An aerial photograph of the Cold Spring Harbor Laboratory campus. The image shows a large, multi-story building with a brown roof in the foreground, surrounded by lush green trees. In the background, there are more buildings, a large green lawn, and a body of water (Long Neck Bay) with several sailboats. The sky is clear and blue. The text 'ANNUAL REPORT 1985' is overlaid on the right side of the image.

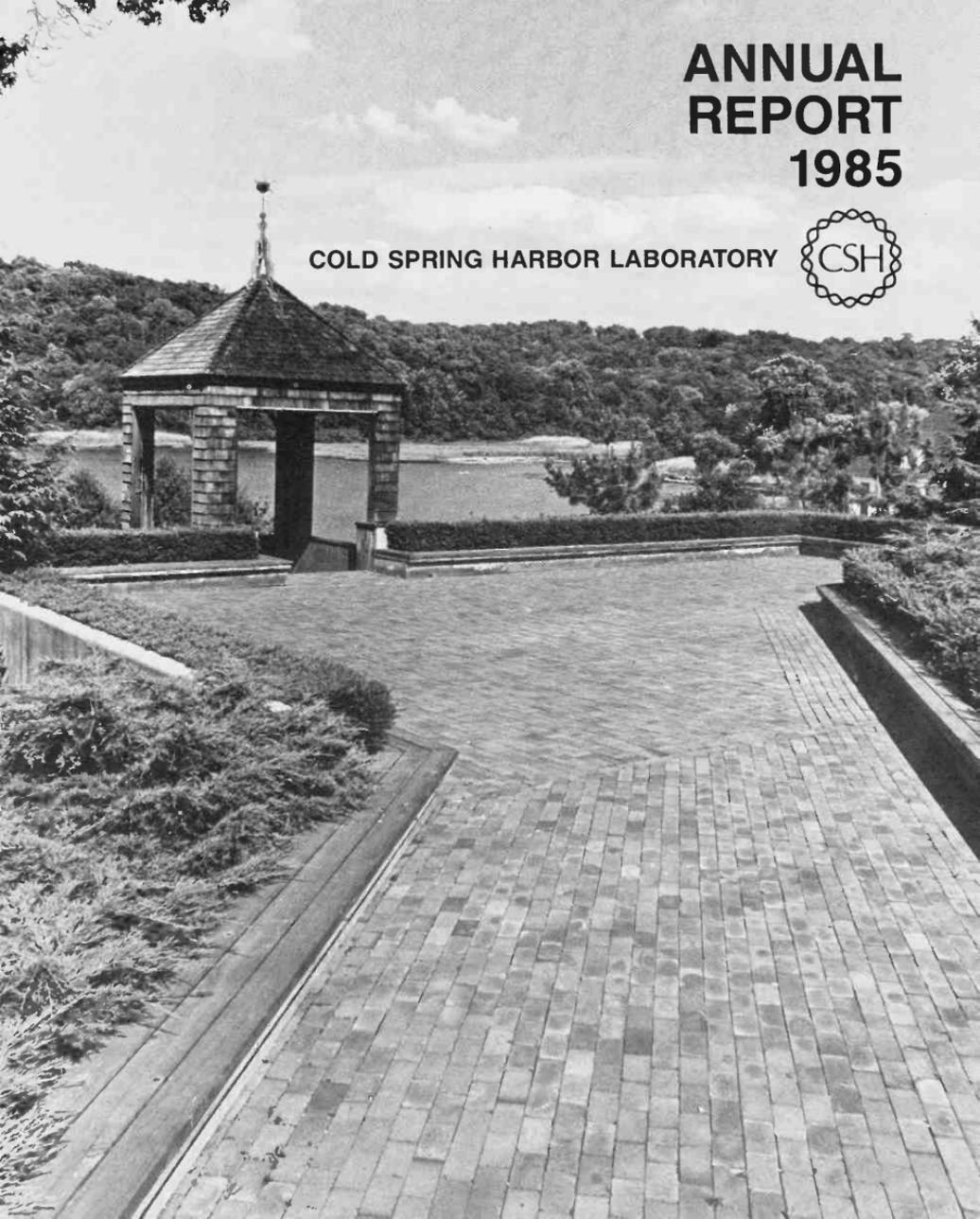
ANNUAL REPORT 1985



COLD SPRING HARBOR LABORATORY

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Cold Spring Harbor Laboratory
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Recto: Gazebo with model of adenovirus perched atop
stands sentinel overlooking Cold Spring Harbor.

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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, Duke University, Princeton University, Massachusetts Institute of Technology, Memorial Sloan-Kettering Cancer Center, New York 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 450 member families 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 Edward Pulling

The retirement of Edward Pulling as Chairman of the Long Island Biological Association (LIBA) in January 1986 marks the end of an era. Over the past 18 years, he has quietly worked to galvanize public support of the Laboratory and understanding of its mission. In the whole history of Cold Spring Harbor, there are few who have done more to advance the cause of science than Edward Pulling.

Born in the London suburb of Ealing in 1898 and reared in the English countryside, Pittsburgh, and Baltimore, Mr. Pulling was educated at Princeton and Trinity College, Cambridge. Choosing the career of an educator, he went on to teach at Groton and Avon Old Farms Schools.

In the year following his marriage to Lucy Leffingwell in 1928, the Pullings decided to found the Millbrook School, which opened in 1931. Upon retirement as headmaster, Mr. Pulling became a LIBA Director and Vice-Chairman of the Board of Trustees of the Laboratory in 1968. In joining the LIBA board, he followed in the tradition of his father-in-law, Russell C. Leffingwell, a founding director of LIBA.

Mr. Pulling assumed the Chairmanship of LIBA in 1969, and within one year had doubled membership to nearly 500 families. During his tenure, LIBA successfully completed four major capital fund drives. The first, conducted during 1972-1973, raised \$250,000 toward construction of the west addition to James lab for cancer virus research, winterization of Blackford Hall, and purchase and

renovation of the Takami residence as a dormitory, now called Olney House. In 1977, with \$225,000 from LIBA, Williams House was completely rebuilt as a year-round residence with five apartments.

Just three years later, in 1980, LIBA provided \$200,000 to purchase 20 acres of land from the Carnegie Institution of Washington. The Harris Building, completed in 1982, and the new Oliver and Lorraine Grace Auditorium are located on this property. The most recent LIBA fund drive (1982–1985) yielded \$600,000 toward construction of the Grace Auditorium.

A mere chronicling of LIBA fund drives, however, does not take into account the many important gifts that came, in large measure, through his bidding—for example, the creation of Banbury Center, the Robertson Research Fund, and the Doubleday Professorship for Advanced Cancer Research.

We have benefited not only from his help, but also from the example of his wisdom and integrity. Consummate teacher, mentor, and friend, Edward Pulling has immeasurably enriched our lives.

DIRECTOR'S REPORT

That our forthcoming 51st *Cold Spring Harbor Symposia on Quantitative Biology* will focus on the molecular biology of *Homo sapiens* indicates the extraordinary advances now resulting from the application of molecular biology to human beings. As with most new major technological breakthroughs, however, these advances are accompanied by social, economic, and moral implications that should not be ignored. In particular, we must begin to consider the consequences of our newly found abilities to analyze human DNA molecules, capabilities that over the next several decades should witness the working out of the complete sequence of the some 3×10^9 base pairs that comprise the human genome.

Now we are just beginning to see the details of how we humans, as well as other forms of life, are the products of random evolution of DNA molecules that began when the first forms of life emerged in an early phase of the earth's existence some four billion years ago. Then, as now, the various enzymatic steps through which DNA is replicated were constantly generating a broad range of new DNA molecules. These mutant DNAs in turn created genetic variants, some better adapted for existence on the earth as it now exists and others terminal misfits, unable to survive under any conditions. This constant production of new variability is itself under genetic control and now expresses itself most strongly among the larger higher plants and animals whose generation times are relatively long and whose populations are relatively small. Such species have to possess a high degree of inherent variants so that even in times of reduced numbers they contain sufficient genetic variants to survive when confronted with new forms of environmental stress. Humans are thus extraordinarily polymorphic animals, with each individual (except for identical twins) being highly unique, not only at the level of external appearance, but also at the level of the structure of their individual genes.

Already it is clear that each of us has easily computerizable DNA fingerprints that distinguish us from all other individuals and that, for example, can unambiguously establish our hereditary relationship to others. As each of us possesses perhaps some hundred thousand different genes, it is clear that none of us possesses only "good" examples of these genes. Each of us contains a mixture of genes, some of which help greatly to favor our existence and reproduction; others, perhaps the large majority, are evolutionally neutral, and still others diminish our chances to have functionally meaningful lives. In some cases, a given gene will always be bad, but in many cases the final effects will depend upon where and how we live. Those genes, for example, that bring about light-color skin help to promote necessary vitamin D synthesis in the skin of those who live in colder northern regions but promote skin cancer for those individuals who live near the equator.

Although initially it was possible to talk about good and bad genes largely only in the abstract, the arrival of recombinant DNA procedures, in particular the use of DNA polymorphisms in gene mapping, has transformed the field of human genetics into a virtual beehive of activity. Each week more and more genes are being mapped to precise chromosomal locations, and long-intractable genetic problems such as epilepsy, schizophrenia, and manic-depressive syndromes may be resolved over the next decade. Even more important, many of the genes

behind the already well-defined genetic diseases like sickle cell anemia are being isolated and their protein products identified. Once we have the culprit proteins, we shall be able to zoom in much more effectively on the pathogenesis of still very mysterious diseases like cystic fibrosis. Equally important, once a bad gene has been isolated, tests can be developed to diagnose its presence in newly developing fetuses. Antenatal diagnosis, for example, will soon almost routinely reveal whether a given fetus is doomed to develop into a child with muscular dystrophy or give the reassuring good news that the respective bad gene is lacking and that the arrival of a healthy baby should be anticipated. The possibility thus will soon be at hand of greatly reducing the long-term social and financial burdens placed on society by genetic diseases, particularly those manifested in childhood.

In accepting genetic disease as an inevitable consequence of human evolution, we are accepting the premise that the human beings who exist on earth today are not the result of any predetermined plan that has given human beings a manifest destiny to dominate the earth. Instead, our unique capabilities as humans come from the evolutionary events that have allowed our species to develop our very powerful brains. It is this specialized collection of nerve cells that allows us to be so conscious of the outside world, memorizing some of this information, and later selectively recalling some of these facts as we think. None of these cognitive attributes by themselves, however, are unique to humans. There is every reason to believe that many higher animals think in some ways like humans, possessing senses of beauty, having friends and enemies, and being anxious, happy, or sad, depending upon how their respective lives go. What makes us so remarkable as higher animals is not our ability to perceive pleasure or pain and act accordingly but our capacity to develop highly structured languages both spoken and written. Through the use of these languages, we can share deeply the experiences of other humans as well as collect this information in forms that can be passed from one generation to another in ever-increasing sophistication. Without the capability that languages provide to transmit complex thought patterns, the amazingly complex, if not wonderful, civilizations in which the temples of Greece could be designed and built, in which Bach and Beethoven could compose, or in which Newton, Darwin, and Einstein could think would never have developed.

As extraordinary as we are, however, there is no reason to assume that we will necessarily continue to exist. No guarantee exists that the human species shall not be eliminated by some virulent disease entity or through the action of man-made toxic products, in particular the radioactive compounds that would be unleashed as a result of an unlimited nuclear exchange. Thus man, like all other forms of life, has no guaranteed "right" of perpetual existence. Only if as a species we continue to behave sensibly will we have a realistic chance to go on. Our survival, at least within the lifetime of the earth's current solar environment, thus always must be seen as in our own hands.

Our rights as humans thus come to us not from any inherent order (purpose) within the universe but as reflections of religious and legal edicts that we have acquired during the course of human history. The Ten Commandments, for example, express sensible rules imposed to let us work together effectively toward the common goals that have made our Western civilization so successful. Not surprisingly, human rights have varied from one civilization to another, and while each of us as individuals may have very strong ideas as to what types of freedom are generally needed for meaningful lives, those freedoms we wish the most vary from time to time depending upon the respective environments in which we find ourselves.

Just as it has been useful to convey specific rights to ourselves, we have also

conveyed them to selected animals like the dog, cat, or cow that have long coexisted with humans and whose absence would bring us sorrow or economic distress if they were to be arbitrarily eliminated by other humans. Until recently, however, virtually no one regarded all forms of even higher life (vertebrates) as inherently worthy of protection. Pests were pests that should be removed if at all possible from interactions with human civilization. Rattlesnakes, coyotes, and rats were invariably perceived as animals to be eliminated, not protected. Today, however, ironically, most likely because of a gradual subconscious acceptance of the theory of evolution, there is a growing tendency among many diverse groups of people to regard the human imposition of harm (be it death or merely pain) on any form of life to be an essentially wicked act. For such people, the killing of the fox is incarnate evil, even though this means more chickens will be eaten by foxes. And even the mice and rats that we used to call vermin now have very vocal protectors who are disturbed if we use these rodents to search for cancer-causing chemicals or for experiments to discover the molecular uniqueness of the various forms of cancer. To such people, mice and rats have inherent rights that must be taken into account when we experiment upon them. I, however, find this concept totally without any merit. If it is not effectively challenged, even its partial acceptance will impede further medical research and limit its potential for reducing human misery.

Unfortunately, the current mood among government officials is to compromise with the animal-rights fanatics, with much credence given to the use of "alternative forms of testing" that do not involve living animals. I fear that such temporizing capitulation will lead to even further capitulation. Instead, we should stand our ground and label nonsense for what it is. To do so, we must have the courage to say that all forms of life are not necessarily good per se and that if the quality of human life is to be improved, we must experiment upon animals like mice. We should get on with our jobs without feeling that we are in any sense heartless because we lack compassion for the mouse. Our survival as humans demands that we put ourselves first, and to think for a long time in a different fashion would be to invite extinction. Of course, this is not to say that we condone any senseless diminution of the earth's still multitudinous forms of plants and animals. I, for example, object greatly to reducing the numbers of those most remarkable of animals, the whales.

Mankind's current inability to feel nonmystically about animal life manifests itself in its current unease with recombinant DNA experimentation. Just as most people still want to believe that there is something more extraordinary than molecules at the heart of living existence, they cannot easily accept that DNA is all that important, being the crucial difference between the various forms of life, without endowing it with virtual genie-like properties that we will never be totally able to handle. Many individuals thus fear that in subjecting the potential hidden geni to the abnormal manipulations of recombinant DNA, we may stir them into revengeful acts that would incalculably harm the human species. As a result of such worries, the mere process of using recombinant DNA is now being singled out as much more potentially dangerous than other breeding and selection processes, which, of necessity, also yield new gene combinations that have never existed before. Thus, a herbicide-resistant plant, if created through recombinant DNA procedures, must be subjected to painstaking safety checks, whereas no such checks are required if it were generated, say, through the recently developed somatoconal selection procedures. The ultimate irrationality of this situation is now more than clear not only to scientists but also to many giant industrial corporations who are attempting to create commercially useful new plants.

This preoccupation with DNA as a potentially uncontrollable genie explains, in part, the concern that many individuals have about current attempts to develop procedures for curing genetic diseases using DNA therapy. Also behind much of this hesitation is the feeling of many people that the DNA within us is not the result of stochastic processes, but of design, and that we have no right to interfere with the destiny that lies behind our individual existences. To me, however, the moral question to be settled is whether such gene therapy procedures have reasonable chances of effecting real cures. Hopefully, this dilemma will evaporate once it becomes clear that gene therapy is a valid procedure for curing sick individuals.

Much more difficult to resolve will be the question of whether selective abortion of genetically disabled fetuses should occur. Here again, the heart of the problem lies in how we perceive of human life per se. Does its very existence demand that we cherish and protect it? Those of us who are scientifically knowledgeable about DNA instinctively act on the premise that the random processes that govern the replication of DNA are bound to lead to some human fetuses that can never grow up into happy, functional individuals. Must these genetically damaged fetuses be allowed to develop into babies whose suffering is bound to bring unmitigated pain not only to themselves, but also to their parents and all others who must try to help them? Should we deny the existence of what we perceive to be the essence of a real human life, the capacity to develop into a person who by interacting successfully with others helps make this a more interesting and compassionate world?

With time, we can hope that those individuals who give us worldwide governmental and religious leadership will, through their respective paths of either reason or revelation, resolve the dilemma of whether a life without a realistic chance to develop into an effective person must go out into the daunting conditions that the earth will always provide.

HIGHLIGHTS OF THE YEAR

The Joe Sambrook Era Draws to a Close

On September 5th, we bade farewell to Joe Sambrook and dedicated to him a 6400-square-foot addition to the James lab which he directed so effectively for some 16 years. The dedication of the Sambrook Laboratory gave us good reason to call together many of the past inhabitants of James Lab to reflect on the Sambrook era, when the universe of the tumor virus world was still small enough that Cold Spring Harbor could lie at its exact center.

Before coming to the Laboratory in the summer of 1969, Joe organized the writing of our first DNA tumor virus grant to the National Cancer Institute. Only two years later, he led the team that put together a major \$1-million program project grant which began in 1972, emphasizing the DNA tumor viruses. Renewed in 1977 and 1982, this large grant (\$2.1 million of direct support in 1985) has long been the backbone of our cancer effort here. Its long-term program support allowed James lab to play a major role in helping to establish the small DNA tumor viruses as the first good model system for studying eukaryotic genes. From James lab came a succession of techniques for isolating and analyzing DNA and RNA at the molecular level.

Joe's success, over the years, in attracting talented young scientists was attested



Sambrook Laboratory dedication

to by those who returned to honor him at a meeting on "DNA Tumor Viruses: Gene Expression and Replication," during which the dedication ceremonies occurred. No institution of this era has helped train more outstanding scientists in academic cancer research than Cold Spring Harbor, and we are proud that so many of them now hold leading academic positions throughout the world at institutions such as MIT, Harvard, Berkeley, Rochester, Yale, Princeton, Zurich, Heidelberg, and Melbourne.

One such alumnus, Mike Botchan, now at the University of California at Berkeley, provided a personal view of the James experience for the 300 scientists and visitors who gathered in 100-degree heat for the dedication ceremony. Renato Dulbecco of the Salk Institute, under whom Joe spent several of his postdoctoral years, then spoke on the development of tumor virus research and made a plea for a multinational effort to determine the DNA sequence of the entire human genome.

We wish Joe every success in his new role as Chairman of the Department of Biochemistry at the Southwestern Medical School of the University of Texas at Dallas.

Joint Agreement with Pioneer Hi-Bred to Study Corn Genetics

On August 29th, the Laboratory signed a five-year, \$2.5-million agreement to conduct cooperative research with Pioneer Hi-Bred International, Inc. of Des Moines, Iowa. Research will center on the use of recombinant DNA techniques to genetically manipulate maize (corn), the most important crop in the United States and a staple food throughout much of the world. The Pioneer accord follows other Cold Spring Harbor research agreements with Exxon Research & Engineering Company (1982, \$7.5 million) and Monsanto Company (1984, \$2.06 million).

Pioneer is the world's largest producer of hybrid seed corn, with 1985 revenues of \$821 million. Merging the Laboratory's expertise in recombinant DNA techniques with Pioneer's experience in plant breeding should accelerate the development of superior corn plants. Pioneer's nationwide network for testing and



Jonathan Wood, farm manager, pollinating corn at Uplands facility

marketing new plant varieties ensures that advances in the laboratory can be most quickly brought to farmers.

The Cold Spring Harbor–Pioneer program is especially fitting in light of the significant role each organization has played in the development of hybrid corn. Cold Spring Harbor's involvement in plant genetics dates to 1908, when Dr. George Shull grew the first high-yielding hybrid corn on a small plot on the Laboratory grounds. His method of "pure-line" breeding marked the beginning of modern agricultural genetics and has already been called one of the 20 greatest discoveries of this century. However, it was Henry A. Wallace—editor of *Wallaces Farmer* and later Secretary of Agriculture and Vice President of the United States—who applied Shull's breeding methods to the commercial production of hybrid seed. Founded by Wallace in 1926, Pioneer (initially called the Hi-Bred Corn Company) first popularized the use of hybrid seed corn by American farmers.

Expansion of Plant Research Facilities

The Pioneer agreement is part of a major effort to make Cold Spring Harbor a center for advanced plant research in the northeast. Thus, in 1984, we began a \$3-million capital program to develop specialized research facilities to accommodate an expanded staff. Using a \$700,000 grant from the National Science Foundation, in 1985 we substantially completed the Uplands Farm Genetics Field Experiment Station, where we have 11 acres of fields and farm buildings. The former garage has been renovated into a 6000-square-foot cytogenetics laboratory for the microscopic examination and storage of corn kernels. Also completed was a new 2200-square-foot research greenhouse, tall enough for growing a winter corn crop.

Included in the Pioneer funding is a \$750,000 grant toward the second phase of the capital program: renovating 1800 square feet of lab space in Delbrück and building a 6500-square-foot extension to the north. To make room for the extension, in late March 1986 we moved the historic Firehouse (now the site of three staff apartments) some one hundred feet down Bungtown Road. This is the second relocation of the Firehouse; it was barged over to the Laboratory from Cold Spring Harbor Village in 1929.

To date, we have received \$435,000 in pledges from private sources, including the Pritchard Charitable Trust, DeCamp Foundation, Culpeper Foundation, Surdna Foundation, Hearst Foundation, and Griggs and Burke Foundation. In addition, the Long Island Biological Association has pledged \$110,000. Although an aggregate of \$2 million has already been raised for the Plant Genetics Capital Fund—the largest amount ever raised for a single project here—we will need to find an additional \$1 million by early 1987 if facilities development is to proceed on schedule. Also, we ultimately will need a further \$700,000 to purchase the Uplands Farm property from The Nature Conservancy, with payments scheduled over an eight-year period.



Firehouse being moved

Making Plans for an X-ray Structural Analysis Group

No matter how well we understand a gene at the DNA level, there is no way we can go from a DNA sequence to the three-dimensional structure of its respective protein. To find a protein structure, there is still no alternative to growing crystals whose structures can be solved by X-ray crystallographic analyses. Until recently,

however, establishing an X-ray crystallographic group seemed beyond our resources. We were not strong in computers or the instrument-making facilities that are much more the mark of chemistry than biology labs.

Now, however, the needed rotating anode X-ray tubes and associated area detectors can be bought off the shelf, admittedly at no modest cost. Moreover, the cost and size of high-powered computers have also become manageable by an organization of our scope. Even more important, the average time necessary to solve a protein of average complexity by X-ray analysis has fallen to the two-to-five-year interval that we associate with many other research objectives at Cold Spring Harbor.

The time thus seemed propitious to start up an X-ray structure group to give us the capability to work out the detailed structures of oncogene proteins such as *ras*, *myc*, and E1A, as well as the receptors for the growth factors that give the signals to commence cell growth and division.

Already we have appointed two highly experienced young crystallographers to start this effort. Coming here in the fall of 1986 will be John Anderson, trained in the laboratories of Steve Harrison and Mark Plashne of Harvard, and Jim Pflugrath, a product of F. Quiochos' laboratory at Rice University and who now has a postdoctoral position with Robert Huber in Munich. They will use lab space in Demerec as well as occupy newly created facilities for computer-graphic analysis in the Hershey Building.

Preparing of Plans for a New Neurobiology Building

Regretably, the absence of a suitable year-round facility for neurobiology made virtually inevitable the departure last fall of Susan Hockfield for a position in the Neurobiology Department of Yale Medical School. Fortunately, we were able to persuade her to remain as Program Director for our Summer Neurobiology Courses, thereby ensuring their continued existence for the foreseeable future.

Susan's departure emphasized the fact, already known to us for several years, that we cannot build up a stable neurobiology effort until our summer teaching space in Jones lab is supplemented by a major new building that will give us modern year-round research space, as well as still more labs for advanced teaching in neurobiology. Strongly encouraged by the Neurobiology Committee of our Board of Trustees, we have drawn up schematic plans for a 16,000-square-foot building to be located behind James lab on the site presently occupied by several of our more than 50-year-old unheated summer cabins. We estimate the cost of the building and equipment will be approximately \$5 million. Raising this sum will not be easy, but we must, if we are to stay at the forefront of biology.

Happily, Daniel Marshak, who will join Demerec lab in April 1986 to head our protein chemistry efforts, has among his major research interests the isolation of a glial cell growth factor. Hopefully, he will soon attract to work with him a trained neurobiologist who can use the Jones lab facilities when they are not occupied by our summer courses. Greatly facilitating the rapid start-up of Dan's research will be funds provided by the Marie Robertson Fund for Neurobiology, which will initially cover his growth factor research until appropriate federal grant support is obtained.

Holding of Our 50th Symposium on Quantitative Biology

The 50th meeting gave us cause to celebrate our Symposium's major role in the development of molecular biology over the last half century. During the first



Sydney Brenner, François Jacob, and Seymour Benzer at the 50th Symposium

decades of this century, biology was considered a descriptive science, intellectually inferior to the "hard numbers" of physics and chemistry. The published "red book" proceedings of the Symposium have done much to change this image and to promote the development of biology as a quantitative discipline. In a real way, therefore, the founding of the Symposium in 1933 by Reginald Harris marked a watershed in the development of modern biology.

For the 50th Symposium we wanted to focus on a topic clearly to be of importance over the next half century. So, we chose molecular biology of development, which concerns itself with how a fertilized egg cell develops into a complex organism composed of many highly specialized tissues and structures. Joe Sambrook was responsible for the organization of the meeting, which brought back to Cold Spring Harbor many of the scientists whose papers at earlier Cold Spring Harbor Symposia helped found molecular biology. In particular, it was a pleasure to again have here François Jacob, Sydney Brenner, and Seymour Benzer.

A Record Number of Summer Course Applicants

Our summer courses continue to remain very popular, with a total of 684 applications received in 1985 compared with 598 in 1984. Most popular remain those courses that emphasize recombinant DNA procedures. The Molecular Cloning Course (16 places) was requested by 240 students and Advanced Cloning (16 places) by 114 students, numbers so large that the selection process by the respective instructors had to be arbitrated in many cases. Greatly aiding the teaching of the Cloning Courses was the total renovation of the teaching laboratory on the top floor of James lab, as well as the use of the new areas for centrifuges and coldrooms provided by the adjacent Sambrook lab space. For the first time, we have facilities equal to the teaching of such qualified students.

