccus denitrificans* grown under aerobic and denitrifying conditions.

D. Robertson, University of Pennsylvania Medical School, Philadelphia: Quinones and electrogenic reactions of the bc_1 complex.

R. Prince, Exxon Research and Engineering Company, Annandale, New Jersey: Genetic and biophysical approaches to elucidating the mechanism of the cytochrome bc_1 complex.

R. B. Gennis, University of Illinois, Urbana: Structure and function of the *E. coli* cytochrome *d* terminal oxidase.

T. G. Frey, University of Pennsylvania School of Medicine, Philadelphia: Cytochrome *c* oxidase–Structure and function.

SESSION 4 ATP AND LIGHT DRIVEN PROTON PUMPS

Chairperson: R. M. Pearlstein, Indiana-Purdue University, Indianapolis

P. Pedersen, Johns Hopkins University School of Medicine, Baltimore, Maryland: Structure of F_1 ATPase-metal binding.

H. S. Penefsky, Public Health Research Institute, New York, New York: Mechanism of action of mitochondrial ATPase.

J. E. Walker, MRC Laboratory of Molecular Biology, Cambridge, England: Genes for ATP synthases from photosynthetic bacteria, chloroplasts, and mitochondria.

R. H. Fillingame, University of Wisconsin, Madison: Mutants and function of *E. coli* H^+-ATP synthase.

C. W. Slayman, Yale University Medical School, New Haven, Connecticut: H^+-ATPase of the *Neurospora* plasma membrane.

SESSION 5 TRANSPORT AND CHEMOTAXIS

Chairperson: M. D. Kamen, University of California, San Diego, La Jolla

M. Saier, University of California, San Diego, La Jolla: The bacterial phosphotransferase system–Structure, evolution, and mechanisms of action.

H. R. Kaback, Roche Institute of Molecular Biology, Nutley, New Jersey: Passage to permease.

R. M. Macnab, Yale University, New Haven, Connecticut: Bacterial flagellar motor.

G. R. Moe, University of California, Berkeley: Transmembrane signaling through the aspartate receptor.

M. I. Simon, California Institute of Technology, Pasadena: The role of the receptor in signal transduction.

Mechanisms of Yeast Recombination

March 9–March 12

ARRANGED BY

A. Klar, Cold Spring Harbor Laboratory, New York

SESSION 1 RECOMBINATION HOT SPOT. I

Chairperson: J. R. Broach, Princeton University, New Jersey

M. M. Cox, University of Wisconsin, Madison: The FLP protein of the yeast 2-micron plasmid.

P. D. Sadowski, University of Toronto Faculty of Medicine, Ontario, Canada: The FLP site-specific recombinase of the 2-micron circle of yeast.

J. R. Broach, Princeton University, New Jersey: Function and mechanism of site-specific recombination in yeast plasmids.

M. Jayaram, Research Institute of Scripps Clinic, La Jolla, California: Cuts, gaps, and recombination.

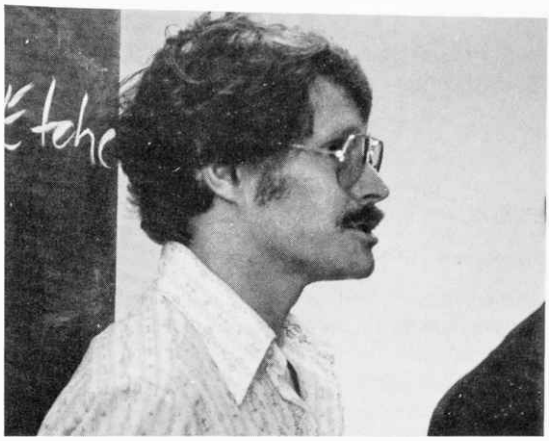
R. A. Butow, University of Texas Health Science Center, Dallas: Mitochondrial DNA recombination.

S. Roeder, Yale University, New Haven, Connecticut: A recombination-stimulating sequence in the yeast ribosomal RNA gene cluster.

SESSION 2 RECOMBINATION HOT SPOT. II

Chairperson: A. Klar, Cold Spring Harbor Laboratory, New York

H. Gutz, Technische Universität, Braunschweig, Federal Republic of Germany: (a) Switching genes in *Schizosaccharomyces pombe*; (b) DNA rearrangements in the mating-type region of *Schizosaccharomyces pombe*.
R. Egel, University of Copenhagen, Denmark: Asymmetries in mating-type switching in *Schizosaccharomyces pombe*.
A. Klar, Cold Spring Harbor Laboratory, New York: Developmental switches of the fission yeast mating-type locus.
J. N. Strathern, Frederick Cancer Research Facility, National Cancer Institute, Maryland: Mating-type switching in *Saccharomyces cerevisiae*.
F. Heffron, Research Institute of Scripps Clinic, La Jolla, California: Recombination in yeast is increased near a synthetic HO recognition sequence.
F. W. Stahl, Massachusetts Institute of Technology, Cambridge: Double-strand breaks—Thinking about them in phage and fungi.



J. Strathern

SESSION 3 *REC* GENES AND THEIR PRODUCTS

Chairperson: M. Esposito, University of California, Berkeley

R. E. Esposito, University of Chicago, Illinois: Genes controlling meiotic recombination.
P. J. Hastings, University of Alberta, Edmonton, Canada: Screening for recombination-defective mutants with a positive selection system for plasmid excision.
M. A. Resnick, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Sister chromatid and meiotic recombination.
R. Malone, University of Iowa, Iowa City: Initiation of meiotic gene conversion in yeast.
M. Esposito, University of California, Berkeley: *Rec* mutants and their DNA-binding proteins.

SESSION 4 GENETIC CONSEQUENCES OF RECOMBINATION

Chairperson: F. W. Stahl, Massachusetts Institute of Technology, Cambridge

H. Klein, New York University Medical Center, New York: Recombination between repeated genes.
T. D. Petes, University of Chicago, Illinois: Generation of translocations by meiotic recombination.
J. Haber, Brandeis University, Waltham, Massachusetts: Physical monitoring of recombination events in mitosis and meiosis.
J. Wallace, Columbia University College of Physicians & Surgeons, New York, New York: Genetic control of repeat sequence recombination.
S. Fogel, University of California, Berkeley: Recent molecular-genetic studies on meiotic recombination.
L. Roman, University of Washington, Seattle: Gene conversion and associated recombination in heterozygous vs. heteroallelic diploid cells.

SESSION 5 PHYSICAL CONSEQUENCES OF RECOMBINATION

Chairperson: J. N. Strathern, Frederick Cancer Research Facility, NCI, Frederick, Maryland

S. Kunes, Massachusetts Institute of Technology, Cambridge: Synapsis-dependent mechanism of illegitimate recombination.
D. Kaback, UMDNJ-New Jersey Medical School, Newark: Is there distributive pairing in yeast?
R. Kolodner, Dana Farber Cancer Institute, Boston, Massachusetts: Genetic recombination and mismatch correction catalyzed by cell-free extracts of yeast.
D. J. Garfinkel, Frederick Cancer Research Facility, NCI, Frederick, Maryland: Retrotransposition of Ty elements.
A. Nicolas, Massachusetts General Hospital, Boston: Comparison of yeast and *Ascobolus* recombination.

Evolution and Environmental Spread of Antibiotic Resistance Genes

March 31–April 3

ARRANGED BY

S. B. Levy, Tufts University School of Medicine, Boston, Massachusetts
R. P. Novick, The Public Health Research Institute of the City of New York, New York

SESSION 1A BREADTH OF ENVIRONMENTAL INTERSPECIES GENE EXCHANGE IN THE NATURAL ENVIRONMENT

Chairperson: P. Voytek, U.S. Environmental Protection Agency, Washington, D.C.

T. O'Brien, Brigham and Women's Hospital, Boston, Massachusetts: Global surveillance of the deployment of antibiotic resistance genes and plasmids.

S. B. Levy, Tufts University School of Medicine, Boston, Massachusetts: Ecology of antibiotic resistance determinants.

B. E. Murray, University of Texas Medical School, Houston: Plasmid-mediated penicillinase in enterococci.

F. White, Kansas State University, Manhattan: The exchange of genetic material between higher plants and *Agrobacterium*.

B. R. Levin, University of Massachusetts, Amherst: Population biology of plasmids and transposons—Gene exchange in natural environments.

L. Chao, Northwestern University, Evanston, Illinois: Correlations between antibiotic resistances.

SESSION 1B MICROBIAL ECOLOGY AND GENE EXCHANGE

Chairperson: B. Wiedemann, University of Bonn, Federal Republic of Germany

R. G. Freter, University of Michigan, Ann Arbor: Parameters that are important in the colonization of natural habitats by bacteria, using the large intestine as an example, and the relation between colonization and plasmid transfer.

W. Witte, Institut für Experimentelle Epidemiologie, Wernigerode, German Democratic Republic: Occurrence, development, and spread of antibiotic resistance in *Staphylococcus aureus*, studied in man and in animal husbandry.

P. Smith, University of Galway, Ireland: Uptake of antibiotic resistance plasmid during antibiotic therapy in a salmon hatchery.

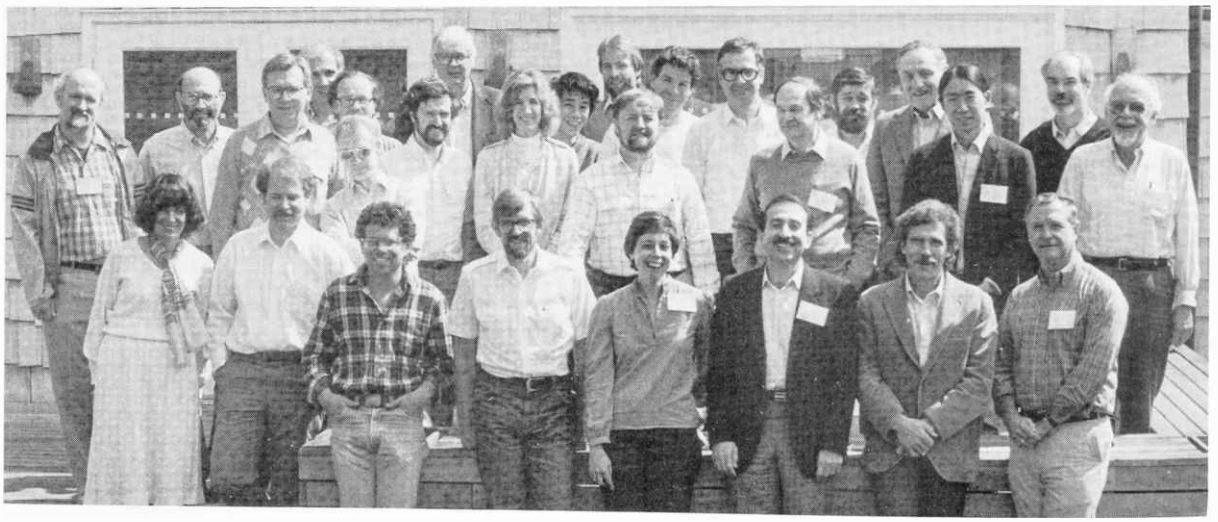
SESSION 2 MOLECULAR MECHANISMS OF GENE TRANSFER

Chairperson: J. R. Scott, Emory University School of Medicine, Atlanta, Georgia

W. Paranchych, University of Alberta, Edmonton, Canada: Comparison of the transfer genes of F-like conjugative plasmids.

D. B. Clewell, University of Michigan, Ann Arbor: Conjugation in streptococci.

H. O. Smith, Johns Hopkins University School of Medicine, Baltimore, Maryland: Genetic transformation in *H. influenzae*.



SESSION 3 FACTORS AFFECTING THE FATE OF TRANSFERRED GENES

Plasmid Replication

Chairperson: D. Sherratt, University of Glasgow, Scotland

J. R. Scott, Emory University School of Medicine, Atlanta, Georgia: Replication regulation in the plasmid prophage P1.
K. Nordstrom, University of Uppsala, Sweden: Molecular aspects on control of replication of plasmid R1.
D. R. Helinski, University of California, San Diego, La Jolla: Regulation of replication of the narrow-host-range plasmid R6K and the broad-host-range plasmid RK2.
M. Bagdasarian, Umea University, Sweden: Proteins required for the broad-host-range mode of replication by the plasmid RSF 1010.
R. H. Rownd, Northwestern University, Chicago, Illinois: IncFII plasmid replication control and stable inheritance.
R. P. Novick, The Public Health Research Institute of the City of New York, New York: Host-specific factors affecting plasmid maintenance.

Plasmid Stability and Partitioning

Chairperson: R. P. Novick, The Public Health Research Institute of the City of New York, New York

S. N. Cohen, Stanford University School of Medicine, California: Chromosomal and extrachromosomal functions that affect plasmid stability in *E. coli*.
D. Sherratt, University of Glasgow, Scotland: Novel recombination mechanisms in the maintenance and propagation of plasmid genes.
S. Molin, The Polytechnical University, Copenhagen, Denmark: Plasmid stabilization in populations of cells.
S. Austin, Frederick Cancer Research Facility, NCI, Frederick, Maryland: The *cis*-acting site responsible for the partition of P1 miniplasmids.

Plasmid and Gene Expression

Chairperson: D. Dubnau, The Public Health Research Institute of the City of New York, New York

J. C. Rabinowitz, University of California, Berkeley: Determinants of transcription and translation in gram-positive microorganisms.
G. C. Walker, Massachusetts Institute of Technology, Cambridge: Plasmid biology of pkM101—The role of the *mucAB* genes.
M. Bibb, John Innes Institute, Norwich, England: Gene expression in the streptomyces.

SESSION 4 ORIGIN AND EVOLUTION OF GENES AND GENE TRANSFER SYSTEMS

Chairperson: S. B. Levy, Tufts University School of Medicine, Boston, Massachusetts

B. Wiedemann, University of Bonn, Federal Republic of Germany: Gene alterations leading to resistance to β-lactam antibiotics.
P. M. Bennett, University of Bristol, England: Transposition and plasmid evolution—Variations on a theme.
D. Dubnau, The Public Health Research Institute of the City of New York, New York: Regulation and evolution of MLS resistance.
S. Harayama, University of Geneva, Switzerland: Mechanisms of and constraints to laboratory evolution of plasmid-specified metabolic pathways.

Nongenotoxic Mechanisms in Carcinogenesis

April 13–April 16

ARRANGED BY

B. E. Butterworth, Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina
T. J. Slaga, University of Texas System Cancer Center, Smithville

SESSION 1 PROMOTION

Chairperson: A. Sivak, Arthur D. Little, Inc., Cambridge, Massachusetts

T. J. Slaga, University of Texas System Cancer Center, Smithville: Skin tumor promotion.
J. DiGiovanni, University of Texas System Cancer Center, Smithville: Studies on the skin-tumor-promoting action(s) anthrone derivatives.
H. C. Pitot, University of Wisconsin Medical School, Madison: Liver tumor promotion.
S. M. Cohen, University of Nebraska Medical Center,

Omaha: Bladder tumor promotion.
B. F. Trump, University of Maryland School of Medicine, Baltimore: Calcium cell injury and tumor promotion.

SESSION 2 FORCED CELL PROLIFERATION

Chairperson: D. S. R. Sarma, University of Toronto, Ontario, Canada

W. Parzefall, University of Vienna, Austria: Measurement and role of stimulation of liver growth in hepatocarcinogenesis.
R. H. Reitz, Dow Chemical Company, Midland, Michigan: Regenerative growth of liver following toxic injury and its role in hepatocarcinogenesis.
D. J. Loury, Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina: The value of measuring cell replication as a predictive index of tissue-specific carcinogenic potential.
E. D. Wachsmuth, Ciba-Geigy AG, Basel, Switzerland: Chemically induced cell turnover in the kidney and its possible role in carcinogenesis.
J. A. Swenberg, Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina: The influence of cytotoxicity in the appearance of induced and spontaneous tumors.

SESSION 3 RODENT BIOASSAYS

Chairperson: P. N. Magee, Temple University School of Medicine, Philadelphia, Pennsylvania

P. M. Newberne, Massachusetts Institute of Technology, Cambridge: Nongenotoxic mouse liver carcinogens.
A. K. Ghoshal, University of Toronto, Ontario, Canada: The induction of liver cancer by dietary deficiency without added carcinogens.
F. J. C. Roe, London, England: The problem of pseudocarcinogenicity in rodent bioassays.

SESSION 4 SOLID STATE CARCINOGENESIS

Chairperson: H. C. Pitot, University of Wisconsin Medical School, Madison

K. G. Brand, Timmendorfer Strand, Federal Republic of Germany: Solid-state carcinogenesis.
M. M. Coombs, Imperial Cancer Research Fund Laboratories, London, England: Biogenic silica fibers and skin cancer.
H. Heck, Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina: The induction of calculi and hyperplasia in weanling rats by terephthalic acid and dimethyl terephthalate—An apparent threshold.

SESSION 5 EXAMPLES OF NONGENOTOXIC CARCINOGENS

Chairperson: H. S. Rosenkranz, Case Western Reserve University, Cleveland, Ohio

W. F. Greenlee, Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina: TCDD—Mechanisms of growth regulation and their potential role in carcinogenicity.
B. E. Butterworth, Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina: DEHP.
R. L. Anderson, The Procter & Gamble Company, Cincinnati, Ohio: The mechanism of nitrilotriacetate (NTA)-associated urinary tract tumorigenesis.

SESSION 6 CELL CULTURE MODELS

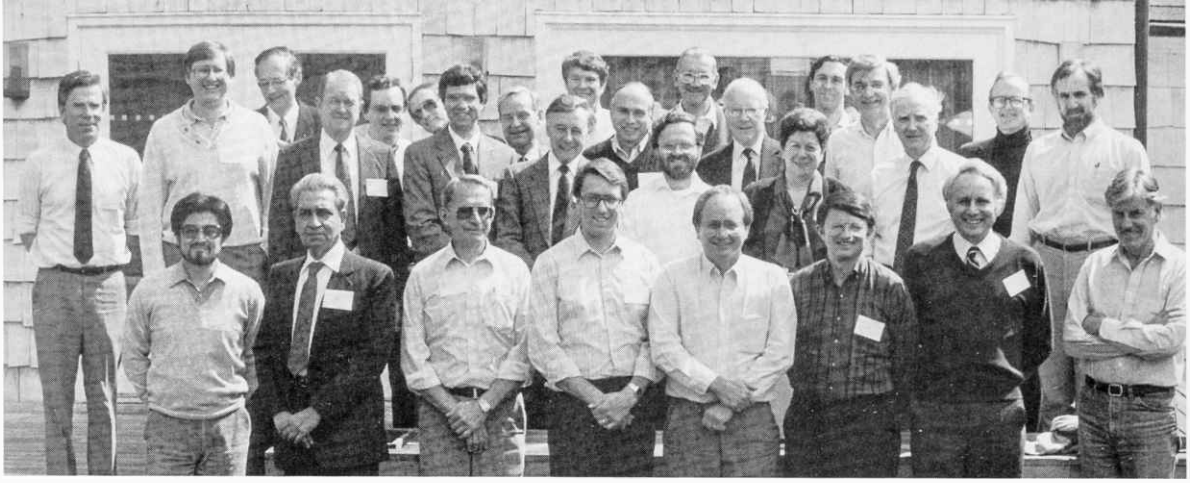
Chairperson: L. Diamond, Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania

Craig J. Boreiko, Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina: Modulation of transformed focus formation in cultures of C3H/10T1/2 cells.
H. Yamasaki, International Agency for Research on Cancer, Lyon, France: The role of cell-to-cell communication in promotion.
J. C. Barrett, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Epigenetic and genetic mechanisms of presumed nongenotoxic carcinogens.
P. A. Cerutti, Swiss Institute for Experimental Cancer Research, Lausanne: Mechanisms of action of prooxidant promoters.