Our Neurobiology Courses included for the first time one on Computational Neuroscience, which was supported by the Sloan Foundation and led by Tony Movshon of New York University and Emilio Bizzi of MIT. Covering material that until recently has gone under the name of Artificial Intelligence, the course's new designation indicates the growing optimism that the mathematical models for certain cognitive processes may reflect what is actually happening in our neural circuitry as opposed to being proposed without any expectation that they reflect

reality. Most encouraging was the exceptional quality of the student body, which led the Sloan Foundation to give us a further grant for three more years of operation.

Toward the general support of neurobiology teaching, we have again received a large grant (\$150,000) from the Klingenstein Foundation, as well as much needed funds from the Grass Foundation for scholarship support. Equally important has been the awarding of major federal training grant support in 1985 from the National Institute of Mental Health and in 1986 from the Institute of Neurological Diseases and Blindness. Thus, if we do not experience serious recisions in already awarded federal funds, we have the resources now available to guarantee very high level teaching in neurobiology over the next several years.

Greatly aiding our potential to ensure that no qualified applicant is denied admission for lack of funds is the new grant made to us in May by the Markey Foundation. Over a three-year interval, it provides \$50,000 per year of support, primarily to offset student costs.

Undergraduate Research Expands

In 1985, the Undergraduate Research Program was expanded to allow 15 students to experience a summer of research under the tutelage of Cold Spring Harbor staff scientists. Previously, 11 students had been funded.

This expansion was made possible by receipt of a four-year grant from the Sloan Foundation (\$74,000), which also helped us to challenge other foundations. Joining with Sloan to provide \$39,000 for the 1985 program were the Burroughs Wellcome Fund and Metropolitan Life Foundation. As is traditional, the Olney Fund provided support for one fellow.

Looking ahead to the Undergraduate Research Program's 30th Anniversary, in 1989, we need to find a benefactor to endow this worthwhile activity. One of the oldest in the nation, our program plays a significant role in developing the research of future scientists.

We Extend DNA Education into Secondary Schools

For many years, the Laboratory has functioned as a specialized university in DNA science, bringing high-level training to graduate students and practicing researchers. Through a new program, begun in 1985 by David Micklos, we are now lending our unique expertise to train high school science teachers so they are better able to teach about molecular biology and its social implications. The Cold Spring Harbor Curriculum Study, initiated with strong support from Cold Spring Harbor Superintendent of Schools Fran Roberts and seed money from Citibank, N.A., and eight local school districts, has already grown to involve 19 school districts on Long Island and in Westchester County.

With the help of Postdoctoral Fellow Greg Freyer, now at Sloan-Kettering Cancer Center, Dave has fashioned a laboratory course that gives students hands-on experience with the tools of biotechnology. With grants from Citibank, the J.M. Foundation, New England Biolabs Foundation, Amersham Corporation, and Fotodyne, Inc., we are initiating in the summer of 1986 a series of teacher-training workshops to bring this unique course to teachers in New York State, New Hampshire, Massachusetts, Wisconsin, Illinois, and California. So enthusiastically has this program been received, both by scientists and lay people alike, that we are now making plans for a DNA Education Center to be located in Cold Spring Harbor Village.



Syosset school teachers Steve Sponenberg and Lois Miller at Curriculum Study workshop



Michael Shodell

Another Very Successful Year for the Banbury Center

Our long-running Risk Assessment Program that emphasizes cancer prevention continues to hold important meetings of relevance to human health. Very timely this year was an April meeting on Human Genital Cancer, which explored the involvement of several human papillomaviruses, and a September meeting that analyzed the molecular ways in which tobacco is a carcinogen. Also highly successful was our workshop for Congressional staff, which explored newer reproductive technologies, and the journalism workshop that focused on the possibility that tissue implants might be used to treat degenerative neurological disorders.

Each of these occasions attested to the innovative leadership of Michael Shodell, and it is with regret that I report that Mike came to me in September saying that he wished to return to his teaching and writing careers. Fortunately, we were able to persuade Mike to stay on until his replacement could be found and move here. Starting on April 1, 1986, the Banbury Director will be Steve Prentis, currently editor of *Trends in Genetics* published by Elsevier out of Cambridge, England.

In choosing Steve, we have decided to focus initially on integrating the publication aspects of the Banbury program more closely into our main publishing program on the lab grounds. So, Steve will also function as the Executive Director of our entire publication program, in charge of overseeing which books to publish. In this capacity, Steve will take on the role long played by me, but no longer possible because of my increasingly complex duties as Director.

To oversee directly our Risk Assessment Program, we shall be making a new staff position specifically for this task. Hopefully, this appointment can be made during the fall of 1986. Greatly aiding this search is a recent grant of \$100,000 per year for three years from the James S. McDonnell Foundation that will not only cover the costs of two meetings per year, but also ensure the publication of their proceedings.



Steve Prentis

More Important Uses of the Robertson Research Fund

A continued key to my functioning as Director is the availability of monies generated each year by the investments held by the Robertson Research Fund. Particularly important this year was my ability to draw upon them to undertake the extensive renovations to James lab necessary for its continued functioning for research on vertebrate gene expression. In addition, Robertson funds helped support the stipends of three visiting scientists and seven postdoctoral fellows, as well as supplementing many other postdoctoral and student stipends to meet the ever-increasing high costs of living on Long Island, recently emphasized as one of the most desirable places to live in the United States. We also used Robertson monies to cover the shortfall created by the underfunding of our summer meetings grant, as well as to provide support for two undergraduates (URPs) who did summer research here. The decreasing availability of federal funds for research over the next several years makes it very likely that the Robertson monies will be more important than ever in maintaining our research potential.

Grace Auditorium Readied for 1986 Meeting Season

During this year we have worked feverishly to complete the 360-seat Grace Auditorium in time for the spring 1986 meetings. Financed through a major gift

from Oliver and Lorraine Grace and the proceeds from a \$600,000 fund drive by the Long Island Biological Association, the more than 20,000-square-foot Grace Auditorium will be formally dedicated on June 1, 1986.

Inside, three massive dormers and several skylights flood the lobby with light and lend to it the quality of an atrium continuously connected with the Long Island landscape. Equipped with the latest in audiovisual equipment, including wireless microphones, xenon-lamp projectors, and closed-circuit television, the Grace Auditorium should be technically equal to the high-level science present at our meetings.

In addition to its conference function, the auditorium will be home to some 18–20 staff members. Located just off the main lobby is the Development and Public Affairs Office, headed by David Micklos. Downstairs is the Meetings Office, which administers our summer conferences and courses, under the direction of Barbara Ward.

Also on the lower level is the Samuel Freeman Computer Center, the hub of a distributed computing network that serves the entire scientific staff. It was created by a major grant from the Samuel Freeman Charitable Trust, which has allowed us to totally upgrade our major computer facilities, including six interconnected SUN workstations. Here, Mike Balamuth, Chris Keller, and their group of programmers are developing state-of-the-art software for deciphering the genetic information stored in DNA. Allied with this Freeman grant will be a grant from the Donaldson Charitable Trust to purchase the computer equipment that we will need to use X-ray and computer-graphic techniques to solve the structures of a variety of oncogenic proteins and DNA-binding proteins.

Another section of the lower level is occupied by the QUEST Biotechnology Resource Center, headed by Jim Garrels. This facility, funded through a major grant from the National Institutes of Health, will allow visiting scientists from many major research universities to apply computerized analysis of two-dimensional protein gels to biomedical research.

We Welcome G. Morgan Browne as Administrative Director

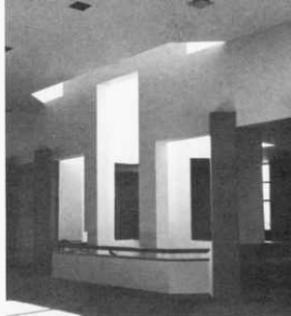
On June 2nd, G. Morgan Browne, Jr., joined the Laboratory as Administrative Director, filling a vacancy created by Bill Udry's departure in January. Morgan brings financial experience essential to running the business operation of the Laboratory which has become increasingly complex over the last several years.

Prior to coming here, Morgan had worked for 25 years as a management consultant to a variety of technically based companies. Interested in what he terms "business architecture," Morgan worked within the organizational structure of his client companies to provide business focusing and financial expertise needed for corporate growth. Serving as part of top management for extended periods of up to five years, he was involved in corporate and legal planning, joint ventures, and patent licensing.

Morgan received a B.A. in history and political science from Yale. No stranger to the Laboratory, Morgan and his wife JoAnn have been LIBA members for several years. They reside in Locust Valley and have three sons—Doug, Tenney, and Morgan.

Professorship and Honors for Michael Wigler

In June, Michael Wigler became one of only 18 investigators nationwide to receive an Outstanding Investigator Grant from the National Cancer Institute. Expected to



Grace Auditorium foyer



G. Morgan Browne and Assistant Administrative Director, John Maroney



Michael Wigler

total over \$8 million in support over the next seven years, this is the largest single-investment grant ever awarded to a Cold Spring Harbor scientist. Then, in December, Mike was appointed American Cancer Society Research Professor of Molecular Genetics, which provides \$50,000 per year until the year 2012. He also became the fifth recipient of the Pfizer Biomedical Research Award, which provides \$500,000 in unrestricted support over five years.

Using the tools of genetics, recombinant DNA, and biochemistry, Mike has done much to usher in the new era in basic cancer research that directly studies human cancer genes. While still a graduate student at the College of Physicians and Surgeons of Columbia University, Mike developed powerful methods to introduce foreign genes into animal cells growing in culture. In 1981, he and his associates here were among the first to show that human cancer has its origins in changes in a normal human gene.

In this research, they isolated several of the *ras* oncogenes that transform healthy cells into malignant ones. In 1984, he began an intensive study of homologs to the *ras* oncogenes in the yeast *Saccharomyces cerevisiae*. Yeasts provide a much simpler biochemical and genetic environment in which to dissect the role of *ras* in normal cell life than any form of higher vertebrate cell. So yeast may actually provide the ideal system in which we can first understand at the molecular level the functioning of a *ras* protein.

Promotion of Bruce Stillman to the Rolling-Five Position

In May, the Trustees of the Laboratory approved the appointment of Bruce Stillman as Senior Scientist in recognition of his incisive experimentation on DNA replication in eukaryotic cells. Coming to us as a postdoctoral fellow after his Ph.D. research on adenovirus DNA at the Australian National University, Bruce soon established himself as a totally independent scientist capable of focusing effectively not only on his own research, but also on that of many others in the lab. Especially important recently have been Bruce's investigations of the *in vitro* replication of SV40 DNA and on the cell-free formation of SV40 chromatin that goes in parallel with DNA replication.

The Senior Scientist position carries with it a rolling five-year appointment, which means that his salary is now guaranteed by the Laboratory for a five-year period that continuously moves forward with time. Unfortunately, this guarantee falls short of the academic tenure offered by many universities, and if we are to retain key scientists like Bruce for most of their research careers, we must greatly increase endowment funds specifically earmarked to fund truly tenured positions.

Changes in Our Scientific Staff

Senior Staff Investigator Stephen Blose accepted a position as Research and Development Director at Protein Databases, Inc. Steve came to the Laboratory in 1978 from the University of Pennsylvania, where he had been an assistant professor. Initially a member of the McClintock Cell Biology group under Guenter Albrecht-Buehler, he helped develop and characterize many useful monoclonal antibodies against cytoskeletal proteins.

Four other Senior Staff Investigators left the Laboratory in 1985 for positions in academia: Susan Hockfield, Fuyuhiko Tamanoi, Russell Malmberg, and Mary-Jane Gething.

Susan Hockfield, who used monoclonal antibodies to study cell-specific markers

that may play a role in the development of the visual system, accepted an assistant professorship in the Neuroanatomy Department of Yale University School of Medicine. Susan came to Cold Spring Harbor in 1980, after completing a postdoctoral fellowship at the University of California in San Francisco. We are indebted to Sue for setting the Neurobiology Training Program on a solid financial footing.

Fuyuhiko Tamanoi is now an assistant professor in the Department of Biochemistry at the University of Chicago. Fuyuhiko was a research fellow at Harvard University until he came to the Laboratory in 1980. Along with Bruce Stillman, he set up the DNA synthesis lab to study the enzymatic mechanisms of DNA replication in eukaryotes.

Russell Malmberg, who used cell-culture mutagenesis to dissect the polyamine pathway in tobacco, has become an associate professor in the Department of Botany at the University of Georgia. Russell was an assistant professor at Michigan State University before coming to the Laboratory in 1981 to help establish a plant research group in Delbrück lab.

Mary-Jane Gething moved to the Department of Biochemistry at the University of Texas Health Sciences Center. Mary-Jane came to the Laboratory in 1982 to work with Joe Sambrook on the transport and secretion of the hemagglutinin protein. She was formerly with the Imperial Cancer Research Fund in London.

Staff Investigators John Lewis and Fumio Matsumura also left the Laboratory. John, who came to the Laboratory in 1980 as a postdoctoral fellow from Memorial Sloan-Kettering Cancer Center to work on cell-cycle-dependent gene expression, is now with Smith Kline & French Laboratories in Philadelphia. Fumio, who came to the Laboratory in 1980 as a postdoctoral fellow, has become an assistant professor in the Biochemistry Department at Rutgers University. While at Cold Spring Harbor, he studied the differential expression of several forms of tropomyosin in transformed cells.

New Staff Members

Joining the Laboratory this year are David Spector as a Senior Staff Investigator and Andrew Rice as a Staff Investigator. Dave comes to us from Baylor College of Medicine, where he was an assistant professor. He will be in charge of a new electron microscopy facility that will occupy the former "Mouse House," which has been enlarged and entirely renovated. Andy, who came from Ian Kerr's lab at the Imperial Cancer Research Fund, now works with the protein synthesis group in Demerec. Ronald Guggenheimer also came to the Laboratory as a Staff Investigator from a postdoctoral fellowship at Memorial Sloan-Kettering Cancer Center, but left in December to join his family's publishing business.

Visiting scientists who completed their studies at Cold Spring Harbor during the year and returned to their previous or new positions were Eric Hunter, University of Alabama; Daniel Birnbaum, Centre d'Immunology, INSERM-CNRS de Marseille-Luminy in Paris; and Timothy Harrison, University of Leicester in England.

Three other visiting scientists who moved on to new positions were Ling Pai Ting, who is now a graduate instructor of microbiology and immunology at the National Yang-Ming Medical College in Taiwan; Asao Fujiyama, who is continuing his studies with Fuyuhiko Tamanoi at the University of Chicago; and Ottavio Fasano, who has moved to the European Molecular Biology Laboratory, Heidelberg, Germany.

Newcomers on the visiting scientist roster are Marcello Siniscalco, on sabbatical from Memorial Sloan-Kettering Cancer Center; Massimo Romani from Italy's

National Cancer Institute in Genoa; Diane Esposito, who received her Ph.D. from Fordham University and is continuing her studies with Marcello; Krystyna Slaska-Kiss from the Biological Research Center in Szeged, Hungary; Esa Kuismanen from the University of Helsinki in Finland; and Junichi Nikawa from Gunma University School of Medicine in Osaka, Japan. Seth Grant, who received his medical degree from the University of Sydney in his native Australia, came to the Laboratory as a postdoctoral fellow working with Jim Hicks but decided in October to stay on as a visiting scientist in Doug Hanahan's lab.

Staff Promotions

In recognition of his work in developing one of the only computerized systems to analyze two-dimensional protein gels, Jim Garrels was promoted from Senior Staff Investigator to Senior Staff Scientist. Jim came to the Laboratory in 1978 after doing postdoctoral work at the Salk Institute.

Promoted from Staff Investigator to Senior Staff Investigator were Steve Dellaporta, Doug Hanahan, Ed Harlow, Winship Herr, and Bill Welch. Steve, who has worked out an elegant method to clone maize genes using the Ac transposon, was instrumental in attracting Pioneer Hi-Bred's support of our plant genetics research. Doug has effectively used fusion genes to study tissue-specific development and oncogenesis in transgenic mice. Ed, who set up the new monoclonal antibody facility in Sambrook lab, has developed a host of antibodies to study adenovirus E1A proteins and the human p53 tumor antigen. Winship has done important fine-structure analysis of the SV40 enhancer region. Bill has purified and characterized several of the mammalian stress proteins.

Visiting scientist Tohru Kataoka, from Osaka University, became a Staff Investigator in February. Working in conjunction with Mike Wigler, Tohru has cloned genes for key proteins in the *ras* cyclic AMP pathway in the yeast *Saccharomyces cerevisiae*.

Postdoctoral Fellows

Leaving after completion of their postdoctoral terms were Tim Adams to the School of Veterinary Sciences at the University of Melbourne; Tom Flanagan and Susan Watts to the University of North Carolina at Chapel Hill; Greg Freyer to Dr. Jerry Hurwitz's lab at Memorial Sloan-Kettering Cancer Center; Howard Fox to the Medical School at the University of California at San Francisco; John Ivy to the Oceanic Institute in Hawaii; Mike Krangel to Jack Strominger's lab at the Dana-Farber Cancer Center; Janet MacInnes to the Department of Veterinary Microbiology and Immunology at the University of Guelph in Canada; Shigeko Matsumura to the Department of Biochemistry at Rutgers University; Reza Sadaie to Bob Galló's lab at the National Institutes of Health; Masao Yamada for the National Children's Medical Research Center in Japan; and Patricia Cotton to pursue other interests.

Postdoctoral fellows Beth Friedman and Sam Zaremba moved with Susan Hockfield to Yale University. Philip Bird, Patricia Gallagher, and Michael Roth moved with Joe Sambrook and Mary-Jane Gething to the University of Texas Health Sciences Center.

Receiving their doctorates were Carolyn Doyle and Kevin Van Doren from SUNY at Stony Brook. After a brief postdoctoral period with Guenter Blobel at Rockefeller University, Carolyn accepted a position at Oncogene Sciences in Mineola. Kevin has moved to David Hirsch's lab at Synergen in Boulder, Colorado.

Graduate students Ramaninder Bhasin and Madan Rao have returned to SUNY

at Stony Brook to continue their studies, and William Costello is with the Law School of Stanford, California.

Changes to Our Board of Trustees

One of the strongest assets of the Laboratory is the diversity of the backgrounds and experience of the members of the Board of Trustees. Not only do I have the counsel of scientists from our ten participating institutions, but I also have the advice and support of Trustees from the public sector for a multiplicity of problems.

In November, the statutory six-year terms of three institutional Trustees expired. Dr. Norton Zinder from Rockefeller University, Dr. Boris Magasanik from MIT, and Dr. Charles F. Stevens from Yale University have all served the Laboratory with distinction. In particular, I want to recognize Dr. Zinder, who served as Secretary of the Board and as Trustee from 1967 to 1975.

At the same time, we welcomed three distinguished scientists as institutional Trustees: Dr. David Botstein from MIT, Dr. James E. Darnell, Jr., from Rockefeller University, and Dr. Frederic M. Richards from Yale University.

Dr. Botstein has long been associated with the Laboratory. We showed bad judgement in not choosing him for the Undergraduate Research Program in 1960, but he nevertheless began to attend our summer meetings on phage as a graduate student and presented his first paper at the 1968 Symposium. While on sabbatical from MIT in 1974, he formed the first yeast group here in the then Davenport lab with Gerry Fink and John Roth. For five years, beginning in 1976, he taught the advanced bacterial genetics course each summer with Ron Davis and John Roth. As Professor of Genetics in the Department of Biology at MIT, he teaches and does research on yeast and human genetics.

Dr. Darnell is serving as Trustee for the second time. His first appointment was as a representative of Columbia University. He is now the Vincent Astor Professor at Rockefeller University, with research interests in animal cell biology and virology. He, too, has long been associated with Cold Spring Harbor. In conjunction with his early work in animal viruses, he came to lecture every summer for 15 years, beginning in 1958.

Since 1963, Dr. Richards has been a professor with the Department of Molecular Biophysics and Biochemistry at Yale. In 1971, with David Phillips, he helped organize our Symposium on the "Structure and Function of Proteins at the Three-dimensional Level." His experience in protein chemistry and X-ray crystallography will benefit us as we expand our research into this field.

In September, the Laboratory mourned the death of Ambassador John P. Humes, who joined the Board in 1981 and served with distinction on the Finance and Investment Committee. Most recently, he headed a special fund drive that led to over \$100,000 in grants and gifts for the plants genetics program. His commitment to basic biological research has provided a legacy of promise for a healthier world. He was appointed Ambassador to Austria by President Richard M. Nixon in 1969, and before his retirement in 1975, he was awarded the Great Golden Medal of Honor, the highest honor the Austrian government can bestow on a civilian. Before his appointment as Ambassador, he was a partner in the law firm established by his father.

Appointed in February 1986 to fill Ambassador Humes' position is David L. Luke III, the CEO of the Westvaco Corporation and a resident of Locust Valley. Mr. Luke has previously served on the Board of the American Museum of Natural History and as Chairman of the Board of Trustees of The Hotchkiss School.

Retirement of Edward Pulling from LIBA Chairmanship

In January 1986, the 1985 Annual Meeting of the Long Island Biological Association (LIBA) and the retirement of Ed Pulling as LIBA's Chairman marked the first public showing of the Oliver and Lorraine Grace Auditorium. It was a marvelous occasion, with an overflowing audience coming together to thank and pay tribute to Ed Pulling. His extraordinarily effective leadership of 17 years brought into existence four highly successful fund drives, which made possible the urgently required construction, purchase, or renovation of many Laboratory facilities, including Williams House, Olney House, Blackford Hall, the James West Annex, and the land on which the Harris Building and the Grace Auditorium are sited. The Grace Auditorium itself is perhaps the greatest achievement of LIBA and Ed to date.

LIBA's Annual Meeting marked the end of Ed's chairmanship, and to show our affection and admiration, short tributes to him were made by myself, George Hosstfeld, the Vice Chairman of LIBA, Clarence Galston, and Reese Alsop. In response, Ed pointed out the enormous pleasure he experienced in leading LIBA and thanked the audience for giving him the opportunity to serve a cause in which he so ardently believed.

Succeeding Ed as the new Chairman of LIBA is George Cutting, a director of LIBA and a long-time resident of Oyster Bay Cove. We remain very fortunate in that we still continue to receive the effective support and close friendship of so many nearby residents.

Maintaining Cold Spring Harbor as a Haven for Bright Minds

Science became a full-time concern at Cold Spring Harbor in 1904, when funding from the Carnegie Institution of Washington created a safe haven here for serious science. Its unflagging support, for example, allowed Barbara McClintock and Alfred Hershey the freedom to pursue ideas—once considered esoteric and arcane—that would lead to their Nobel Prizes. Since the withdrawal of the Carnegie Institution in 1962, the Laboratory no longer enjoys the relative luxury of a full-time, externally sited mentor. Our funding now comes from many disparate sources, which subtly steer the course of our research and ultimately limit our freedom to explore new realms of bioscience.

Thus, we increasingly need to offer the financial incentives for key staff members to stay on at the Laboratory and the security for them to aggressively pursue novel ideas—regardless of trends in government or foundation funding. Unfortunately, we now have internal funding for only one tenured research position, the Doubleday Chair. Over the next several years, we must establish four or five additional endowed positions, if we are to remain competitive with our sister research institutions and universities.

Ten years ago, the lack of tenured positions was not such a serious problem. Molecular biologists could only aspire to relatively poor-paying jobs in academia. There was no great disparity in salary offered here or at other institutions. However, the explosive growth of biotechnology has upped the ante. Now both specialty biotechnology firms and established pharmaceutical and chemical companies offer handsome salaries to molecular biologists. Many well-endowed universities have responded to this competition by offering salaries in line with those offered by industry. The economics of the situation make it difficult for us to both find and keep bright young scientists.

The problem will only be compounded if the budget-cutting axe of Gramm-Rudman falls severely on the National Institutes of Health, our major source of

research support. If that happens, all too often bright young scientists will not almost automatically acquire the grants needed to support their independent research. In addition to endowed research positions, we need to create a staff-development endowment to support young scientists at the beginnings of their research careers.

Under our newly established Cold Spring Harbor Fellows Program, exceptional postdoctoral fellows are offered the opportunity to accelerate into funded junior-staff positions. During the three-year period, fellows work independently to establish their research credentials prior to seeking competitive grants. First appointed to one of these positions is Adrian Krainer, whose Ph.D. research was done at Tom Maniatis' lab at Harvard on the problem of RNA splicing.

We Must Retain Our Sense of Being a Closely Knit Village of Science

The intellectual tie that so easily brings our staff together and makes it so natural for them to attend each other's seminars is our focus on DNA. Whenever possible, we go for the gene behind the biological phenomenon that intrigues us, and much more often than not we are surprised by what we find.

Now, through our return to a serious interest in plants, and hopefully through our not-too-distant acquisition of the year-round neurobiology building we need so badly, we are inevitably acquiring the attributes of a graduate university of DNA. Though we lack degree-granting ability, our joint program in genetics with the State University of New York at Stony Brook now gives us the presence of some 20 graduate students.

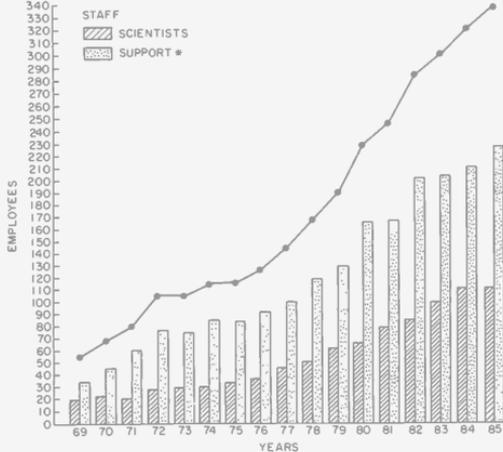
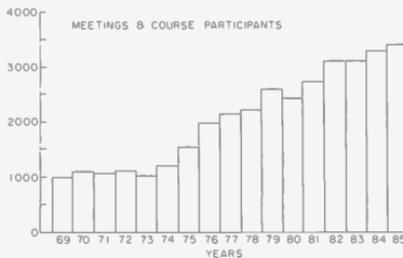
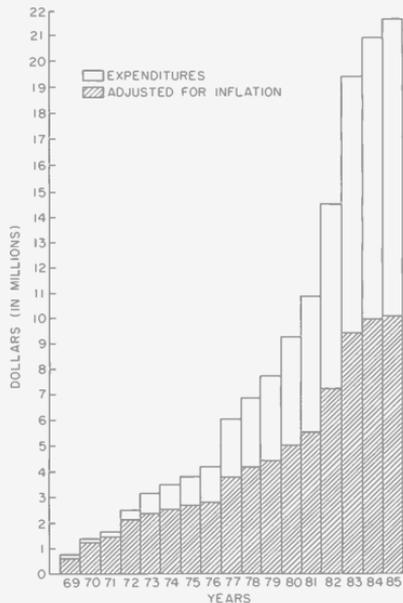
Most importantly, our intensive set of summer courses and our combined set of large and small meetings that collectively number some 20 each year provide an educational experience and exposure to first-class scientists, matched at best in only a few other institutions throughout the world. In recruiting new staff and postdocs, we have not only the success of the past to argue from, but, more important, the probability that their scientific future at Cold Spring Harbor will be highly productive.

Always attractive bait in recruitment has been our ability to offer many of our key scientists, especially when they first arrive, the opportunity to live on the Laboratory grounds. It not only lets them be minutes away from their experiments, but greatly facilitates the speed of forming friendships with other Laboratory members. With the expansion of our science, however, we have begun to lose housing units that we formerly had in the Hershey Building, Urey Cottage, the Power House, and McClintock lab. So, our ability to house our staff has not at all been commensurate with our expansion in science. Increasingly, we have had to rent off-campus dwellings for our postdoctoral fellows and graduate students, which of necessity generates commuting trips not faced by those who live on the Laboratory grounds.

We badly need to formulate a long-term housing policy that optimally will let us acquire dwellings in the very immediate neighborhood. Given, however, the extraordinarily rapid increase in real estate values on Long Island, this objective will require much financial ingenuity. To the outside world, Cold Spring Harbor has always stood as a village of resident, as opposed to commuting, scientists. So, we must not only retain our semi-19th century appearance, but also maintain some of the inherent tranquility that went with that era.



**DEPARTMENTAL
REPORTS**



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

Recto: Staff members relax by the quiet pond situated behind Delbruck Lab and the Firehouse (right).

ADMINISTRATION

Whatever the fate of Gramm-Rudman, it is clear that efforts to reduce the federal deficit will increasingly restrict the federal funding of scientific research and, particularly, the recovery of associated indirect costs. The Laboratory is fortunate, therefore, to have in place a staff of experienced managers fully capable of controlling costs while continuing to provide the fast, flexible, and innovative services that our scientific community expects.

As a newcomer to the Laboratory at mid-year, my task was made infinitely easier by the dedication and support of these individuals, who managed so well during the approximately six-month period when the Laboratory was without an Administrative Director. Special recognition is due to John Maroney, our Assistant Administrative Director, who served so effectively during that period, and to our Treasurer, Robert Cummings, who was most generous with his time, energy, and experience.

During 1985, after the rapid growth of recent years as portrayed by the charts on the facing page, operations of the Laboratory stabilized at a high level. This provided an opportunity to prove and refine the new and more automated management systems that had been installed with considerable foresight in prior years to manage finances, purchasing, grant control, personnel, and other functions. Core services operated for a full year under the centralized management system established in 1984. These vital services, which include the safety department, machine shop, electronic and equipment repair, animal care, photographic and technical illustrating, and scientific computing, all operated more efficiently and will be increasingly useful to our scientific staff in future years.

The Laboratory has long been distinguished by its ability to attract the finest scientific minds. We have first-rate facilities and much state-of-the-art equipment. The world-class science that results has proved to be a powerful magnet for the needed funding. In the future, however, more of this funding is going to have to come from corporate and private sources, lessening our dependence on the uncertainties of government support. During 1985, the Commercial Relations Committee, in its second year of existence, played a key role in reviewing and guiding the Laboratory in its increasing numbers of commercial partnerships. A good beginning was made on the difficult task of writing a commercial relations manual to assist our scientific staff in managing the often complex issues that arise out of their involvement with commercial organizations. We are deeply indebted to Townsend Knight, chairman of this committee, for the extraordinary amount of time he devoted to these efforts.

Already, early in 1986, we can see that the brief pause in the Laboratory's expansion will soon give way to renewed growth. The administrative staff will need to manage this growth with the limited resources currently at hand and with the present number of support personnel. It is essential that we continue to decrease indirect costs as a percentage of overall research costs. We are most fortunate to have the kinds of individuals who will accomplish this through competence, dedication, and hard work.

G. Morgan Browne

BUILDINGS AND GROUNDS

With the new construction, renovations, installations, extensions, landscaping, and planning for future buildings, 1985 must go on record as one of our busiest years so far.

The work on Grace Auditorium is nearing completion. Final touches are being added to the exterior of the building and the first floor. Office furniture for the basement-level offices has been planned and ordered and landscaping has been started. A gazebo donated to the Laboratory was transported to its new location at the top of the hill on the north side of Grace Auditorium. To accommodate the expected increase in parking, construction of a new road and parking lot was begun on the west side of the Motel.

The exterior of Hooper House was sandblasted and painted a pale beige with light-mauve trim; the house now blends well with the others along Bungtown Road. Davenport House exterior was also redone, retaining its Victorian color scheme. A small extension was begun on the old Mouse House, which will be converted into an electron microscopy facility.

Earlier in the year, fighting the clock to complete the project in time for the summer courses, the entire top floor of James lab was gutted, the labs reworked, and new heating and air-conditioning systems installed. At the same time, the finishing touches were being done on Sambrook lab in preparation for the Dedication.

In the fall, two labs on the first floor of James lab were completed and new heating and air-conditioning systems were installed. In Demerec, two labs and one-half of another were completed, the kitchen was renovated, and a coldroom was installed. In addition, a second-floor laboratory in McClintock was renovated, and in the Buildings and Grounds building, a new Safety/Core office was completed.

The first floor of the south wing of the Nichols administration building was reworked and a new lobby and new offices were added. Installation of built-in furniture is scheduled for next year. A large section of the ongoing electrical loop system was completed when electrical lines were buried between Grace Auditorium and James lab. To make the exchange of information by computer more efficient, new Ethernet cable has been installed throughout the Laboratory.

At Uplands Farm, the garage was converted into a Field Station, and the top-floor apartment was renovated. A potting shed and greenhouse were started, with an estimated completion date of January 1986. Over at Banbury, exterior lighting was installed.

A complete reorganization of the custodial staff resulted when Charlie Marshall left after five years of service. Willie Gardner is now in charge of the custodial staff, and Dorothy Youngs now has charge of the housekeeping staff. As a result, we have seen a great improvement in both departments and in all of our buildings.

We mourn the loss of two good friends and fellow workers: Tom Lyden, who died in June of this year, and Joe Brodawchuck, who died in September. They were the best and we will miss them both.

Despite the completion of so much work, we will be starting the new year with the expectation of a heavier workload than ever. May it ever be so.

Jack Richards

DEVELOPMENT AND PUBLIC AFFAIRS

Science Phobia and the "Two Cultures"

Although essayist C.P. Snow argued that the schism between the science system and the social system is so large that they constitute two separate cultures, science is merely one phase in the continuum of human endeavor. Science does not exist in isolation from society; all scientific advances take place within the context of culture.

The impact of science and technology on our society is so ubiquitous that we tend to take it for granted: Concorde jets whisk away to London in three hours, computers disgorge \$20 checks and accurately debit accounts, sonic waves pulverize gallstones with only a scant incision necessary, microwave ovens produce hot meals in minutes, satellites beam live events from halfway around the globe, and ultrasound and gene probes detect birth defects antenatally.

Although we are immersed in technology, too many people have too little ken about the workings of science. So, in a world increasingly dictated by science, many suffer from a sort of "science phobia," which makes them believe that they cannot possibly understand this culture of researchers. This attitude prevents them from participating in the excitement of our scientific age and, in a sense, alienates them from their own technological life-styles.

Science is no different from any specialized cultural endeavor, such as orchestra conducting or French cooking. How many of us understand the intricacies of meter in Beethoven's Fifth Symphony or the lift in a seafood soufflé? But we need not be conductors or chefs to enjoy these cultural activities; our technical ignorance does not thwart our enjoyment. Indeed, appreciation of any cultural activity begins in relative ignorance—the potential to understand and master the intricacies only adds depth to our enjoyment.

Nor should the technicalities of science prevent us from enjoying its intellectual challenge. There is no excuse for scientific illiteracy in today's society. Most major newspapers employ professional science writers who make it their business to explain research in terms a nonscientist can understand; many have weekly "Part II" sections devoted entirely to science and technology. There now exist several popular magazines, such as *Science 86* and *Discover*, that accurately present an amazing array of scientific activities. Excellent science programming, such as *Nova* and *Innovation*, is available almost nightly on PBS channels.

A serious attempt to attend to any of these mass media channels can lead any *intelligent person* to an appreciation and understanding of modern science. When intelligent people spend as much time attending to science articles and telebroadcasts as they do to the Dow Jones Index and Wall Street Week, then the "two cultures" will become one.

Looking toward a Second Century

On July 7, 1990, the Laboratory will celebrate the 100th anniversary of its founding as one of the first American field stations for experimental biology. During 1985, preliminary plans were laid for a major development program to coincide with the centennial celebration.

As a center for advanced research and education, the Laboratory has exerted an influence on the development of modern science that is far out of proportion to

its small size. Now is the proper time to reflect on this great success as one of the few independent research institutions in this nation and to take action to ensure a second century of scientific excellence at Cold Spring Harbor.

Critical to the success of the program will be increased volunteerism on the part of all who believe in the importance of basic research and who recognize the special place Cold Spring Harbor occupies in the history of modern genetics. We will depend on our friends to introduce us to individuals, foundations, and corporations that can aid in the Laboratory's continued development as a village of science where researchers can work relatively free from distractions.

The Biological Revolution Documentary

In commemoration of the centennial, we recently completed a documentary video, *The Biological Revolution: 100 Years of Science at Cold Spring Harbor*. Produced under a special grant from the Long Island Biological Association, the 30-minute video presents a kaleidoscopic history of Cold Spring Harbor—from quiet camp site of Matinecock Indians, to bustling milling and whaling center, to enclave of scientists who seek to explain life in terms of the molecular interactions that take place within cells.

Through events that occurred at Cold Spring Harbor, the video recounts the quest to understand the nature of the genetic code, beginning with Darwin's theory of evolution and culminating with powerful techniques to precisely manipulate the DNA molecule. Laboratory Director James Watson and four other Nobel Prize-winning scientists lend their own insights on the genetic revolution they helped launch.

Coproducer and director Gregory McLaughlin brought to the production a fast-paced news style learned through extensive work as editor and field producer for Cablevision and Cable News Network. Peter Thomas, one of the truly great voices in the mass communications industry, breathed life into narration written by coproducer David Micklos. Arranged through the generosity of Helen and Charles Dolan, the donation of editorial and dubbing facilities by Cablevision reduced production costs by one-half. The documentary will air on the Cablevision system in spring 1986. VHS and Beta copies can be purchased or borrowed through the Library.

Training Science Communicators

Scientific advances reorder our life-styles at a bewildering rate. Although our involvement in technology is increasing at an exponential rate, public understanding of the scientific factors that speed us into brave new worlds lags far behind. This widening "knowledge gap" breeds individual anxiety and, in a real sense, threatens the informed decision-making essential to democratic politics. Thus, in a society increasingly dominated by science-related issues, we need more people who can translate the specialized jargon of science into language understandable to nonscientists. Their job is essential: to narrow the "knowledge gap."

In 1985 we instituted an internship to give promising journalism students the practical training to become effective science communicators. While at Cold Spring Harbor, an intern interacts directly with our scientists, having the dual challenge of learning firsthand about the process of discovery and of distilling that experience for a lay audience.

Our first intern, Laurel Vanderkleed, came to us from the graduate science-writing program at Miami University (Ohio). Laurel played a critical role in the



Greg McLaughlin and editor
Jayne Grote in the editing
suite at Rainbow
Network Communications

development of the Cold Spring Harbor Curriculum Study, testing laboratory procedures, assisting with workshops, and writing reference appendices. She also was key to the conversion of the *Harbor Transcript* to an attractive external newsletter. After a nine-month stay, Laurel left in the fall to become associate editor at Technical Insights, Inc., which publishes technically oriented newsletters.

We were also sorry to bid goodbye in December to Jenny Flanagan, who, for the past year, had performed with good grace the many hectic chores required of a public affairs assistant. Standing behind the scenes with lists in hand, she was the organizing factor of many public events, including the annual LIBA dinner parties. She was key to the development of the Corporate Sponsor Program and computerization of our development and public affairs efforts.

David Micklos

LIBRARY SERVICES

Neurobiology Library Moved, Storage Facility Established

Space needs dictated the integration of the McClintock lab's neurobiology library into the main library collection. The neurobiology library was established in 1971 to support the receipt of a Sloan Foundation grant that funded the summer-long neurobiology program for ten years. In 1981, our year-round neurobiology program was established in Jones lab. The limited space in Jones did not accommodate the 1800-volume collection, and it remained in McClintock until this year, when the space it occupied was badly needed for scientific offices.

To provide space in the main library, which reached its capacity in late 1984, we arranged to share that portion of East Side School in Cold Spring Harbor leased by the Publications Department for its book shipping and warehouse operations. About 7700 older bound volumes from the library's journal collection were relocated to 1600 linear feet of shelving in remote storage. The move was completed over a weekend in August by the library staff in conjunction with a specialized moving company; the library remained open throughout. This arrangement eased the overcrowded conditions while still providing for retrieval within 24 hours.

Reference Service and Archives Use Increases

In 1985, patron/reference services increased by another 12%. Our continued effort to provide faster information service for the Laboratory's scientific staff is seen in a two-year growth of 53% in reference service with an over-all decrease in staff of 0.5. The continued excellence of a well-trained staff accounts for this sustained savings. Genemary Falvey, Librarian, has developed a variety of processes to accommodate this continued growth.

An ever-increasing demand has been placed on the Archives Department, which loaned 538 photographs in 1985. Requests came from 38 outside organizations representing 12 countries and a variety of institutions. Largest among our on-grounds consumers are the Public Affairs Office and the Publications Department.

Cathy Garg, Archives secretary, was actively involved in the research for the Laboratory's Centennial film, the Sambrook dedication brochure, and the exhibit for the 50th anniversary of the Symposium. Organization of the 470 entries in the portrait collection, which includes files on such notables as Darwin and Mendel as well as a total file of staff and alumni, was completed. Additionally, all scrapbooks, ledgers, and photo albums have been preserved in acetate sheets and acid-free boxes to ensure the greatest shelf life.

Annual Growth of Permanent Collection

In 1985, the book collection grew by a net total of 1799 bound volumes, bringing the total number of volumes to 29,098. This 22% increase is due in part to the large number of advanced textbooks and reference tools that were added to the collection this year. The journal collection remained stable at 434. This year, 15 titles were added and 14 were withdrawn.

Staff Member Celebrates 12th Year

Laura Hyman celebrated her 12th year at the Laboratory. Along with handling myriad details for the entire staff, Laura functions as my administrative assistant for budgets, personnel, and scheduling, in addition to her library job as binder and interlibrary loan expert. An invaluable asset, she has managed to streamline the binding of our 434 current titles; bindery turnaround has been reduced to one month and rush materials can be readied in two weeks. Laura also recommended renegotiation of our subscription service contract, managing to secure a reduction from 6% to 4%, a savings of \$1200.

There are three new faces on the Library staff. Wanda Felker, Library Clerk, replaced Michaela McBride, who transferred to the Meetings Office. Carol O'Shea, Library Assistant, stepped in for Margaret (Peggy) Main, who entered library school in September. Cathy Garg was hired as Archives Assistant in May to fill the vacancy left by Caroline Albinson.

Susan Gensel Cooper

PUBLICATIONS

Expanding Our Audience with Paperback Editions of Monographs

With the aim of easing the financial burden on students, we began 1985 by issuing paperback editions of two of our previously published monographs: *Lambda II* and *Gene Function in Prokaryotes*. This trend was continued later in the year with the release in paperback of the two-volume monograph *The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance and Metabolism and Gene Expression*, which was followed by the publication of an updated paperback edition of our *Nucleases* monograph.

With a sigh of relief and a publication dinner held for the editors and principal contributors who were attending the RNA Tumor Virus meeting in May, we celebrated the completion of the second volume, *Supplements and Appendixes*,

of the paperback edition of the *RNA Tumor Virus* monograph. Conceived two years earlier, at a previous RNA Tumor Virus meeting, this massive 2525-page work stands as a tribute to the unselfish dedication of all who persevered through numerous rewrites and burgeoning sequence additions as we strove to keep current with a field that was exploding with new data almost daily.

By the close of 1985, combined unit sales of these paperbacks equaled almost 40% of the total units sold of their hardcover predecessors, a strong indication that there is a potentially substantial market for these lower-priced editions. Although release of the paperback editions expanded the audience for our existing monographs, we note that for the second year in a row, no new monograph was added to our list. Two new monographs are, however, planned for publication in 1986, and a major effort is being made to revitalize this series, which has been highly regarded by the scientific community since it began in 1971 with the publication of *The Bacteriophage Lambda*.

Strong Comeback for Current Communications Series

After a hiatus in 1984, the *Current Communications in Molecular Biology* series, which was begun in 1983 with two titles, came back strongly in 1985 with four new titles: *Eukaryotic Transcription: The Role of cis- and trans-acting Elements in Initiation, Protein Transport and Secretion, Immune Recognition of Protein Antigens, and Plant Cell/Cell Interactions*. With the publication of these volumes, we feel we are well under way to achieving our goal of producing a minimum of six titles in this series annually. Priced attractively between \$27 and \$30, cumulative sales of all volumes in this series exceeded the \$150,000 mark by the close of 1985.

Cancer Cells Gains Acceptance and Series on Vaccines Launched

New in our just one-year-old *Cancer Cells* series was volume 3 *Growth Factors and Transformation*. With first-year sales of this title 29% higher than the averaged sales of the two inaugural issues in 1984, it is clear that the series has gained the confidence of our customers, and we look forward to its continuation for many years to come.

We are optimistic that our new series on vaccine development, launched in 1985, will achieve the same acceptance. The inaugural issue, *Vaccines 85*, addressed the molecular and chemical basis of resistance to parasitic, bacterial, and viral diseases. Future issues will incorporate immunological approaches and chart the potential, and hopefully the success, of developing a vaccine against AIDS.

Neuroscience and Plant Molecular Biology Are Featured in Two New Volumes

Topics currently of interest in the neurosciences and plant molecular biology were also featured in our 1985 publications, the former in *Gap Junctions* and the latter in *Molecular Biology of the Photosynthetic Apparatus*. Responding to the many requests from our customers for a copy of the manual used in the *Molecular Biology of Plants* course here at CSH, we decided to publish this interim manual as a service to the scientific community while work continues on producing a comprehensive lab manual in 1986. The Laboratory's summer courses have always been fertile ground for the development, refinement, and testing of the latest

techniques, and we look forward to harvesting the best that these courses yield to continue our highly successful program of lab manuals.

In conjunction with our manual program, we will continue to offer, when needed, a complete kit of strains to facilitate the use of a manual. In keeping with this, a fully tested Strain Kit to accompany our *Experiments with Gene Fusions* manual became available in 1985.

50 Years of Publishing: The Symposia on Quantitative Biology

The opening days of 1985 found us firmly pushing volume 49 of the *Symposia, Recombination at the DNA Level*, out of the printer and into the hands of our customers while busily preparing for the 50th Anniversary of the Cold Spring Harbor Symposia on Quantitative Biology, soon to be upon us in May. As we closed 1985 we found ourselves in a not much different situation, putting to bed the last issue of our 50 years of publishing history while reaching ahead to May and the start of our second half-century, our aim unaltered from that expressed by Reginald G. Harris when he ushered in this publishing program in 1933: "to make available to all workers the methods and ideas which will be set forth in the group meetings."

Production Stabilized at 1984 Level while Expenses Declined

With a total in 1985 of 11 new titles, 5 new editions, and 7 reprintings of existing titles, the production output of the publications program remained approximately equal to that of 1984. Since 73% of our new titles, however, were issued in the lower-priced paperback format (as compared with 40% of our 1984 titles), it is not surprising that dollar sales declined by approximately 5% in 1985. This decline in income was more than offset by an almost 20% decrease in total expenditures, which resulted in a net increase in revenues after expenses from 1984 to 1985 of \$166,815. Several significant factors contributed to this savings, including the almost total use of in-house computers for typesetting, lower binding costs for paperbacks, and staff reductions through attrition.

Although 1985 was a year of consolidation and evaluation, the prospects for a sustained period of growth over the next several years seem bright. *Cancer Cells* and *Current Communications* show every indication of having established consistent sales records, several high-quality lab manuals long in development now appear to be likely candidates for publication over the next two years, and efforts already under way to develop several definitive monographs within the same time frame all contribute to this optimistic outlook.

Publications Services to the Laboratory

As we draw from the vast resources of the Laboratory's meetings, course, and research programs to provide the seeds for our growing publications program, we in turn provided our services to the Laboratory in 1985 by producing the abstracts booklets for the meetings program (10 in 1985), the program booklet for the dedication of the Joseph Sambrook Laboratory, and the 1984 Annual Report, as well as the Annual Reports for LIBA and Banbury Center. Last year's CSH Annual Report was a particularly complex undertaking as the entire design and production were revamped to achieve a more contemporary look while highlighting the unique character of CSH through the use of numerous informal

photographs in conjunction with striking photographs of details of the rich architectural heritage of our grounds.

A New Vehicle for Disseminating Information

We undertake the task given to the Publications Department by Dr. Harris to be the conduit for the dissemination of information not only in the books we produce, but also in our marketing efforts. In 1985 the Marketing Department developed the *CSHL Notebook*, a triennial newsletter designed to bridge the gap between direct mail campaigns and to inform students and researchers the world over about our meetings and course programs. That such information was clearly needed was reflected in the responses to a detailed questionnaire included in the first issue: 93% of the respondents endorsed the newsletter as a welcome addition. We also learned from the 82% of the respondents who have the responsibility of buying textbooks for their institutions that there are many courses for which no textbook is available. We are presently analyzing these responses with a view to how we might develop materials to meet these needs.

The Pace of Science Demands Rapid Order Processing

Over the last five years, we have taken several steps to decrease the turnaround time for filling an order. The first step was the introduction of a new computer system that generates packing slips within 24 hours. This year, we have taken the final step by bringing our shipping and warehousing totally in-house. Depending on the type of shipping requested by a customer, this allows us to get a book to our domestic customers in as little as 48 hours and to our foreign customers within two weeks.

Foreign Marketing and Distribution Agreements

Supplying timely information and making our titles available worldwide are constant goals. Accordingly, we completed negotiations for an exclusive distributorship in Japan with Maruzen Co., Ltd., in Tokyo. Through their extensive advertising program and excellent distribution channels, Maruzen is well-equipped to serve our Japanese customers efficiently.

Our own marketing capabilities have been made available to the John Innes Institute under an agreement with them to market and distribute in North America their new lab manual *Genetic Manipulation of Streptomyces*.

Marketing Techniques Reduce Costs and Increase Sales

Two steps were taken in 1985 to make the most effective use of our marketing dollar. Our house mailing list was meticulously pruned by 23%, from 57,000 to 44,000 entries, by eliminating addresses inactive since January 1982. In addition, to lessen the impact of the escalating cost of journal ads, each ad now features several titles, with the exception of ads for the Symposium.

With these economies, we were able to reallocate funds to produce our first four-color flyer, which featured volume 3 in our *Cancer Cells* series, *Growth Factors and Transformation*, and highlighted the previous volumes *The Transformed Phenotype* and *Oncogenes and Viral Genes*. The result was a

marked upswing in sales of the earlier volumes and a quickening in the pace of sales of volume 3.

Video Becomes Part of Our Book Exhibits

In the process of displaying our books at key professional meetings throughout the year, the Marketing Department disseminates much useful information about the Laboratory's overall program of research and education. Following the completion in 1985 of a video documentary of the history of CSH, it was decided that this video will become an integral part of all our book exhibits in 1986. Prepared in anticipation of the centennial of the Laboratory in 1990, *The Biological Revolution: 100 years of Science at Cold Spring Harbor* traces, through events that happened at CSH, the development of the biotechnology revolution. It is thus a fitting backdrop for the display of our books, which have chronicled those events for the last 50 years.

Nancy Ford

RESEARCH



Recto: Corn thrives during the winter months in the newly completed greenhouse at Uplands Farm. Construction of the facility is part of a \$3-million capital program to reestablish plant genetics as a major concern at Cold Spring Harbor.

TUMOR VIRUSES

In the field of eukaryotic gene regulation, the DNA tumor viruses remain a main-spring. Study of adenovirus and simian virus 40 (SV40) has illuminated areas of molecular biology ranging from DNA replication to translational control and from cellular transformation to messenger RNA synthesis and maturation. From its origins nearly 15 years ago, the Tumor Virus group has played a seminal part in the development of the pattern and style of research at Cold Spring Harbor. The following pages record the continued progress of this highly interactive group of investigators.