SESSION 7 REGULATORY CONSIDERATIONS

Chairperson: D. E. Stevenson, Shell Development Company, Houston, Texas

R. W. Tennant, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Some implications of National Toxicology Program genetic toxicity test results on chemicals tested in rodents for carcinogenicity and noncarcinogenicity.
H. E. Scribner, Rohm and Haas Company, SpringHouse,



Pennsylvania: Practical approaches to evaluating nongenotoxic carcinogens.

G. N. Williams, American Health Foundation, Valhalla, New York: Definition of human cancer risk.

I. B. Weinstein, Columbia University College of Physicians & Surgeons, New York, New York: Uniform risk assessment policy for all carcinogens.

Applications of DNA Probes

April 20–April 23

ARRANGED BY

L. S. Lerman, Genetics Institute, Cambridge, Massachusetts

SESSION 1 HUMAN GENETIC DISEASE AND CHROMOSOME MAPPING. I

K. E. Davies, John Radcliffe Hospital, Oxford, England: Diagnosis of Duchenne muscular dystrophy, X-linked phosphataemic rickets, and X-linked mental retardation.

L. M. Kunkel, Children's Hospital Medical Center, Boston, Massachusetts: Molecular genetics of Duchenne muscular dystrophy.

C. T. Caskey, Baylor College of Medicine, Houston, Texas: Molecular basis on origin of Lesch-Nyhan mutations.

J.-L. Mandel, Faculté de Medicine de Strasbourg, France: DNA probes and linkage analysis in the region of the fragile X-mental retardation locus.

S. A. Latt, Children's Hospital Medical Center, Boston, Massachusetts: Use of DNA probes to study chromosome deletions and amplification.

T. B. Shows, Roswell Park Memorial Institute, Buffalo, New York: Mapping chromosome II and cancer gene markers.

D. W. Russell, University of Texas Health Science Center, Dallas, Texas: The molecular genetics of familial hypercholesterolemia.

SESSION 2 HUMAN GENETIC DISEASE AND CHROMOSOME MAPPING. II

U. Francke, Yale University School of Medicine, New Haven, Connecticut: Detection of microdeletions with bytogenetics and DNA probes.

A. J. Jeffreys, University of Leicester, England: Hypervariable DNA and genetic "fingerprints."

R. L. White, University of Utah School of Medicine, Salt Lake City: Linkage maps of human chromosomes.

G. Ruvkun, Massachusetts General Hospital, Cambridge: Polymorphism mapping using repetitive elements.

H. Lehrach, European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: Molecular approaches to mammalian genetics.

F. S. Collins, University of Michigan, Ann Arbor: Chromosome hopping.

H. Donis-Keller, Collaborative Research, Lexington, Massachusetts: Application of RFLP probes to genetic mapping and clinical diagnosis in humans.

SESSION 3 PHYSICAL SEPARATIONS, NEW PROBE TECHNOLOGY, AND MICROBIOLOGY. I

J. W. Gray, Lawrence Livermore National Laboratory, Livermore, California: Flow cytogenetics—
(1) Chromosome classification and purification;
(2) production of chromosome-specific recombinant DNA libraries; (3) chromosome-specific fluorescence DNA-DNA hybridization.

C. Smith, Columbia University College of Physicians & Surgeons, New York, New York: Macrorestriction

mapping by pulsed-field gel electrophoresis.
M. Olson, Washington University School of Medicine, St. Louis, Missouri: Electrophoretic separations of large DNA molecules.
R. W. Davis, Stanford University Medical Center, California: Separation and mapping of large-molecular-weight DNA by alternating homogeneous electric fields.
D. C. Ward, Yale University, New Haven, Connecticut.
H. A. Erlich, Cetus Corporation, Emeryville, California: Genetic analysis using enzymatic amplification of specific genomic consequences.
A. T. Haase, University of Minnesota Medical Center, Minneapolis: Analysis of infections and pathological conditions by in situ hybridization.

L. Lerman

SESSION 4 PHYSICAL SEPARATIONS, NEW PROBE TECHNOLOGY, AND MICROBIOLOGY. II

M. Ranki, Orion Genetic Engineering Laboratory, Helsinki, Finland: Nucleic acid sandwich hybridization: Methodology and applications to microbial diagnosis.
R. B. Wallace, Beckman Research Institute/City of Hope, Duarte, California: Synthetic DNA probes.
R. M. Myers, University of California, School of Medicine, San Francisco: Assays for detecting single base changes in cloned and genomic DNA.
M. Collins, Genetics Institute, Cambridge, Massachusetts: DNA strand displacement—A novel diagnostic approach.
A. E. Smith, Integrated Genetics, Framingham, Massachusetts: Development of diagnostic DNA probes.
F. C. Tenover, Veterans Administration Medical Center, Seattle, Washington: Use of DNA probes for epidemiologic studies of antibiotic resistance genes.
L. S. Lerman, Genetics Institute, Cambridge, Massachusetts: Helic stability and genetic analysis.

SESSION 5 VIRUSES, CANCER, AND PARASITES

L. Gissmann, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Human papillomavirus DNA in genital cancer.
M. A. Israel, National Cancer Institute, Bethesda, Maryland: Molecular approaches to the diagnosis of cancer.
J. Sklar, Stanford University School of Medicine, California: DNA arrangements in lymphoid neoplasia and their application to diagnosis.
D. H. Spector, University of California, San Diego, La Jolla: Molecular analysis of cytomegalovirus infections.
D. F. Wirth, Harvard School of Public Health, Boston, Massachusetts: DNA probes in the detection of parasitic infections.

K. Davies, A. Jeffreys

Gene Transfer Vectors for Mammalian Cells

October 14–October 17

ARRANGED BY

J. H. Miller, University of California, Los Angeles

SESSION 1

Mutagenesis

M. P. Calos, Stanford University School of Medicine, California: Analysis of mutation in human cells using shuttle vectors.
J. H. Miller, University of California, Los Angeles, and Phaik Mooi-Leong, Yale University, New Haven, Connecticut: Comparison of mutagenesis in bacterial and mammalian cells.
K. Dixon, National Institute of Child Health and Human

Development, Bethesda, Maryland: Use of a SV40-based shuttle vector to analyze spontaneous and UV-induced mutations arising in mammalian cells.

M. Seidman, Otsuka Pharmaceutical Co., Ltd., Rockville, Maryland: Mutagenesis of a shuttle vector plasmid in normal human and xeroderma cells.

F. Hutchinson, Yale University, New Haven, Connecticut: Mutagenesis in an *E. coli gpt* gene stably incorporated in the genome of CHO cells.

N. Drinkwater, University of Wisconsin, Madison: Use of EBV shuttle vectors for analysis of mutagenesis in human cells.

SESSION 2

Mutagenesis (continued)

E. Dogliotti, Massachusetts Institute of Technology, Cambridge: Construction of shuttle vectors for studying the genetic effects of defined chemical carcinogen-DNA base adducts in mammalian cells.

V. M. Maher, Michigan State University, East Lansing: Kinds of mutations formed when a shuttle vector containing adduct of BPDE replicates in human cells.

M. Hoekstra, Scripps Clinic and Research Foundation, La Jolla, California: A method of transposon mutagenesis in yeast.

Bacterial Vectors

N. Pagratis, University of Chicago, Illinois: Construction of broad-host-range expression vectors for bacteria.

Vaccinia

B. Moss, National Institutes of Health, Bethesda, Maryland: Vaccinia virus vectors.

Adenovirus

Y. Gluzman, Cold Spring Harbor Laboratory, New York: Adenovirus vectors.

M. Calos, Y. Gluzman

SESSION 3

Mammalian Vectors

Retroviruses

C. Cepko, Harvard Medical School, Boston, Massachusetts: Gene transfer into primary neural cells using retrovirus vectors.

J. Dougherty, University of Wisconsin, Madison: Retrovirus vectors and their variation.

BPV

M. Botchan, University of California, Berkeley: (1) Negative regulation of papillomavirus replicon. (2) Use of retroviruses for the study of DNA tumor virus transformation.

G. N. Pavlakis, N.C.I.-Frederick Cancer Research Facility, Maryland: Applications of BPV and retroviral vectors.

Insect Baculovirus

G. Ju, Hoffmann-La Roche Inc., Nutley, New Jersey: Use of an insect baculovirus vector system.

SESSION 4

EBV

A. J. Levine, Princeton University, New Jersey: Epstein-Barr virus plasmids.

Expression in Mammalian Cells

C. Gorman, Genentech, Inc., South San Francisco, California: Factor VIII expression in mammalian cells.

N. Sarver, Meloy Laboratories, Springfield, Virginia: Expression of complete and abridged F.III using BPV shuttle vectors.

L. McConlogue, Cetus Corporation, Emeryville, California: Amplification vector based on ODCase.

T. V. Ramabhadran, Monsanto Company, St. Louis, Missouri: Host cell-specific variation in the posttranslational processing of engineered proteins.

R. Kaufman, Genetics Institute, Cambridge, Massachusetts: Translational control in transfected mammalian cells.

J. Miller

SESSION 5

Gene Therapy

D. St. Louis, The Salk Institute, San Diego, California: Transfer of genes in whole animals.

Plants

J. Schell, University of Gent, Belgium: Regulation of expression of genes introduced in plants.

Transgenic Mice

S. Camper, The Institute for Cancer Research, Philadelphia, Pennsylvania: Developmental regulation of the α-fetal protein gene in transgenic mice.

G. Lozano, Princeton University, New Jersey: Regulation of expression of SV40 early genes in transgenic mice.

Mechanistic Approaches to Developmental Toxicology

October 19–October 22

ARRANGED BY

J. A. McLachlan, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina

R. M. Pratt, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina

C. L. Markert, North Carolina State University, Raleigh

SESSION 1 IN VITRO APPROACHES

Chairperson: R. M. Pratt, National Institute of Environmental Sciences, Research Triangle Park, North Carolina

N. Bournais-Verdiabasis, City of Hope Medical Center, Duarte, California: Altered differentiation and induction of heat-shock proteins in *Drosophila* embryonic cells associated with teratogen treatment.

A. Braun, Massachusetts Institute of Technology, Cambridge: Teratogen metabolism.

T. H. Shepard, University of Washington, Seattle: Whole embryo culture—Normal and abnormal development.

L. Saxen, University of Helsinki, Finland: Renal development in vitro.

Discussants: R. E. Morrissey, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, and F. Welsch, Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina.

SESSION 2 MOLECULAR AND EXPERIMENTAL EMBRYOLOGY

Chairperson: J. A. McLachlan, National Institutes of Environmental Health Sciences, Research Triangle Park, North Carolina

R. Pedersen, University of California, San Francisco: Cell lineage of mammalian germ layers.

P. M. Iannacone, Northwestern University, Chicago, Illinois: Models of organogenesis based on mosaic pattern analysis in chimeric rats.

M. Levine, Columbia University, New York, New York: Spatial regulation of homeobox gene expression in *Drosophila*.

G. Edelman, Rockefeller University, New York, New York: Role of cell recognition in normal development.

C. L. Markert, North Carolina State University, Raleigh: Are heat-shock proteins involved in development?

Discussants: J. G. Scandalios, North Carolina State University, Raleigh, and L. Saxen, University of Helsinki, Finland.

J. M. Rice, N.C.I.-Frederick Cancer Research Center, Maryland: Mechanisms of transplacental carcinogenesis: Mutation, oncogene activation, and tumor promotion.

SESSION 3 NON-MAMMALIAN MODELS

Chairperson: C. L. Markert, North Carolina State University, Raleigh

J. G. Scandalios, North Carolina State University, Raleigh: Expression of developmentally regulated genes in maize, under normal and stressed conditions.

E. M. Johnson, Jefferson Medical College, Philadelphia, Pennsylvania: Patterns of developmental toxicity evaluated in hydra "embryos."

M. Solursh, University of Iowa, Iowa City: Studies on normal and abnormal differentiation of the chick limb mesenchyme.

T. D. Sabourin, Battelle Columbus Division, Ohio: Comparative evaluation of a short-term test for development effects using frog embryos.

Discussants: D. Kochhar, Jefferson Medical College, Philadelphia. Pennsylvania, and L. Dencker, University of Uppsala, Sweden.

SESSION 4 EXPERIMENTAL ANIMAL-HUMAN COMPARISONS

Chairperson: R. L. Brent, Jefferson Medical College, Philadelphia, Pennsylvania

R. M. Pratt, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Receptor-dependent mechanisms of retinoid-induced craniofacial malformations.

E. J. Lammer, Massachusetts General Hospital, Boston: Patterns of malformations among fetuses and infants exposed to retinoic acid (isotretinoin) in utero.

J. A. McLachlan, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Mechanisms for induction of differentiation defects associated with diethylstilbestrol.

A. F. Haney, Duke University Medical Center, Durham, North Carolina: Structural and functional malformations in humans exposed in utero to diethylstilbestrol.

Discussants: W. J. Scott, Jr., Children's Hospital Medical Center, Cincinnati, Ohio, and R. Miller, University of Rochester School of Medicine, New York

R. L. Brent, Jefferson Medical College, Philadelphia, Pennsylvania: Etiology of unknown causes of birth defects.

SESSION 5 RISK ASSESSMENT

Chairperson: G. P. Oakley, Centers for Disease Control, Atlanta, Georgia

J. Manson, Smith, Kline and French Laboratories, Philadelphia, Pennsylvania: Biological considerations for risk estimation in developmental toxicology.

J. Springer, Food and Drug Administration, Washington, D.C.: Regulatory perspectives of teratogenic risk assessment.

N. Kaplan, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Quantification of risk in teratology.

J. Hanson, University of Iowa, Iowa City: Teratogen information systems and their use in assessment of human risk.

Discussants: C. A. Kimmel, U.S. Environmental Protection Agency, Washington, D.C., and G. P. Oakley, Centers for Disease Control, Atlanta, Georgia.

Angiogenesis

November 2-November 5

ARRANGED BY

D. B. Rifkin, New York University Medical Center, New York
M. Klagsbrun, Children's Hospital Medical Center, Boston, Massachusetts

SESSION 1 FGF (OR HBGF); PROTEIN STRUCTURE, GENE STRUCTURE AND RECEPTORS. I

Chairperson: P. A. D'Amore, Children's Hospital Medical Center, Boston, Massachusetts

M. Klagsbrun, Children's Hospital Medical Center, Boston, Massachusetts: Opening remarks.

K. A. Thomas, Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey: Structure and activities of acidic fibroblast growth factor.

M. Petitou, Institut Choay, Paris, France: A heparin hexasaccharide fragment able to bind to anionic endothelial cell growth factor—Preparation and structure.

M. Klagsbrun, Children's Hospital Medical Center, Boston, Massachusetts: Multiple forms of basic FGF.

J. C. Fiddis, California Biotechnology Inc., Mountain View: Genes for the angiogenic growth factors—Basic and acidic FGF.

A. Sommer, Synergen, Inc., Boulder, Colorado: Primary structure of human basic fibroblast growth factor derived from protein and cDNA sequencing.

SESSION 2 FGF (OR HBGF); PROTEIN STRUCTURE, GENE STRUCTURE AND RECEPTORS. II

Chairperson: R. D. Rosenberg, Massachusetts Institute of Technology, Cambridge

T. Maciag, Biotechnology Research Center, Rockville, Maryland: Endothelial cell growth factor and its receptor.

Y. Courtois, INSERM, Paris, France: Modulation of growth factor (EDGFs and FGFs) activity and fixation in development and pathogenesis of the eye.

D. Barritault, Université Paris, France: Eye-derived growth factors—Early events and receptor studies.

D. Moscatelli, New York University Medical Center, New York: Presence of basic fibroblast growth factor in a variety of cells and its binding to cells.

I. Vlodavsky, Hadassah University Hospital, Jerusalem, Israel: Heparin-binding growth factors produced by normal and malignant cells are sequestered and stabilized by the subendothelial extracellular matrix.

SESSION 3 ANGIOGENESIS FACTORS

Chairperson: T. Maciag, Biotechnology Research Center, Rockville, Maryland

P. E. DiCorleto, Cleveland Clinic Institute, Ohio: Production of PDGF-like protein by endothelial cells.

A. B. Roberts, National Cancer Institute, Bethesda, Maryland: Type-β-transforming growth factor—Stimulator or inhibitor of angiogenesis?

C. Haudenschild, Boston University School of Medicine, Massachusetts: Nonpeptide angiogenesis factors.

J. Castellot, Harvard University Medical School, Boston, Massachusetts: Differentiation-dependent stimulation of angiogenesis by 3T3-adipocyte.

S. Kumar, Christie Hospital and Holt Radium Institute, Manchester, England: Hyaluronic acid and angiogenesis.

M. J. Banda, University of California, San Francisco: Regulation of endothelial cell metalloproteinase activity.

SESSION 4 ANGIOGENESIS INHIBITORS, BIOLOGY OF VASCULAR CELLS

Chairperson: D. B. Rifkin, New York University Medical Center, New York

J. Folkman, Children's Hospital Medical Center, Boston, Massachusetts: Inhibitors of angiogenesis—Angiogenic steroids.

D. B. Rifkin, New York University Medical Center, New York: Studies on the regulation of protease activity during cell invasion and angiogenesis.

B. M. Glaser, Johns Hopkins Hospital, Baltimore, Maryland: Retinal pigment epithelial cell release inhibitors of neovascularization.

P. A. D'Amore, Children's Hospital Medical Center, Boston, Massachusetts: Role of pericytes in microvascular growth control.

P. Bohlen, University of Zurich, Switzerland: Inhibitors of endothelial cell proliferation.

R. Auerbach, University of Wisconsin, Madison: Endothelial cell specificity and angiogenesis.

D. Rifkin

SESSION 5 ANGIOGENESIS IN VIVO

Chairperson: J. Folkman, Children's Hospital Medical Center, Boston, Massachusetts

W. Risau, Max-Planck Institut, Tubingen, Federal Republic of Germany: Regulation of blood-vessel development.

M. M. Sholley, Virginia Commonwealth University, Richmond: Proliferation and migration of irradiated endothelial cells.

J. M. Davidson, Vanderbilt University School of Medicine, Nashville, Tennessee: Wound repair, growth factors, and connective tissue metabolism.

D. R. Knighton, University of Minnesota Hospital, Minneapolis: Environmental regulation of macrophage angiogenesis.

H. M. Jensen, University of California School of Medicine, Davis: Angiogenesis induced by "normal" human breast tissue—A probable marker for precancer.

J. Folkman, Children's Hospital Medical Center, Boston, Massachusetts: Closing remarks.

Congressional Workshop on Radon in the Home

November 12–November 14

ARRANGED BY

S. Prentis, Cold Spring Harbor Laboratory, New York

SESSION 1

J. Harley, Hoboken, New Jersey: Introduction/history/ measurement procedures.
B. L. Cohen, Department of Physics, University of Pittsburgh, Pennsylvania: Distribution of exposures in the United States.

SESSION 2

N. H. Harley, Department of Environmental Medicine, New York University, New York: Epidemiology and risk estimates.
J. T. Tappan, ARIX Sciences Incorporated, Grand Junction, Colorado: Mitigation procedures and results.

SESSION 3

E. Hotte, New Jersey Department of Environmental Protection, Trenton: State responses to the radon problem.
R. Guimond, Office of Radiation Programs, U.S. Environmental Protection Agency, Washington, D.C.: Federal responses to the radon problem.

The Neurochemistry of Aging

November 30–December 3

ARRANGED BY

P. Davies, Albert Einstein College of Medicine, Bronx, New York
C. E. Finch, University of Southern California, Los Angeles

SESSION 1 NEUROCHEMISTRY

Chairperson: P. Davies, Albert Einstein College of Medicine, Bronx, New York

D. M. Bowen, Institute of Neurology, London, England: Neurochemical identity of degenerate (tangle-bearing) cortical neurones.
D. G. Morgan, University of Southern California, Los Angeles: Neurotransmitter receptors in Alzheimer's disease and normal aging.
S. I. Rapoport, National Institute on Aging, Bethesda, Maryland: Functional assessment of altered neurochemistry in aging and Alzheimer's disease—Tissue loss and metabolic deficits as measured with CT and positron emission tomography.
F. H. Gage, University of California, San Diego: Intracerebral neuronal grafting of identified cell types into the aging brain.
C. W. Cotman, University of California, Irvine: Excitatory amino acid receptors and Alzheimer's disease.
G. E. Gibson, Burke Rehabilitation Center, White Plains, New York: Interactions of calcium and neurotransmitter metabolism during aging.
J. Goldman, Albert Einstein College of Medicine, Bronx, New York: Altered calcium metabolism in Alzheimer's disease.

SESSION 2 NEUROENDOCRINOLOGY

Chairperson: C. E. Finch, University of Southern California, Los Angeles

C. E. Finch, University of Southern California, Los Angeles: Neuroendocrine aging—Human brain mRNA.

J. R. Sladek, Jr., University of Rochester Medical Center, New York: Fetal neuronal transplants reverse Parkinsonism symptoms in MPTP-treated monkeys.

J. W. Rowe, Harvard Medical School, Boston, Massachusetts: Sympathetic nervous system activity in aging man.

P. M. Wise, University of Maryland School of Medicine, Baltimore: Hypothalamic monoamine function during aging—Its role in the onset of reproductive infertility.

R. E. Brinton, Rockefeller University, New York, New York: Neurochemical dissection of the mneumonic process—Where the system can falter.

R. M. Sapolsky, Salk Institute, San Diego, California: Protecting the injured hippocampus by attenuating glucocorticoid secretion.

J. H. Morrison, Research Institute of Scripps Clinic, La Jolla, California: Plaque and tangle distribution and cortico-cortical degeneration in Alzheimer's disease.

SESSION 3 FIBROUS PROTEINS

Chairperson: D. J. Selkoe, Brigham and Women's Hospital, Boston, Massachusetts

D. J. Selkoe, Brigham and Women's Hospital, Boston, Massachusetts: Molecular comparison of intraneuronal paired helical filaments and extracellular amyloid fibrils in Alzheimer's disease.

S. B. Prusiner, University of California School of Medicine, San Francisco: Prion proteins and degenerative neurologic disorders.

S.-H. Yen, Albert Einstein College of Medicine, Bronx, New York: Alzheimer's neurofibrillary tangles.

H. M. Wisniewski, Institute for Basic Research in Developmental Disabilities, Staten Island, New York: Ultrastructure, immunology, and biochemistry of paired helical filaments and plaque amyloid.

P. Gambetti, Case Western Reserve University, Cleveland, Ohio: Aging and neuronal cytoskeleton.

F. Gaskin, Oklahoma Medical Research Foundation, Oklahoma City: Autoantibodies to neurofibrillary tangles and brain tissue in Alzheimer's disease and aging.

SESSION 4 CHROMOSOME 21

Chairperson: C. J. Epstein, University of California School of Medicine, San Francisco

C. J. Epstein, University of California School of Medicine, San Francisco: Pathogenic relationships between Down's syndrome and Alzheimer's disease.

D. Patterson, Eleanor Roosevelt Institute for Cancer Research, Denver, Colorado: Somatic-cell genetic and molecular dissection of chromosome 21.

A. C. Warren, Johns Hopkins University, Baltimore, Maryland: DNA polymorphism haplotypes of human chromosome 21—Molecular analysis of the mechanism of nondisjunction.

M. L. Oster-Granite, Johns Hopkins Hospital, Baltimore, Maryland: The trisomic 16 mouse and Down's syndrome—Relevance of Alzheimer's disease.

G. G. Glenner, University of California, San Diego: The amyloid fibril protein(s) of Alzheimer's disease and adult Down's syndrome.

H. M. Wisniewski, Institute for Basic Research in Developmental Disabilities, Staten Island, New York: Neuropathology and dementia in people with Down's syndrome.

SESSION 5 MOLECULAR AND GENETIC APPROACHES TO AGING

Chairperson: L. L. Heston, University of Minnesota, Minneapolis

L. L. Heston, University of Minnesota, Minneapolis: Family studies in Alzheimer's disease.

D. Goldgaber, National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, Maryland: Isolation, characterization, and chromosomal localization of human brain cDNA clones coding for the precursor of the amyloid of Alzheimer's disease and aging brain.

J. F. Gusella, Massachusetts General Hospital, Boston: Investigation of familial Alzheimer's disease of DNA markers.

A. Roses, Duke University Medical Center, Durham, North

Carolina: Molecular genetic strategies in Alzheimer's disease.

P. Davies, Albert Einstein College of Medicine, Bronx, New York: Molecular studies of a new protein in Alzheimer's disease.

Discussants:

D. Aswad, University of California, Irvine

J. H. Morrison, Scripps Clinic and Research Foundation, La Jolla, California

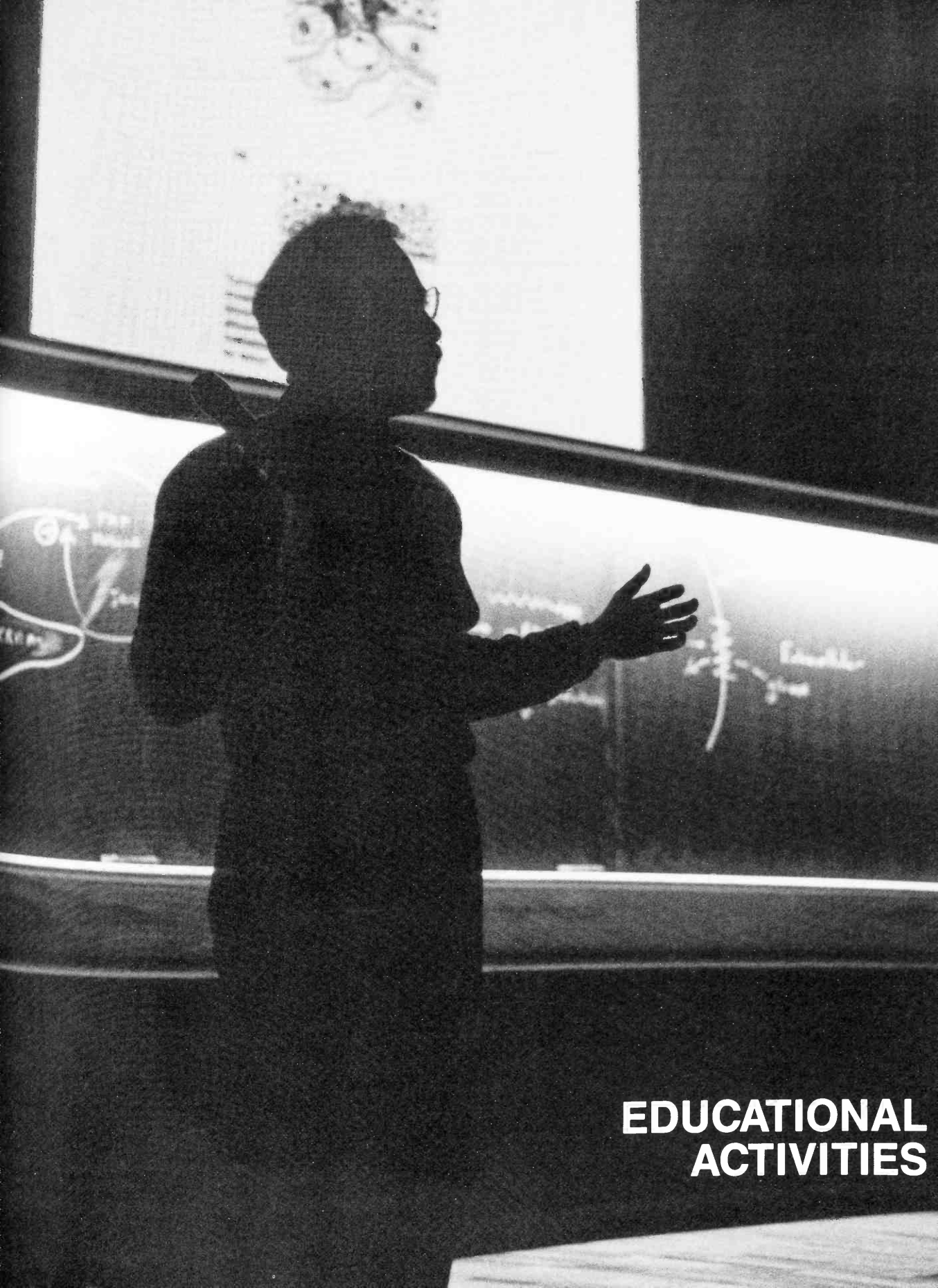
K. O'Malley, Washington University Medical School, St. Louis, Missouri

M. L. Oster-Granite, Johns Hopkins University, Baltimore, Maryland

S. B. Prusiner, University of California, San Francisco, School of Medicine

R. D. Terry, University of California, San Diego

L. P. Weiner, University of Southern California, Los Angeles



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.

Single-channel Methods: Expression and Recording

June 6–June 26

INSTRUCTORS

Dionne, Vincent E., Ph.D., University of California, San Diego
White, Michael M., Ph.D., University of Pennsylvania, Philadelphia
Snutch, Terry, Ph.D., California Institute of Technology, Pasadena
Corey, David, Ph.D., Harvard University, Cambridge, Massachusetts
Yellen, Gary, Ph.D., University of Maryland, College Park

ASSISTANT

Stevens, Meg, B.A., Harvard University, Cambridge, Massachusetts

The technologies of molecular biology and patch-clamping promise major revisions and novel approaches for the examination of many neurobiological problems. Applications of these methods to study the physiology and biophysics of ion channels were taught in this intensive laboratory and lecture course. Students concentrated initially on the basic aspects of these two methods, then proceeded to more integrated studies. Single-channel recording: the fabrication of patch electrodes, giga-seal formation, cell-attached, cell-free and whole-cell recordings, the design and implementation of recording equipment, and the theory and analysis of single-channel currents. Single-channel expression: mRNA purification and handling, the use of Xenopus oocytes and cultured cells as expression systems, the injection of foreign mRNA into oocytes to express ion-channels, and the characterization of newly expressed ion-channels using voltage-clamp and patch-clamp methods on the oocyte. There were opportunities during the final week of the course for students to undertake special projects of their own design which utilized the methods being taught.

PARTICIPANTS

Adamek, Gloria, Ph.D., University of Cincinnati, Ohio
Bender, Alex, Ph.D., University of Saskatchewan, Canada
Edge, Mark, B.S., University of Alabama, Birmingham
Golowasch, Jorge, B.A., Brandeis University, Waltham, Massachusetts
LaMotte, Robert, Ph.D., Yale University, New Haven, Connecticut
Marban, Eduardo, Ph.D., Johns Hopkins University, Baltimore, Maryland
Marcus, Emilie, B.A., Yale University, New Haven, Connecticut
Oesterle, Elizabeth, Ph.D., Purdue University, Lafayette, Indiana
Pietrobon, Daniela, Ph.D., University of Padova, Italy
Pradier, Laurent, B.S., University of California, Davis

SEMINARS

Beam, K., Colorado State University. Measuring ion channel distribution and density with the loose-patch clamp.
Sahley, C.L., Yale University. Single ion channels and behavioral plasticity.
Moczydlowski, E.G., University of Cincinnati. In vitro expression: Application of planar bilayers to ion.
Stevens, C.F., Yale University. Glutamate receptors in the central nervous system.

Molecular Embryology of the Mouse

June 6–June 26

INSTRUCTORS

Rossant, Janet, Ph.D., University of Toronto, Canada
Pedersen, Roger, Ph.D., University of California, San Francisco
Beddington, R., Ph.D., University of Oxford, England
Robertson, Liz, Ph.D., University of Cambridge, England

ASSISTANT

Watson, A., M.S., University of Western Ontario, London, Canada

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 germ cells and preimplantation and postimplantation embryos, embryo transfer, establishment of embryo-derived stem

cell lines, germ layer separation, chimera formation, nuclear transplantation, microinjection of DNA into eggs, retroviral infection of embryos, microinjection of cell lineage tracers, *in situ* hybridization and immunofluorescence and immunoperoxidase techniques.

PARTICIPANTS

Bikoff, Elizabeth, Ph.D., Mt. Sinai School of Medicine, New York, New York
Black, Douglas, B.A., Yale University, New Haven, Connecticut
D'Agostaro, Giacomo, Ph.D., University of Toronto, Canada
Darling, Susan, Ph.D., Imperial College Research Fund, London, England
DePinho, Ronald, M.D., Columbia Presbyterian Medical Center, New York, New York
Fienberg, Allen, B.A., Yale University, New Haven, Connecticut
Fotedar, Rati, M.A., University of Alberta, Canada
Lilly, Frank, Ph.D., Albert Einstein College of Medicine, Bronx, New York
Merlino, Glenn, Ph.D., NCI, National Institutes of Health, Bethesda, Maryland
Richman, Adam, B.A., Yale University, New Haven, Connecticut
Tsukamoto, Ann, Ph.D., University of California, San Francisco
Tucker, Gordon, Ph.D., CNRS, Paris, France
Tyner, Angela, B.A., University of Chicago, Illinois
Windle, Jolene, Ph.D., Salk Institute, LaJolla, California

SEMINARS

Chalfie, M., Columbia University. Lineage analysis in *C. elegans*.
Costantini, F., Columbia University. Gene expression in transgenic mice.
Fox, N., Wistar Institute. Stage-specific antigens.
Hanahan, D., Cold Spring Harbor Laboratory. Oncogenes in transgenic mice.
Jaenisch, R., Whitehead Institute. Retroviruses and insertional mutagenesis.
Magnusson, T., Case Western Reserve. Developmental mutations.
Mahon, K., National Institutes of Health. Lens development and tumor formation.
McLaren, A., MRC Mammalian Development Unit. Sex determination.
Papaidannou, V., Tufts University. Comparative embryology.
Petersen, A., McGill University. Neurological mutants and chimeras.
Ruddle, F., Yale University. Homeo box genes.
Sargent, T., National Institutes of Health. Molecular embryology of *Xenopus*.
Schatter, G., Florida State University. Cytoskeleton.
Schultz, G., University of Calgary. Gene expression in early embryos.
Solter, D., Wistar Institute. Nuclear transplantation.
Wassarman, P., Roche Institute. ZP proteins.
Struhl, G., Harvard University. *Drosophila* developmental genetics.
Hogan, B., NIMR. Endoderm differentiation.

Advanced Bacterial Genetics

June 6–June 26

INSTRUCTORS

Berget, Peter B., Ph.D., University of Texas Medical School, Houston
Maurer, Russel, Ph.D., Case Western Reserve University School of Medicine, Cleveland, Ohio
Weinstock, George, Ph.D., University of Texas Medical School, Houston

ASSISTANTS

Bultema, Sarah Highlander, Ph.D., University of Texas Medical School, Houston
Lancy, Edward, M.S., Case Western Reserve University School of Medicine, Cleveland, Ohio
Weiseman, Jane, M.S., University of Texas Medical School, 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 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.

PARTICIPANTS

Caillet, Joel, M.S., Institut de Biologie, Paris, France
Collier, David, B.S., University of North Carolina, Chapel Hill
Daeschel, Mark, Ph.D., U.S. Department of Agriculture, Raleigh, North Carolina
Garcia-Bustos, José, Ph.D., Rockefeller University, New York, New York
Gött, Peter, M.S., University of Konstanz, Federal Republic of Germany
Hirschberg, Rona, Ph.D., University of Missouri, Kansas City
Hughes, Edward, Ph.D., Yale University, New Haven, Connecticut
Kinder, Susan, M.D., University of Texas, San Antonio
Lenstra, Reijer, Ph.D., Hôpital St. Louis, Paris, France
Libertin, Claudia, M.D., University of Illinois, Chicago
Merlin, Dale, M.S., Indiana University, Bloomington
Moore, Karen, B.A., University of California, Los Angeles
Romesser, James, Ph.D., I.E. du Pont de Nemours & Company, Wilmington, Delaware
Solomon, Kimberly, M.S., University of Maryland, College Park
Tropp, Burton E., Ph.D., Queens College, Flushing, New York
Wower, Iwona, Ph.D., University of Massachusetts, Amherst

SEMINARS

Sternberg, N., E.I. du Pont de Nemours & Co. Processing the bacteriophage P1 packaging site is regulated by adenine methylation.

Mekalanos, J., Harvard Medical School. Molelular genetics of cholera toxin.

Berg, D., Washington University School of Medicine. Mechanism and control of transposition.

Shortle, D., Johns Hopkins School of Medicine. Genetic analysis of the stability of staphylococcal nuclease to reversible denaturation.

Miller, C., Case Western Reserve University School of Medicine. Oxygen regulation of peptidase T and other *Salmonella* genes.

Manoil, C., Harvard Medical School. Using gene fusions to study membrane protein topology.

Gross, C., University of Wisconsin. The heat-shock response in *E. coli*.

Molecular Neurobiology of Human Disease

June 10–June 20

INSTRUCTORS

Black, Ira B., M.D., Cornell University Medical Center, New York, New York

Breakefield, Xandra, Ph.D., E.K. Shriver Center and Harvard Medical School, Boston, Massachusetts

Gusella, James, Ph.D., Massachusetts General Hospital, 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 psychiatric 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 lipidoses and possible means of gene therapy, autoimmune diseases, brain imaging and metabolism, epilepsy and seizure disorders, viral infections of the nervous system and amyloidosis, experimental models of learning and memory.

PARTICIPANTS

Blumberg, Benjamin M., Ph.D., University of Medicine and Dentistry of New Jersey, Newark

Cattaneo, Roberto, B., Ph.D., University of Zurich, Switzerland

Chen, Karen S., B.A., University of California, San Diego

Cooper, David N., Ph.D., University of London, England

Crowley, Joan C., Ph.D., University of Medicine and Dentistry of New Jersey, Newark

Evans, Bradley K., M.D., Rockefeller University, New York, New York

Garofalo, Lorella, P.S., McGill University, Montreal, Canada

Gaston, Sandra M., Ph.D., Massachusetts Institute of Technology, Cambridge

Hsu, Yun-Pung, Ph.D., Massachusetts Institute of Technology, Cambridge

Joho, Rolf H., Ph.D., Baylor College of Medicine, Waco, Texas

MacDonald, Margaret, B.S. Washington University, St. Louis, Missouri

Marchionni, Mark, University of Michigan, Ann Arbor

McDonald, John W., B.S., University of Michigan, Ann Arbor

Schmidt, Robert W., M.D., Ph.D., Washington University School of Medicine, St. Louis, Missouri

Shaw, Marlene V., Ph.D., University of Southern Indiana, Evansville

Shivers, Brenda D., Ph.D., Tulane University Medical School, New Orleans, Louisiana

Short, Priscilla M., M.D., Mt. Sinai Medical Center, New York, New York

Streichenberg, Catherine M., Sc. Nat. Eth, University of Zurich, Switzerland

Whaley, William, Ph.D., University of Arkansas, Fayetteville

SEMINARS

Schwarcz, R., Maryland Psychiatric Research Center. Movement disorders and degenerative diseases—Experimental models.

Wexler, N., Hereditary Disease Foundation. Huntington disease in Venezuela.

McKelvy, J., State University of New York. Neuropeptides.

Reichardt, L., University of California School of Medicine. Nerve growth factor—Possible roles in human disease and the central nervous system.

Olsen, L., Karolinska Institute. Nerve transplantation.

Aguayo, A., Montreal General Hospital. Nerve regeneration.

Caskey, T., Baylor College of Medicine. Molecular lesions in the Lesch-Nyhan syndrome.

Prusiner, S., University of California. Molecular biological insight into slow viral infections.