MOLECULAR BIOLOGY OF TUMOR VIRUSES

Y. Gluzman	T. Adams	B. Ondek	R. Chisum
T. Grodzicker	K. Buchkovich	M. Quinlan	J. Clarke
E. Harlow	M. Chapman	M. Romani	J. Cossetti
W. Herr	R. Cone	A. Shepard	R. Gensel
M. Siniscalco	D. Esposito	C. Stephens	M. Goodwin
	H. Fox	K. VanDoren	K. Marcinczak
	R. Gerard	P. Whyte	R. McGuirk
	J. Harper	M. Yamada	C. Schley
	D. McVey	C. Bautista	L. Vanderwal
	I. Mohr	D. Chao	J. Wiggins

The major focus of research in the Tumor Virus Section continues to be the analysis of the transforming genes and proteins of SV40 and adenoviruses as well as studies on regulation of viral gene expression. Many projects are concerned with the role of transforming proteins in cell proliferation, growth control, and transformation; with the interaction of transforming proteins with other cellular proteins; with modifications and cell localization of transforming proteins and their role in protein function; and with comparisons of viral and cellular transforming proteins. The properties of wild-type and mutant T antigens in *in vitro* replication systems are also being actively explored. Many experiments are concerned with the dissection of controlling elements, such as viral promoters and enhancers, their interaction with viral and cellular proteins, and the effects of these interactions on transcription and replication. Yeast cells, as well as a variety of mammalian cells, are being explored as host systems to study the effects of viral regulatory

proteins. Viral vectors that can be used to over-produce transforming proteins, new monoclonal antibodies directed against transforming proteins, new mutants in viral transforming proteins and controlling elements have been produced and are valuable reagents for many of the genetic and biochemical studies.

Production of a Cell Proliferation Factor by Baby Rat Kidney Cells Infected with Ad5 12S Virus

M. Quinlan, T. Grodzicker

Primary cultures of cells isolated from baby rat kidney (BRK) consist of fibroblast and epithelial cells. Within 1 week after plating, the epithelial cells die and the fibroblasts remain viable for about 2 weeks. However, when these cultures are infected with the adenovirus 5 (Ad5) 12S virus, the epithelial cells un-

dergo rapid proliferation and dominate the cultures. This effect is detectable within the first 24 hours after infection and results in an eight- to tenfold increase in the level of [³H]thymidine incorporated. The proliferation of epithelial cells continues for up to at least 1 month after infection. Ad5 12S is a mutant that only encodes the 12S gene product of early region 1A (E1A) in the form of a cDNA copy of the 12S mRNA. The 12S gene product has been shown to immortalize primary cells but not to activate gene expression from the viral genome. The 12S sequence is transcribed and translated by these cells between 9 and 18 hours after infection. Cells infected by the Ad5d1312 mutant virus, which has a deleted E1A region, resemble mock-infected cultures. Infection by Ad5d1309, which contains a wild-type E1A region, or by Ad5 13S, which only encodes the 13S gene product within the E1A region, results in the death of the majority of the cells within 48 hours.

The conditioned medium from Ad5-12S-infected cells is able to produce a similar proliferative effect on epithelial cells when added to uninfected primary BRK cultures. Pure populations of epithelial cells can be produced when BRK cells are plated in serum-free, hormonally defined media. The infection of such a population with Ad5 12S results in rapid cellular proliferation, even in the absence of serum or growth factors. The conditioned medium from Ad5-12S-infected BRK cells (maintained in media containing a variety of different growth factors, or none) induces the proliferation of uninfected epithelial cells; conditioned medium from cultures infected with Ad5d1312 or mock-infected does not. The conditioned medium is also able to extend the life of the primary cultures for several weeks. This and other data suggest that some factor is produced by BRK cells infected with Ad5 12S. The continued presence of this factor seems to be necessary for the continuous proliferation of the epithelial cells. However, the conditioned medium is unable to complement *Ha-ras* to produce transformed foci, which are produced when the 12S and *Ha-ras* genes are cotransfected. At present, we are analyzing the nature of the growth factor(s) produced, by analyzing both proteins and RNAs induced in 12S-infected cells; we are also investigating the range of cell types that can be stimulated by the 12S product. We are also studying E1A mutants to determine what region of the 12S protein is necessary to stimulate cell growth and to correlate this property with other parameters of transformation.

Adenovirus Vectors: Downstream Sequences That Affect Transcription Initiation from the Adenovirus 2 Major Late Promoter

T. Grodzicker [in collaboration with S. Mansour and R. Tjian, University of California, Berkeley]

We have used *in vitro* and *in vivo* recombination to position the coding sequences for SV40 T antigen at various locations downstream from the adenovirus major late promoter. Previously, we had constructed hybrid viruses that had T-coding sequences in the middle of the third segment of the tripartite leader, in the intron between the first and second leaders, in the auxiliary *i* leader segment (located in the intron between the second and third leader), and in the intron between the second and third leader but not within the *i* leader. All viruses made equal and very high levels of SV40 mRNAs that carried various amounts of the tripartite leader at their 5' ends, depending on where the T-antigen-coding sequences were located. The SV40 mRNA from one recombinant virus, Ad-SVR284, which carried a nearly complete tripartite leader, was translated ten times more efficiently than hybrid SV40 mRNAs from the other viruses. The poorly translated SV40 mRNAs, which carried only one or two segments of the tripartite leader, also lacked the normal T-antigen initiation codon as a consequence of splicing from unpaired 5' splice sites to a cryptic 3' site within the T-antigen-coding sequences. The 3' splice removed 48 nucleotides of SV40 RNA, including the normal AUG, so that translation initiated at an in-frame initiator AUG 13 codons downstream from the normal translation start site. Therefore, we could not determine whether their translational defect was due to the leader structure at the 5' ends of these mRNAs or to the use of an AUG triplet downstream from, but in-frame with, the normal T-antigen initiation codon.

To distinguish between these possibilities, we constructed two new adenovirus-SV40 hybrid viruses in which the T-antigen-coding region is fused to either the first leader segment or the second leader segment to eliminate the possibility of cryptic splicing events. Together with Ad-SVR284, these new viruses form a matched set encoding hybrid SV40 mRNAs that differ only in the number of tripartite leader segments attached to their 5' ends. Moreover, one of these viruses contains the

most extensive deletion of DNA sequences 3' to the late promoter that we have yet tested. We have measured the transcription and translation of the SV40 sequences carried by these hybrid viruses. To our surprise, we found that there was no detectable effect of altering the 5' leader sequences on the translation of hybrid SV40 mRNAs. However, DNA sequences that lie downstream from the major late promoter (starting at +33 relative to the 5' end of the mRNA) and extend into the intron following the first leader (to nucleotide +190) contain an element that increases, by about tenfold, the frequency of initiation from the adenovirus major late promoter. In addition, we have used a transient expression assay to demonstrate that the effect of these downstream sequences is mediated by a virus-encoded or virally induced *trans*-acting factor. We plan to map these downstream sequences further and to investigate what viral products mediate transcription stimulation.

Analysis of the Adenovirus E1A Proteins Using Monoclonal Antibodies

E. Harlow, C. Stephens, P. Whyte, C. Schley

The proteins encoded by the E1A region of Ad5 have been analyzed using a series of monoclonal antibodies. These antibodies bind to at least six distinct epitopes located in both the first and second exons of the 13S polypeptide. Immunoprecipitations from adenovirus-infected HeLa cells using these antibodies have shown that the E1A products are heterogeneous in size and charge. When the E1A proteins are analyzed by two-dimensional isoelectric focusing, approximately 80 polypeptide species can be resolved. About half of these polypeptides are products of the 12S and 13S mRNAs. The patterns of 12S and 13S proteins on two-dimensional gels are similar, although the 12S products have more acidic isoelectric focusing points and migrate somewhat faster in the second dimension. Pulse-chase experiments have shown that these spots are normal intermediates in E1A synthesis. Using short labeling times, we have been able to identify the primary translation product for both the 12S and 13S mRNAs. The modification pathway for these proteins moves the polypeptides to a more acidic pI and a higher molecular weight. In addition to the 12S and 13S polypeptides, a number of other E1A-related protein species can be seen

using two-dimensional analyses. These include polypeptides with relative molecular weights of 35,000, 30,000, 25,000, 20,000, and 15,000. The 30,000-molecular-weight polypeptide appears to be encoded by an E1A-specific mRNA. The origin of the other polypeptides is at present unknown.

The monoclonal antibodies also coprecipitate a series of bands with relative molecular weights of approximately 300,000, 130,000, 110,000, 80,000, and 60,000. Several lines of evidence suggest that these proteins are host polypeptides that are complexed to the E1A products. (1) These bands are not precipitated from cells that do not contain E1A polypeptides. (2) The bands are still seen when antibodies that bind to separate epitopes on E1A are used. (3) After denaturation, the E1A proteins, but not the other polypeptides, are specifically recognized by the monoclonal antibodies. (4) The 300,000- and 110,000-molecular-weight proteins are precipitated when lysates of labeled HeLa cells and unlabeled 293 cells are mixed together and precipitated with an E1A-specific antibody.

Overexpression of the Human Cellular Tumor Antigen p53

T. Adams, K. Buchkovich, E. Harlow

A full-length cDNA clone for the human tumor antigen p53 has been isolated. This clone has been inserted behind several different mammalian promoters, including the SV40 early promoter, the metallothionein promoter, and the mouse mammary tumor virus promoter to begin studies of the function of the p53 protein. Transient expression studies have shown that these constructs are correct and that they synthesize an authentic p53 protein. However, attempts to obtain stable high-expressing cell lines have failed to date. We have also compared the ability of these clones to collaborate with an activated *ras* gene to transform normal rat primary cells. All three of these p53 expression plasmids fail to cooperate in this assay under conditions where the adenovirus E1A gene will complement the *ras* gene. In an attempt to circumvent these problems, the cDNA has been cloned into an adenovirus expression vector provided by Y. Gluzman (this section). We have assayed the level of transcription from this vector and have found that this construct produces approximately 50 times more RNA than

the highest expressing cell line we have analyzed. We are currently checking for the expression of the p53 protein itself.

SV40 DNA Replication

R. Gerard, Y. Gluzman

Development of an in vitro SV40 DNA replication system (see B. Stillman, this section) allowed us to assess the effect of mutations in the origin of DNA replication and in a variety of mutant SV40 T antigen proteins.

T-antigen mutants. Five mutant T antigens containing amino acid substitutions known to affect SV40 replication in vivo have been purified. Human cells were infected with helper-independent adenovirus vectors carrying different mutant SV40 early genes, the mutant T antigens were purified by immunoaffinity chromatography, and their in vitro properties were compared with those of the purified wild-type protein. One mutant protein (C11) is completely defective in T-antigen ATPase activity but still binds to the origin sequences. Three other T-antigen proteins (C2, C6-2, and T22) are defective in their ability to bind to origin DNA but retain ATPase activity. Finally, the fifth altered T antigen (C8A) binds to origin sequences and retains ATPase activity. All five proteins fail to support SV40 DNA replication in vitro. However, in mixing experiments, all five proteins efficiently compete with the wild-type protein and reduce the amount of DNA replication. These data suggest that an additional function of T antigen other than origin binding or ATPase activity is required for initiation of DNA replication, and this hypothesis is currently under investigation.

Origin mutants. Plasmid DNAs containing the SV40 origin of DNA replication support efficient DNA synthesis in vitro and in vivo. Deletion of DNA sequences that had been previously defined by studies in vivo to constitute the minimal core origin sequence is also necessary for DNA synthesis in vitro. In the process of this investigation, the importance of the AT-rich region (n.p. 15–31) on the late side of SV40 origin became obvious: removal of 4–6 A-T nucleotides (n.p. 25–31) renders the origin defective. We constructed SV40 viral genomes that contain these lesions and confirmed that these constructs are defective for replication and did not

produce any plaque, although T-antigen synthesis was not affected by the mutations in the AT region. We were able to isolate revertants and found that they always contain an increase in the length of the AT region. This was due either to insertions or to point mutations. We are going to isolate and analyze a large number of revertants to draw firm conclusions about the role of the AT-rich region in SV40 origin function.

Properties of the SV40 T Antigen Produced in Mouse Cells

R. Gerard, Y. Gluzman

To address the question of why SV40 DNA fails to replicate in mouse cells (nonpermissivity), wild-type SV40 T antigen was expressed from the mouse metallothionein-I promoter in C127 mouse cells using a bovine papilloma virus (BPV)-based vector system. Induction of the transformed mouse cells with heavy metals resulted in the production of three times more SV40 T antigen than is found in COS monkey cells. Nevertheless, after adding a variety of exogenous templates containing an SV40 origin, no SV40 DNA replication was observed in the mouse cells; however, abundant replication was obtained in COS cells. Fusion of T-antigen-producing mouse cells with C6 cells (permissive monkey cells transformed by UV-irradiated SV40 that make a T antigen that is defective for viral replication but contain a functional origin) demonstrated that the T antigen produced in mouse cells was capable of replication of SV40 DNA in monkey cells. Furthermore, T antigen purified from mouse cells was active in an in vitro SV40 DNA replication system (in collaboration with R. Guggenheimer, this section). From this, we conclude that mouse cells transformed with a BPV vector carrying SV40 T antigen are deficient in replicating SV40 DNA because they are missing some positive factor(s) present in permissive cells.

Properties of *Saccharomyces cerevisiae* Cells Expressing SV40 T Antigen

D. McVey, Y. Gluzman [in collaboration with J. Hicks]

The possibility that SV40 T antigen expressed in yeast cells would interact with yeast components

(proteins?) and would affect some metabolic processes prompted us to undertake these experiments. Expression of T antigen in yeast cells was done to determine whether T antigen could produce any phenotype, particularly one affecting the life cycle and replication of *Saccharomyces cerevisiae*.

T-antigen cDNA was cloned into the YEp51 vector, and its expression was driven by the regulated *GAL10* promoter. No morphological changes were observed when yeast transformants were grown on glucose-containing medium (repressed conditions). However, when the yeast transformants were grown on galactose-containing medium (induced conditions), 5–15% of the cells exhibited a “shmooing” phenotype, reminiscent of the behavior of some cell-division-cycle mutants (e.g., *cdc28*). This phenotype was not exhibited by cells expressing truncated (aminoterminal 272 amino acids) T antigen, although much larger quantities of the truncated polypeptide were produced, as detected by immunoprecipitation.

The major problem with this system is the low frequency (5–15%) with which T-antigen-producing yeast manifests the shmooing phenotype. This makes genetic analysis very difficult. To overcome this problem, we are trying to express larger quantities of T antigen using a variety of yeast vectors and strains.

Further Modifications of Helper-independent Adenovirus Vectors

K. VanDoren, Y. Gluzman

CONSTRUCTION OF A DOUBLE REPLACEMENT VECTOR

The helper-independent double replacement vector allows the propagation of two different genes (usually one selectable marker and one gene of interest) inserted into two different areas of the adenovirus genome (early regions 1 and 3). This vector makes it possible to introduce both genes into the host genome by isolating drug-resistant cells after infection: Because the defective adenovirus genome usually integrates in toto, the second gene will also be present with high frequency. We constructed double recombinant viruses containing the human β -globin gene or a human γ - β chimeric globin gene

in place of the E1 region and a neomycin resistance gene in place of the E3 region. The human β -globin/*neo'* virus was used to transform human (K562) or mouse (MEL) erythroleukemia cells, and approximately 25% of the *neo'* cells also contained the complete human β -globin gene. In collaboration with D.S. Carlsson (National Institutes of Health), we are investigating the regulation of expression of the β -globin gene in these cells.

EXPRESSION OF GENES INSERTED IN THE ADENOVIRUS E3 REGION

Previously, we demonstrated that recombinant viruses that carry insertions in the E3 region can be efficiently propagated. The inserted gene was a neomycin resistance marker driven by the SV40 promoter. Viruses with inserts in either orientation were equally viable and could convert recipient cells to the neomycin-resistant state with equal efficiency. However, we obtained a different result when we inserted the SV40 T antigen gene driven by the major late adenovirus promoter into the E3 region. The only viruses we obtained contained the insert oriented so that transcription proceeded from right to left relative to the adenovirus genome (anti-E3 orientation). There are a few possible explanations for this discrepancy and they are now under investigation.

Pre-mRNA Splice-site Selection: In Vitro Splicing of Adenovirus E1A Precursor RNA

J. Harper, W. Herr

Maturation of many eukaryotic mRNAs involves removal of intervening sequences by RNA splicing. This process must be carried out with precision to produce mRNAs that encode proteins faithfully. Major advances in our understanding of the splicing process have resulted from the faithful reproduction of mRNA splicing in vitro (for review, see Keller, *Cell* 39: 423 [1984]). Analysis of the products and intermediates generated by in vitro splicing reactions has defined a pathway for the removal of intervening sequences and exon ligation. The first step is cleavage of the precursor at the 5' splice site and attachment of the 5' end of the intron to an adenosine residue near the 3' end of the

intron, generating a free 5' exon and a branched circular molecule, referred to as a lariat. The second step is cleavage at the 3' splice site and ligation of the two exons, producing spliced RNA and the intron sequences in a shortened lariat structure. Despite this elucidation of the mechanism of splicing, the signals that direct accurate excision of introns are not fully understood. Much of the specificity resides in conserved sequence elements found at the 5' and 3' splice sites, but this is not sufficient to direct accurate splicing because many pre-mRNAs contain similar sequences that are not normally used as splice sites. Some other factors must modulate the accuracy and efficiency of splice-site selection.

We are addressing this question by examining splice-site selection during *in vitro* splicing of precursor RNA containing multiple functional splice sites. For our initial studies, we have chosen the adenovirus E1A gene, which encodes three overlapping mRNAs that are produced by splicing from different 5' splice sites to a common 3' splice site. The accumulation of these RNAs is differentially regulated during the course of lytic infection by adenovirus. Two of these RNAs, the 12S and 13S species, are expressed throughout the lytic cycle, whereas the 9S species is not expressed until intermediate and late times after infection.

We have examined the relative efficiency with which the 13S, 12S, and 9S 5' splice sites are utilized in an *in vitro* splicing reaction. An E1A gene construct was made in which the bacteriophage SP6 promoter replaced the normal adenovirus E1A promoter and enabled precursor RNA to be prepared *in vitro* using SP6 RNA polymerase. This precursor was incubated in nuclear extracts from uninfected HeLa cells under conditions shown to be optimal for pre-mRNA splicing (Kraimer et al., *Cell* 36: 993 [1984]). This reaction generates a large number of products, including correctly spliced 13S RNA and the expected intermediates of 13S splicing (i.e., 5' exon and lariat intermediates), but no detectable 12S or 9S RNAs. The 13S RNA produced in these reactions can undergo a further processing event. This reaction involves selection of the 9S 5' splice site and a cryptic lariat attachment site in the 13S mRNA, resulting in an accurately excised 9S 5' exon and a lariat intermediate, but no detectable final spliced product. This reaction is probably analogous to that observed in cells transfected with a 13S cDNA plasmid, in which aberrant 12S and 9S RNAs were produced by processing of the 13S pri-

mary transcript (see Svensson et al., *J. Mol. Biol.* 165: 475 [1983]).

These results indicate that the three 5' splice sites are differentially recognized during the *in vitro* splicing reactions; the 13S splice site is recognized in the E1A precursor, resulting in correct 13S RNA splicing, the 9S splice site is recognized in a 13S precursor, resulting in an incomplete splicing reaction, and the 12S does not appear to be recognized in these reactions. These differences could be the result of competition between the three 5' splice sites for common splicing factors or for the 3' splice site, lack of specific splicing factors (e.g., 12S specific factors) from the splicing extracts, or a combination of the two. We are currently testing the idea of splice-site competition using a mutant E1A precursor that contains a deletion of the 13S 5' splice site. Future experiments will examine the possible role of precursor modification (e.g., adenine methylation) in splice-site selection and will explore different cells and extraction methods for making splicing extracts.

The SV40 Enhancer Is Composed of Multiple Functional Elements That Can Compensate for One Another

W. Herr, J. Clarke, H. Fox, B. Ondek, A. Shepard

We are studying the structure and function of elements within the SV40 enhancer that are responsible for activation of transcription. Our strategy has been to make point mutations within specific regions of the enhancer that weaken enhancer function and growth of SV40. We then select for and analyze the structure of SV40 growth revertants in which enhancer function has been restored. Last year, we described the structure of 18 revertants of the enhancer mutant *dpm12*. This mutant contains one copy of the 72-bp element and two point mutations within each of two different 8-bp stretches of alternating purines and pyrimidines (PU/PY); each revertant contains a tandem duplication ranging in size from 45 bp to 135 bp within the mutated enhancer region. A 15-bp region, called element C, that spans the "core" consensus sequence, GTGGAAAG, was present in all the duplications, suggesting that this region plays a critical role in restoring activity to the *dpm12* mutant.

To identify sequences that can compensate for point mutations within the C element, we selected

growth revertants of the mutant *dpm6*, which contains a mutated core element (GTGGAAAG to GTCCAAAG). Revertants of this mutant likewise contain tandem duplications. As shown in Figure 1, the duplications span either one or both of two separate regions that we refer to as elements A and B. Each of these elements contains one of the PU/PY sequences that is mutated in the *dpm12* mutant. These results suggest that the SV40 enhancer contains multiple elements capable of functioning independently and compensating for one another. Consistent with this hypothesis, revertants of the double mutants *dpm16* and *dpm26* containing mutated A and C and B and C elements, respectively, consistently duplicate the unmutated B or A domains.

When all three elements are mutated as in the mutant *dpm126*, no new element appears to compensate for loss of function; instead, enhancer function is effectively restored by "double duplications," in which the first duplication event recreates one of the sequences destroyed by the point mutations and the second event then duplicates this newly created sequence. This result illustrates one mechanism by which a strong enhancer can be created, i.e., by apparent creation of functional sequences at duplication junctions.

To determine whether or not the C element can activate transcription in the complete absence of the A and B domains, we synthesized a 17-bp-long SV40 fragment (GGGTGTGGAAAGTCCCC) containing 14 bp (underlined) of the 15-bp C element. Four and seven tandem copies of this element efficiently stimulate transcription of the β -globin gene in both CV-1 and HeLa cell transient expression assays. These results show that at least one of the three elements we have described can function independently of the other two. We plan to use these synthetic enhancers as probes to isolate and identify *trans*-acting cellular factors.

Transcriptional activation by the three elements (A, B, and C) may involve interactions with different cellular *trans*-activating factors. If this is the case, the revertant enhancers that contain duplications of different active elements may display different host-cell preferences, depending on the relative concentration of the *trans*-acting factors present in the cell. To date, we have examined the relative efficiency of the various mutants and revertants to activate β -globin gene expression in the simian cell line CV-1 and in HeLa cells. We find that both the *dpm6* and *dpm12* mutant enhancers yield

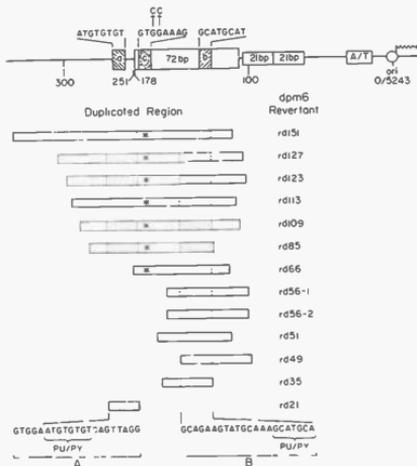


FIGURE 1 Tandemly duplicated sequences in 13 revertants of the SV40 enhancer mutant, *dpm6*. The diagram at the top of the figure illustrates the structure of the SV40 early promoter. Shown from right to left are the major early transcription start site (zig-zag arrow), the origin of replication (ori), the AT-rich TATA-like element (A/T), the two perfect 21-bp repeats, and a single copy of the 72-bp element. Numbers below the diagram refer to the nucleotide positions in the SV40 sequence. The positions of the two 8-bp PU/PY sequences (hatched boxes a and b) and the 8-bp core sequence (box c) are indicated with the respective nucleotide positions shown above each box. The two *dpm6* point mutations are shown above the sequence of the SV40 core element. The regions that are tandemly duplicated in the *dpm6* revertants are shown in order of size by the rectangular boxes. The series of double Xs show the location of the two "core" point mutations when included in the duplication. The two stippled areas show the location within the duplicated sequences of the two elements A and B, the sequences of which are shown at the bottom of the figure with brackets identifying the PU/PY sequences corresponding to the a and b hatched boxes at the top of the figure.

similar levels of β -globin RNA after transient expression in either cell line. Revertants of these two mutants exhibit two- to sixfold higher activity in CV-1 cells. In HeLa cells, the *dpm12* revertants are also two- to sixfold more active, but revertants of the *dpm6* mutant are scarcely more active than the parent mutant. These results indicate that the revertant enhancers are differentially selective with respect to the cell lines in which they will function effectively. By examining the behavior of the revertant enhancers in a variety of different cell lines, we

hope to identify cells that are likely to be rich sources of particular *trans*-acting enhancer factors.

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DNA SYNTHESIS

B. Stillman	R. Guggenheimer	E. White	J. Brody
	P. Cotton	G. Prelich	P. Newman
	J. Diffley	B. Faha	S. Penzi
		N. Byrne	

Research in this laboratory is aimed at elucidating (1) the mechanism and regulation of DNA replication in eukaryotic cells and (2) the functions of the adenovirus early region 1B (E1B)-encoded tumor antigens. In previous years, we have concentrated on understanding the mechanism of replication of one DNA replicon, the human adenovirus genome. These studies have now focused on a cellular encoded, site-specific, DNA-binding protein (nuclear factor I), which is required for adenovirus DNA

replication *in vitro* by binding to the origin sequences. The work on nuclear factor I has stimulated a search for cellular site-specific, DNA-binding proteins that may initiate DNA replication at specific origins in cellular chromosomes. To this end, we have detected a protein that binds to an autonomously replicating sequence (ARS1), which is present in the genome of yeast and is a candidate for an origin of chromosomal DNA replication.

In 1984, we began studies on the replication of

SV40 DNA in vitro and progress in this area has been rapid. We have characterized the kinetics and products of this cell-free reaction and have analyzed mutations in both origin sequences and the SV40 tumor antigen, which is another site-specific, DNA-binding protein that is required for initiation of SV40 DNA replication. During the course of these studies on SV40 DNA replication, we discovered reaction conditions that enabled replicating DNA to be preferentially assembled into a chromatin structure that resembles the structure of SV40 minichromosomes that are found in infected cells. This is the first such system described, and it may have far-reaching consequences for future biochemical studies on chromatin structure and function.