Clark, J. and R. Gravel. Hospital for Sick Children. Molecular and biochemical studies of the lipidoses.

McNamara, J.O., Duke University Medical Center. Ion channels in epilepsy and seizure disorders.

Crill, W., University of Washington School of Medicine. Ion channels in epilepsy and seizure disorders.

Lindstrom, J., Salk Institute. Autoimmune diseases—Myasthenia gravis.

Raichle, M.E., Washington University School of Medicine. Neuroimaging.

Worton, R., Hospital for Sick Children. Molecular approaches to the defective genes in muscular dystrophy and demyelination syndromes.

Willard, H., University of Toronto. Molecular approaches to the defective genes in muscular dystrophy and demyelination syndromes.

Schwab, M., University of California. DNA changes in neural tumors.

Cavenee, W., University of Cincinnati Medical School. DNA changes in neural tumors.

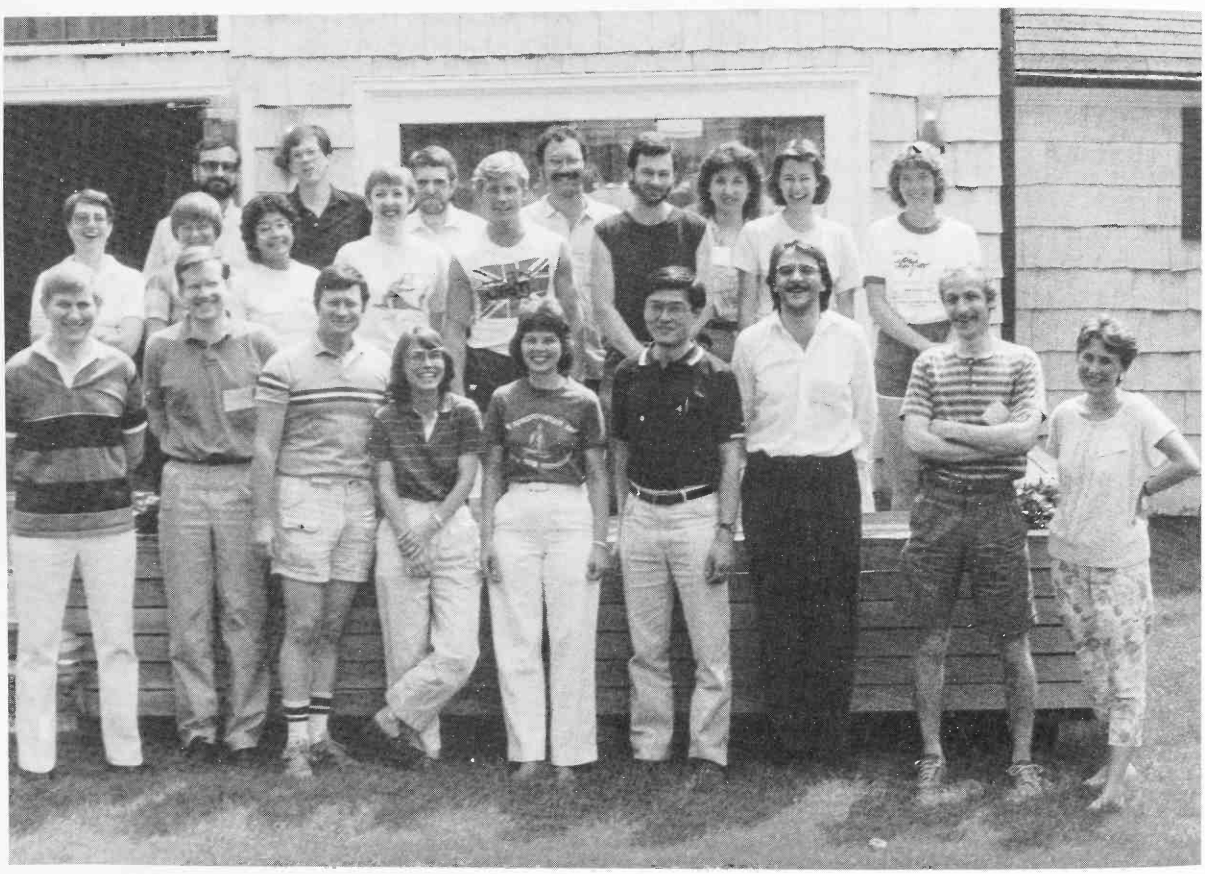
Haase, A., University of Minnesota. Viral infections of the nervous system.

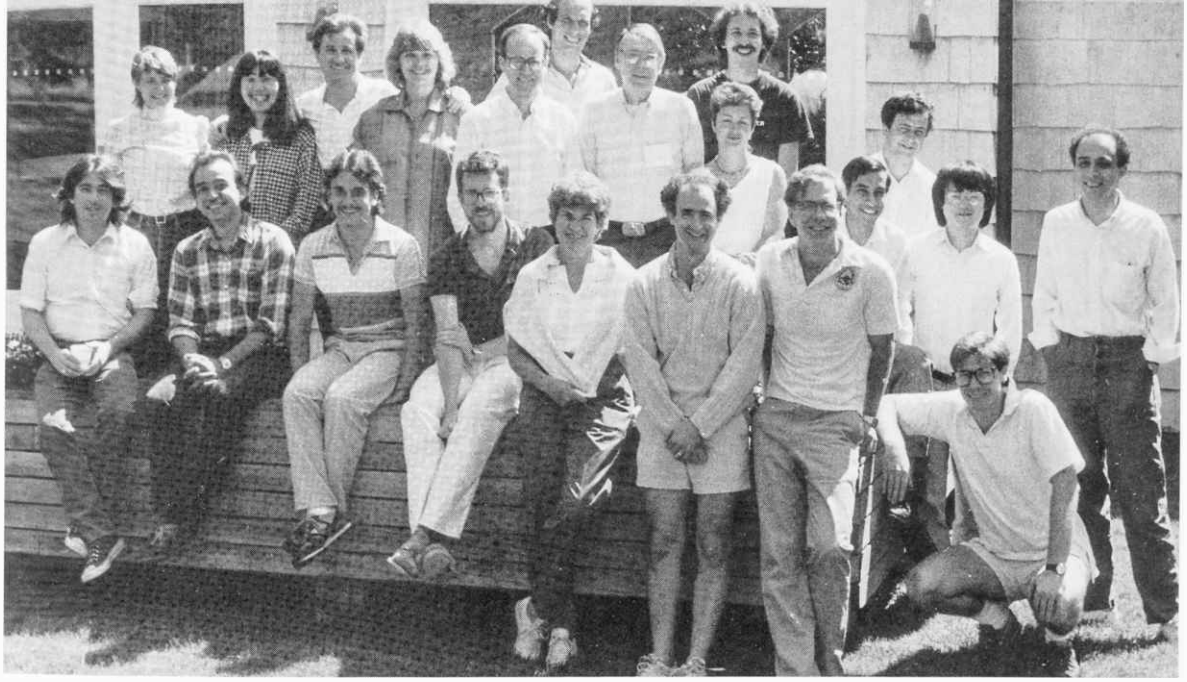
Fields, H., University of California School of Medicine. Pain syndromes and neuropeptides.

Jessell, T.M., Harvard Medical School. Pain syndromes and neuropeptides.

Sejnowski, T., Johns Hopkins University. Computer models.

Mishkin, M., National Institutes of Health. Learnings and memory—Primate models.





Molecular Biology of the Nervous System

June 22–July 6

INSTRUCTORS

McKay, Ronald D., Ph.D., Massachusetts Institute of Technology, Cambridge
Zipursky, Larry, Ph.D., University of California, Los Angeles
Reichardt, Louis, Ph.D., University of California, San Francisco
Evans, Ronald M., Ph.D., Salk Institute, San Diego, California

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

Abe, Miyako, Ph.D., Dana Farber Cancer Institute, Boston, Massachusetts
Coleman, Paul D., Ph.D., University of Rochester, New York
Erlander, Mark, M.S., University of California, Los Angeles
Finocchiaro, Gaetano, M.D., Yale University, New Haven, Connecticut
Fults, Daniel W., M.D., University of North Carolina, Chapel Hill
Haas, Carola A., M.S., Max Planck Institute, Martinsried, Federal Republic of Germany
Jones, Kathryn J., Ph.D., Rockefeller University, New York, New York
Kushner, Leslie, Ph.D., Albert Einstein School of Medicine, Bronx, New York
Lad, Rajnikant, M.D., National Institutes of Health, Bethesda, Maryland
Lendahl, Urban, M.S., Karolinska Institute, Stockholm, Sweden
Seizinger, Bernd, M.S., Massachusetts General Hospital, Boston
Soule, Christophe, Ph.D., CNRS, Paris, France
Suzue, Toshihiko, Ph.D., California Institute of Technology, Pasadena
Walton, Kevin, B.A., Johns Hopkins University, Baltimore, Maryland
Woodford, Barbara, M.A., University of California, Los Angeles
Zagotta, William, B.S., Stanford University, California
Zaremba, Sam, Ph.D., Yale University, New Haven, Connecticut

SEMINARS

Green, M., Harvard University. RNA processing.
Roeder, R., Rockefeller University. Mechanisms of gene transcription.
Zipursky, S.L., University of California, Los Angeles, Gene expression in the developing *Drosophila* eye.
Rothman, J., Stanford University. Signals controlling the organization of cells.
Manser, T., Princeton University. The immune system.
Tsein, R., Yale University. Electrophysiology of calcium channels.
Agnew, W., Yale University. Biochemistry of sodium channels.
Stroud, R., University of California, San Francisco. Structure of the acetylcholine receptor.
Schwarz, J., Columbia University. Second message systems and synaptic modulation.
Rutishauser, U., Case Western Reserve University. Cell adhesion molecules.
Levine, M., Columbia University. Segmentation and homeotic genes in *Drosophila*.
Greenberg, M., Harvard Medical School. Oncogenes in the nervous system.
Bate, M., Cambridge University. Neurogenesis.
Bentley, B., University of California, Berkeley. Cellular mechanisms of neurite outgrowth.

Neurobiology of *Drosophila*

June 29–July 19

INSTRUCTORS

Bate, Mi, Ph.D., University of Cambridge, England
Jan, Lily, Ph.D., Howard Hughes Medical Institute and University of California, San Francisco
Jan, Yuh Nung, Ph.D., Howard Hughes Medical Institute and University of California, San Francisco

ASSISTANT

Schwarz, Tom, Ph.D., Howard Hughes Medical Institute and University of California, San Francisco

The use of genetics as a tool in addition to physiological, anatomical and biochemical techniques has opened up questions in neurobiology that are otherwise difficult to address. *Drosophila* is an ideal organism for such studies because of its well-developed classical and molecular genetics. This laboratory/lecture course provides an introduction to current research in neuronal function and development in *Drosophila*. It is intended for researchers at all levels who may want to use *Drosophila* as an experimental system for studying neurobiology.

The course began with a crash course of lectures and laboratory exercises on *Drosophila* genetics and other techniques which make *Drosophila* research distinctive, such as cytogenetics and DNA transformation. The main emphasis, however, was on studies of the nervous system.

The course covered basic electrophysiological techniques, as applied to mutants with altered ion channels or sensory physiology. It also included mutant analysis of complex behaviors, such as courtship, circadian rhythm, 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

Bodary, Sarah, Ph.D., University of California, San Francisco
Brand, Michael, M.A., University of Cologne, Federal Republic of Germany
Ellis, Hilary, Ph.D., University of California, San Diego
Mecklenburg, Kirk, B.S., Ohio State University, Columbus
Provost, Nicole, M., M.S., University of Washington, Seattle
Schejter, Eyal D., B.A., Weisman Institute, Rehovot, Israel
Schneider, Lynne, B.A., Washington University, St. Louis, Missouri
Stein, David, B.S., Stanford University, California
Weinzierl, Robert, B.S., University of Cambridge, England
Ramos, Ricardo, M.D., Yale University, New Haven, Connecticut

SEMINARS

Timpe, L., University of California, San Francisco. Introduction to behavioral mutants.
———. Mutations affecting synaptic transmission.
Ganetzky, B., University of Wisconsin. *Drosophila* genetics.
Pardue, M.L., Massachusetts Institute of Technology. Cytogenetics.
Spradling, A., Carnegie Institution of Washington. Hybrid dysgenesis and p element transformation.
———. Chorion gene amplification.
Quinn, W., Massachusetts Institute of Technology. Learning mutants.
Fischbach, K.F., Institut für Biologie 3. Mutations affecting the adult central nervous system.
Nüsslein-Volhard, C., Max Planck Institut für Entwicklungsbiologie. Early embryogenesis.
Martinez-Arias, A., MRC Laboratory of Molecular Biology. Segmentation.
Doe, C., Stanford University. Neurogenesis in embryos.
Campos-Ortega, J., Institut für Entwicklungsphysiologie. Mutations affecting embryonic neurogenesis.
Palka, J., University of Washington. Neurogenesis in imaginal wing disks.
———. Projection of sensory neurons in adults.
Hall, J., Brandeis University. Mutations affecting sexual behavior and circadian rhythm.
Ready, D., Princeton University. Neurogenesis in imaginal eye disks and the sevenless mutation.

Advanced Techniques in Molecular Cloning

June 29–July 19

INSTRUCTORS

Zoller, Mark, Ph.D., Cold Spring Harbor Laboratory, New York
Brosius, Jurgen, Ph.D., Columbia University, New York

ASSISTANTS

Williamson, Nicky, B.S., Imperial College, London, England
Johnson, Karen, B.S., Cold Spring Harbor Laboratory, New York

This laboratory and lecture course on advanced aspects of molecular cloning was designed for scientists who are familiar with basic recombinant DNA techniques. The course consisted of experiments in the construction of cDNA libraries, screening of libraries with oligonucleotides and antibodies, *in vitro* mutagenesis of a cloned gene by oligonucleotide directed mutagenesis, M13 cloning and sequencing, gene synthesis, preparation of RNA from SP6 vectors, the use of computers for DNA and protein sequence analysis, and expression of foreign genes in *E. coli.*

Guest instructors presented the application of these techniques to the analysis of cloned genes as well as discussing alternative approaches not covered in the laboratory section. Knowledge of basic techniques of recombinant DNA was a prerequisite for admission to the course.

PARTICIPANTS

Acuto, Oreste, Ph.D., Dana-Farber Cancer Institute, Boston, Massachusetts
Cesarman, Ethel, M.D., New York University, New York
Cline, Thomas W., Ph.D., Princeton University, New Jersey
Fuqua, Suzanne A., Ph.D., University of Texas Health Science Center, San Antonio
Georgiou, George, Ph.D., Cornell University, Ithaca, New York
Hatch, Christopher, L., Ph.D., NCI, National Institutes of Health, Bethesda, Maryland
Kenyon, Cynthia J., Ph.D., Medical Research Council, Cambridge, England
Kiss, Tamas, B.S., Hungarian Academy of Science, Szeged, Hungary
Lotan, Ilana, Ph.D., Columbia University, New York, New York
Mansour, Tag, Stanford University, California
O'Connor, Daniel, M.D., University of California, San Diego
Raehse, Regina, B.S., Paul Ehrlich Institute, Frankfurt, Federal Republic of Germany
Server, Alfred C., Ph.D., University of Texas Health Science Center, Houston
Smith, Chari D., B.A., Brandeis University, Waltham, Massachusetts
Staneloni, Roberto J., Ph.D., Rockefeller University, New York, New York
Yadven, Mitchell W., B.S., George Washington University, Washington, D.C.

SEMINARS

Cone, R., Cold Spring Harbor Laboratory. Immortalization of epithialium-like cells by E1a.
Gubler, U., Hoffmann-La Roche. Posttranslational processing of cholecystokinin in pig brain.
Brosius, J., Columbia University. A small brain-specific RNA.
Roberts, R., Cold Spring Harbor Laboratory. The applications of computers to molecular biology.
Helfman, D., Cold Spring Harbor Laboratory. Isolation of specific cDNAs by antibody screening.
Henikoff, S., Fred Hutchinson Cancer Center. Peculiar features of a locus encoding purine pathway activities in *Drosophila*.
Wallace, B., City of Hope. Principles of oligonucleotide hybridization.
Zoller, M., Cold Spring Harbor Laboratory. Strategies for site-directed mutagenesis.
Cate, R., Biogen. Cellular and molecular biology of Mullerian inhibiting substance.
Wallner, B., Biogen. Isolation and characterization of two human phospholipase-A2-inhibiting proteins.
Ulmer, K., University of Maryland. Protein engineering.
Studier, W., Brookhaven National Laboratory. Expression of protein T7 vectors.
Schatzman, A., Smith Kline and Beckman. Expression of foreign proteins in *E. coli*.
Urdea, M., Chiron Corporation. Strategies in gene synthesis.
Smith, M., University of British Columbia. Use of oligonucleotides as primers and probes.

Molecular and Developmental Biology of Plants

June 29–July 19

INSTRUCTORS

Horsch, Robert, Ph.D., Monsanto Corp., St. Louis, Missouri
Messing, Joachim, Ph.D., Waksman Institute, Rutgers University, Piscataway, New Jersey
Sussex, Ian, Ph.D., Yale University, New Haven, Connecticut

ASSISTANTS

Chereskin, Barbara, Ph.D., Waksman Institute, Rutgers University, Piscataway, New Jersey
Kirihara, Julie, B.S., Waksman Institute, Rutgers University, Piscataway, New Jersey
Kirk, Nancy, B.S., Yale University, New Haven, Connecticut
Smith, Alan, Ph.D., Monsanto Corp., St. Louis, Missouri

This course provided an intensive overview of current topics and techniques in plant biology, with emphasis on molecular and developmental biology and genetics. It was designed for scientists with working knowledge of molecular techniques who are either working with plant systems or wish to. The course consisted of a rigorous lecture series, a hands-on laboratory, and informal discussions, not to mention a wonderful picnic. 18 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

Anthony, Janice L., M.S., Texas A & M University, College Station
Arias, Jonathan A., M.S., University of Colorado, Boulder
Bagg, Ann, B.S., University of California, Berkeley
Baumann, Goetz, B.S., University of Bielfeld, Federal Republic of Germany
Beeching, Jon, Ph.D., University of Bath, England
Breitenberger, Caroline A., Ph.D., Massachusetts Institute of Technology, Cambridge
Destefano, Luis Julio, M.S., Louisiana State University, Baton Rouge
Fouser, Lynette, A., Ph.D., University of Toronto, Canada
Gal, Susannah, B.A., National Institutes of Health, Bethesda, Maryland
Hatzios, Kriton K., Ph.D., Virginia Polytechnic Institute, Blacksburg
Jen, George C., Ph.D., Massachusetts Institute of Technology, Cambridge
Rodaway, Shirley J., Ph.D., American Cyanamid Co., Princeton, New Jersey
Sheahan, John J., B.S., University of Wisconsin, Madison
Signer, Ethan R., Ph.D., Massachusetts Institute of Technology, Cambridge
Stuitje, Antoine, Ph.D., Free University, Amsterdam, The Netherlands

SEMINARS

Alberscheim, P., University of Georgia. Oligosaccharides in cell-to-cell interactions.
Burr, B., Brookhaven National Laboratory. The use of RFLP in mapping plant genes.
Nasrallah, J., Cornell University. Molecular biology of self-incompatibility.
Sommerville, C., Michigan State University. Genetic manipulation of the Arabidopsis genome.
Lamb, C., Salk Institute. Cellular responses to stress.
Quail, P., University of Wisconsin. Phytochrome-induced genes.
Crouch, M., Indiana University. Seed development.
Beachy, R., Washington University. Soybean storage protein genes.
———. TMV resistance in transgenic plants.
Hall, T., Texas A & M. Molecular functions of plant RNA viruses.
Staskowizc, B., University of California. Molecular approaches to pathogenesis.
Dellaporta, S., Yale University. Transposable elements in maize.
Goodman, H., Massachusetts General Hospital. Gene amplification in plants.
Gronenborn, B., Max-Planck Institute. Wheat dwarf virus replication.
Palmer, J., University of Michigan. The organization of plant mitochondrial DNA.
Hanson, M., Cornell University. Organelle genetics.
Bogorad, L., Harvard University. Chloroplast structure and function.
Theologis, A., Washington University. Hormone-regulated genes.

Developmental Neurobiology

July 16–July 28

INSTRUCTORS

Patterson, Paul, Ph.D., California Institute of Technology, Pasadena
Goodman, Corey, Ph.D., Stanford University, California

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; cell adhesion and cell recognition; 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

Brugg, Bernard, M.S., Friedrich Miescher Institute, Basel, Switzerland
Carpenter, Ellen M., B.A., University of Chicago, Illinois
Cepko, Constance, Ph.D., Harvard Medical School, Boston, Massachusetts
Ernsberger, Uwe, B.S., Max-Planck Institute, Martinsried, Federal Republic of Germany
French-Constant, Charles, M.A., University College London, England
Garcia Alonso, Luis A., M.S. University Autonoma, Madrid, Spain
Hashimoto, Yasuhiro, M.D., University of Pennsylvania, Philadelphia
Hen, Rene, Ph.D., Columbia University, New York, New York

Jay, Daniel, Ph.D., Harvard Medical School, Boston, Massachusetts
Lim, Tit, B.S., Cambridge University, England
Mahanthappa, Negesh, B.A., California Institute of Technology, Pasadena
Minth, Carolyn, Ph.D., Purdue University, Lafayette, Indiana
Nicolas, Jean-François, Ph.D., Institut Pasteur, Paris, France
Nybroe, Ole, Ph.D., University of Copenhagen, Denmark
Petursdottir, Gudrun, M.A., University of Oslo, Norway
Rydel, Russell, M.S., New York University, New York
Sharpe, Arlene, Ph.D., Whitehead Institute, Cambridge, Massachusetts
Thomas, Steven, B.A., University of Michigan, Ann Arbor
Tiemeyer, Michael, B.A., Johns Hopkins University, Baltimore, Maryland
Wayne, Denise, B.A., University of Florida, Gainesville
Zak, Naomi, Ph.D., Weizmann Institute, Rehovot, Israel
Zuniga, Martha Celilia, Ph.D., University of Texas, Austin

SEMINARS

Spitzer, N., University of California, San Diego. Early vertebrate neural development.
Rakic, P., Yale University. Cell proliferation and migration during neuronal development.
Hortiz, R., Massachusetts Institute of Technology. Neuronal development in the nematode.
Bray, D., Medical Research Council. Mechanisms of growth cone extension.
Landmesser, L., University of Connecticut. Axon guidance in vertebrates.
Sanes, J., Washington University. Synapse formation.
Purves, D., Washington University. Neural specificity and synapse rearrangement in the PNS.
Fraser, S., University of California, Irvine. Neural specificity in the retinotactal system.
Bonhoeffer, F., Max-Plank Institute, Tubingen. Specificity of retinal growth cones in vivo and in vitro.
Rutishauser, U., Case Western Reserve. Cell adhesion molecules.
Jessell, T., Columbia University. Cell recognition during sensory neuron development.
Shatz, C., Stanford University. Development of the mammalian visual system.
Abrams, R., Columbia University. Mechanisms of learning in *Aplysia* and hippocampus.
Nottebohm, F., Rockefeller University. Plasticity, learning, and neurogenesis in song birds.

Immunoglobulins: Molecular Probes of the Nervous System

July 21–August 10

INSTRUCTORS

Silberstein, Laura, Ph.D., Stanford University, California
Evans, Christopher, Ph.D., Stanford University, California
Hockfield, Susan, Ph.D., Yale University, New Haven, Connecticut

ASSISTANT

Treloar, Allison, Yale University, New Haven, Connecticut

Over the last decade antibodies have become powerful and popular reagents in neurobiological research. This course, intended for research scientists of all levels, was designed to provide an advanced understanding of the power and limitations of immunoglobulins both as biochemical and anatomical reagents. Through laboratory work and lectures we will explore immunochemical and immunohistochemical techniques in detail, and the application of these techniques to current issues in neurobiology.

The laboratory work will include: generation of monoclonal antibodies; preparation and characterization of antibodies to synthetic peptides and to complex antigens; solid phase immunoassays; antibody characterization and purification; antigen purification using antibody affinity methods; Western blotting; antibody conjugation; biochemical characterization of neuropeptides using RIA and HPLC; and light and electron microscopic immunohistochemistry.

A series of lectures by invited speakers will cover: structure and function of immunoglobulins; molecular and cellular immunology; hybridoma technology; studies using synthetic peptides; immunological characterization of the acetylcholine receptor; and immunological characterization of neuronal diversity.

PARTICIPANTS

Bicker, Gerd G., Ph.D., Free University, Berlin, Federal Republic of Germany

DeAzeredo, Fernando, Ph.D., Federal Fluminense University, Brazil

Keshishian, Haig, Ph.D., Yale University, New Haven, Connecticut

Levi, Andrea, Ph.D., National Institutes of Health, Bethesda, Maryland

Lowenstein, Pedro R., Ph.D., Johns Hopkins University, Baltimore, Maryland

Martin, Kathleen, B.A., Yale University, New Haven, Connecticut

Paterson, Jean, Ph.D., University of Manitoba, Canada

Rice, Margaret E., Ph.D., New York University Medical Center, New York

Smyth, Joan, B.A., Trinity College, Dublin, Ireland

SEMINARS

Steiner, L., Massachusetts Institute of Technology. Immunoglobulins.

———. Immunoglobulin superfamily.

Woodland, B., University of Massachusetts Medical Center. Cellular immunology—Introduction.

———. Cellular immunology—Conclusion.

Padlan, E., National Institutes of Health. Three-dimensional structure of immunoglobulin.

Dwyer, D., University of Alabama. Regulation of the immune response by anti-idiotypes.

Janeway, C., Yale University School of Medicine. Role of T cells in the generation and regulation antibody responses.

———. Lymphocytic receptor repertoires: Genetic origin and selective influences on expression.

Alt, F., Columbia University College of Physicians & Surgeons. Development of the immunoglobulin heavy-chain variable region genes repertoire.

Lindstrom, J., Salk Institute. Probing acetylcholine receptor with monoclonal antibodies.

Levitt, P., Medical College of Pennsylvania. Antibodies reveal partitions and boundaries in CNS development.

Barald, K., University of Michigan Medical School. No-flow cytometer isolation of neural crest subpopulations with monoclonal antibodies.

Scharff, M., Albert Einstein College of Medicine. Tailormaking monoclonal antibodies: Modifications of the technology and the generation of mutant antibodies.

Carlson, S., University of Washington. TAP-1, a nerve terminal anchorage protein from electric organ.

Van Den Pol, A., Yale University School of Medicine. Use and interpretation of ultrastructure immunocytochemistry.

Hockfield, S., Yale University. A monoclonal antibody that demonstrates organization and development of mammalian central visual area.

Evans, C., Stanford Medical School. Antibodies as probes for neuroactive peptides.

Molecular Cloning of Eukaryotic Genes

July 21–August 10

INSTRUCTORS

Bothwell, Al, Ph.D., Yale School of Medicine, New Haven, Connecticut
Alt, Fred, Ph.D., Columbia University, New York, New York
Lehrach, Han, Ph.D., European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany

ASSISTANTS

LeClair, Ken, B.A., Yale University, New Haven, Connecticut
Lutzker, Stuart, B.A., Columbia University, New York, New York
Craig, Alister, Ph.D., European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany

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: 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 and by recombination, 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 will be discussed. Guest lecturers discussed the application of molecular cloning procedures to the study of specific eukaryotic gene systems.

PARTICIPANTS

Adeyemo, Oyewole, Ph.D., University of Ibadan, Nigeria
Braciale, Thomas, Ph.D., Washington University, St. Louis, Missouri
Burridge, Keith, Ph.D., University of North Carolina, Chapel Hill
Conrad, Patricia Ann, Ph.D., International Laboratory for Research on Animal Diseases, Nairobi, Kenya
Ekblom, Peter, Ph.D., Max-Planck Institut, Tubingen, Federal Republic of Germany
Fu, Xiao Chang, Ph.D., Sichuan Cancer Institute, Chengdu, People's Republic of China
Goldberg, Alfred, Ph.D., Harvard Medical School, Cambridge, Massachusetts
Good, Michael, Ph.D., National Institutes of Health, Bethesda, Maryland
Kondor-Koch, Claudia, Ph.D., University of Frankfurt, Federal Republic of Germany
Maher, Pamela, Ph.D., University of California, San Diego
Merisko, Elaine, Ph.D., University of Kansas Medical Center, Kansas City
O'Donnell, Paul, Ph.D., Memorial Sloan Kettering Cancer Center, New York
Podolsky, Daniel, M.D., Massachusetts General Hospital, Boston
Rock, Kenneth, M.D., Harvard Medical School, Boston, Massachusetts
Saling, Patricia, Ph.D., Duke University, Durham, North Carolina
Smith, Helene, Ph.D., Peralta Cancer Research Institute, Oakland, California

SEMINARS

Hood, L., California Institute of Technology. Structure and function of antigen receptor genes.
———. Biotechnology
McKnight, S., Fred Hutchinson Cancer Center. Regulation of transcription.
Mulligan, R., Whitehead Institute. Use of retrovirus vectors to analyze hematopoietic differentiation.
Sen, R., Whitehead Institute. DNA sequences involved in tissue-specific expression in lymphocytes.
Costantini, F., Columbia University. Gene regulation in transgenic mice.
Goff, S., Columbia University. Definition of retrovirus genes by in vitro mutagenesis.
Gluzman, Y., Cold Spring Harbor Laboratory. Eukaryotic cloning vectors.
Kellems, R., Baylor University. Isolation and characterization of the adenosine deaminase gene and gene therapy.
Hanahan, D., Cold Spring Harbor Laboratory. Oncogenesis and transgenic mice.

LeClair, K., Yale University. Molecular cloning of the murine Ly6 T-cell differentiation antigen.
———. Mutational analysis of the Ig heavy-chain promoter.
———. Development of a T-cell-dependent and T-cell-independent immune response.
Lee, F., DNAX Corporation. Molecular characterization of hematopoietic growth factors.
Schimke, R., Stanford University. On the generation of gene amplification and other chromosomal rearrangements. I.
———. On the generation of gene amplification and other chromosomal rearrangements. II
Murray, N., University of Edinburgh. Lambda cloning vectors.
Snyder, M., Yale University. Use of λgtII to clone and study yeast gene products.
Wigler, M., Cold Spring Harbor Laboratory. Structure and function of cellular oncogenes.
Kaufman, R., Genetics Institute. Molecular genetics of factor VIII.
Tucker, P., University of Texas Southwestern Medical School. Regulation of Ig gene assembly and isotype expression.

Yeast Genetics

July 21–August 10

INSTRUCTORS

Sherman, Fred, Ph.D., University of Rochester, New York
Fink, Gerald, Ph.D., Massachusetts Institute of Technology, Cambridge
Hicks, James, Ph.D., Scripps Clinic and Research Foundation, La Jolla, California

ASSISTANTS

Bulgaz, Elena, Ph.D., Scripps Clinic and Research Foundation, La Jolla, California
Dietrich, Fred, B.A., Whitehead Institute, Cambridge, Massachusetts

The major laboratory techniques used in the genetic analysis of yeast were studied, including the isolation and characterization of chromosomal and mitochondrial mutants, tetrad analysis, chromosomal mapping, mitotic recombination, and test of allelism and complementation. Micromanipulation used in tetrad analysis was carried out by all students. Recombinant DNA techniques, including yeast transformation, filter hybridization, and gel electrophoresis were applied to

cloning and genetic analysis of yeast DNA. Lectures on fundamental aspects of yeast genetics were presented along with seminars given by outside speakers on topics of current interest.

PARTICIPANTS

Barnes, Larry, D., Ph.D., University of Texas Health Science Center, San Antonio
Benton, Bret, State University of New York, Stony Brook
Bretscher, Anthony, Ph.D., Cornell University, Ithaca, New York
Brown, Julie A., B.S., University of Virginia, Charlottesville
Cunningham, Kyle, B.A., University of California, Los Angeles
Gross, David S., Ph.D., Louisiana State University, Shreveport
Horwich, Arthur L., M.D., Yale University, New Haven, Connecticut
Jansen, Kathrin, Ph.D., Cornell University, Ithaca, New York
Kline, Connie J., B.S., Yale University, New Haven, Connecticut
Kwon-Chung, K.J., Ph.D., National Institutes of Health, Bethesda, Maryland
Maguire, Michael E., Ph.D., Case Western Reserve University, Cleveland, Ohio
Skalka, Anna, Ph.D., Roche Institute, Nutley, New Jersey
Smith, John A., Ph.D., Massachusetts General Hospital, Boston
Thomason, Lynn C., B.S., University of Oregon, Eugene
Toyn, Jeremy J., B.S., EMBL, Heidelberg, Federal Republic of Germany
Yarbough, Patrice O., Ph.D., University of Texas Health Science Center, Dallas

SEMINARS

Wigler, M., Cold Spring Harbor Laboratory. Growth regulation in yeast.
Broach, J., Princeton University. Mechanisms of plasmid propagation in yeast.
Zoller, M., Cold Spring Harbor Laboratory. Expression on yeast cAMP-dependent protein kinase in *E. coli*.
Carlson, M., Columbia University. Glucose repression controlled by a protein kinase.
Guarente, L., Massachusetts Institute of Technology. Regulation of the yeast *cycl* gene.
Klar, A., Cold Spring Harbor Laboratory. Developmental switches of mating type in fission yeast.
Tye, B.-K., Cornell University. DNA replication in yeast.
Guthrie, C., University of California, San Francisco. RNA processing and snRNAs in yeast.
Mason, T., University of Massachusetts. Mitochondrial and cytoplasmic histidine tRNA synthetase.
Sternglanz, R., State University of New York, Stony Brook. Yeast DNA topoisomerases.
Warner, J., Albert Einstein College of Medicine. Ribosomal genes and their regulation.
Petes, T., University of Chicago. Structural and functional flexibility of the yeast genome.
Prakash, L., University of Rochester. Structure and function of yeast DNA repair genes.
Douglas, M., University of Texas, Dallas. Genetic analysis of protein localization in mitochondria.
Tzagoloff, A., Columbia University. Genetics of mitochondrial biogenesis.

Computational Neuroscience

July 31–August 11

INSTRUCTORS

Atkeson, Christopher, Ph.D., Massachusetts Institute of Technology, Cambridge
Bizzi, Emilio, M.D., Massachusetts Institute of Technology, Cambridge

ASSISTANT

McIntyre, Joe, Massachusetts Institute of Technology, Cambridge

This intensive lecture/laboratory 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 can 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

Arista, Francesco, B.S., University of Louivain, Belgium
Belczynski, Carl, B.S., University of Michigan, Ann Arbor
Bootsma, Reinoud, Ph.D., Free University, Amsterdam, The Netherlands
Drucker, Steven, B.S., Brown University, Providence, Rhode Island
Faye, Ian, B.S., Massachusetts Institute of Technology, Cambridge
Flanagan, John R., M.A., McGill University, Montreal, Canada
Hartman, Leo, B.S., University of Rochester, New York
Jaeger, Robert J., Ph.D., Chicago Institue of Technology, Illinois
Lou, Jau-Shin, M.D., Barrow Neurological Institute, Phoenix, Arizona
MacFarland, Jenny L., B.S., University of Washington, Seattle
Miller, Crispin, B.S., Massachusetts Institute of Technology, Cambridge
Moody, Charles James, Ph.D., Southern Illinois University Medical School, Springfield
Otten, Egbert, Ph.D., University of Groninger, The Netherlands
Pelisson, Denis, M.S., INSERM, Paris, France
Sachdev, Robert, B.S., University of Michigan, Ann Arbor
Schwarz, Urs, M.S., University Hospital, Zurich, Switzerland
Wang, Hongbin, B.S., University of Minnesota, St Paul
Weir, Donald, B.A., University of Oxford, England
Weytjens, Jan, M.D., University of Groningen, The Netherlands
Wylie, Richard M., Ph.D., Walter Reed Army Institute, Washington, D.C.

SEMINARS

Hollerbach, J., Massachusetts Institute of Technology. Multijoint kinematics: Human results.
Kalaska, J., University of Montreal. Neural representations for motor control.
Hogan, N., Massachusetts Institute of Technology. Using optical control theory to model behavior.
———. Minimum jerk movements.
Mussa-Ivaldi, F., Massachusetts Institute of Technology. Multijoint arm studies.
Humphrey, D., Emory University School of Medicine. Central control of voluntary arm and hand movements.
Hinton, G., Carnegie Mellon University. Connectionist approaches to motor control.
Getting, P., University of Iowa. Neural network reconstruction: Mechanisms for motor pattern generation.

Abbs, J., University of Wisconsin. Temporal and spatial coordination of multiarticulate movements: Potential neural mechanisms.

Hollerbach, J., Massachusetts Institute of Technology. Tactile sensing and hand control.

Robert, M., Carnegie Mellon University. The dynamics of running in robots.

Loeb, G., National Institutes of Health. Models of the cat hindlimb.

Ghez, C., Columbia University. Motor psychophysics.

Lisberger, S., University of California, San Francisco. Visual motion processing and sensory-motor integration for smooth pursuit eye movements.

Optican, L., National Institutes of Health. Adaptive control of saccadic eye movements.

Sparks, D., University of Alabama. The role of the superior colliculus and other brainstem areas in the control of saccadic eye movements.

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.

1985–1986

September

Jacek Skowronski, NCI, National Institutes of Health, Bethesda, Maryland: Long interspersed repeated sequences in mammals searching for function.

H. Robert Guy, NCI, National Institutes of Health, Bethesda, Maryland: Approaches to predicting membrane channel protein structure from their sequences.

John Anderson, Harvard University, Cambridge, Massachusetts: The structure of a phage repressor-operator complex.

Tom Poulos, Genex Corporation, Rockville, Maryland: Haem enzyme structure and function.

October

Luis Parada, The Whitehead Institute, Cambridge, Massachusetts: Oncogene effects on primary rodent cells.

Walter Schaffner, University of Zurich, Switzerland: Enhancers and tissue specificity of transcription.

November

Arthur Horwich, Yale University School of Medicine, New Haven, Connecticut: Genetic analysis of a mitochondrial targeting sequence.

Steve Mayfield, University of Geneva, Switzerland: *Chlamydomonas* transformation vectors.

James W. Pflugrath, Max-Planck Institute, Martinsried, Federal Republic of Germany.: Amylase inhibitor—X-ray structure and modeling studies.

Kenneth Marians, Memorial Sloan-Kettering Cancer Center, New York, New York: The role of topoisomerase I in pBR322 DNA replication in vitro.

Frank McCormick, Cetus Corporation, Emeryville, California: New aspects of *ras* structure and function.

Robert Simpson, Arko Plant Research Center, Dublin, California: Multiple uses of agrobacteria to alter plant phenotypes.

Hsin Ming Yang, Oncogene Science, Seattle, Washington: Characterization and biosynthesis of a human melanoma associated proteoglycan.

James Manley, Columbia University, New York, New York: Processing of animal cell mRNA.

Richard Garber, University of Washington, Seattle: Molecular functions and misfunctions of the *Drosophila* homeotic gene Antennapedia.

December

Rodrigo Bravo, European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: Early genes induced by growth factors in quiescent cells.

Ueli Aebi, Johns Hopkins University, Baltimore, Maryland: Study of protein structure and protein-protein interactions by high-resolution electron microscopy and digital imaging processing.