Finally, our studies on the adenovirus E1B-encoded tumor antigens have continued to yield surprising results. The E1B 19K protein was previously shown to localize predominantly in the nuclear envelope of infected cells. We have now demonstrated that this protein is a negative regulator of early gene transcription and controls the expression of other early gene products during infection of various human cells. The overproduction of early gene products in 19K⁻ mutant-infected cells may cause the wide variety of phenotypes that were described in last year's Annual Report.

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

J. Diffley, B. Stillman

The efficient initiation of adenovirus DNA replication in vitro requires, in addition to the virus-encoded preterminal protein-DNA polymerase complex (pTP-pol), a HeLa cell protein called nuclear factor I (NFI). The stimulatory effect of NFI on the initiation of adenovirus DNA replication is mediated through the binding of NFI to a specific sequence within the origin of adenovirus replication. Although a role for NFI in adenovirus replication is clear, any role for NFI in cellular DNA metabolism remains obscure. For example, NFI has been shown by investigators in several laboratories to bind to other viral DNA sequences, including transcriptional enhancers and potential cellular

regulatory sequences. To understand its cellular function, we have set out first to purify NFI. In last year's Annual Report, we described a sensitive assay for NFI based on its ability to bind specifically to a small DNA fragment containing the origin of adenovirus DNA replication. We have used this assay in the purification of NFI to apparent homogeneity. The purified protein exhibits one dominant band with a molecular weight of 160K on SDS-polyacrylamide gels and some minor proteins of lower molecular weight. The stoichiometry of DNA binding is consistent with two moles of the 160K polypeptide binding per mole of binding site. We have raised and are currently characterizing polyclonal antibodies directed against the 160K polypeptide that will be useful for further characterization of this polypeptide and its relationship to NFI activity.

Since the binding of proteins to specific DNA sequences appears to be a common motif in the organization of DNA replication origins from both prokaryotes and eukaryotic viruses, we are investigating whether these observations can be extended to eukaryotic cells and have thus begun to look at sequence-specific DNA-protein interactions at potential cellular origins of DNA replication. The ability of certain sequences (known as autonomously replicating sequences, ARS) to confer, in *cis*, upon any DNA the ability to be maintained extrachromosomally in yeast makes these sequences good candidates for eukaryotic origins of replication. One well-studied ARS, ARS1, has been shown to consist of at least three sequence elements important for full ARS function. Using a variation of the DNA-binding assay described for NFI, we have recently identified a factor in whole-cell extracts from the yeast *Saccharomyces cerevisiae*, which binds specifically to ARS1. After partial purification, we have shown that the protein protects approximately 25 bp of ARS1 DNA from DNase-I digestion. This 25-bp sequence is contained entirely within a 63-bp region previously determined by deletion analysis (Celniker et al., *Mol. Cell. Biol.* 4: 2455 [1984]) to be important in ARS function. Our current efforts involve (1) site-directed mutagenesis of the protein-binding site to determine if protein binding is required for ARS activity; (2) the complete purification of this protein; (3) the identification of the gene encoding this protein; and (4) elucidation of any role played by this protein in cellular DNA replication.

SV40 DNA Replication

R. Guggenheimer, G. Prelich, J. Brody, N. Byrne,
B. Stillman

Recently, we have initiated studies that utilize SV40 as a model system for the replication of eukaryotic chromosomes. Our work on SV40 DNA replication *in vitro* was performed in collaboration with Y. Gluzman's laboratory and has initially focused on (1) developing a detailed understanding of the function of *cis*-acting DNA sequences that comprise a functional origin of DNA replication; (2) the characterization of the protein requirements, both viral and cellular, for *in vitro* DNA replication of the SV40 genome; and (3) analysis and characterization of the products of the *in vitro* DNA replication system.

ESTABLISHMENT OF THE *IN VITRO* DNA REPLICATION SYSTEM

We have used an *in vitro* DNA replication system that carries out efficient and extensive synthesis of exogenously supplied plasmid DNAs containing the entire SV40 genome or fragments of the viral genome that span the origin of replication as determined by *in vivo* studies. The *in vitro* SV40 DNA replication system utilizes soluble extracts of adenovirus-transformed human embryo kidney cells (293 cells) as a source of replicative enzymes. SV40 DNA synthesis has been shown to be completely dependent on an intact SV40 origin of replication (see below) and requires the virus-encoded large tumor (T) antigen as well as crude cytoplasmic extracts from 293 cells. DNA synthesis also requires Mg²⁺, ATP, an ATP regenerating system (creatine phosphate and creatine phosphokinase), and the four deoxynucleoside triphosphates. Addition of the ribonucleoside triphosphates other than ATP only marginally stimulates DNA synthesis *in vitro*.

DNA synthesis has been shown to initiate at or near the origin of replication and proceeds bidirectionally. Density gradient centrifugation analysis of products of *in vitro* reactions synthesized in the presence of bromodeoxyuridine has demonstrated that replication proceeds semi-conservatively, with multiple rounds of replication occurring.

CHROMATIN ASSEMBLY *IN VITRO*

Initial analyses of the products of *in vitro* replica-

tion reactions by high-resolution agarose gel electrophoresis indicated that the products of these reactions were covalently closed, relaxed circular DNA molecules and topoisomers migrating between form I and form II DNA markers. These results were confirmed by agarose gel electrophoresis performed in the presence of the intercalating agent chloroquine. Furthermore, we have demonstrated that these intermediates of the replication process can be converted to negatively supercoiled (form I) DNA molecules upon the addition of a nuclear extract prepared from 293 cells. The supercoiling reaction requires the SV40 T antigen, and varying the amount of T antigen can modulate the level of supercoiling. Electron microscopic (Fig. 1) and biochemical analyses have revealed that the negative supercoiling of newly replicated DNA is due to the formation of stable nucleoprotein complexes *in vitro* and that these nucleoprotein complexes are indistinguishable from SV40 minichromosomes. Interestingly, formation of chromatin *in vitro* occurs concomitantly with replication and requires host-cell factors derived from the cytoplasmic and the nuclear extracts of 293 cells. The ability to synthesize chromatin during DNA replication *in vitro* should advance future studies on the structure and assembly of chromatin and its role in cell replication and transcription.

CIS-ACTING DNA SEQUENCES

The DNA sequences that comprise the origin of SV40 replication have been defined by studies *in vivo*, and we have demonstrated that the origin sequences required for efficient DNA synthesis *in vitro* are essentially the same as these. A 65-bp region of the SV40 genome has been defined as the minimal origin of replication by analysis of the ability of deletion mutants of SV40 DNA to function as templates in the DNA replication reaction. Within this minimal origin, four subregions have been defined. The first consists of a portion of the early side of T-antigen binding-site I. The second, a region between T-antigen binding-sites I and II, is essential and overlaps with a region that is the transition point between leading- and lagging-strand synthesis *in vivo*. A third region essential for template function consists of T-antigen binding-site II that is centered in a 27-bp palindrome. Finally, a stretch of 17 A.T base pairs that lie on the late side of the palindromic sequence is required. These results are summarized in Figure 2.

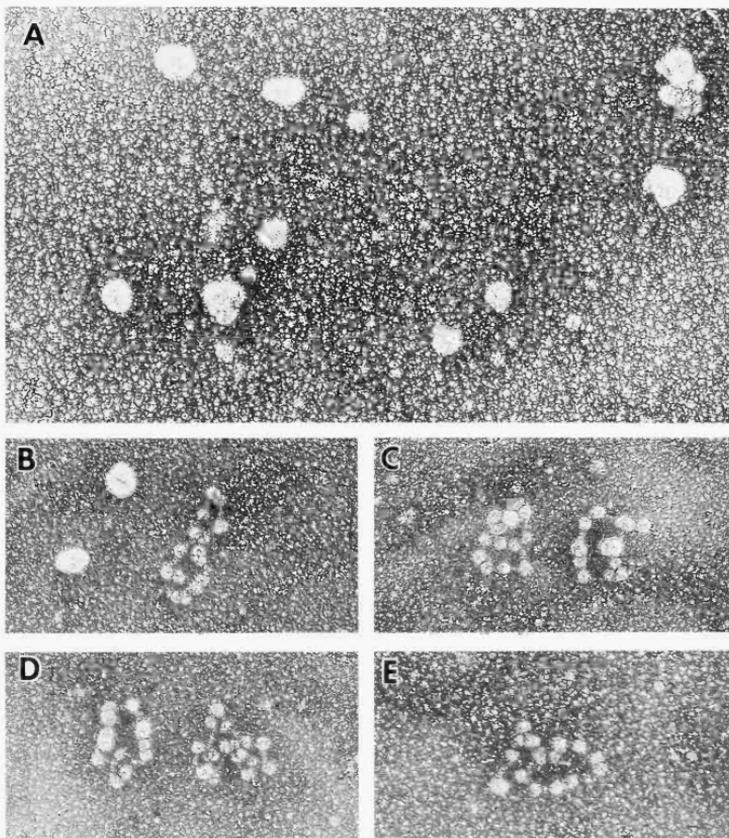


FIGURE 1 Electron microscopy of SV40 DNA replication products. Reactions were done with a nuclear extract that promotes the assembly of replicated DNA into a chromatin structure. The products were isolated by sucrose gradient centrifugation, and the peak fractions were pooled, dialyzed, and then fixed directly (*A*) or after sitting on ice for 48 hr, followed by heating at 30°C for 15–30 min (*B–E*). After fixation and staining, the samples were viewed in the electron microscope. The figure shows the image of the negative. Bar, 0.1 μ m.

T-ANTIGEN FUNCTION

We have also analyzed the effect of mutations in the T-antigen coding region on the ability of the resultant altered proteins to (1) support DNA synthesis *in vitro*, (2) bind to the origin sequences, and (3) hydrolyze ATP to ADP and P_i. All three activities are known to be intrinsic to wild-type T antigen.

The results of studies on five such mutants suggest that both ATPase and origin binding activities are necessary but not sufficient for the replication activity of T antigen. One mutant, C8A, which contains a lysine to glutamic acid change at residue 224, retains wild-type levels of origin binding and ATPase activities but is replication-defective. Ad-

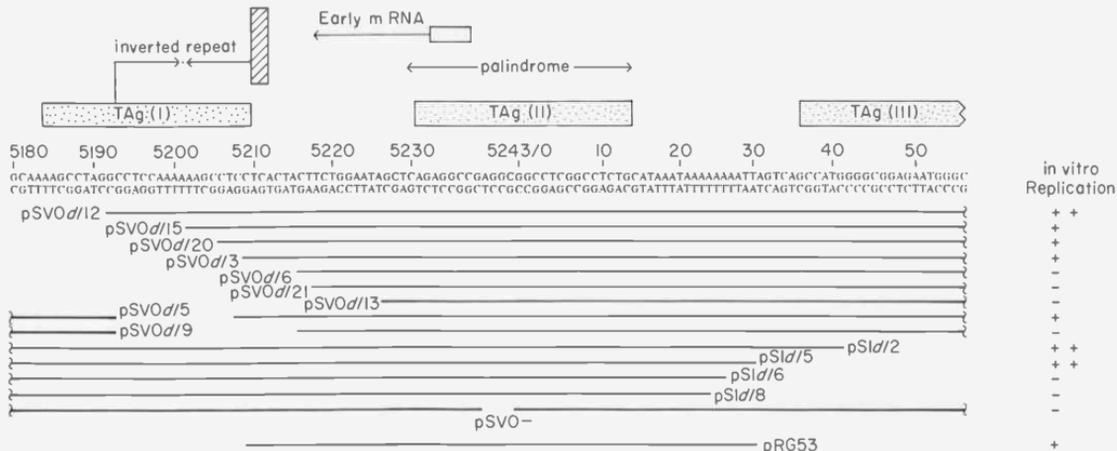


FIGURE 2 Definition of the SV40 DNA replication origin. The nucleotide sequence surrounding the SV40 origin region is shown. Above the sequence, the three T-antigen binding sites (I, II, and III) are indicated, together with the start site for early gene transcription (open box) and the transition point between leading- and lagging-strand synthesis (vertical hatched box). Below the line are the deletion endpoints for plasmids in the pSV0 and pS1 series, together with plasmids pSV0⁻ and pRG53. The construction and analysis of these plasmids were described previously (Stillman et al., *EMBO J.* 4: 2933 [1985]). The solid line represents sequences present in the plasmid DNAs. The ability of these plasmids to support SV40 DNA replication in vitro is indicated at the right: ++ indicates 100% of wild-type activity; + indicates 40–50% of wild-type activity; - indicates no detectable activity.

ditionally, we have demonstrated that several replication-defective mutant T antigens compete efficiently with wild-type T antigen in DNA replication reactions. On the basis of these results, we suggest that an additional function of T antigen, possibly involving a protein-protein interaction, is an essential feature of this multifunctional protein with respect to its replication activity.

GENETICS OF DNA REPLICATION IN VITRO

An interesting feature of the SV40 DNA replication system is that DNA synthesis only commences after a 10-15-minute lag but then remains linear for 90 minutes at 37°C. Experiments have demonstrated that this lag period could be abolished by preincubation of the template DNA, T antigen, and 293-cell cytoplasmic extracts for 15 minutes at 37°C in the absence of deoxynucleoside triphosphates. Omission of any of the aforementioned components during the preincubation, or preincubation of all required components at 4°C, 23°C, or 30°C, resulted in the reappearance of the lag in DNA synthesis. By ammonium sulfate fractionation of the 293-cell cytoplasmic extract, we have been able to demonstrate that a fraction, devoid of both DNA polymerase α and DNA primase activities, is competent in abolishing the kinetic lag in DNA synthesis.

Similar kinetic lags in DNA synthesis have been observed in a variety of DNA replication systems in vitro, most notably the *Escherichia coli* *oriC* and the phage ϕ X SS \rightarrow RF systems. In both cases, it has been demonstrated that this lag period corresponds to the time required for the assembly of a multi-enzyme prepriming complex required for generation of DNA primers used to initiate synthesis of nascent DNA chains. Because we have not yet been able to determine the nature of the primers synthesized in the SV40 replication system, we refer to the kinetic lag as a "presynthesis" stage. This presynthesis stage does not require the function of either DNA polymerase α or DNA primase and may represent the formation of a multi-enzyme complex functionally similar to the preprimosome of *E. coli*. Alternatively, the lag may reflect the time required to alter and prepare the template DNA for efficient use in the replication system. With respect to the latter possibility, we have demonstrated that the topological state of the input DNA is crucial in determining the efficiency with which this DNA is utilized as a template in the in vitro reactions. Spe-

cifically, covalently closed, negatively supercoiled DNA (form I) is at least 20-fold more efficient as a template than linear duplex (form III) DNA molecules.

It is our intention to pursue these studies on the in vitro replication of SV40 DNA. Our approach at the present time is to fractionate the 293-cell cytoplasmic extract and then purify and characterize the required activities, with a long-term goal to reconstitute SV40 DNA replication in vitro with purified components. Due to the lack of suitable eukaryotic cell replication-defective mutants, it is only through this biochemical approach that we may ask questions concerning the functional, mechanistic, and regulatory aspects of DNA replication in higher organisms.

Adenovirus E1B Tumor Antigens

E. White, B. Faha, P. Cotton, P. Newman, B. Stillman

The main focus of our work on adenovirus during the last year has been to determine the function of the E1B 19K tumor antigen in transformation and productive infection. It is clear that this protein has a pivotal role in oncogenic transformation by adenovirus; however, the precise mechanism behind this phenomenon is not known. We have approached this problem (1) by determining the intracellular localization of the 19K protein in adenovirus-transformed and lytically infected cells, (2) through the characterization of adenovirus mutants carrying defined genetic lesions within the coding region for this protein, (3) by investigating the interaction of the 19K protein with the other viral early region gene products through the construction of double-mutant viruses, and (4) by examining the effect of E1B 19K gene mutations on viral gene expression in different human host-cell lines.

INTRACELLULAR LOCALIZATION OF THE E1B 19K PROTEIN

Biochemical fractionation, indirect immunofluorescence, and immunoelectron microscopy of adenovirus-infected and -transformed cells has established that the E1B 19K tumor antigen is associated with the nuclear envelope and cytoplasmic membranes, probably those of the endoplasmic reticulum (see last year's Annual Report). The 19K protein is found in both the inner and outer nuclear

membranes of the nuclear envelope and the nuclear lamina, and this association with the nuclear envelope appears to be required for preserving the integrity of the host-cell chromosomal DNA during productive infection (see below). We have detected a small fraction of the 19K tumor antigen on the cell surface of adenovirus-transformed cells and are currently investigating its possible functional significance.

E1B 19K GENE MUTANTS

Adenovirus mutants have been isolated and have been shown to contain either point mutations or a deletion within the coding region for the E1B 19K tumor antigen. Infection of HeLa or KB cells with these mutant viruses causes the degradation of host-cell chromosomal DNA and enhanced and unusual cytopathic effects, the *deg* and *cyt* phenotypes, respectively. DNA degradation directly correlates with the absence of 19K protein in the nuclear envelope, indicating that its proper intracellular localization is required to prevent the DNA degradation in infected cells. The initial characterization of these mutants has formed the foundation for our current work on the interaction of the 19K protein with the other adenovirus early proteins and the role of the 19K protein in the control of early gene expression.

INTERACTIONS BETWEEN THE E1B 19K GENE PRODUCT AND OTHER VIRAL PROTEINS

DNA degradation is the result of early gene function, since it occurs in the absence of DNA replication and late gene expression. A viral mutant that does not synthesize a 19K gene product due to an out-of-frame deletion has the *deg* phenotype. This suggests that the DNA degradation, which occurs after infection with an E1B 19K gene mutant virus, is caused directly or indirectly by the action of another viral early gene product. We have postulated that the functional 19K protein in the nuclear envelope prevents the host-cell chromosomal DNA from becoming degraded by modulating the activity of this other early viral gene product(s) during the course of a normal infection. To this end, we have constructed double-mutant viruses that contain alterations in the 19K coding region as well as in other early viral genes. These double-mutant viruses have been assayed for their ability to induce the *deg* and *cyt* phenotypes in infected HeLa cells.

An involvement of the E1A proteins has been

tested by constructing viruses that express only the 12S (243R) or 13S (289R) E1A gene products in conjunction with an E1B 19K gene mutation. Both viruses are capable of causing the *deg* and *cyt* phenotypes, precluding the individual involvement of the 243R or 289R proteins. However, a double mutant that deletes both E1A proteins remains to be tested. Another virus that has been constructed contains the temperature-sensitive mutation in the E2A 72K DNA-binding protein from Ad5ts125 in conjunction with an altered E1B 19K gene. Infection with this virus also induces DNA degradation at the restrictive temperature where the DNA-binding protein is not functional. Experiments to test the effects of expression of the remaining early region gene products on DNA degradation are currently under way.

HOST-RANGE PHENOTYPE OF THE E1B 19K GENE MUTANTS

We have identified three classes of human cell lines that respond differentially to E1B 19K gene mutations. The first class is represented by HeLa cells, where the growth of E1B 19K mutant viruses is not much different from that of the wild-type adenovirus 2 (Ad2). Infection of HeLa cells with the mutant viruses causes the *deg* and *cyt* phenotypes (see above). The second class is represented by KB cells, where the mutant viruses grow substantially poorer than the wild-type virus (>100-fold reduction in virus yield). The DNA degradation is even more severe, and the cytopathic effect is more pronounced than in HeLa cells. The third class is represented by W138 and human embryo kidney (HEK) cells, where there is no DNA degradation after infection with mutant E1B 19K viruses, and in fact the mutant viruses possess a substantial growth advantage over that of the wild-type Ad2. In virus-yield experiments, the E1B 19K gene mutant viruses grow to 500-fold higher titers than the wild-type virus. We have sought to determine the nature of the reduced ability of the wild-type virus to grow in these cells by examining viral DNA, RNA, and protein synthesis in infected W138 cells.

Viral DNA replication is reduced in Ad2 but not E1B 19K mutant-infected W138 and HEK cells. The levels of viral DNA synthesized after infection with Ad2 have been quantitated by hybridization to a labeled adenovirus DNA probe and are tenfold lower, when compared to mutant-infected cells. This effect can be overcome, however, by infecting the

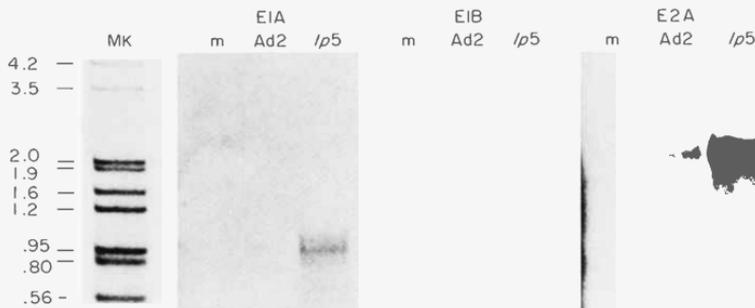


FIGURE 3 Northern analysis of cytoplasmic poly(A)⁺ mRNA isolated from mock wild-type (Ad2)-infected and mutant (lp5)-infected WI38 cells. Filters were hybridized to SP6-generated RNA probes for E1A, E1B, and E2A region transcripts.

wild-type virus at high multiplicities of infection (>50 pfu/cell). Synthesis of early and late viral proteins has been examined by Western blotting proteins from wild-type and mutant-infected cell extracts using antibodies directed against E1A, E1B 19K, E2A DNA-binding protein, and hexon. Both late protein synthesis and early protein synthesis are reduced. Similar results were obtained when infected cells were labeled *in vivo* with [³⁵S]methionine and labeled extracts were examined for early and late viral protein synthesis.

The reduced synthesis of early proteins in Ad2-infected WI38 cells was further investigated at the RNA level. Cytoplasmic mRNA was isolated from mutant- and wild-type-infected WI38 and HEK cells and translated *in vitro*. The levels of translatable mRNA for viral polypeptides are tremendously reduced in Ad2-infected cells when compared to mutant-infected cells. Northern analysis of poly(A)⁺ mRNA from these cells has revealed that the reduced levels of early viral gene expression in Ad2-infected WI38 cells are the result of reduced levels of cytoplasmic message encoding these proteins (Fig. 3). To establish whether the effects on early viral gene expression occur at the level of mRNA processing, transport, or transcription, nuclei from wild-type- and mutant-infected WI38 cells were assayed in run-on transcription reactions *in vitro*, and the RNA was analyzed by hybridization to adenovirus DNA probes for each of the early region genes. We conclude from these experiments that the reduced level of early viral gene ex-

pression in WI38 cells infected with the wild-type adenovirus is due to reduced levels of transcription of the viral genes.

The E1B 19K tumor antigen must therefore be acting as a negative regulator of early viral gene expression in human WI38 cells, thereby accounting for viral gene expression only in the absence of functional 19K protein. Experiments to define the molecular mechanism behind this important phenomenon are being pursued.

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NUCLEIC ACID CHEMISTRY

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Cloned Restriction-Modification Genes from Bacilli

A. Kiss

We have completed the sequence of the gene coding for the *BsuRI* endonuclease and have determined, by SI-nuclease mapping, the initiation sites of the *BsuRI* endonuclease and methylase transcripts. The predicted amino acid sequences of the *BsuRI* enzymes were compared, in a computer search, with each other and with other type II restriction and modification enzymes for which sequence information is available. The following are the main conclusions of this search: (1) There is no homology between the *BsuRI* endonuclease and methylase, suggesting that these two enzymes recognize their common target sequence (GGCC) by different mechanisms. (2) Extensive homology was found between the *BsuRI* methylase and the *BspRI* methylase, another enzyme recognizing GGCC. Parts of this homology are shared by two other modification enzymes: the SPR methylase (specificity: GGCC, CCGG, and CCA/TGG) and the *MspI* methylase (CCGG). (3) Regions of partial homology were found between the *Eco* dam (GATC), T4 dam (GATC), *DpnII* (GATC), and *EcoRV* (GATATC) methylases. The correlation between the sequence similarities and the recognition specificities suggests that the conserved regions may play a role in the recognition process.

Comparison at the amino acid sequence level of different modification methylases recognizing the same or related sequences provided information

that hopefully will guide further experiments (e.g., site-directed mutagenesis) to determine how these proteins recognize the specific DNA sequence. Unfortunately, due to the lack of cloned genes, no such comparison between restriction enzymes of identical specificity was possible. Therefore, we started experiments to clone the gene coding for the *BspRI* endonuclease, an isoschizomer of *BsuRI*. We tried several cloning strategies using selection either for the methylase or for the endonuclease, but so far we have not been successful in cloning this gene. These experiments yielded an unexpected clone that exhibits *in vivo* restriction and modification but with a specificity different from that of *BspRI*. *In vivo*, the clone restricts nonmodified bacteriophage λ . However, *in vitro*, using cell-free extracts of the clone under assay conditions generally used for type II restriction enzymes, we failed to detect specific cleavage of λ DNA. Thus, we conclude that this clone probably codes for a previously undetected type I or type III restriction-modification system of *Bacillus sphaericus*.

Experiments are in progress to isolate mutations that alter the recognition specificity of the cloned restriction and modification enzymes.

5-Azacytidine Sensitivity of *Escherichia coli*

A.S. Bhagwat

5-Azacytidine (5-azaC) is an analog of cytidine in which the C—H group at the 5-position of cytidine

is replaced by an azide group. It is an inhibitor of growth for both prokaryotes and eukaryotes. Its action depends on the presence of a deoxycytidine methylase in the cell. In *Escherichia coli*, the analog is taken up by the cells, phosphorylated, and incorporated into RNA. It is also converted to its deoxynucleotide form by the enzyme ribonucleotide reductase and incorporated into DNA. Such incorporation into DNA or RNA is not toxic by itself; however, it is highly toxic when a deoxycytidine methylase is present in the cell. It has been shown that a deoxycytidine methylase, in trying to methylate the 5-azadC residue in DNA, covalently attaches itself to DNA. This presents a block in DNA replication, leading to cell death.