Michele Sawadogo, Rockefeller University, New York, New York: DNA binding specificity of USF: A human gene-specific transcription factor required for maximum expression of the adenovirus major late promoter.

Steve Whitehead, Harvard Medical School, Cambridge Massachusetts: Molecular biology of the human acute phase reactant C-reactive protein.

January

Don Cleveland, Johns Hopkins University, Baltimore, Maryland: Tubulin gene expression—Regulation at two levels.

Martha Mutchler, Cornell University, Ithaca, New York: Two experimental systems in tomato.

Chris Norbury, Imperial Cancer Research Fund, London, England: Cellular sequences that influence 3′ processing of viral mRNA in polyoma-transformed cell lines.

David Frendewey, Institute of Cell and Tumor Biology, Heidelberg, Federal Republic of Germany: Assembly of a pre-mRNA splicing complex and mechanistic complications.

Ray Gesteland, University of Utah, Salt Lake City: Ribosomal frameshifting.

Ron Evans, Salk Institute, San Diego, California: The structure and expression of the glucocorticoid receptor.

February

Richard Morimoto, Northwestern University, Chicago, Illinois: Multiple regulatory controls for human HSP70 expression.

Yasuji Oshima, Osaka University, Japan: Regulatory circuit for phosphatase synthesis in *Saccharomyces*.

March

Keith Burridge, University of North Carolina, Chaple Hill: Transmembrane linkage between the extracellular matrix and actin cytoskeleton.

Venketesan Sundaressen, University of California, Berkeley: The molecular genetics of Robertson's mutator—A maize transposable element.

Claire Morre, Massachusetts Institute of Technology, Cambridge: In vitro cleavage and polyadenylation of eukaryotic mRNA.

James Lees-Miller, University of Alberta, Canada: Purification and characterization of two novel proteins from smooth muscle.

Jonathan Jones, Advanced Genetic Sciences, Inc., Oakland, California: Maximizing the expression of a bacterial chitinase gene in plant cells.

Giuseppe Gavizzi, University of Milan, Italy: Genetics of Sn—A tissue-specific, light-sensitive genetic element in maize.

Tom O'Halloran, Massachusetts Institute of Technology, Cambridge: Overproduction, purification, and characterization of the MerR metallo-regulatory protein.

Ken Lipson, Memorial Sloan-Kettering Cancer Center, New York, New York: Regulation of the insulin receptor.

April

Craig Rosen, Dana-Farber Cancer Center, Boston, Massachusetts: Regulation of HTLV-I and HTLV-II gene expression.

Ed Stavnezer, University of Cincinnati Medical Center, Ohio: Products and activities of the v-*ski* oncogene.

Kenneth Ferguson, University of Texas, Dallas: Guanine nucleotide binding to G proteins.

R. Bertolotti, CNRS, Gif-sur-Yvette, France: Normal and neoplastic differentiation—Programming genes, gene dosage, and shuttle vectors.

Bernardo Nadal-Ginard, Harvard University, Cambridge, Massachusetts: Promoter selection and alternative RNA splicing—Mechanisms used to generate contractile protein diversity.

Nouria Hernandez, Yale University, New Haven, Connecticut: Are small snRNA genes transcribed by a specialized RNA polymerase?

May

Jim Roberts, Fred Hutchinson Cancer Center, Seattle, Washington: Negative control of DNA replication in the composite SV40-BPV plasmid.

Wilson H. Burgess, Meloy Laboratories, Gaithersburg, Maryland: Structural evidence that β-endothelial cell growth factor is the precursor of α-ECGF and acidic-FGF.

Heidi Ernst, University of California, Davis: Cloning and characterization of sequences encoding the α subunit of translational initiation factor eIF-2.

David Foster, Rockefeller University, New York, New York: The transformation potential of the *fps* gene of Fujinami sarcoma virus and its cellular progenitor.

Jeremy Paul, Massachusetts Institute of Technology, Cambridge: Structure-function analysis of fibronectin.

Clark Tibbetts, Vanderbilt University, Nashville, Tennessee: A functional domain and target for adenovirus E1A autorepression.

Michael Gilman, Whitehead Institute, Cambridge, Massachusetts: Intracellular mediators of c-*fos* induction.

June

Masafumi Tanaka, Jackson Laboratories, Bar Harbor, Maine: A cellular enhancer element which is active in embryonal carcinoma (EC) cells.

David Shore, Medical Research Council, London, England: Characterization and isolation of proteins involved in control of the silent yeast mating-type genes.

Mike Fried, Imperial Cancer Research Foundation, London, England: The use of "expression selection" in the study of gene regulation in mammalian cells.

Franz Thiébaut, Laboratoire d'histologie du Cerchăr, Verneuil-en-Halatté, France: Use of backscattered electron imaging for studying nuclear or nucleolar structure.

July

Moshe Oren, Weizmann Institute, Rehovot, Israel: Properties of p53 oncogenes.

August

Elizabeth Gateff, Johannes Gutenburg Universitat, Mainz, Federal Republic of Germany: Recessive oncogenes of *D. melanogaster.*

September

Dennis Carroll, University of Massachusetts, Amherst: Signal transduction and the regulation of membrane-cytoskeleton associations of the human erythrocyte.

Benny Shilo, Weizmann Institute, Rehovot, Israel: The *Drosophila* EGF receptor.

Susan P. Williams, University of Alberta, Canada: Structural and functional analysis of phosphoproteins.

Susan Lobo, Florida State University, Tallahassee: Expression of mouse U1B genes in vivo and in vitro.

Bruce Futcher, McMaster University, Hamilton, Canada: Recombinational amplification of 2-micron plasmid of yeast.

Carol W. Greider, University of California, Berkeley: Telomere elongation in vitro.

October

Leslie Goodwin, New York University, New York: Isolation and characterization of a cell resembling prothymocytes.

Emily Wilson, Emory University, Atlanta, Georgia: Role of protein kinase C in neutrophil activation—Studies utilizing sphingold long-chain bases as inhibitors.

William Hanratty, University of Pittsburgh, Pennsylvania: Conditional mutants of *Drosophila* oncogenes.

November

Paul Walton, University of Pennsylvania, Philadelphia: Translocation of the Mg^{++}-dependent phosphadidate phosphohydrolase between the cytosol and endoplasmic reticulum as a method of enzymic control.

Thomas Peterson, CSIRO, Canberra, Australia: Exploring the maize *P* locus—A molecular and genetic analysis.

Bob Kingston, Massachusetts General Hospital, Boston: Regulation of human HSP70 transcription by heat and by oncogenes.

Gordon Foulkes, National Institute for Medical Research, London, England: Extracellular and intranuclear events in Albelson-transformed cells.

Robert Rjian, University of California, Berkeley: Interplay of multiple sequence-specific transcription factors at enhancers and promoters.

David Knipe, Harvard Medical School, Boston, Massachusetts: Herpes simplex virus nuclear proteins and the regulation of viral gene expression.

December

Hiroto Okayama, National Institutes of Health, Bethesda, Maryland: A cDNA expression cloning system.

Adolf Graessmann, University of Berlin, Federal Republic of Germany: DNA methylation, chromatin structure, and regulation of gene expression.

Pam Silver, Princeton University, New Jersey: Nuclear protein localization in yeast.

Peter Ruvolo, Albert Einstein College of Medicine, Bronx, New York: Single-stranded DNA-binding proteins from conjugative plasmids in *E. coli*.

Robert H. Singer, University of Massachusetts, Amherst: In situ hybridization—Analysis of the intracellular localization of mRNAs for cytoskeleton proteins.

Mark Diamond, University of Buffalo, New York: Synthesis and turnover of a rat hepatocyte canaliculus glycoprotein.

Art Bruskin, University of California, San Francisco: Transformation of mammalian cells by retroviruses containing *erb* or *myc*.

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, 290 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) 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 a large number of applicants, took part in the program, which was supported by Alfred P. Sloan Foundation, Burroughs Wellcome Fund, American Cyanamid Company, Robertson Research Fund, and Pfizer Inc.

Peter Argani, Princeton University
Research Advisor: **Yakov Gluzman**
Origin-specific binding of SV40 large T antigen.

Sean Burgess, University of Colorado
Research Advisor: **Winship Herr**
Late transcription in SV40.

Nina Caplin, Duke University
Research Advisor: **William Welch**
Purification of the major mammalian glucose-regulated proteins.

Mark Eisner, Stanford University
Research Advisor: **Scott Powers**
Localization and characterization of *supC*: A suppressor of the heat-shock sensitivity phenotype induced by the *Ras2*^{val19} mutation in yeast.

Irene Griff, Massachusetts Institute of Technology
Research Advisor: **Fevzi Daldal**
Isolation and identification of stigmatellin-resistant mutations in the Pet operon in *R. capsulata*.

Martin Horvath, Brown University
Research Advisor: **Bruce Stillman**
Characterizing the ABF1 binding site of ARS1 in vivo.

Brad Johnson, Yale University
Research Advisors: **Ashok Bhagwat** and **Richard Roberts**
Sequencing of *Eco*RII endonuclease gene.

Ethel Johnson, Vanderbilt University
Research Advisor: **David Helfman**
Sequence determination and analysis of introns in rat embryonic fibroblast tropomyosin 1.

Abhijeet Lele, Jesus College, Cambridge
Research Advisor: **Edward Harlow**
Identification of cellular mediators of E1A action by random mutagenesis of E1A-transformed cells.

William Moomaw, State University of New York, Albany
Research Advisor: **Stephen Dellaporta**
Identification of *Ac2* elements in the DNA of maize stocks showing responder element activity.

Nicholas Morrissey, University of Rochester
Research Advisor: **Andrew Rice**
Characteristics of double-stranded RNA required for the activation of the protein kinase, DAI.

Roya Namvar, New York University
Research Advisor: **Douglas Youvan**
Oligonucleotide-directed site-specific mutagenesis of the light-harvesting I antenna genes of *Rhodobacter capsulata*.

Scott Panzer, Harvard University
Research Advisor: **Douglas Hanahan**
Expresson patterns of papilloma viruses in transgenic mice.

Henry Stapp, Hampshire College
Research Advisor: **Pablo Scolnik**
Study of in vitro carotenoid biosynthesis and in vitro assembly of functional light-harvesting II complex with *Rhodobacter capsulata*.

Wendy Weiher, University of Pennsylvania
Research Advisor: **Roger Cone**
Efficiency of oncogene transfection into E1A immortalized rat cells.

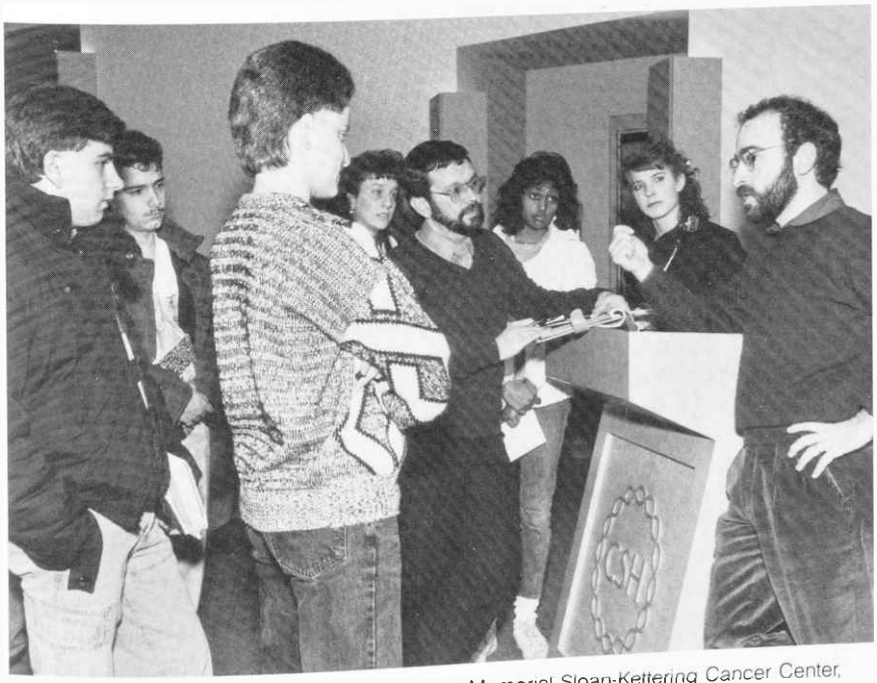
DNA Literacy Program

Since the explosion of information made possible by the invention of the printing press, a democracy has needed to be a society of literates. Similarly, the growing importance of molecular biology demands a society of *DNA literates*. Unfortunately, DNA science is advancing so fast that the gap between biotechnological progress and public understanding widens every day.

The overreaching goal of Cold Spring Harbor Laboratory's DNA Literacy Program is to close this biotechnology understanding gap. Our primary target audiences are secondary and freshman college instructors who teach both general biology and advanced elective courses. Since biology constitutes the most widely studied science in the United States, its instruction offers a major channel through which science literacy in general and DNA literacy in particular can be spread.

We believe that DNA literacy must become biology teachers' foremost concern. The current failure to emphasize DNA science in our schools means failure to teach the most exciting topics biology has to offer; failure to present socially and personally relevant issues; and, in the final analysis, failure to fulfill teaching's most important function—to prepare citizens capable of informed votes on policy issues.

The DNA Literacy Program now consists of two elements: the local Cold Spring Harbor Curriculum Study and the national Vector DNA Science Workshop Program. Close interaction, over the past two years with 250 educators from 12 states and Great Britain, has helped us crystallize program goals and evaluate our progress toward reaching them.



Dr. Morris Birnbaum, right, research associate at Memorial Sloan-Kettering Cancer Center, talks with students and teachers after his lecture "Molecular Regulation of Glucose Transport," the fourth of six *Great Moments* Lectures.

The Vector Mobile DNA Laboratory Staff: (l. to r.) Program Director David Micklos, DNA Workshop Coordinator Christine Bartels, summer lab aide Jeff Mondschein, and Technical Advisor Dr. Greg Freyer (below).

Cold Spring Harbor Curriculum Study

The Curriculum Study was founded in January 1985 as a consortium between Cold Spring Harbor Laboratory and eight local school districts. The program, which has grown to include 19 school districts on Long Island and in Westchester County, uses local resources and teaching systems as a proving ground for novel educational materials on DNA science.

Program Director David Micklos and Dr. Greg Freyer, now a research associate at Memorial Sloan-Kettering Cancer Center, developed an articulated set of lab experiences introducing teachers to the major concepts and techniques of molecular biology. Their protocols, presuming no specific prior knowledge, are the basis of a five-day workshop, *Recombinant DNA for Beginners*, which has become the central component of our educational efforts.

Our experience indicates that the hands-on summer workshop is a good format for acquainting teachers with the laboratory materials and training them to initiate lab-teaching at their own schools. After holding a regional workshop both in 1985 and 1986 for about 40 individuals each, we estimate that at least 600 students have now performed experiments under the supervision of participating teachers. Participants setting up DNA science labs for their classes receive further support through joint equipment purchase options, telephone consultation, and site visits by Cold Spring Harbor staff. In the *Great Moments* lecture series for students and teachers held at Grace Auditorium, renowned speakers from both the Laboratory and outside institutions cover a number of notable achievements and current issues in molecular biology.

Vector DNA Science Workshop Program

The successful Curriculum Study experience in New York State and interest from numerous educators around the country suggested the feasibility of a mobile laboratory which would carry all equipment and reagents necessary to administer *Recombinant DNA for Beginners* workshops at any location the United States. In summer 1986 a grant from Citibank, N.A., allowed us to equip our first Vector van and take the teacher-training course on the road. Vector Workshops were held in seven locations: Huntington and Irvington, New York; Boston, Massachusetts; Concord, New Hampshire; Chicago, Illinois; Milwaukee, Wisconsin; and Davis, California.

Thus, the complete set of experiments was successfully performed by over 200 enthusiastic high school and college teachers nationwide. Survey data indicate that all of them felt excited and reinvigorated in their profession, gained increased awareness and understanding of DNA science, and were stimulated to seek more information on their own. Within months of the Vector experience, many of them have been able to restructure their generally outdated syllabi to accommodate molecular biology topics, and a significant number have actually started to introduce DNA labs to their classes.

We have found, however, that regional teaching infrastructures—cluttered syllabi, meager equipment funds, lack of teacher support and incentives, and inadequate class time—are major obstacles to successful lab implementation. In the future, many of our activities will be directed at increasing administrators' awareness and creating follow-up activities for workshop participants.

Students and teachers receive supplies for an experiment during a Vector Workshop.

Prospectus

For summer 1987, we have scheduled double the number of teacher-training courses and purchased a second Vector van. Again, our excursions will take us to various parts of the country, from New York City and New England to sites in the Southeast, Midwest and West. We are now in the process of recruiting staff members for Vector II.

The lab manual accompanying the workshop, *Recombinant DNA for Beginners: A First Laboratory Course in Molecular Biology*, will be jointly published by Cold Spring Harbor Laboratory Press and Carolina Biological Supply Company this year. Carolina Biological, with its well-established distribution network, will help us retool science classrooms by marketing both the manual and all necessary reagents.

It has become apparent that crucial to widespread biotechnology will be a national clearinghouse and teacher-training center specializing in DNA science education. We are now looking for major support from foundations, biotechnology-oriented companies, and public agencies that will make reality our dream to develop a DNA Learning Center at Cold Spring Harbor. Proposed features of the facility include a teaching laboratory, computer graphics laboratory, DNA library, and science media development unit, all aimed at bringing the excitement of molecular biology to the lay public.

Proposed DNA Learning Center in Cold Spring Harbor Village.

Teachers prepare to separate DNA fragments using gel electrophoresis.

Support

Support of the DNA Literacy Program has been provided by a consortium of foundations, companies, and private individuals, including Citibank, N.A., the J.M. Foundation, the Josiah Macy, Jr. Foundation, Bethesda Research Laboratories, the Richard Lounsbery Foundation, Fotodyne Incorporated, Amersham Corporation, Cetus Corporation, New England Biolabs Foundation, Eli Lilly Research Laboratories, Pioneer Hi-Bred International, Inc., the Dorcus Cummings Memorial Fund of the Long Island Biological Association, The Harris Trust, and Mr. and Mrs. Oliver R. Grace.

Our activities are coordinated with numerous educational and research institutions, including the New York State Bureau of Science Education, Science Teachers Association of New York State, Science Supervisors Association, National Science Teachers Association, New York City Board of Education, State University of New York, California State Board of Education, Biotechnology Program of the University of California at Davis, Macy Bioprep Program, Marquette University, Cleveland Clinic Foundation, Argonne National Laboratory, North Carolina Biotechnology Center, Utah State University, Bronx High School of Science, Thomas Jefferson High School for Science and Technology, St. Paul's School, Choate Rosemary Hall, and the Winsor School.

The Curriculum Study consists of the following school districts: Cold Spring Harbor Central School District; Commack Union Free School District; East Williston Union Free School District; Great Neck Union Free School District; Half Hollow Hills Central School District; Herricks Union Free School District; Huntington Union Free School District; Irvington Union Free School District; Jericho Union Free School District; Lawrence Union Free School District; Manhasset Union Free School District; North Shore Central School District; Northport-East Northport Union Free 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.

Students practice methods for bacterial cuture.

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 1986 a total of 320 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, Shu Swamp Preserve, 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 the 100 foot Sloop Clearwater chartered from Hudson River Sloop Clearwater, Inc. of Poughkeepsie, New York. 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 six-mile canoe trip on the Nissequogue River, and a day of sailing on the Sloop Clearwater.

PROGRAM DIRECTOR

William M. Payoski, M.A., Adjunct Professor,
Nassau Community College

INSTRUCTORS

Ruth Burgess, B.A., naturalist, Nassau County BOCES
Joanna Cardinali, M.A., naturalist, Nassau County BOCES
Steven Lander, M.S., science coordinator, Harborfields School District
Fred Maasch, M.S., science teacher, Islip High School
Maryann Manzilillo, M.A., naturalist, Nassau County BOCES
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
Vertebrate Biology
Marine Biology
Nature Photography
Adventure Education
Marine Biology Workshops

FINANCIAL STATEMENT

FINANCIAL STATEMENT

BALANCE SHEET—year ended December 31, 1986 with comparative figures for year ended December 31, 1985

ASSETS

COLD SPRING HARBOR LABORATORY

	1986	1985
CURRENT FUNDS		
Unrestricted		
Cash and Short-term investments	$12,044,160	$11,567,359
Accounts Receivable	452,317	286,511
Prepaid expenses and other assets	502,059	480,909
Inventory of books	246,453	218,043
Total unrestricted	13,244,989	12,552,822
Restricted		
Marketable securities (quoted market 1986—$2,000,000; 1985—$850,000)	212,500	212,500
Grants and contracts receivable	3,283,509	6,648,896
Due from unrestricted fund	1,803,052	501,239
Total restricted	5,299,061	7,362,635
Total current funds	$18,544,050	$19,915,457
ENDOWMENT FUNDS		
Robertson Research Fund		
Cash	1,104,670	231,649
Marketable securities (quoted market 1986—$24,573,649; 1985—$22,240,523)	20,533,544	18,393,860
Total Robertson Research Fund	21,638,214	18,625,509
Olney Memorial Fund		
Cash	3,191	1,138
Marketable Securities (quoted market 1986—$48,044; 1985—$41,928)	30,593	32,101
Total Olney Memorial Fund	36,784	33,239
Unrestricted Endowment Fund		
Cash	139,074	—
Total Unrestricted Endowment Fund	139,074	—
Total endowment funds	$21,814,072	$18,658,748
PLANT FUNDS		
Investments	718,012	879,871
Due from unrestricted fund	2,741,895	3,594,835
Land and improvements	1,471,432	1,340,375
Buildings	17,858,736	16,374,820
Furniture, fixtures and equipment	4,550,467	3,846,120
Books and periodicals	365,630	365,630
Construction in progress	631,106	406,541
	28,337,278	26,808,192
Less allowance for depreciation and amortization	6,947,936	5,896,757
Total plant funds	$21,389,342	$20,911,435
Total Cold Spring Harbor Laboratory funds	$61,747,464	$59,485,640

LIABILITIES AND FUND BALANCES

	1986	1985
CURRENT FUNDS		
Unrestricted		
Accounts payable	$ 815,477	$ 854,619
Deferred income	165,000	150,000
Due to Banbury Center	227,668	148,944
Due to plant fund	2,741,895	3,594,835
Due to restricted fund	1,803,052	501,239
Fund balance	7,491,897	7,303,185
Total unrestricted	13,244,989	12,552,822
Restricted		
Accounts payable	137,520	275,171
Fund balance	5,161,541	7,087,464
Total restricted	5,299,061	7,362,635
Total current funds	$18,544,050	$19,915,457
ENDOWMENT FUNDS		
Fund balance	$21,814,072	$18,658,748
PLANT FUNDS		
Loan payable	8,000,000	8,000,000
Fund balance	13,389,342	12,911,435
Total Plant Funds	$21,389,342	$20,911,435
Total Cold Spring Harbor Laboratory funds	$61,747,464	$59,485,640

ASSETS

BANBURY CENTER

	1986	1985
CURRENT FUNDS		
Unrestricted		
Cash	$ 700	$ 700
Prepaid and deferred expenses	14,148	5,726
Inventory of books	62,605	56,299
Due from CSHL unrestricted fund	191,758	103,322
Total unrestricted	269,211	166,047
Restricted		
Grants and contracts receivable	125,003	99,141
Due from CSHL unrestricted fund	35,910	45,622
Total restricted	160,913	144,763
Total current funds	$ 430,124	$ 310,810
ENDOWMENT FUNDS		
Robertson Maintenance Fund		
Cash	221,406	10,707
Marketable securities (quoted market 1986 – $3,930,272; 1985 – $3,637,079)	3,292,985	3,013,692
Total endowment funds	$ 3,514,391	$ 3,024,399
PLANT FUNDS		
Land	772,500	772,500
Buildings	846,028	846,028
Furniture, fixtures and equipment	191,383	191,383
	1,809,911	1,809,911
Less allowance for depreciation	419,112	387,505
Total plant funds	$ 1,390.799	$ 1,422,406
Total Banbury Center funds	$ 5,335,314	$ 4,757,615
Total Cold Spring Harbor Laboratory and Banbury Center funds	$67,082,778	$64,243,255

LIABILITIES AND FUND BALANCES

	1986	1985
CURRENT FUNDS		
Unrestricted		
Accounts payable	$ 34,756	$ 8,142
Fund balance	234,455	157,905
Total unrestricted	269,211	166,047
Restricted		
Accounts payable	2,181	4,577
Fund balance	158,732	140,186
Total restricted	160,913	144,763
Total current funds	$ 430,124	$ 310,810
ENDOWMENT FUNDS		
Fund balance	$ 3,514,391	$ 3,024,399
PLANT FUNDS		
Fund balance	$ 1,390,799	$ 1,422,406
Total Banbury Center funds	$ 5,335,314	$ 4,757,615
Total Cold Spring Harbor Laboratory and Banbury Center funds	$67,082,778	$64,243,255

CURRENT REVENUES, EXPENSES AND TRANSFERS
year ended December 31, 1986
with comparative figures for year ended December 31, 1985

COLD SPRING HARBOR LABORATORY

	1986	1985
REVENUES		
Grants and contracts	$13,819,752	$15,869,438
Indirect cost allowances on grants and contracts	4,533,243	4,406,047
Contributions		
Unrestricted	243,906	332,423
Restricted, Capital and Endowment	1,082,585	278,483
Long Island Biological Association	160,000	100,000
Robertson Research Fund Distribution	650,000	575,000
Royalty and licensing fees	105,861	197,000
Summer programs	721,951	553,395
Laboratory rental	20,732	20,732
Marina rental	63,423	61,278
Investment income	905,418	903,132
Publications sales	1,356,928	1,432,527
Dining Hall	776,767	638,690
Rooms and apartments	303,484	248,854
Other sources	19,716	22,968
Total revenues	24,763,766	25,639,967
EXPENSES		
Research*	12,055,370	11,710,764
Summer and training programs*	1,999,031	1,660,496
Publications sales*	1,013,374	1,029,060
Dining hall*	854,967	769,574
Research support	359,542	400,341
Library	285,024	267,341
Operation and maintenance of plant	2,442,498	2,413,788
General and Administrative	2,031,514	1,965,647
Depreciation	1,061,477	1,046,630
Total expenses	22,102,797	21,263,641
TRANSFERS		
Capital building projects	2,248,761	3,805,100
Total expenses and transfers	24,351,558	25,068,741
Excess of revenues over expenses and transfers	$ 412,208	$ 571,226

* Reported exclusive of an allocation for research support, operation and maintenance of plant, general and administrative, library, and depreciation expenses.

BANBURY CENTER

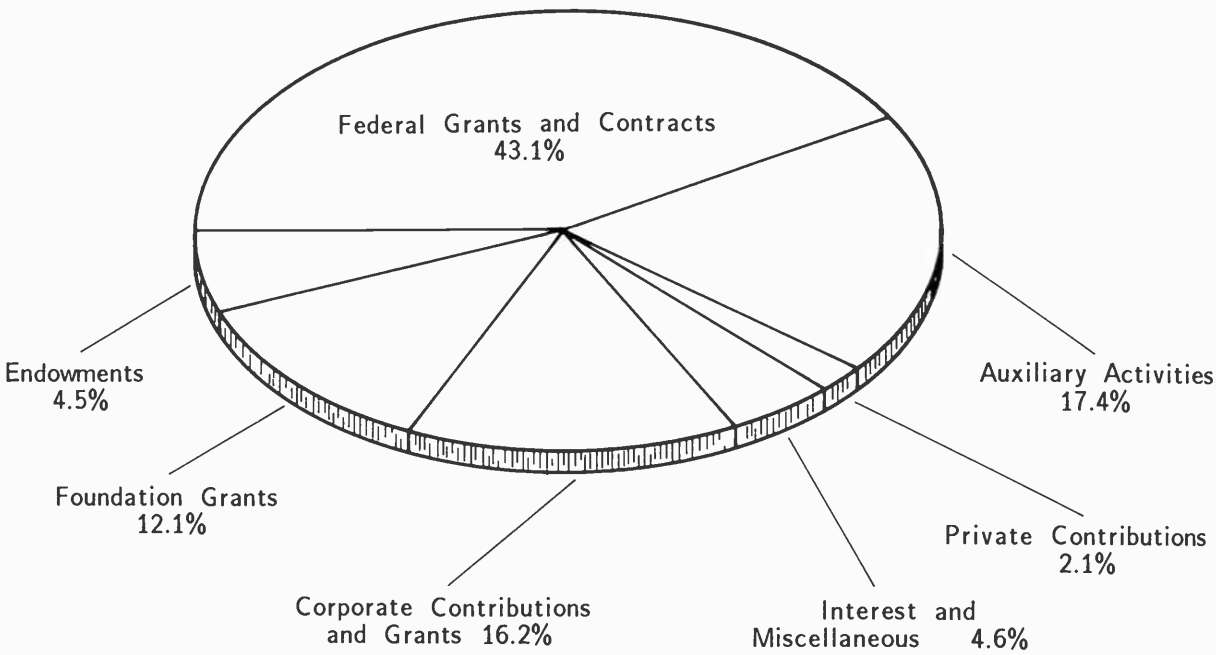
	1986	1985
REVENUES		
Endowment income	$ 120,000	$ 112,000
Grants and contributions	431,642	335,210
Indirect cost allowances on grants and contracts	21,265	28,445
Rooms and apartments	124,000	97,026
Publications	142,300	133,164
Conference fees	27,980	22,086
Dining Hall	60,985	42,314
Total revenues	928,172	770,245
EXPENSES		
Conferences	264,932	188,559
Publications	123,156	124,563
Operation and maintenance of plant	154,278	124,760
Program administration	252,629	222,715
Depreciation	31,607	30,486
Capital plant	–	11,000
Total expenses	826,602	702,083
Excess of revenues over expenses	$ 101,570	$ 68,162

NOTE: Copies of our complete, audited financial statements, certified by our independent auditors, Peat, Marwick, Mitchell & Co., are available upon request from the Comptroller, Cold Spring Harbor Laboratory.

COLD SPRING HARBOR LABORATORY

SOURCES OF REVENUE

YEAR ENDED DECEMBER 31, 1986



GRANTS AND CONTRIBUTIONS

FINANCIAL SUPPORT OF THE LABORATORY

Cold Spring Harbor Laboratory is a nonprofit research and educational institution chartered by the University of the State of New York. Contributions 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.

Because its endowment is limited and the uses of research grants are formally restricted, the Laboratory depends on generous contributions from private foundations, sponsors, and friends for central institutional needs and capital improvements.

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, moveable genetic elements, yeast genetics, and molecular neurobiology. However, the continued development of new research programs and training courses requires substantial support from private sources.

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.

Bequests Probably most wills need to be updated. Designating Cold Spring Harbor Laboratory as beneficiary ensures that a bequest will be utilized as specified for continuing good.

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.

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 Public Affairs and Development, Cold Spring Harbor Laboratory, Box 100, Cold Spring Harbor, N.Y. 11724, or call 516-367-8455.

GRANTS FOR RESEARCH AND EDUCATION

January 1, 1986—December 31, 1986

COLD SPRING HARBOR LABORATORY

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*Total Award*
FEDERAL GRANTS			
NATIONAL INSTITUTES OF HEALTH			
Program Projects	Cancer Research Center, Drs. Watson, Sambrook, Roberts and Mathews	1/77–12/86	$18,604,426
	Gene Organization, Drs. Watson and Sambrook	4/81– 3/86	5,626,997
Research Support	Dr. Beach	12/84–11/87	361,511
	Dr. Beach	9/86– 8/89	578,929*
	Dr. Blose	12/78–11/87	1,094,594
	Dr. Dellaporta	7/85– 6/88	534,805
	Dr. Feramisco	7/80– 7/86	686,968
	Dr. Feramisco	4/85– 3/88	535,534
	Dr. Franza	9/85– 7/88	478,689
	Dr. Garrels	1/85–12/89	2,028,833
	Dr. Helfman	9/85– 8/88	572,760
	Dr. Hicks	7/81– 6/86	1,623,433
	Dr. Klar	7/81– 3/87	1,572,978
	Dr. Kurtz	4/80– 3/86	606,389
	Dr. Mathews	4/80– 3/86	729,283
	Dr. Roberts	9/86– 8/89	303,642*
	Dr. Stillman	7/83– 6/92	1,869,678
	Dr. Watson	4/85– 3/86	146,986
	Dr. Watson	4/86– 3/87	144,681*
	Dr. Welch	4/84– 3/87	314,933
	Dr. Wigler	7/85– 6/92	8,426,929
	Dr. Zoller	9/85– 8/88	545,752
Fellowships	Dr. Bar-Sagi	9/85– 8/87	47,000
	Dr. Cheley	10/86– 3/88	37,494*
	Dr. Bautch	9/83– 8/86	57,244
	Dr. Field	10/86– 9/89	63,996*
	Dr. Gerard	5/84– 4/86	38,204
	Dr. Livi	10/83–10/86	57,244
	Dr. McLeod	2/85– 1/88	63,996
	Dr. Morris	11/86–10/89	82,008*
	Dr. Quinlan	6/84– 5/87	57,244
	Dr. Potashkin	10/85– 9/88	64,886
Training	Institutional, Dr. Grodzicker	7/78– 8/89	1,291,646
Course Support	Advanced Bacterial Genetics, Drs. Hicks and Klar	5/80– 4/88	302,523
	Cancer Center, Dr. Grodzicker	6/82–12/86	657,174
	Neurobiology, Dr. Hockfield	6/79– 3/86	646,143
	Neurobiology, Dr. Hockfield	5/82– 4/90	723,939

* New grants awarded in 1986

Grantor	Program/Principal Investigator	Duration of Grant	Total Award
Meeting Support	51st Symposium: Molecular Biology of *Homo sapiens*	1986	24,719*
	Hepatitis B Virus	1986	5,000*
	Papilloma Virus	1986	12,000*
	DNA Topology	1986	1,500*
	G-Proteins and Signal Transduction	1986	5,500*
	RNA Processing	1986–1987	3,500*
	Eukaryotic Transposable Elements	1986	2,000*
NATIONAL SCIENCE FOUNDATION			
Research Support	Dr. Klar	8/86– 7/89	330,000*
	Dr. Roberts	11/83– 5/85	120,908
	Dr. Roberts	3/83– 8/86	40,592
	Dr. Roberts	1/83– 5/90	640,000
	Dr. Roberts	7/83–12/86	252,000
Course Support	Plant Molecular Biology, Dr. Klar	5/84–12/86	121,676
	Plant Molecular Biology, Dr. Klar	8/86– 1/90	137,490*
Meeting Support	RNA Processing	1986–1987	12,400*
	G-Proteins and Signal Transduction	1986	2,000*
	Papilloma Viruses	1986	2,100*
	Hepatitis B Viruses	1986	2,100*
	Eukaryotic Transposable Elements	1986	5,000*
	DNA Topology	1986	9,000*
	51st Symposium: Molecular Biology of *Homo sapiens*	1986	7,000*
DEPARTMENT OF AGRICULTURE			
Research Support	Dr. Dellaporta	9/85– 8/86	21,000
	Dr. Dellaporta	9/84– 8/86	100,000
	Dr. Hiatt	8/86– 7/89	165,000*
Meeting Support	Eukaryotic Transposable Elements	1986	8,000*
DEPARTMENT OF ENERGY			
Research Support	Dr. Dellaporta	9/85– 8/86	75,000
Meeting Support	Symposium: Molecular Biology of *Homo sapiens*	1986	9,000*
NONFEDERAL GRANTS			
Research Support			
A.B.C. Foundation	Dr. Wigler	5/82– 4/87	600,000
Rita Allen Foundation	Dr. Herr	9/85– 8/90	150,000
Rita Allen Foundation	Dr. Stillman	1/83–12/87	150,000
American Cancer Society	Dr. Beach	1/85–12/86	70,000
	Drs. Stillman, Gluzman—Institutional Award	7/82– 6/87	185,000

* New grants awarded in 1986

Grantor	Program/Principal Investigator	Duration of Grant	Total Award
	Dr. Wigler	7/84- 6/86	200,000
	Dr. Wigler, Professorship	1986-2012	1,333,333*
	Dr. Wigler	1986	10,000*
Amersham International plc	Dr. Welch	10/85- 9/87	50,000
	Dr. Harlow	11/86-10/87	148,780*
Cancer Research Institute	Dr. Franza	4/86- 3/87	25,000*
Cornell University	Dr. Siniscalco	1/86- 8/86	31,115*
Diabetes Research & Education Foundation	Dr. Hanahan	5/86- 4/87	19,960*
Russell and Janet Doubleday Fund	General Support	1986	16,750*
Exxon Research and Engineering Company	Cooperative Research	1/82-12/86	7,500,000
International Foundation for Cancer Research	Dr. Hanahan	10/84- 9/86	50,000
Juvenile Diabetes Foundation	Dr. Hanahan	9/86- 9/88	66,000*
Leo Model Foundation	General Support	1986	1,000*
McKnight Foundation	Dr. Malmberg	3/83- 2/86	105,000
Merck & Co., Inc.	Dr. Cone	1986	20,000*
Monsanto Company	Cooperative Research	10/84- 9/90	2,089,200
Muscular Dystrophy Association	Dr. Helfman	7/86- 6/89	94,500*
	Dr. Mathews	7/83-12/86	88,776
New England Biolabs, Inc.	Dr. Roberts	1986	10,000*
Pfizer, Inc.	Dr. Wigler	1985-1990	500,000
Phillips Petroleum Company	General Support	1986	3,000*
Pioneer Hi-Bred International, Inc.	Cooperative Research	8/85- 4/91	2,500,000*
Protein Databases Inc.	General Support	1986	25,000*
Marie Robertson Memorial Fund	Neurobiology	1986	125,000*
Scripps Clinic	Dr. Hanahan (subcontract)	1/86- 9/88	557,137*
Fellowships			
Alberts Heritage Foundation for Medical Research	Dr. Mizzen	7/86- 6/87	19,730*
American Cancer Society	Dr. Young	8/84- 7/85	16,500
Arthritis Foundation	Dr. Morris	7/86-11/86	6,000*
Cancer Research Institute	Dr. Cone	10/85- 9/87	48,000
	Dr. Young	8/85- 8/87	46,000
	Dr. Sturm	9/86- 8/88	51,000*
	Dr. Efrat	9/86- 8/88	51,000*
	Dr. Field	10/85- 9/86	18,000
Anna Fuller Fund	Dr. Hanahan	7/84- 6/86	44,280
Leukemia Society of America	Dr. Colicelli	9/86- 8/89	105,000*
Life Science Research Foundation	Dr. Bautch	9/86- 8/87	19,000*
NYS Health Research Council	Undergraduate Research	1986	4,733*
Robert H.P. Olney Memorial Fund	Dr. Hicks	9/84- 8/86	25,900
Rockefeller Foundation			
Damon Runyon-Walter Winchell Cancer Fund	Dr. Broek	6/84- 5/86	38,000
	Dr. Hearing	9/84- 8/86	38,000
	Dr. Hiatt	8/84- 5/86	34,750
	Dr. Sass	9/84- 8/86	38,000
Helen Hay Whitney Foundation	Dr. Kostriken	3/86- 2/89	56,000*
Training			
American Cyanamid Company	Undergraduate Research Program	1986	7,000*
Bristol-Myers Company	Fellowship Support	6/86- 5/91	500,000*
Burroughs Wellcome Fund	Undergraduate Research Program	1986	10,500*