I have found that the sensitivity of *E. coli* to 5-azaC depends on two factors—the amount of deoxycytidine methylase present in the cell and the state of DNA repair in the cell. *E. coli* K-12 strains carry a DNA methylase, *dcm*, that methylates the second C within the sequence 5'-CC(A/T)GG-3'; but most *dcm*⁺ *E. coli* strains are not sensitive to 5-azaC. They become sensitive to the drug either when a plasmid carrying the *dcm* gene is introduced in the cell or when the chromosome carries a mutation in *recA*, the gene central to inducible DNA repair in *E. coli*. The plasmid carrying the *dcm* gene is known to produce 30-fold more methylase than the chromosomal *dcm*. The current hypothesis is that when the cell is *recA*⁺, a small number of enzyme-DNA complexes are repaired by a *recA*-dependent system. If this hypothesis is verified by further studies, it would constitute the only known repair system for DNA damage involving covalently linked DNA-protein complexes.

Regulation of Transposon Tn3: Choice between Intermolecular and Intramolecular Transposition

A.S. Bhagwat

Tn3, a bacterial transposon that carries the ampicillin resistance gene, undergoes a replicative mode of transposition. Its transposition to another replicon (intermolecular transposition) results in two replicons, each carrying one copy of Tn3. Alternatively, it can transpose within its host replicon (intramolecular transposition), resulting in either a deletion of part of the replicon or the insertion of a second, inversely repeated, copy of Tn3 within the

same replicon. Although the intermolecular transposition is expected to aid the survival of the transposon and the spread of the ampicillin resistance gene, the intramolecular transpositions are expected to cause deleterious rearrangements within the host replicon.

I have determined the relative frequencies of the two modes of Tn3 transposition using a system in which both types of events take place in the same cell and have the same DNA sequence as target. I have found that the intermolecular mode is 15 times more frequent than the intramolecular mode. Furthermore, eliminating much of the intermolecular transposition in the cell does not increase the frequency of intramolecular transposition.

The experiment involves "mating-out" Tn3 insertions that are conditionally lethal to the recipient. These are Tn3 insertions in the *EcoRII* methylase gene on a plasmid that also carries an active *EcoRII* endonuclease gene. Cells that carry, or receive through conjugation, such plasmids are killed by the endonuclease unless the cell also carries the methylase gene. In the donor, cell death is avoided by maintaining the plasmid carrying the *EcoRII* endonuclease and methylase genes (pR209), along with the plasmid carrying Tn3 (pR209::Tn3). When such a donor is mated with a recipient lacking the *EcoRII* methylase, and transconjugants are selected for Tn3 (Amp^R) as well as for the outside marker on pR209 (Tet^R), two major types of transconjugants are expected. One type involves two intermolecular transpositions—pR209::Tn3 to pR209, followed by cointegrate formation with the F-factor, and pR209::Tn3 to the F-factor, followed by cointegrate formation with pR209. These cointegrates are transferred to the recipient during mating and resolved. They have the phenotype of either restriction⁺ (R⁺) modification⁺ (M⁺) or R⁻ M⁻. Alternatively, the transconjugants can arise through one intramolecular transposition and one intermolecular transposition of Tn3; an intramolecular transposition within pR209::Tn3 making it R⁻, followed by cointegrate formation with the F-factor. The cointegrates are then transferred to the recipients during mating and resolved, resulting in an R⁻ M⁻ phenotype. Therefore, the two pathways are expected to result in transconjugants with plasmids that have different phenotypes and different structures. Thus, a restriction enzyme analysis of plasmids in the transconjugants can ascertain the pathway by which each has arisen.

When such an analysis was carried out on 110

transconjugants, 94 were found to have arisen by the first pathway (2 intermolecular transpositions), whereas only 3 were found to have arisen by the second pathway (1 intramolecular and 1 intermolecular transposition). At least half of the transpositions in the first pathway could be shown to have been mutually independent. Thus, the first pathway was used by Tn3 15- to 30-fold more frequently than the second pathway. As both pathways involve one transposition to the F-factor, the preference for the first pathway must reflect a preference for intermolecular transposition to pR209 as opposed to intramolecular transpositions within pR209. This result has been found to be independent of the orientation of the donor Tn3. When the plasmid carrying the active methylase gene in the donor (pR209) is replaced by a plasmid carrying the right end of Tn3 in addition to the methylase gene (pR222), no transposition can take place to pR222 because of the "transposition immunity" property of Tn3. Thus, much of the intermolecular transposition within the cell is inhibited. Under these conditions, the choice between the pathways described above is reversed. In this case, no transconjugants arise by the first pathway, whereas most of the transconjugants arise by the second pathway. More interestingly, the overall frequency at which Tn3 undergoes intramolecular transposition remains unchanged. It appears that intramolecular transposition of Tn3 is maintained at a low frequency, regardless of the state of intermolecular transposition within the cell.

Restriction Endonucleases

P.A. Myers, A. Sohail, R.J. Roberts

The collection of restriction enzymes continues to grow, and more than 600 such enzymes are now known; 137 different specificities have been characterized, including 21 new ones within the last year. Among these new specificities, the recognition sequences for *BspMI* (ACCTGC[4/8]), *BspMII* (T ↑ CCGGA), *EagI* (C ↑ GGCCG), *PpuMI* (PuG ↑ G[A/T]CCPy), *PfIMI* (CCAN[N]₂TGG), and *PpaI* (GAGACC) have been characterized as part of a collaborative program with I. Schildkraut and D. Comb (New England Biolabs). In addition, more than 20 other enzymes have been characterized that are isoschizomers of previously known re-

TABLE 1 Simple Hexanucleotide Palindromes

	2nd	A	C	G	T	3rd
1st						
A	—	—	—	<i>BglII</i>	—	
C	—	—	<i>NcoI</i>	<i>PvuI</i>	—	A
G	<i>EcoRI</i>	—	<i>SphI</i>	<i>BamHI</i>	<i>SnaI</i>	
T	—	—	—	<i>BclI</i>	—	
A	—	—	—	<i>Eco47III</i>	<i>ClaI</i>	
C	<i>PmaCI</i>	—	<i>SmaI</i>	—	<i>XhoI</i>	
G	<i>AatII</i>	—	<i>NaeI</i>	<i>NarI</i>	<i>SalI</i>	C
T	<i>SnaBI</i>	—	<i>BspMII</i>	<i>MstI</i>	<i>AsuI</i>	
A	<i>HindIII</i>	—	<i>MluI</i>	<i>StuI</i>	<i>AvaIII</i>	
C	<i>PvuII</i>	—	<i>SacII</i>	<i>XmaIII</i>	<i>PstI</i>	
G	<i>SacI</i>	—	<i>BsePI</i>	<i>ApaI</i>	<i>ApaLI</i>	G
T	—	—	<i>NruI</i>	<i>BalI</i>	—	
A	<i>SspI</i>	—	<i>SpeI</i>	<i>ScaI</i>	—	
C	<i>NdeI</i>	—	<i>AvrII</i>	<i>SplI</i>	<i>AflII</i>	
G	<i>EcoRV</i>	—	<i>NheI</i>	<i>KpnI</i>	<i>HpaI</i>	T
T	—	—	<i>XbaI</i>	—	<i>AhaIII</i>	

striction enzymes. As shown in Table 1, 47 of the possible 64 enzymes that could recognize hexanucleotide palindromes have now been discovered.

In Vitro Splicing of Adenovirus 2 Late RNA

G.A. Freyer, E. Grubman

We have continued our studies of RNA splicing in vitro using extracts from HeLa cell nuclei. The substrate that contains the first and second exons and first intron of the tripartite leader of adenovirus 2 (Ad2) major late RNA is synthesized in vitro using the *Salmonella* phage RNA polymerase, SP6. The transcripts contain a modified version of the first intron that contains only 86 nucleotides (6 nucleotides from the 5' end of the intron and 18 nucleotides from the 3' end of intron). The products of splicing of this RNA have been completely analyzed and all intermediates have been characterized, including both intron lariats and intron/exon lariats. Analysis has been performed by hybridizing the products to appropriate cDNA, trimming with ribonuclease T1, and fingerprinting the oligonucleotides. It has been possible to identify unambiguously each of the products of the reaction. The lariat attachment site has been shown to involve an A residue 24 nucleotides upstream of the 3' splice site, in agreement with the results of Konarska et al. (*Nature* 313: 552 [1985]).

One interesting mutant version of the substrate has been prepared in which two A residues, one of which is the normal lariat attachment site, have been converted into G residues. This mutant clone was prepared by oligonucleotide-directed, site-specific mutagenesis. When RNA is transcribed from this clone *in vitro* and used in the splicing reaction, all of the usual intermediates are found, including the final splice product. However, the efficiency with which the final splice is produced is reduced at least 40-fold as compared with wild type. As with the wild type, all intermediates of this reaction have been characterized. The lariat intermediates are present in quantities similar to those observed with the wild-type precursor. We have shown that the nucleotide used for the lariat attachment site is now the G residue that replaced the wild-type A at the normal site. Analysis of the lariat attachment by T1 fingerprinting and by redigestion with RNase P1 shows unambiguously that the lariat forms through one of the newly introduced G residues. Primer-extension experiments using oligonucleotides that hybridize to the handle of the lariat also confirm the position of the lariat attachment site. This is in contrast to other lariat attachment sites that have been characterized in other systems in which the branch point has always been found to involve an A residue. It is of interest that the debranching enzyme (Ruskin and Green, *Science* 229: 135 [1985]), which is able to cleave the lariat specifically at the branch point when an A residue is present, also works successfully when a G residue is present.

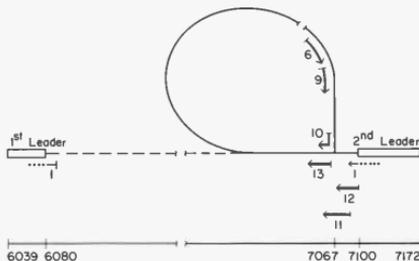


FIGURE 1 Position of oligonucleotides used to probe for the first intron of the Ad2 major late transcript.

either as hybridization probes on Northern blots or as sequencing primers for reverse transcriptase.

When oligonucleotides 6, 9, 10, or 13 were used to probe Northern blots, strong signals were obtained from two RNA species with apparent sizes around 1000 nucleotides. The slower moving of these two species shows abnormal mobility on different concentration agarose gels and is believed to be a circular version of the faster-moving molecule. Neither of these two molecules can be detected when oligonucleotide 12 is used as probe. These two RNA species are not polyadenylated and are detected in cytoplasmic extracts from infected cells. Time course experiments show that these sequences accumulate early during infection and reach steady-state levels by about 12 hours postinfection. Since late mRNA continues to be synthesized and processed at that time, these intron sequences clearly must turn over. Experiments are now in progress to discover whether these intron sequences are associated with specific proteins.

Fate of Adenovirus 2 Introns In Vivo

G.A. Freyer, K. O'Neill, R.J. Roberts

The main thrust of our work in this area has been to examine extracts of adenovirus 2 (Ad2)-infected HeLa cells at late stages of infection to discover whether any RNA species are present that contain sequences corresponding to the first two intervening sequences removed during the processing of the late mRNA leader sequences. A number of oligonucleotide probes were designed that would be able to detect possible lariat intermediates or their products. The probes were made specifically against the first intron of the Ad2 major late transcript, and their locations are indicated schematically in Figure 1. They were ³²P-labeled at their 5' ends and used

Adenovirus 2 E1A Gene Products

B. Zerler

In collaboration with E. Moran and M. Mathews (Protein Synthesis section), we are continuing to examine the transforming and gene-regulating properties of the adenovirus E1A gene. Previously, to distinguish the roles of individual E1A gene products, we made plasmids and viruses containing 9S, 12S, and 13S E1A cDNAs.

Virus isolates containing the 13S cDNA in place of E1A are competent for lytic functions in HeLa

cells, whereas the 12S viruses are defective. Northern and Western analyses of virus-infected HeLa cells indicate that the adenovirus E2 gene product, the DNA-binding protein, is made in HeLa cells during 13S virus infection but is not detectable during infection with 12S virus, although expression of both the 12S and 13S products is detectable by Northern and Western analyses. Although defective for lytic functions in human cells, the 12S virus transforms primary baby rat kidney cells in culture at very high efficiency. Infection of these cells with 13S or wild-type E1A viruses is cytotoxic. Only the 13S plasmid stimulates expression of the heterologous *cat* gene from the adenovirus E3 promoter in a transient expression assay, whereas both the 12S and 13S plasmids establish primary baby rat kidney cells in culture or cooperate with the *ras* oncogene to transform these cells fully. Thus, it appears that both the 12S and 13S products have transforming functions. However, the transformation function of the 12S product is not dependent on the ability to turn on other adenovirus genes and does not appear to be required for virus lytic growth in HeLa cells. It is not clear whether the lytic functions require the establishment activity of the 13S product.

The kinetics of expression of the 9S mRNA in 9S cDNA virus-infected HeLa cells are interesting, since the 9S mRNA normally does not appear at appreciable levels until late in wild-type infection. It is noteworthy that the level of 9S mRNA in HeLa cells is increased when the 9S virus is coinfecting with the 13S virus, suggesting a possible role for the 13S RNA or protein products in 9S expression. The enhancement of the 9S mRNA level occurs even in the presence of cycloheximide, but the mechanism of action of cycloheximide on mRNA levels in virus-infected cells is not certain. Transcription of other early genes dependent on E1A is readily detectable in cycloheximide-treated cells and may depend on residual protein synthesis. It is also interesting that 293 cells do not normally express 9S mRNA from the endogenous E1A sequences they contain, but 9S mRNA is expressed during adenovirus infection of 293 cells. We have observed the production of 9S mRNA during infection of 293 cells with the 9S cDNA virus and are continuing to investigate the regulation of 9S mRNA expression.

In addition to the above studies, we have expressed the 12S and 13S E1A cDNA products in *Saccharomyces cerevisiae*. The cDNAs were cloned into a yeast expression vector under transcriptional control of the inducible *GAL10* promoter. Western

blot analysis shows the presence of two 13S proteins and three 12S proteins. The largest of the 13S and 12S proteins made in *S. cerevisiae* have the same apparent molecular weight as the largest of the 13S and 12S proteins present in adenovirus-infected HeLa cells. There is also a major lower-molecular-weight 12S protein made in *S. cerevisiae* that is not detected in virus-infected HeLa cells. In addition, there is a protein of the same apparent molecular weight made in both 12S- and 13S-containing *S. cerevisiae* that is not detected in virus-infected HeLa cells. The 12S protein is found in the soluble fraction when yeast are lysed by either glass-bead homogenization or French Press, whereas only 50% of the 13S protein is found in the soluble fraction using either method of cell lysis. Western analysis shows that the amount of 12S or 13S protein made in 10^7 cells of *S. cerevisiae* in mid-log growth is about equal to the amount of 12S and 13S protein made in 10^7 HeLa cells infected with adenovirus at a multiplicity of infection of 10.

When grown under inducing conditions, the *S. cerevisiae* containing either the 13S or 12S E1A cDNAs do not exhibit any observable phenotype. Generation time and morphology appear to be similar to those of the controls. We have also found that mating-type *a* (*MATa*) cells expressing E1A proteins are not altered in their susceptibility to the cell-cycle-arrest effect of α -factor. In other experiments, it was found that adenovirus E1A proteins do not alter the ability of *S. cerevisiae* to sporulate, and diploid strains expressing E1A do not exhibit any differences in growth and morphology from diploids not expressing E1A.

We are currently purifying the E1A proteins from yeast based on an isolation protocol applied to adenovirus E1A proteins obtained from *Escherichia coli*. Purified E1A protein from yeast will be assayed for function by microinjection into mammalian cells and will be used to screen mammalian cell extracts for factors that interact with E1A.

Besides expressing the adenovirus E1A proteins in *S. cerevisiae*, the E1A proteins have also been successfully expressed in DHFR⁻ CHO cells following transfection of a plasmid containing E1A and the amplifiable *dhfr* gene. By stepwise amplification with methotrexate, we hope to establish a mammalian cell line that expresses large amounts of E1A protein.

We have also recently expressed E1A cDNA protein in *E. coli*. The 13S cDNA was cloned into a bacterial expression vector under transcriptional con-

tol of the T7 promoter. From earlier studies in other laboratories, it is known that E1A protein produced in *E. coli* is not properly modified and is inactive. Therefore, the bacterially made E1A protein provides a good substrate to analyze the modifications necessary for activity. The bacterial system also provides a large amount of protein that, in combination with E1A protein isolated from *S. cerevisiae*, can be used to screen mammalian cell extracts for factors that interact with E1A.

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PROTEIN SYNTHESIS

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	E. Moran	M. Freedman	

Research in the Protein Synthesis laboratory continued along the lines described in last year's Annual Report. The main themes are the regulation of gene expression in adenovirus-infected human cells both at the translational level and at the transcriptional level, autoantibodies and autoimmunity, and the heat-shock (stress) response of human cells.

VA RNA and Translational Control

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

Last year, we reported that the VA RNAs are essential in adenovirus-infected HeLa (human) cells to preserve the activity of certain protein synthesis initiation factors. Eukaryotic initiation factor 2 (eIF-2) ferries the initiator tRNA, methionyl-tRNA_F, to

the ribosome in one of the first steps of protein synthesis. At the completion of the series of initiation reactions, eIF-2 emerges complexed with GDP, and the cofactor must be replaced by GTP before eIF-2 can reload with methionyl-tRNA_F in preparation for another initiation event. The cofactor substitution reaction is itself mediated by an enzyme, the guanine nucleoside exchange factor (GEF, also known as eIF-2B), and work in other systems has shown that the interaction between eIF-2 and GEF constitutes an important site for the regulation of polypeptide chain initiation. Phosphorylation of eIF-2 on its α -subunit (it contains three different polypeptide chains) results in formation of a tight complex between the two enzymes, preventing GEF from fulfilling its role.

Adenovirus encodes two small RNAs, the virus-associated (VA) RNAs, transcribed by RNA polymerase III. These RNAs (~160 nucleotides long)

are produced abundantly at late times of infection and accumulate in the cytoplasm. The deletion mutant Ad5d/331 described by Thimmappaya et al. (*Cell* 31: 543 [1982]) fails to make the major species of VA RNA, VA RNA₁, and protein synthesis is severely depressed during the late phase of infection with this virus. Work in cell-free translation systems from d/331-infected cells indicated that a mechanism of the kind outlined above must be at play. In extracts of d/331-infected cells, (1) initiation of protein synthesis is restored by addition of either eIF-2 or GEF; (2) GEF activity is lacking; and (3) a kinase capable of phosphorylating eIF-2 on its α -subunit is present.

This kinase phosphorylates the serine residue that comprises the regulatory site on the α -subunit of eIF-2. Identification of the protein kinase was a vital step in elucidating the mechanism of VA RNA action. Of the two enzymes known to share this substrate specificity, one (known as the heme-controlled inhibitor, HCI) is activated by the absence of heme and the other (known as the double-stranded RNA-activated inhibitor, DAI) is activated by the presence of double-stranded RNA (dsRNA). The enzyme present in d/331-infected HeLa cells resembles DAI in several diagnostic ways: sensitivity to a sulfhydryl reagent; binding to a dsRNA affinity matrix; and association with a phosphoprotein of approximately 70 kD, which is thought to be a subunit of DAI. Furthermore, when infected with d/331, cells deficient in DAI were permissive for protein synthesis. This evidence left no doubt that DAI is the kinase responsible for the inhibition of protein synthesis and immediately suggested how VA RNA might function. VA RNA₁ is highly structured and contains several duplex regions, although these are not sufficiently extensive or unbroken to activate the enzyme as long, perfect dsRNA molecules do. Short duplexes, however, block activation of DAI by long dsRNA molecules, and we have shown that VA RNA₁ can indeed interfere with the activation of DAI by authentic dsRNA in a model system. Indirect evidence suggests that the activator of DAI found in virus-infected cells is probably dsRNA produced by symmetrical transcription of both viral DNA strands.

As illustrated in Figure 1, it appears that VA RNA protects against activation of DAI and the consequent cessation of all protein synthesis in the infected cell. But it is also possible that VA RNA subserves additional functions in the infected cell;

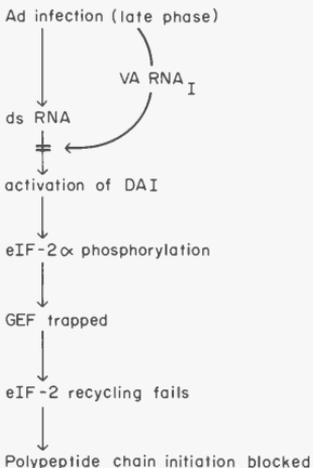


FIGURE 1 The role of VA RNA₁ in maintaining protein synthesis in adenovirus cells. (Reprinted, with permission, from O'Malley et al., *Cell* 44: [1986] in press.)

for example, it may counter the antiviral action of interferon, which induces synthesis of DAI to higher levels, or perhaps it may participate in the process that favors the translation of viral mRNAs relative to cellular species at late times of infection. Investigation of these possibilities is under way.

Functions of the E1A Gene

E. Moran, C.V. Dery, C. Herrmann, J.M. Langstaff, M.B. Mathews

The E1A gene occupies the extreme left end of the viral chromosome and plays a key role in regulating viral transcription (a process known as *trans*-activation) and in cellular transformation by adenovirus. The extent to which these functions are interrelated and the mechanism whereby they are achieved by the E1A proteins are issues that continue to attract intensive research in many laboratories. Our work in this field has been pursued partially in collaboration with B. Zerler, R. Roberts, and T. Grodzicker (see Nucleic Acid Chemistry and Molecular Biology of Tumor Viruses) and has taken a genetic approach. We have made and analyzed E1A mutants of three classes: (1) variants in

which the intact genomic EIA sequence is replaced by cDNAs equivalent to the three differently spliced EIA mRNAs (9S, 12S, and 13S), (2) deletions removing portions of the EIA sequence bordered by convenient restriction sites, and (3) point mutants altering selected individual amino acids.

In last year's Annual Report, we discussed the roles of the products of the 9S, 12S, and 13S EIA mRNAs and described the properties of a mutant that lacks amino acids 121–150 of the 13S product. We have now constructed an additional deletion mutant that lacks residues 86–120. A virus carrying this deletion grew as well in HeLa cells as in 293 cells, indicating that it is competent in *trans*-activation. The corresponding EIA plasmid was, as expected, able to stimulate E3-*cat* expression in HeLa cells and was able to cooperate with *ras* in transforming baby rat kidney (BRK) cells. Taken together with earlier findings, these data suggest that amino acids following position 140 are not required for transformation, residues 140–187 are required for *trans*-activation, residues 86–120 are not essential for either the *trans*-activation or transformation functions, and residues 121–150 are important for both. This region is highly conserved between adenovirus serotypes, shows homology with the *myc* oncogene product, and includes a string of acidic amino acids between residues 133 and 138. We are studying this region in more detail through the use of oligonucleotide-directed mutagenesis to generate specific point mutations in conserved amino acids. Specific point mutations have been introduced at base pair 928 (to change the amino acid from cysteine to glycine at position 124), base pair 946 (proline to alanine at position 130), base pair 952 (serine to glycine at position 132), and base pair 961 (glutamic acid to lysine at position 135).

In addition, because the sequence Cys-X-X-Cys, which occurs twice within the 13S unique region (from amino acid positions 154–157 and 171–174), has been proposed to be an active site in some proteins, we have introduced a single base change at nucleotide 1072 to change the serine residue at position 172 to alanine. This mutation did not impair the *trans*-activation of E3-*cat* in HeLa cells or the ability to cooperate with *ras* in transformation of BRK cells, although changes at nearby amino acid positions 173 and 176 have been reported to impair the *trans*-activation function severely (Glenn and Ricciardi, *J. Virol.* 56: 66 [1985]).

Analysis of the amino acid changes in the region between residues 121 and 150 is in progress. Results

obtained so far indicate that the 946 and 952 mutations retain the *trans*-activation function. The 961 and 928 mutations also retain the *trans*-activation function but are impaired for transformation. Both of the latter have been transferred to 12S cDNA virus constructs to study the transformation defect in more detail. The mutant proteins are expressed at about the same levels as wild-type 12S protein; however, immortalization activity of the 12S/961 virus was about 40-fold less than that of wild-type 12S virus on primary BRK cells, whereas immortalization activity of the 12S/928 virus was at least 100-fold reduced. The EIA/928 mutation was also transferred to virus to analyze its growth properties in more detail. This virus formed plaques as efficiently on HeLa cells as on 293 cells, and the time course of infection was the same as that of wild-type virus. Thus, we have detected no impairment of the lytic functions in the EIA/928 virus, but the plasmid pEIA/928 was reduced more than 50-fold compared with wild-type in its ability to cooperate with *ras* to transform BRK cells. Since both the 12S and 13S plasmids were active in the transformation assay, this defect in the pEIA/928 plasmid suggests that the 13S product has lost its establishment activity while retaining *trans*-activation function. We are presently making a 13S/928 construct to test directly the implication that the early adenovirus gene *trans*-activation functions of the 13S product do not require an intact establishment activity.

To expand our study of *trans*-activation and to explore the mechanisms involved in regulating expression of early and late genes, we have placed the bacterial chloramphenicol acetyltransferase (*cat*) gene under the control of individual adenovirus promoters. The E2 and E3 constructs were already available from Weeks and Jones (*Mol. Cell. Biol.* 3: 1222 [1983]); we completed the early set (E1A, E1B, and E4) and generated constructs carrying the major late promoter (MLP) and other late promoters (IX, IVa₂, and E2L). The constructs are being checked by sequence analysis and assayed by transfection into a variety of mammalian cell lines. In HeLa cells, only the early promoters were active. With the exception of the E1A promoter itself, all of the early promoters were stimulated by cotransfection of the EIA gene or its derivative containing the 13S cDNA; the 12S cDNA repressed their expression, and the 9S cDNA either repressed slightly or was ineffective. In HeLa cells, the late promoters were not active either in the presence or in the absence of EIA products. In 293 cells, which express

both EIA and EIB products, the early promoters and the MLP were active, but the other late promoters (IX, IVa₂, and E2L) were inactive or active only to a small or variable extent. The EIA promoter itself was active in both cell lines and was repressed by the 12S and 9S products but not by the 13S product. These results indicate that the early promoters are autonomously active and are EIA-sensitive, whereas the late promoters apparently require additional factors (possibly including EIA but not limited to EIA) for their activity. The nature of these additional factors is currently under investigation.