* New grants awarded in 1986

Grantor	Program/Principal Investigator	Duration of Grant	Total Award
Grass Foundation	Neurobiology Scholarships	1980–1986	101,670
Lucille P. Markey Foundation	Scholarship Support	1985–1988	150,000
Pfizer Inc.	Undergraduate Research Program	1986	7,000*
Rockefeller Foundation	Scholarship Support	1985–1986	16,000
Alfred P. Sloan Foundation	Undergraduate Research Program	1985–1988	74,000
Course Support			
The Esther A. and Joseph Klingenstein Fund	Neurobiology Support	1986–1988	300,000
Alfred P. Sloan Foundation	Computational Neuroscience Course	1986–1989	140,000*
Meeting Support			
Rita Allen Foundation	Symposium: Molecular Biology of *Homo sapiens*	1986	15,000*
Banbury Fund	Symposium: Molecular Biology of *Home sapiens*	1986	15,000*
Sanford C. Bernstein Foundation	Papilloma Virus	1986	10,000*
Burroughs Wellcome Co.	Papilloma Virus	1986	6,500*
Council for Tobacco Research	Papilloma Virus	1986	2,500*
Johnson & Johnson	Vaccines	1986	5,000*
Life Technologies, Inc.	Poxvirus	1986	100*
March of Dimes Birth Defects Foundation	Regulation of Liver Gene Expression	1986–1987	2,000*
Otolaryngology Foundation	Papilloma Virus	1986	5,000*
Rockefeller Foundation	Vaccines	1986–1988	45,000*
DNA Literacy Program			
Amersham Corporation	Vector Workshop	1986	10,000*
Citicorp/Citibank	Vector Van	1986	30,000*
Dorcas Cummings Memorial Fund of Long Island Biological Association	DNA Education Center	1986	10,000*
Fotodyne Incorporated	Vector Workshop	1986	9,000*
GIBCO/Bethesda Research Laboratories	Vector Workshop	1986	10,000*
Mr. and Mrs. Oliver R. Grace	DNA Education Center	1986	20,000*
The Harris Trust	DNA Education Center	1986	10,000*
The J.M. Foundation	Program Support	1986	30,000*
Richard Lounsbery Foundation	Vector Workshop	1986	10,000*
Miller Brewing Company	Vector Workshop	1986	1,000*
New England Biolabs Foundation	Vector Workshop	1986	7,000*
University of California, Davis	Vector Workshop	1986	10,000*

* New grants awarded in 1986

BANBURY CENTER

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*Total Award*
FEDERAL GRANTS			
NATIONAL SCIENCE FOUNDATION			
Meeting Support	Evolution and Environmental Spread of Antibotic Resistance Genes	1986	$25,000*
DEPARTMENT OF ENERGY			
Meeting Support	Evolution and Environmental Spread of Antibotic Resistance Genes	1986	25,000*
NONFEDERAL GRANTS			
Meeting Support			
American Cancer Society	DNA Adduct Workshop	1986	11,253*
American Industrial Health Council Inc.	Nongenotoxic Carcinogenicity Conference	1986	25,000*
James S. McDonnell Foundation	Conference Support	1985–1987	300,000*
Risk Science Institute	DNA Adducts Workshop	1986	10,000*
Shearson-American Express Foundation	Conference Support	1986	64,752*
Alfred P. Sloan Foundation	Journalists' and Congressional Workshops	1984–1986	162,000

* New grants awarded in 1986

ENDOWMENTS AND GIFTS FOR FACILITIES

ENDOWMENTS

	Market Value*
Cold Spring Harbor Laboratory	
Cold Spring Harbor Laboratory Unrestricted Endowment Fund	$ 139,075
1986 Contributors: Mr. G. Morgan Browne, Jr.	
Mrs. Sinclair Hatch	
Mr. and Mrs. George N. Lindsay	
Mr. and Mrs. David L. Luke III	
Mr. Taggart Whipple	
The Doubleday Professorship for Advanced Cancer Research	1,500,000
Robert H.P. Olney Memorial Fund	51,235
1986 Contributors: The Rev. Linda Peyton Hancock	
Mr. Richard Olney III	
Ms. Pamela Peyton Post	
Mrs. Robert W. Tilney, Jr.	
Robertson Research Fund	25,678,319
Robertson Maintenance Fund	4,151,678
Total Cold Spring Harbor	$31,520,307
Long Island Biological Association (LIBA)	
Dorcas Cummings Memorial Lecture Fund	$58,633
Other Endowments	37,487
Total LIBA	$96,120

FACILITIES

Oliver and Lorraine Grace Auditorium			
Mr. & Mrs. Oliver R. Grace	Construction	1986	$ 91,047†
Protein Databases Inc.	Construction	1986	25,000†
Mrs. Elizabeth B. Schneider	Construction	1986	3,591†
Arthur and Walter Page Laboratory of Plant Genetics			
Anonymous	Construction	1986	25,000†
Charles E. Culpeper Foundation	Construction	1985–1987	75,000
Ira W. DeCamp Foundation	Construction	1986	50,000†
Long Island Biological Association	Construction	1986	130,000†
National Science Foundation	Construction	1983–1988	690,715
North Country Garden Club	Construction	1986	1,000†
Dr. David B. Pall	Construction	1986	105,057†
William and Maude Pritchard Charitable Trust	Construction	1986	75,000†

			Market Value*
X-Ray Crystallography Laboratory			
Mr. & Mrs. Oliver R. Grace	Equipment	1986	50,000†
Lucille P. Markey Charitable Trust	Equipment	1986–1988	863,500†
Jones Laboratory			
McKnight Foundation	Equipment for Neurobiology Course	1984–1986	110,000
Samuel Freeman Computer Center			
Cancer Research Institute	Equipment	1986	50,000†
Oliver S. and Jennie R. Donaldson Charitable Trust	Equipment	1986	50,000†
Samuel Freeman Charitable Trust	Equipment	1985–1986	350,000
Lodge Building Fund			
Dr. Mark Ptashne	Construction	1986	45,625†‡

* As of December 31, 1986
† New grants awarded in 1986
‡ Value of stock on December 31, 1986

ANNUAL GIVING PROGRAMS

Corporate Sponsor Program

Access to current research presented at high-level professional meetings is an important resource for the biotechnology industry. Since 1984 the Corporate Sponsor Program has encouraged increased interaction between academic and industrial scientists, while at the same time providing financial stability for the Laboratory's meetings program.

The annual $15,000 membership commitment from each Corporate Sponsor has enabled the Laboratory to significantly expand its role as a clearinghouse for high-level biotechnical information. With more than 3,600 scientists attending 25 conferences and 13 advanced training courses at the Laboratory and Banbury Center in 1986, the Laboratory now offers the most comprehensive postgraduate educational program available anywhere in the United States.

Among those attending 1986 meetings were 310 scientists representing 85 companies, an increase in corporate attendance of 48 percent from 1983. During the same period, 1983–86, overall attendance increased 42 percent (from 2,540 to 3,610), and the number of meetings increased 32 percent (from 19 to 25).

Proceeds from the Corporate Sponsor Program allowed initiation of an ongoing series of Special Banbury Conferences that focus on emerging areas of research especially 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 1986 conferences were "Mechanisms of Microbial Energy Transduction," "Recombination Mechanisms in Yeast," "DNA Probes," "Gene Transfer Vectors," and "Tumor Angiogenesis Factor."

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, and recognition in meeting abstracts and publications.

In the past three years, 26 companies have participated in the Corporate Sponsor Program. The membership renewal rate is 90 percent. 1986 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
Becton Dickinson and Company
Cetus Corporation
Ciba-Geigy Corporation
CPC International Inc.
E.I. du Pont de Nemours & Company
Genentech, Inc.
Genetics Institute
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Eli Lilly and Company
Monsanto Company
Pall Corporation
Pfizer Inc.
Schering-Plough Corporation
Smith Kline & French Laboratories
Tambrands Inc.
The Upjohn Company
Wyeth Laboratories

Alumni Cabin Campaign

The Alumni Cabin Campaign was initiated in the fall as an experiment to answer the question: "Do meeting attendees, course instructors, and former staff have enough affection for Cold Spring Harbor to consider themselves alumni?"

The answer was a resounding "yes," as biologists from around the world recalled how their careers had benefited from the Cold Spring Harbor experience. By year's end nearly 200 scientists (one in four asked) had contributed almost $34,000 for construction of "Alumni Cabin," one of six four-bedroom cabins planned to replace existing, dilapidated structures. Gifts continue to be received in 1987, and it is hoped the campaign goal of $100,000 can be reached by the time the cabins come into service in fall 1988. Construction is due to start in fall 1987.

Donations were received prior to December 31, 1986, from:

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Dr. Peggy Anderson
Dr. Ralph B. Arlinghaus
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Dr. Manuel Perucho
Pharmacia AB on behalf of
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Dr. Peter Phillipsen
Dr. Terry Platt
Dr. Dale Purvis
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Dr. Takashi Yura
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In memory of Ethel S. Tessman

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Rosina Cox Boardman
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Jane Gudebrod
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The LONG ISLAND BIOLOGICAL ASSOCIATION (LIBA) and Its Relation to the Cold Spring Harbor Laboratory

Biological research at Cold Spring Harbor began in 1890 when the Brooklyn Institute of Arts and Sciences was looking for a place to set up a summer laboratory as the headquarters of its Department of Zoology. Representatives of the Institute were invited by Eugene S. Blackford, at that time the Fish Commissioner of the State of New York, to inspect a site on Bungtown Road across Northern Boulevard (25A) from the Fish Hatchery. The site was found to be ideal, and so the original Laboratory was organized with Mr. Blackford as president of the Board of Trustees. The land was leased from Mr. John D. Jones, whose family since 1819 had operated various industries including shipbuilding, coopering, and textile manufacture (later whaling, also) at the head of Cold Spring Harbor. Bungtown Road, which runs through the Lab property, got its name from the factory that specialized in making bungs—or stoppers—for barrels.

In 1892 the Laboratory's land was leased for a dollar a year from the Wawepex Society, which Mr. Jones had organized as a corporation for holding real estate and for investing funds for the propagation of fishes and for scientific research. In 1904 the Wawepex Society leased additional land to the Carnegie Institution of Washington, which wanted to locate a Department of Experimental Evolution in the Cold Spring Harbor area. Charles B. Davenport, who had been directing the Laboratory since 1896, assumed the additional duties of director of the Carnegie Institution's experimental station. It is interesting to note that Mr. Davenport lived in what came to be known later as the Carnegie Dormitory, the Victorian house on 25A built by John D. Jones before the turn of the century, and recently repainted in its original colors.

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 Lab's Board of Trustees.

What has happened, in effect, is that LIBA has become an expanding group of local "Friends of the Laboratory" who help support it through annual contributions. Also, from time to time, the Association undertakes campaigns to finance special important projects for which the Lab cannot obtain funds from the Federal Government or from other sources. For instance, in recent years, building the James Laboratory Annex and the renovation of Blackford Hall, the rebuilding of Williams House, the acquisition of the land formerly belonging to the Carnegie Institution, and (in part) the construction of the new Grace Auditorium.

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.

Membership in LIBA requires a minimum annual contribution (tax deductible) of $25 for a family. 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) 367-8319.

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CHAIRMAN'S REPORT

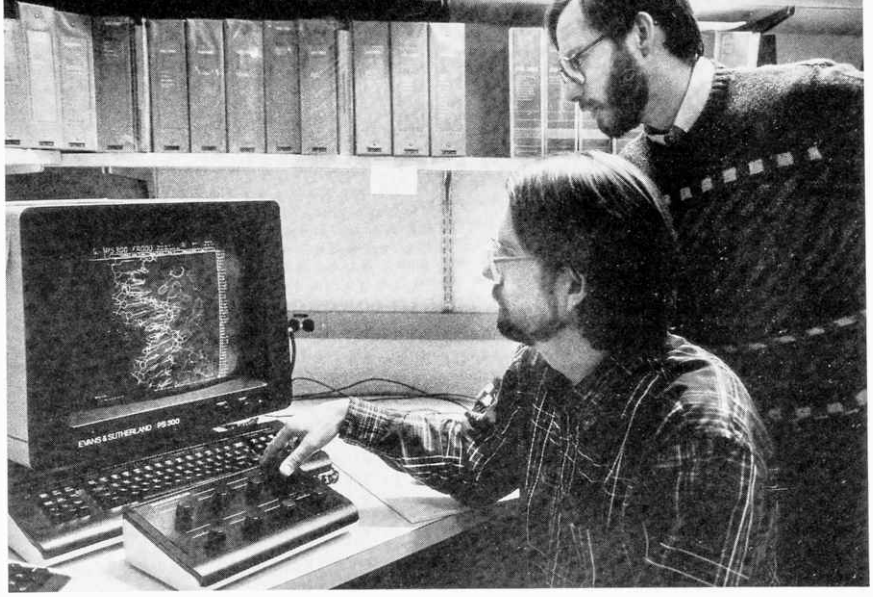
My first year as Chairman has been a busy one. The retirement of Edward Pulling necessitated reassignment of many tasks which he handled so effectively over the years. Fortunately, he has been available to counsel me when I needed it.

We began the year by transferring $30,000 to the Laboratory to complete our pledge to finance production of the video documentary *The Biological Revolution: 100 Years of Science at Cold Spring Harbor.* The video is an important tool for increasing community awareness of the importance of Cold Spring Harbor to the world of molecular biology. Copies of the 30-minute cassette are available for loan or purchase from the Carnegie Library at the Laboratory.

At our winter Directors' meeting, we moved to increase our earlier pledge of $110,000 to the Plant Genetics Program at Uplands Farm. Consequently, in April we transferred $130,000 to the Laboratory for use in this project. LIBA's contribution has been a significant factor in establishing this research center which is certain to play an important part in developing molecular biology in the plant field. One of the immediate objectives is technology for a corn plant that is a more effective food producer, particularly in arid areas.

In May LIBA members heard an authority on the AIDS virus, Dr. Robert Gallo from the National Institutes of Health, give the Dorcas Cummings Memorial Lecture. We read daily about the dangers of this killer virus.

LIBA members at Grace Auditorium dedication



The Laboratory's Symposium, the LIBA dinner parties, and the dedication of the Oliver and Lorraine Grace Auditorium began the most active summer of meetings and courses in the laboratory's history with more than 3,600 participants. At the dedication of the auditorium, Dr. Daniel E. Koshland, Jr., professor of biochemistry at the University of California at Berkeley and editor of *Science* magazine, spoke of the important function the facility will play in increasing the interchange of ideas. We must remember that the Laboratory's importance includes its function as an independent center for meetings and high level courses, as well as its leadership role in research.

As a result of ongoing discussions with the Laboratory's administration regarding its needs and LIBA's support role, two actions were taken. First, we determined that the Laboratory must be able to offer grants to young scientists to help them in their first year while they establish their research credentials to the point where they can apply for competitive grants from foundations and government agencies. To initiate the program we set out to fund four LIBA Fellowships at $25,000 each. This program will give the LIBA membership a real sense of participating in the direct suport of scientists at the Laboratory.

Second, although LIBA has traditionally supported capital projects at the Laboratory, we determined that an organized annual effort would better serve the Laboratory's needs. This decision led to two basic changes: 1) A "giving year" beginning October 1 to meet the desires of the membership for tax planning and 2) Establishment of the Cold Spring Harbor Laboratory Associates. The Associates Program gives special recognition to those who donate at a higher level ($1,000 or more) and offers them the opportunity to expand their knowlege of molecular biology through scientific briefings and hands-on workshops.

For the first time, therefore, we are reporting contributions totalling $104,528.64 from 349 members during the year ending September 30, 1986. Since then, membership has risen to 500, including 84 Cold Spring Harbor Laboratory Associates. Contributors since the beginning of the new giving year will be listed in next year's Annual Report.

I have greatly enjoyed representing the LIBA membership. I have particularly enjoyed working with Jim Watson, Morgan Browne, and the administration in determining how LIBA can best serve the Laboratory.

George W. Cutting, Jr., Chairman

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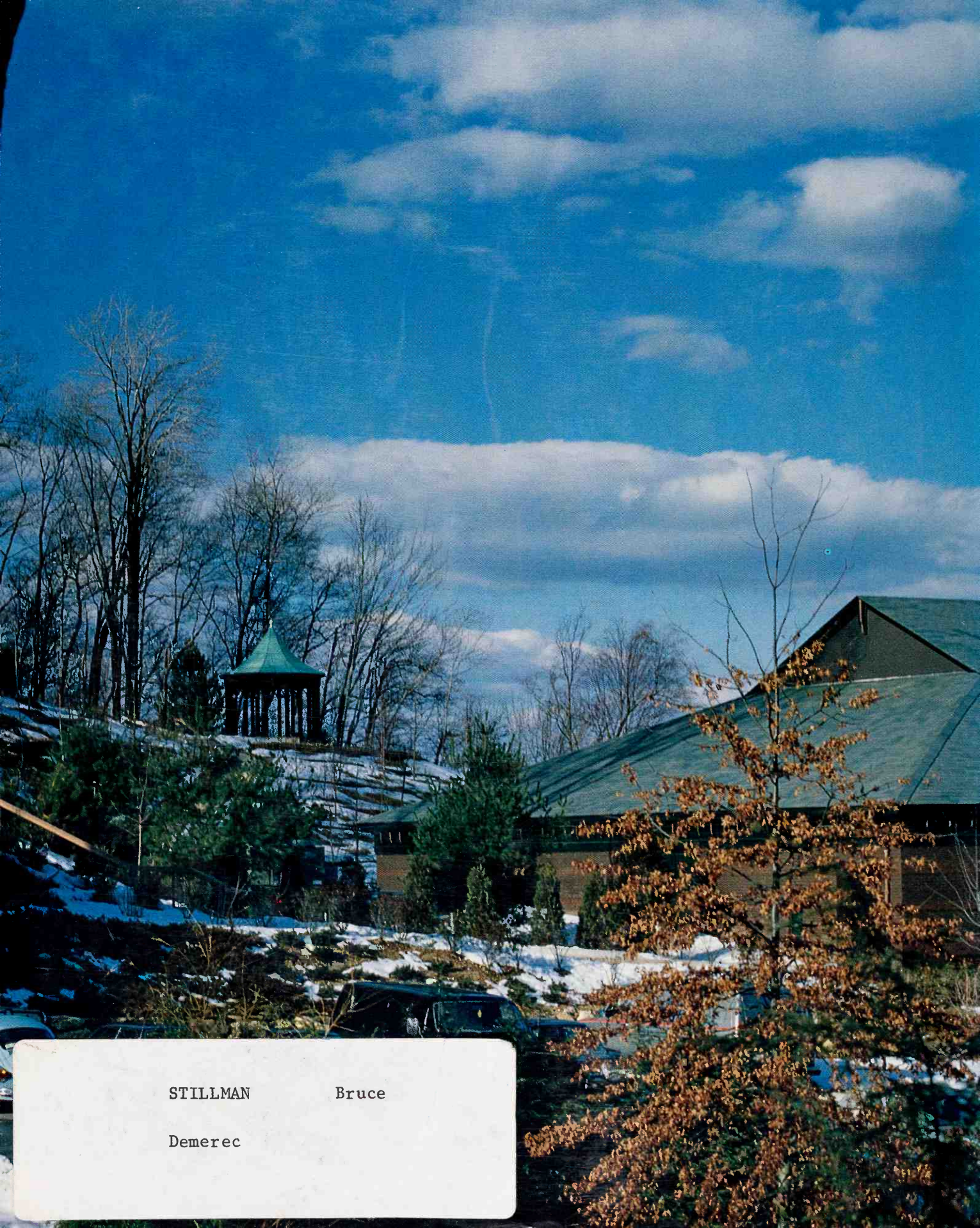

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