None of the promoters tested to the present time is stimulated by the EIA 12S product, but it seems likely that this protein activates some host-cell products during transformation. Although we have not yet succeeded in cloning the cDNA for the proliferating cell nuclear antigen (PCNA), we have obtained preliminary evidence that this cell-cycle-linked protein may represent such a 12S-sensitive product. The synthesis of PCNA occurred at a very low level in uninfected BRK cells but increased rapidly after infection with 12S virus. PCNA synthesis was first detected at about the same time as EIA protein, and it also increased in cells infected with wild-type virus or 13S virus but not with the EIA deletion mutant *d/312*. We are presently extending our analysis to viruses mutant in the EIA gene to elucidate the relationship of this effect with transformation and *trans*-activation.

Ribonucleoprotein Particles and Autoimmunity

C.C. Bunn, L. Manche, M.B. Mathews

Stemming from our work on the cellular protein known as the La antigen, which complexes with the VA RNAs, we have become increasingly interested in problems of autoimmunity. In several diseases, antibodies are generated against "self" (the body's own constituents), and such autoantibodies may in turn contribute to the disease process. Antibodies against the La antigen are often found in diseases such as systemic lupus erythematosus and Sjögren's syndrome, whereas antibodies against tRNA-related proteins are common in muscle-wasting diseases such as polymyositis and dermatomyositis. During the past year, we have concentrated our attention

on the latter group of antibodies, which seem to pick out a subset of myositis patients who are prone to severe lung complications.

As discussed in last year's Annual Report, there are three well-characterized antibody specificities in this group, of which Jo-1, reacting with the charging enzyme histidyl-tRNA synthetase, is the most frequent. The PL-7 and PL-12 specificities, reacting with threonyl-tRNA and alanyl-tRNA synthetases, respectively, are less common, but through screening over 100 new sera, we now have over a dozen examples of these sera. Only one other seemingly distinct tRNA-related antibody specificity emerged from our screening, implying that there may perhaps be few such specificities awaiting discovery. It is intriguing to note that the aminoacyl-tRNA synthetases, which are known targets of autoimmune responses, are distinguished from most other synthetases in that they do not appear to participate in the large macromolecular synthetase complex that has been studied in several laboratories.

The PL-12 system differs from both the Jo-1 and PL-7 systems in that PL-12 antisera contain two distinct sets of antibodies, directed against the charging enzyme, alanyl-tRNA synthetase, and its cognate tRNA, tRNA^{Ala}. To understand in detail the molecular features of an antigen that excite an autoimmune response, we have begun to characterize the tRNAs recognized by PL-12 antibodies at the sequence level. To date, we have fractionated the mixture of tRNA species into three major families and have derived a considerable amount of sequence information. The sequence of two of the tRNA^{Ala} species is nearing completion, and we have begun to explore the antibody-reactive sites on the tRNA. We will compare sera from different patients to discover whether there is a unique antigenic epitope. To shed light on one of the theories of autoimmunity (the anti-idiotypic hypothesis), we will also determine the synthetase-binding site on the tRNA to see if the antibody and synthetase interact with the same site on the RNA molecule.

Human Stress Protein Genes

D.D. Pascucci, G.P. Thomas

Our work on the human stress response (heat-shock) genes has continued to focus on the genes

encoding two of these proteins, the 90K and 70K proteins. Last year we reported the characterization of a 90K gene isolated from a human DNA library in bacteriophage λ . The sequence of a 4.2-kb region was determined from the phage λ 2/59 and compared with published sequences of the whole of one of the yeast 90K genes and half of the *Drosophila* 90K gene. Considerable homology was detected throughout the entire coding regions, with little homology in the untranslated regions. However, we were not able to express polypeptides of approximately 90K from the putative coding regions, and further examination of the gels revealed a number of frameshifts in the "coding region," leading us to suspect that λ 2/59 contained a pseudogene. Comparison with the complete Genbank compendium of sequences revealed two regions of extremely good homology (\sim 95%) with the L1 repeat, the so-called *Kpn1* family of repeated sequences, and it became clear that this isolate represented a processed pseudogene embedded in an L1 repeat, with a 15-bp direct repeat at the site of insertion. Restriction fragments predicted from this clone are present in genomic blots, and thus the phage is unlikely to have undergone gross rearrangements.

Two other clones isolated from the same DNA library appeared identical with the λ 2/59 clone that was sequenced, and thus we resorted to two different DNA libraries in an effort to obtain the authentic gene. These libraries were screened with fragments subcloned from λ 2/59. Among the products was phage λ E9, which was distinct from the original clone by restriction mapping and lacked detectable interruptions of coding sequence. Upon sequence determination, this clone also proved to contain at least two frameshifts, and we are forced to conclude that it probably represents another processed pseudogene. This view is reinforced by the discovery of L1 sequences to the 5' side of the putative coding sequences. The presence of such repeats in close proximity to one end of the λ E9 sequence need not imply that it is embedded in such a repeat, since no L1 sequences have been found to the 3' side, at a distance that, in the pseudogene, would be expected to extend past the poly(A) tail of the 90K mRNA. However, expression studies have yet to be undertaken to determine whether this sequence can be expressed or whether the frameshifts are errors.

Both clones are nevertheless sufficiently close to the 90K mRNA to generate S1-protected hybrids

under very stringent conditions and to generate the same-sized bands in both S1 protection and Northern analyses, using RNA from normal or stressed cells. From this, we assume that the inducible 90K mRNA is practically indistinguishable from its normally expressed counterpart, since other than a mass increase, no difference is observed. It is also possible to derive probable amino acid sequences from these clones based on the most likely initiation and termination codons. These predicted sequences are approximately 95% identical, given that some assignments remain tentative because of the frameshifts. This homology is much greater than the homology of these clones with the yeast or *Drosophila* 90K sequences. In comparisons with the yeast sequence, it appears that the region of greatest divergence lies toward the carboxyl terminus, although even here amino acid homology approaches 70–75%. Thus, in common with the 70K gene family (see below), the stress proteins as a whole rank among the most highly conserved proteins throughout nature, even though as yet there is little indication of their real function under normal growth circumstances or under stress.

Protein studies suggest the existence of at least three families of approximately 70K polypeptides that may be related to one another. One way to resolve this issue is to isolate and characterize the cDNAs corresponding to the 70K proteins. We have completed the sequence of one such cDNA of about 1750 bp. It contains sequences from amino acid 100 to the carboxyl terminus of the protein and into the untranslated region, but it does not contain the poly(A) tail. With the exception of a single base difference, which causes a conserved substitution at residue 148, it is identical with the sequence published last year by Hunt and Morimoto (*Proc. Natl. Acad. Sci.* 82: 6455 [1985]).

Using this clone as a probe, we have detected two differently spliced transcripts in stressed cells. This observation suggests that there may be two genes encoding the 70K sequence that generate transcripts differing at their 3'-untranslated region; alternatively, the primary transcript of one gene is subject to differential processing at a site located near the end of the coding sequence. Screening the cDNA library with this clone as probe indeed led to the isolation of an additional cDNA clone, pDDP1. However, the mRNA product of this gene differs in size from mRNAs hybridizing to the original pDP8 clone. It is likely that pDDP1 corre-

sponds to another member of the 70K family that (unlike the original) is relatively uninducible and is expressed under normal circumstances.

In cultured rat cells, the 70K protein family is seemingly less heterogeneous than in HeLa cells and apparently contains one constitutive and one induced species. We have also isolated cDNA clones for the 70K family from a library from rat REF52 cells. The largest clone, REF19, contains sequences from residue 180 to the untranslated sequence at the 3' end and including some of the poly(A) tail. As judged from RNA analysis, this clone apparently corresponds to a major, induced species in rat cells. Curiously, the rat sequence exhibits better homology with the *Drosophila* gene than with the human pDP8 sequence. With this in mind, we examined interrelationships between the different 70K clones using a combination of Northern blots, S1 protection, and hybridization. It appears that the pDP8 gene, which corresponds to the major induced species in HeLa cells, is the odd man out; the uninducible human gene (pDDP1), the inducible rat gene (REF19), and the *Drosophila* 70K gene are more closely related to one another than to the major induced human gene (pDP8). The significance of this observation is not clear at the moment.

Progress has also been made toward understanding the mechanism of the unusual translational control that operates in stressed cells. Although preexisting normal mRNAs remain intact and functional and are associated with polysomes, they either are not utilized for protein synthesis or are used at a greatly reduced rate. We have isolated from the polysomes of stressed cells a novel population of mRNP particles that contain little or no normal mRNA but a good proportion of newly synthesized mRNA (largely stress-protein mRNAs). These particles are isolated from density gradients after fixation and contain no detectable amounts of the mRNAs for tropomyosin, myosin, actin, or tubulin. The particles are lighter than mRNA particles from polysomes of untreated cells, and they contain ribosomal RNA in addition to mRNA and considerable amounts of protein, but their composition remains to be fully defined.

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PROTEIN CHEMISTRY

C. Slaughter T. Fischer
C.R. Moomaw

The alkaline phosphatases (APs) are a family of cell-surface enzymes that have become very important in clinical chemistry for the diagnosis of certain cancers. Like other ectoenzymes (enzymes with active sites oriented toward the outside of the cell), the role of AP in the physiology of the normal cell is not understood, although the presence of AP in large amounts on epithelial cells with absorptive functions has suggested some direct or indirect role in a transport process yet to be identified. Several tissue-specific forms of AP have been described, and the existence of several differentially expressed AP genes has been inferred. Broadly, the family can be divided into two parts, the heat-stable APs, including placental, testicular, and intestinal APs, and the heat-labile APs, including bone, liver, and kidney APs. (The usefulness of the distinction between heat-stable and heat-labile APs is also supported by a variety of other biochemical and immunochemical studies.) A basis for oncologic testing is that "ectopic" APs similar to one or another of the normal heat-stable APs are frequently expressed in tumors arising from tissues that normally only express heat-labile APs in appreciable amounts. These ectopic APs are shed into the serum, where they can be detected by biochemical means. They have also been used as the basis for direct tumor-imaging studies using immunochemical methods. The genetic basis for normal tissue-specific control of these proteins, the mechanism for their ectopic expression, the structural relationship between ectopic and normal APs, the mechanism of shedding into the serum, and the normal function of these ectoenzymes are all questions predicated upon the detailed knowledge of the complexity of the AP gene family and the structures of the expressed proteins.

AMINOTERMINAL AMINO ACID SEQUENCES

Using monoclonal antibodies specific for placental, intestinal, and liver APs, we isolated small samples of APs from these organs by immunoaffinity chromatography. The proteins were subjected to aminoterminal amino acid sequence analysis, and the first 42 residues of each were identified. All

three sequences were different, confirming that different genes are involved in coding for all three forms of APs as previously inferred. Furthermore, the two relatively heat-stable APs, placental and intestinal, were more closely related to one another than either was to the heat-labile liver AP, reflecting the degree of immunochemical relatedness between these proteins. Comparison of the sequences with the known amino acid sequence of AP from *Escherichia coli* revealed little similarity in the first 35 residues, but the remainder showed homology with an aminoterminal region of *E. coli* AP that forms a β -sheet structure, at one end of which the active site of the enzyme is located. An aspartic acid residue believed to play a role in catalysis is present in the human and the bacterial sequence in this location. This suggests that mammalian and bacterial APs are related functionally and genetically. Two different molecular forms of APs are found in adult and fetal intestine, respectively. These differ in their sensitivity to neuraminidase, reflecting a difference in glycosylation, but were found to show the same aminoterminal amino acid sequences.

CLONING OF THE PLACENTAL AP GENE

Cyanogen bromide digestion and isolation of fragments from a sample of placental AP permitted the sequence analysis of a number of peptides from which oligonucleotide probes could be constructed. In collaboration with H. Harris at the University of Pennsylvania, these have been used to identify a clone containing the gene for human placental AP from a placental cDNA library. This has been analyzed to obtain the complete coding region sequence. A similar series of experiments on liver AP is presently under way.

LARGE-SCALE ISOLATION OF PLACENTAL AP

A convenient procedure for the large-scale isolation of placental AP has been worked out for use in crystallographic studies to be undertaken in collaboration with G. Wykoff at Yale University. The purification procedure involves butanol extraction,

anion exchange chromatography, hydrophobic interaction chromatography, and gel filtration.

MEMBRANE INSERTION OF AP

The structural basis for membrane insertion of AP, and hence the mechanism for its release into the serum, is being approached through studies of certain isozymes of AP found in tissue extracts. From all tissues, native gel electrophoresis resolves two sets of isozymes, one with rapid anodal mobility and the other with very slow anodal mobility. Two sets of isozymes have also been described by other investigators. These are believed to represent dimers and high-molecular-weight aggregates, respectively. We have determined that although the fast electrophoretic components we observe correspond to the simple dimeric species, the slow electrophoretic components have properties different from those of the aggregated form. The slow components therefore represent a third molecular form of the enzyme. This species behaves as though it were more hydrophobic than the fast components, but it has a similar apparent molecular weight. Our working hypothesis is that it may contain a chemical grouping of importance for membrane attachment. A method for large-scale purification of the slow components has therefore been developed for preparation of both slow and fast isozymes from the same placenta. The purified material is currently being used in comparative peptide-mapping studies in an effort to identify the structural differences and hence the mechanism for membrane attachment.

PLACENTAL AP POLYMORPHISM

Human placental AP is an extraordinarily polymorphic enzyme. By native gel electrophoresis, 3 common alleles and more than 20 rare alleles have been identified. By analogy with the major histocompatibility antigens (another family of polymorphic cell-surface glycoproteins that are coded by a multigene family), it is possible that this variation is in part the result of gene conversion events. Studies have therefore been initiated to determine the extent and origin of the allelic diversity; 260 placentas have been collected from a local hospital and their AP electrophoretic phenotypes have

been determined. Selected placentas have also been examined by hydrophobic interaction chromatography, and it has been found that some allelic types differ with respect to their hydrophobicity as well as their net surface charge. Taken together with the extensive antigenic differences revealed in previous studies using monoclonal antibodies, this result underscores the probably complex nature of the allelic differences. The AP from selected placentas will be purified for peptide-mapping studies which, along with sequence analysis of the peptides found to differ from type to type, should localize the areas subject to allelic structural variation.

STRUCTURAL ANALYSIS OF MINOR HEAT-STABLE AP

It has long been a goal of protein chemistry to develop the sensitivity of its methods to the point that the spots generated by two-dimensional gel electrophoresis can be analyzed directly for aminoterminal amino acid sequence. In this way, advantage would be taken of the tremendous discriminative power of two-dimensional gels for isolating pure proteins from very complex mixtures in a single step. Two recent technical developments appear to make this goal a practical possibility. The first is the commercial production of an on-line PTH amino acid analyzer for the Applied Biosystems Gas Phase Sequencer. This instrument allows the identification of phenylthiohydantoin (PTH) amino acids at the level of 1 pmole or less, approximately one order of magnitude improvement in sensitivity compared with this laboratory's present capabilities. The second development is the introduction of procedures for *trans*-blotting proteins on gels onto glass-fiber filter paper compatible with sequencing chemistry. This allows sequence analysis of proteins immobilized on such filters without further processing. Experiments in collaboration with R. Franza (Cell Biology Section) are now being undertaken in this laboratory to realize the goal of sequencing spots from two-dimensional gels using these developments. The first fruits of this effort will be the structural analysis of heat-stable APs expressed in very minor amounts in tissues that contain predominantly heat-labile AP. These minor AP species may be similar to, or identical with, the ectopic APs that are expressed when tumors arise in such tissues.

MOLECULAR GENETICS OF EUKARYOTIC CELLS

This section includes four laboratories that use molecular approaches to understanding aspects of the cellular and organismal biology of eukaryotes. The control of the expression of the α_{2u} globulins (Hormonal and Developmental Control of Gene Expression) is the focus of D. Kurtz's laboratory. 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 and also under complex developmental control. The control of cell growth (Genetics of Cell Proliferation) is the focus of M. Wigler's laboratory. Cellular oncogenes that can positively alter the proliferative capacity of cells are being characterized. A human oncogene encoding a product of novel structure has recently been discovered. Particular attention is given to the function of the *ras* oncogenes that are implicated 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. F. Tamano's laboratory (Biochemistry of Yeast *RAS* proteins) is characterizing the covalent modifications of *RAS* proteins and the role of such modifications in *RAS* function. Finally, 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. 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.

HORMONAL AND DEVELOPMENTAL CONTROL OF GENE EXPRESSION

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Our laboratory has been studying the hormonal control of gene expression in higher eukaryotes. The model system we have been using for the most part is the regulation of the expression of a rat gene family that codes for a protein called α_{2u} globulin. This protein is synthesized in several rat tissues under complex developmental and hormonal control. Sex steroids, glucocorticoids, growth hormone, and insulin all interact to regulate α_{2u} globulin synthesis *in vivo*.

DNA SEQUENCES REQUIRED FOR GLUCOCORTICOID INDUCTION OF α_{2u} GLOBULIN

α_{2u} globulin transcription can be induced by glucocorticoids. This response is "secondary" or indirect; i.e., protein synthesis is absolutely required for the hormone to exert its effect on α_{2u} transcription. This is in contrast to most of the other well-studied glucocorticoid-inducible systems (e.g., mouse mammary tumor virus [MMTV]) in which the response is primary. A primary response to ste-

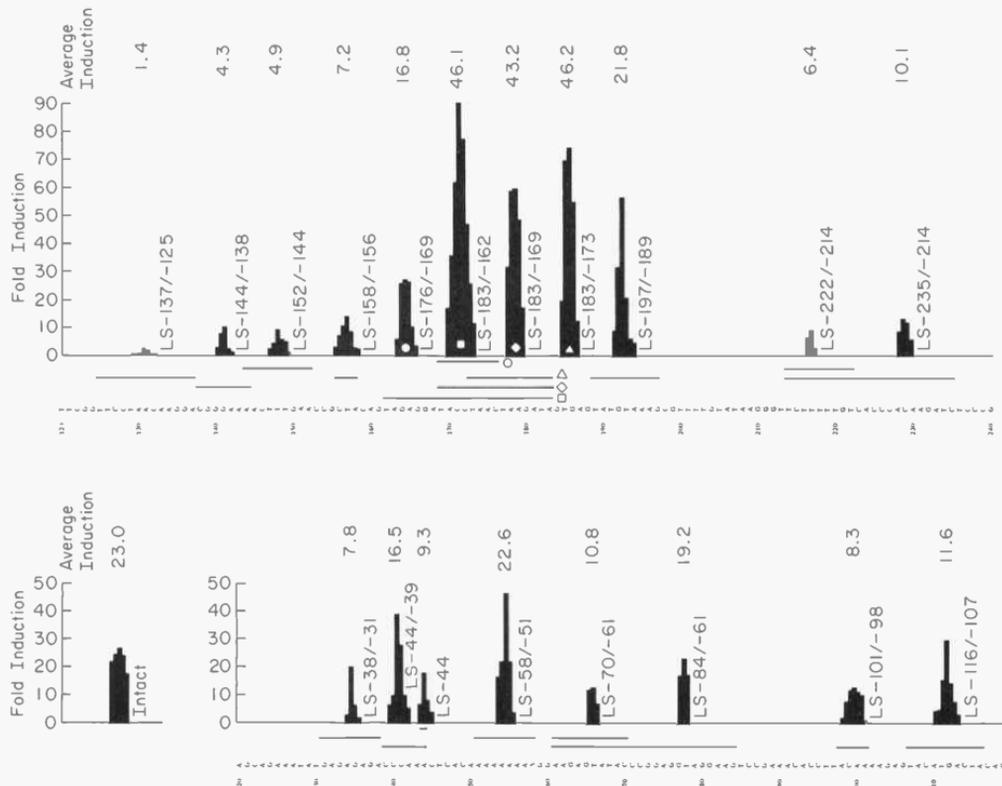


FIGURE 1 Effect of the linker-scanning mutations on α_{2b} globulin mRNA induction in L cells.

roid hormones is believed to occur by a mechanism in which the hormone binds to its receptor, resulting in its "activation." The activated receptor then binds to specific DNA sequences in or near a set of target genes and, by an unknown mechanism, induces transcription. Very little is known about the molecular mechanism of a secondary response to steroid hormones. The most reasonable model postulates that the steroid hormone induces a regulatory protein(s) that is required for the transcriptional activation of the secondary response genes.

We had previously shown that cloned α_{2u} globulin genes retain their responsiveness to glucocorticoids when transfected into mouse L cells in tissue culture. These studies showed that 235 bp of 5'-flanking DNA, relative to the α_{2u} transcription start site, were sufficient to maintain glucocorticoid inducibility. We have saturated this 235-bp region with clustered point mutations using the "linker-scanning" mutagenesis procedure. These mutant α_{2u} genes were then introduced into L cells, and their response to glucocorticoids was assayed (Fig. 1). Mutations between approximately -125 and -160 were found to reduce greatly or abolish α_{2u} induction by glucocorticoids, although they had no effect on basal transcription. One mutant, LS-137/125, was found to be virtually unresponsive to hormones, and several others more distal to this position showed greatly reduced induction. The phenotype of the mutant LS-137/-125, in which induction is completely abolished, indicates that the hormone-responsive region of the α_{2u} promoter is organized very differently from that of MMTV and several other primary response genes, which seem to contain multiple binding sites for the hormone receptor. Mutations in any one of these sites do not abolish induction but only slightly reduce it. The glucocorticoid response region of the α_{2u} promoter seems to be much more compact.

A series of mutations in the α_{2u} promoter further upstream of the glucocorticoid response region, between approximately -165 and -185, were found to increase induction two- to fourfold (Fig. 1) without affecting the basal level of expression. As yet, we have no explanation for this class of "hyperinducible" mutants, nor can we assess their biological relevance, if any.

In the glucocorticoid regulatory region of the α_{2u} promoter, at position -136 to -131, is the hexanucleotide -GGAACA-, which is found at a very similar position in the promoter of the rat α -1

acid glycoprotein gene, another glucocorticoid-inducible gene whose response, like α_{2u} , is secondary. It is obviously premature to attempt to define a "consensus sequence" for secondary hormonal response until more genes of this type have been characterized.

ALTERED REGULATION OF α_{2u} SYNTHESIS IN STRAIN NBR RATS

α_{2u} is synthesized in five tissues in rats: liver, submaxillary gland, lachrymal gland, preputial gland, and mammary gland. Different protein isoforms are produced in the various organs, the result of different α_{2u} globulin gene sets being active in the different tissues (Fig. 2). The mouse analog of α_{2u} , called MUP, is synthesized in these same tissues in mice (with the exception of the preputial gland). The isoforms of MUP that are synthesized in the livers of mice of different strains can vary greatly, and it has been shown that this variation is controlled by an unlinked regulatory locus termed *mupA*. We have screened several rat strains to look for possible differences in α_{2u} globulin synthesis or regulation. Most rat strains showed identical α_{2u} synthesis patterns in all tissues; however, strain NBR rats showed a marked alteration in α_{2u} synthesis: (1) The level of α_{2u} in the liver of NBR rats was approximately 100-fold lower than the level found in Sprague-Dawley (SD) rats (in which it represents 1-2% of total hepatic protein synthesis). (2) In prepubescent animals, the level of α_{2u} in the submaxillary gland of NBR rats is comparable to the level found in SD rats (~0.3% of protein synthesis). In SD rats, this level drops three- to fivefold

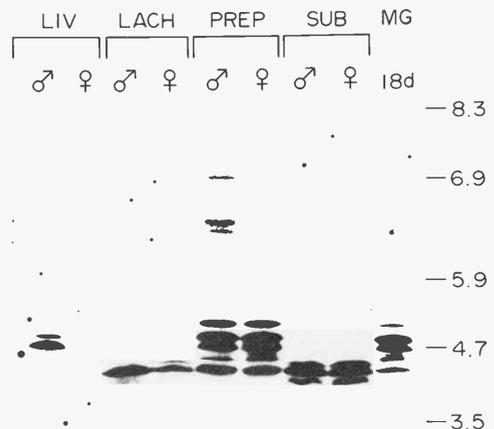


FIGURE 2 α_{2u} globulin protein isoforms produced in rat tissues.

at puberty, and this lower level of synthesis is maintained in adult animals. In NBR rats, α_{2u} synthesis in the submaxillary drops at puberty and becomes undetectable in adult animals. (3) The level of synthesis of α_{2u} in the lachrymal gland in NBR rats is comparable to the level found in SD; however, the protein isoform pattern is "reversed." That is, in SD rats, lachrymal gland α_{2u} is characterized by four isoforms: two major forms with pI values of 4.5 and 4.3 and two minor forms with pI values of 4.7 and 4.1 (Fig. 2). In NBR rats, the pI 4.1 isoform is predominant, and the 4.3 and 4.5 forms are minor species.

We performed crosses between NBR and SD rats and found that the α_{2u} synthesis phenotypes were codominant: Levels of α_{2u} globulin synthesis in the liver and adult submaxillary gland in F_1 animals were approximately 50% of the levels in SD rats, and all lachrymal gland isoforms were found at approximately equal levels in the F_1 animals. This indicates that the altered expression in NBR rats is probably not controlled by an unlinked regulatory locus such as *mupA*. Southern blot analysis of DNA from NBR rats showed that these animals possess the full complement of α_{2u} genes (20–25 copies per haploid genome). However, the α_{2u}

genes in NBR rats were found to be highly methylated compared to the genes with SD rats. In the F_1 animals, the level of methylation of the α_{2u} genes was intermediate, indicating an allelic maintenance of the methylation pattern. Further studies are necessary to determine whether hypermethylation is the cause of the altered expression of the α_{2u} globulin gene family in NBR animals.

In Press, Submitted, and In Preparation

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GENETICS OF CELL PROLIFERATION

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Our laboratory is concerned with the genetic and functional analysis of growth control in eukaryotic cells. We have stressed studies on the function of the *ras* oncogenes, a family of genes found to be activated in perhaps 10–20% of human cancers and which have been highly conserved in evolution. Homologs of the mammalian *ras* exist in the yeast *Saccharomyces cerevisiae* (*RAS1* and *RAS2*), and we have been studying the function of these genes in this species. We have shown that human *ras* proteins can complement the loss of *RAS1* and *RAS2* proteins in yeast and hence are functionally homol-

ogous. Both human and yeast *ras* proteins can stimulate the magnesium and guanine nucleotide-dependent adenylate cyclase activity present in yeast membranes, and hence we also have been studying the adenylate cyclase pathway of yeast. Our studies have led to the important conclusion that although *ras* is an essential controlling element of adenylate cyclase in yeast, it has other essential functions in that organism. Moreover, there may be multiple pathways controlling the growth of yeast that are independently regulated but can have overlapping functions. In this respect, growth control in

yeast may be fundamentally as complex as growth control in mammalian cells and may prove to be a useful model system for studying the latter.

In addition to our studies of yeast, we are continuing studies on oncogenes in mammals. We have developed a useful *in vivo* assay system for the human *ras* proteins: induction of maturation in microinjected frog oocytes. From these studies, we conclude that *ras* proteins do not stimulate the adenylate cyclase of frog oocytes but have powerful biological effects through another pathway. We are studying two other human oncogenes: the human *ros1* gene, which may be a growth-factor receptor, and the human *mas1* gene, which is a new oncogene we discovered that encodes a protein of novel structure.

Studies in *Saccharomyces cerevisiae*

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We have previously demonstrated the existence of two *ras* genes in the yeast *Saccharomyces cerevisiae*, *RAS1* and *RAS2*, which encode proteins that are structurally homologous to the mammalian *ras* proteins (Powers et al., *Cell* 36: 607 [1984]). The studies described below demonstrated that *ras* genes are essential for *S. cerevisiae*, that they are essential controlling elements of adenylate cyclase in *S. cerevisiae*, that the mammalian *ras* can perform this biochemical function, and that yeast *RAS* genes have at least one other essential function in *S. cerevisiae*. The latter result derives from two experimental sources: studies of suppressors of *ras* function and careful genetic analysis of the adenylate cyclase pathway in yeast. We have isolated and characterized several of the genes of the *ras*/adenylate cyclase pathway, some of still unknown function.

EARLY STUDIES OF YEAST *RAS* GENES

We and other investigators have previously shown that at least one functional *ras* gene is essential for the germination of haploid yeast spores (Kataoka et al., *Cell* 37: 437 [1984]; Tatchell et al., *Nature* 309: 523 [1984]). This was demonstrated by constructing

doubly heterozygous diploid yeast cells containing wild-type *RAS1* and *RAS2* alleles and *RAS1* and *RAS2* alleles each disrupted by a different auxotrophic marker. These diploid cells were then sporulated, and the resulting tetrads were analyzed. Only spores containing at least one functional *ras* gene could germinate. We then introduced into these doubly heterozygous diploid cells a *RAS2* gene under the transcriptional control of the galactose-inducible *GAL10* promoter (Kataoka et al., *Cell* 40: 19 [1985b]), linked to a third auxotrophic marker. These diploid cells were then sporulated, and tetrads germinated on either glucose-containing medium (YPD) or galactose-containing medium (YPGal). The results confirm that at least one functional *ras* gene is required for germination. *ras1⁻ ras2⁻ GAL10-RAS2* spores germinate only in the presence of galactose, the inducer of the *GAL10* promoter. Cells with the genotype *ras1⁻ ras2⁻ GAL10-RAS2* were grown in YPGal and then shifted to YPD. Within several cell generations, cell growth ceased and cells lost viability. These experiments indicated that the *ras* genes are needed not only for spore germination, but also for the continued growth and viability of yeast cells (Kataoka et al., *Cell* 40: 19 [1985b]).

Using the approach described above, we have been able to test if expression of the normal human *Ha-ras* protein is sufficient for viability in yeast cells lacking their own endogenous *ras* genes. To this end, we constructed a *GAL10-Ha-ras* transcription unit that utilized a full-length cDNA clone of the human *Ha-ras* mRNA under the control of the galactose-inducible *GAL10* promoter. This unit, closely linked to a *LEU2* marker, was inserted into diploid yeast cells that were doubly heterozygous for their endogenous *ras* genes. Cells were induced to sporulate, and tetrads were examined after germination on YPD or YPGal plates. Approximately 40% of spores with the genotype *ras1⁻ ras2⁻ GAL10-Ha-ras* were capable of germination when plated on YPGal, from which we conclude that the human *Ha-ras* protein can supply essential *ras* function to yeast.

Certain missense mutations drastically alter the biological activity of mammalian *ras* genes. In particular, the human *Ha-ras^{val12}* gene, which encodes valine instead of glycine at the 12th codon of the *Ha-ras* gene, can induce the tumorigenic transformation of NIH-3T3 cells. To test the consequences of a similar mutation of *RAS2* on the properties of

yeast cells, we constructed the *RAS2^{val19}* gene using site-directed mutagenesis (Kataoka et al., *Cell* 37: 437 [1984]). *RAS2^{val19}* thus encodes valine instead of glycine at the 19th codon, which corresponds to the 12th codon of the mammalian Ha-*ras* gene. We then examined the effects of introducing this gene into yeast cells. Our first observation was that diploid cells containing *RAS2^{val19}* could not be induced to sporulate by incubation under conditions of nutritional deprivation (Kataoka et al., *Cell* 37: 437 [1984]). Next, we observed that haploid cells carrying *RAS2^{val19}* lost viability if starved for nitrogen, sulfur, or phosphorus and failed to arrest in G₁ under those conditions (Toda et al., *Cell* 40: 27 [1985]). Wild-type cells normally arrest in G₁ and retain viability for long periods if nutritionally deprived. In addition to these phenotypes, we also noted that cells carrying *RAS2^{val19}* failed to accumulate carbohydrate stores as cells entered the stationary growth phase. Thus, in general, *RAS2^{val19}* cells appeared to show a defective response to nutritional stress (Toda et al., *Cell* 40: 27 [1985]).

The cluster of cellular properties just described closely resembles the phenotype of cells carrying the *bcy1* mutation (Matsumoto et al., *Proc. Natl. Acad. Sci.* 79: 2355 [1982], *Cell* 32: 417 [1983], *Exp. Cell Res.* 146: 151 [1983]; Uno et al., *J. Biol. Chem.* 257: 14110 [1982]). The *bcy1* mutation was first isolated by Matsumoto and co-workers as a mutation that suppresses the growth defect that otherwise results from the disruption of adenylate cyclase (Matsumoto et al., *Proc. Natl. Acad. Sci.* 79: 2355 [1982]). Cells carrying *bcy1* appear to lack the regulatory subunit of the cAMP-dependent protein kinase and hence have lost the requirement for cAMP (Uno et al., *J. Biol. Chem.* 257: 14110 [1982]). These observations suggested that the *ras* genes might be participating in the cAMP pathway. In support of this idea, we found that *bcy1* suppressed the lethality that otherwise results from disruption of both *ras* genes.

The adenylate cyclase activity of the yeast *S. cerevisiae* is stimulated by guanine nucleotides in the presence of magnesium (Casperson et al., *J. Biol. Chem.* 258: 791 [1983]). In this respect, yeast adenylate cyclase resembles the adenylate cyclase of mammalian cells, which can be stimulated by a guanine-nucleotide-binding complex called G_i (Gilman, *Cell* 36: 577 [1984]). Since the yeast *ras* proteins also bind guanine nucleotides (Tamanai et al., *Proc. Natl. Acad. Sci.* 81: 6924 [1984]), we reasoned that they might also modulate adenylate cyclase.

This was tested directly by the assay of membranes from wild-type yeast cells and *ras1⁻ ras2⁻* cells. Membranes from wild-type or *ras1⁻ ras2⁻* strains contained appreciable adenylate cyclase activity when assayed in the presence of manganese ion, but *ras1⁻ ras2⁻* membranes displayed negligible levels of activity when assayed in the presence of magnesium and a nonhydrolyzable guanine nucleotide analog (Toda et al., *Cell* 40: 27 [1985]). These results were confirmed in a striking manner by membrane-mixing experiments. We prepared membranes from *RAS1 RAS2* yeast carrying the *cyr1-1* mutation and membranes from *ras1⁻ ras2⁻* yeast. The *cyr1-1* mutation disrupts the catalytic subunit of adenylate cyclase. Membranes from these two sources were assayed separately and together after membrane mixing and fusion. The data indicate that membrane mixing and fusion regenerate a guanine-nucleotide-stimulated adenylate cyclase activity (Toda et al., *Cell* 40: 27 [1985]).

These studies suggested that it would be possible to develop an in vitro assay for *ras* protein function. The addition of purified yeast *RAS2* protein to membranes from *bcy1 ras1⁻ ras2⁻* cells indeed induces adenylate cyclase activity to about 50-fold above background level (Broek et al., *Cell* 41: 763 [1985]). Adenylate cyclase activity in the *bcy1 ras1⁻ ras2⁻* membranes can also be increased dramatically by addition of yeast *RAS1* protein. Restoration of adenylate cyclase activity by *ras* proteins is dependent on the presence of guanine nucleotide, with the nonhydrolyzable GTP analog, GppNp, yielding twice the activity of that observed in the presence of GDP. Incubation of the *RAS2* protein with GTP prior to mixing with the *bcy1 ras1⁻ ras2⁻* membranes results in adenylate cyclase activity comparable to that restored with *RAS2* bound to GDP. In contrast, preincubation of *RAS2^{val19}* with GTP results in activation of adenylate cyclase to levels identical with those induced by *RAS2* proteins bound to GppNp. These results confirm our genetic studies and indicate that there are proteins in membranes which can distinguish between *ras* proteins complexed with GTP and *ras* proteins complexed with GDP; furthermore, they provide for the first time an in vitro bioassay for the effector function of *ras* (Broek et al., *Cell* 41: 763 [1985]).

Using this approach, we tested whether the human *ras* protein could stimulate yeast adenylate cyclase. For this purpose, we used a bacterial expression system as our source of human Ha-*ras* protein (Gross et al., *Mol. Cell. Biol.* 5: 1015 [1985]). Adeny-

late cyclase activity in *bcy1 ras1⁻ ras2⁻* membranes was dramatically increased by the addition of purified human Ha-*ras* protein. These results indicate that the effector domain of yeast and mammalian *ras* proteins has been conserved in evolution (Broek et al., *Cell* 41: 763 [1985]). In particular, these results urged us to test whether *ras* proteins may be involved in regulating adenylate cyclase in vertebrates. However, these experiments, conducted with *ras*-injected frog oocytes, indicated that *ras* was not acting upon adenylate cyclase but upon some other effector system (Birchmeier et al., *Cell* 43: 615 [1985]; and see below).

The results of our studies in yeast and frog oocytes have left us with an apparent paradox: *ras* stimulates adenylate cyclase in one but not in the other. Several models are consistent with these results. In the first model, *ras* proteins interact directly with a domain of yeast adenylate cyclase. In the course of evolution, the interaction with this domain has been conserved, but the function of adenylate cyclase has changed. In a second model, *ras* interacts directly with another component (X) that directly or indirectly interacts with adenylate cyclase in yeast. In the course of evolution, the interaction of *ras* with X has been conserved, but X interacts with a different effector system in vertebrates. In a third model, *ras* interacts directly or indirectly through more than one effector system, and in evolution, one or more of these interactions have been conserved and/or are operant in different cell types. To decide among these possibilities, we have initiated more detailed studies in yeast. The first major approach has been a detailed genetic analysis of the adenylate cyclase pathway (described in the next section). The second major approach has been an examination of *ras* function by "suppressor" analysis (described in the section following the next).

THE YEAST ADENYLATE CYCLASE PATHWAY

Adenylate Cyclase (CYR1). Mutants in the adenylate cyclase locus, *CYR1*, were obtained from K. Matsumoto. The wild-type gene for *CYR1* was cloned by complementation screening and then sequenced. It contains an open reading frame of greater than 6000 bp, which can encode a protein of 2026 amino acids (Kataoka et al., *Cell* 43: 493 [1985a]). Northern analysis indicates that the entire open reading frame is used. The structure of this large protein is interesting. Centrally located is a 23-

amino-acid unit repeated 25 times. The consensus sequence of this repeat is leucine-rich, and, amazingly, a protein with a similar consensus sequence and periodic structure is found in human serum. Deletion analysis indicates that the catalytic activity is located in the carboxyterminal portion of the molecule, and the aminoterminal portion contains the *ras* responsive domain.

High-level expression of the catalytic portion of adenylate cyclase in yeast is sufficient to suppress the lethality that results from loss of *ras* function. Thus, if *ras* has other essential functions in yeast besides stimulating adenylating cyclase, these functions can be compensated by overexpression of the cAMP effector pathway. Biochemical analysis indicates that only one gene for adenylate cyclase must exist in yeast, since cells containing deletions of this gene do not produce cAMP. Surprisingly, however, complete deletions of the *CYR1* gene are not lethal. By tetrad analysis, approximately half of the spores without cyclase can germinate and give rise to very slow growing colonies (T. Toda et al., in prep.). We estimate that the doubling time of such cells is initially approximately 24 hours, which is in marked contrast to spores lacking *ras* function. These rarely germinate and, if they do, cease division soon after. We conclude from this that *ras* is likely to have a second function essential for yeast cells besides the stimulation of adenylate cyclase. Another line of experiments is consistent with this idea. Cell strains lacking the adenylate cyclase gene have been grown and transformed with a high-copy extrachromosomal plasmid containing the *RAS2^{val19}* gene. Such strains now evince a heat-shock phenotype and sensitivity to starvation.

The Regulatory Subunit of the cAMP-dependent Protein Kinase (BCY1). *bcy1* cells were first obtained as bypass mutants of cells with defective adenylate cyclase (Uno et al., *J. Biol. Chem.* 257: 14110 [1982]). Biochemically, *bcy1* cells appear to lack the regulatory subunit of the cAMP-dependent protein kinase. Phenotypically, *bcy1* cells, like *RAS2^{val19}* cells, are sensitive to nitrogen starvation and heat shock, presumably because they cannot arrest in G₁. *BCY1* was cloned from a centromere-linked library (constructed and generously supplied by M. Rose, Massachusetts Institute of Technology) by transforming *bcy1* cells with the library and selecting cells capable of resistance to nitrogen starvation and heat shock. We obtained one clone that displays all of the genetic features expected of a

BCY1 clone. Gene disruptions of *BCY1* were constructed and used to transform haploid yeast cells by gene replacement. The transformants display a phenotype similar to that of cells containing the spontaneously occurring *bcy1* mutation. In addition to the heat-shock phenotype, such cells do not grow at all on nonfermentable carbon sources or on galactose and grow poorly on glucose.

Sequence analysis confirms that *BCY1* encodes the regulatory subunit of the cAMP-dependent protein kinase. The primary amino acid sequence of the encoded protein is virtually identical with the primary amino acid sequence of the protein purified by E. Krebs and co-workers, which has been demonstrated to be the cAMP-dependent protein kinase of yeast. Moreover, M. Zoller (Genetics Section) has demonstrated that the *BCY1* protein expressed in *E. coli* can act as a cAMP regulatory subunit when combined with the bovine heart catalytic subunit of the cAMP-dependent protein kinase.

Catalytic Subunits of the cAMP-dependent Protein Kinase (TPK1, 2, and 3). While cloning genes that in high copy were able to suppress a temperature-sensitive, cell-division-cycle mutant called *cdc25*, we found a gene with the potential to encode a protein with great sequence similarity to the bovine heart cAMP-dependent protein kinase catalytic subunit. Originally called *CAK1*, in last year's Annual Report, we renamed this gene *TPK1*. Hybridization analysis indicated that *TPK1* was homologous to two related genes that we cloned and called *TPK2* and *TPK3*. Sequence analysis indicated that the three encode proteins that are nearly 90% homologous to each other and show greater than 50% homology with the bovine cAMP protein kinase. Biochemical analysis indicated that *TPK1* indeed encodes a catalytic subunit of the yeast cAMP-dependent protein kinase. *TPK2* and *TPK3* presumably also encode catalytic subunits, although we have not yet proved this. Consistent with this idea, when any of the three genes are present in high copy, they suppress the growth defect that otherwise results from disruption of the gene for adenylate cyclase.

The three *TPK* genes form a complementary triplet. Disruption of any pair has no readily discernible phenotype. Disruption of all three leads to cells that grow very slowly, with a doubling time that we estimate to be 24 hours. This phenotype is consistent with the phenotype of very slow growth

that results from the disruption of the adenylate cyclase gene and differs from the phenotype of *ras*-disrupted cells that cannot grow at all. These results also suggest that *ras* has more effects on yeast than are mediated through the cAMP effector pathway.

High-affinity cAMP Phosphodiesterase (PDE2). A gene encoding a high-affinity cAMP phosphodiesterase, *PDE2*, was cloned by searching for genes that, in high copy, can reverse the heat-shock phenotypes of cells containing the *RAS2^{val19}* gene (see below). Sequence analysis of this gene showed that it encoded a protein with an amino acid composition similar to that of the high-affinity cAMP phosphodiesterase of bakers' yeast. Biochemical analysis of yeast cells containing disruptions of *PDE1* or expressing high levels of *PDE1* confirmed this conclusion. Comparison of the sequence of yeast *PDE1* protein with unpublished amino acid sequences of *Drosophila* and bovine cAMP phosphodiesterases indicated that there is a conserved 12-amino-acid sequence in all three.

SUPPRESSORS OF RAS FUNCTION IN *S. CEREVISIAE*

One fruitful approach to the genetics of *ras* is to examine genes that function either to bypass or to diminish *ras* function. Two different approaches can be taken. First, since *ras* genes are essential for growth in yeast cells, we can look for genes or mutations that relieve this requirement. Second, since yeast strains containing the *RAS2^{val19}* mutation are heat-shock-sensitive, we can look for genes or mutations that restore normal heat-shock sensitivity in such strains.

Genes That Do and Genes That Do Not Suppress Effects of the Loss of ras Function. We previously demonstrated that the loss of the functional *BCY1* gene, encoding the regulatory subunit of the cAMP-dependent protein kinase, renders cells resistant to the loss of adenylate cyclase or *ras* function. This result initially suggested that all the essential effects of *ras* are mediated through the adenylate cyclase system, a conclusion that we now know is wrong. To pursue this observation further, we conducted studies with the individual *TPK* genes, by transforming yeast cells with these genes on high-copy extrachromosomal replicating plasmids. We then examined the ability of such cells to lose adenylate cyclase or *ras* function. Although cells containing any of the *TPK* genes in high copy could grow read-

ily when they lose adenylate cyclase, the same is not true when they lose *ras*. These results suggested that, in addition to stimulating adenylate cyclase, *ras* proteins have other essential functions in yeast. We have since isolated two other genes (which we have provisionally named *SCH1* and *SCH9*) that can partially suppress the growth retardation that results from the loss of adenylate cyclase, but they do not suppress the lethality resulting from the loss of *ras*. These genes were obtained initially as suppressors of *cdc25* (see below).

Genes and Mutations That Suppress the RAS2^{val19} Phenotype. Strains containing the RAS2^{val19} mutation are sensitive to heat shock. We therefore sought genes in high-copy yeast libraries that could suppress the heat-shock phenotype of RAS2^{val19} strains. Two were found. One encoded the high-affinity cAMP phosphodiesterase, *PDE2*, which was described in a previous section. The other, which has been provisionally called *JUNI*, is of unknown function. *JUNI* has been sequenced but shows no homology with phosphodiesterases or other genes of known function. It encodes a 45,000-dalton protein, with two homologous domains of 10,000 daltons at its carboxyl terminus.

We also sought "suppressor" mutations in other genes that would render these cells heat-shock-resistant. Mutations in the RAS2^{val19} gene and mutations in the *CYR1* gene were encountered along with many others. One suppressor, *supC*, has been cloned and shown to be distinct from previously identified genes. Another of these suppressor mutations, called *supH*, is of particular interest. Cells with *supH* were found to be temperature-sensitive. Moreover, they were a-specific steriles. In a collaboration with the Laboratory, I. Herskowitz and co-workers (University of California at San Francisco) confirmed that *supH* was an a-specific-sterile and furthermore that it failed to make biologically active a-factor, although a-factor mRNA was made. Meantime, we showed that *supH* cells made RAS2 proteins, but the adenylate cyclase of such cells behaved as though they lacked *ras* protein. It therefore seems likely that *supH* encodes a protein needed for processing both *ras* and a-factor. Sequence comparisons show that the a-factor gene encodes a protein with the terminal sequence Cys-A-A-X, where A is any aliphatic amino acid and X is the terminal amino acid. This site is probably the site for fatty acid acylation and is required for the proper func-

tioning of *ras* proteins. It is therefore possible that *supH* encodes the enzyme that performs this acylation.

SUPH has been cloned and is now being characterized. The genetic properties of *supH* are themselves of great interest. Although high-copy plasmids expressing RAS2 will suppress the temperature-sensitive defect of *supH*, high-copy plasmids of *TPK1* will not. Since high-copy plasmids of *TPK1* will suppress the growth defect of *ras*⁻ cells in an otherwise wild-type background, *supH* must cause defects in yet other pathways not controlled by *ras* but which nevertheless interact with *ras*-controlled pathways. From our studies, we infer that there are at least three essential pathways controlling growth in *S. cerevisiae*. The first, the cAMP pathway, is regulated by *ras* proteins. A second, which we are provisionally calling the "ras alternative" pathway, is also controlled by *ras*. It is clear that under certain conditions, the first pathway can subsume the essential functions of the second. Genetic data, not presented here, suggest that these two pathways may coordinately control certain cellular phenotypes. A third pathway is not regulated by *ras* but must cooperate with the *ras* alternate pathway in some manner. All three pathways are dependent on a functional *SUPH* gene.

Since *supH* is likely to cause defects in the modification of proteins with the Cys-A-A-X terminal sequence, some proteins of that class are likely to be regulatory proteins in yeast. *supH* may provide a genetic and biochemical entree to such proteins. Moreover, we have not excluded the possibility that *SUPH* itself encodes a regulatory protein, controlling the activity of *ras* and other proteins.

OTHER RELATED STUDIES

In addition to the above studies, which have mainly centered on the *ras*/adenylate cyclase pathways, our laboratory has been studying the cell-division-cycle mutant *cdc25* (in collaboration with M. Zoller and L. Levin. *cdc35* (which is allelic to *CYR1*) and *cdc25* are the two temperature-sensitive mutants that comprise the first group of start mutants, i.e., mutants that arrest in G₁ prior to the sex factor G₁ arrest point. We began by isolating genes that in high copy could suppress the growth arrest of *cdc25*. Two genes were initially isolated. The *TPK1* gene, formerly called *CAK1*, was one. It turned out to be one of the cAMP-dependent protein kinase catalytic subunits and has been described in a previous

section. *TPK1* is not allelic to *cdc25*. The second suppressor gene was shown to be allelic and complementary to *cdc25* and was therefore the *CDC25* gene itself. The nucleotide sequence of *CDC25*, completed by L. Levin, indicates a potential to encode a large protein of 185,000 daltons, with no similarity to other known proteins.

We have investigated genes that in high copy suppress the *cdc25* temperature-sensitive allele or more severely disrupted *CDC25* alleles. Most genes of the known *ras*/adenylate cyclase pathway appear to be capable of suppressing both: high-copy *RAS2^{val19}*, *CYR1*, *TPK1*, *TPK2*, and *TPK3*. High-copy *RAS2* wild type will suppress the *cdc25* temperature-sensitive allele but not the more severely disrupted alleles of *CDC25*. In addition, we have isolated two new genes, *SCH1* and *SCH9*, by screening high-copy libraries that suppress the temperature-sensitive allele. These genes do not suppress the more severely disrupted alleles. More will be said about these genes below.

These data suggest that the *CDC25* pathway and adenylate cyclase overlap in some manner. One possibility is that *CDC25* is a component of the adenylate cyclase pathway. However, biochemical experiments indicate that cells with the *CDC25* disruption have normal adenylate cyclase. These results argue, but do not prove, that the *CDC25* pathway and the *CYR1* pathway are distinct and that high-level expression of the cAMP effector pathway can suppress the need for the *CDC25* pathway, perhaps in a manner analogous to its suppression of the need for the *ras* alternate pathway. Thus, we should seriously consider whether *CDC25* is part of the *ras* alternate pathway.

The genes *SCH1* and *SCH9*, initially isolated because they suppressed the temperature-sensitive defect of *cdc25* cells, are each of considerable interest. Both genes can suppress the growth defects of cells that lack adenylate cyclase. Therefore, they may be parts of pathways with the capacity to assume the functions of the cAMP effector pathway. The striking picture that emerges is that there are multiple pathways in *S. cerevisiae* with potentially overlapping domains of command.

CONCLUSIONS

Table 1 summarizes the genes we have cloned and characterized that interact with the yeast *ras*/cyclase pathways. The overwhelming impression from

TABLE 1 Genes of *S. cerevisiae* That Interact with the *ras*/cyclase Pathway

Gene	Identify or identifying properties
<i>RAS1</i>	activates adenylate cyclase
<i>RAS2</i>	activates adenylate cyclase
<i>CYR1</i>	adenylate cyclase
<i>BCY1</i>	regulatory subunit of cAMP-dependent protein kinase
<i>TPK1</i>	catalytic subunit of cAMP-dependent protein kinase
<i>TPK2</i>	catalytic subunit of cAMP-dependent protein kinase
<i>TPK3</i>	catalytic subunit of cAMP-dependent protein kinase
<i>PDE1</i>	high-affinity cAMP phosphodiesterase
<i>JUN1</i>	suppressor of the <i>RAS2^{val19}</i> phenotype
<i>SUPH</i>	involved in functional modification of <i>ras</i> proteins and α -factor
<i>CDC25</i>	mutants in <i>CDC25</i> are suppressed by genes of the <i>ras</i> /cAMP effector pathway
<i>SCH1</i>	suppressor of <i>cdc25</i> and <i>cyr1</i>
<i>SCH9</i>	suppressor of <i>cdc25</i> and <i>cyr1</i>

our studies is that control of growth in *S. cerevisiae* is complex. There are probably multiple regulatory pathways that in some cases not only can act coordinately, but also can compensate for each other. The complexity of growth control in *S. cerevisiae* will probably rival the complexity now being seen in animal cells and may provide a good model for the latter. What is the need for this complexity? One simple answer is possible. The growth phase of any cell requires the production of cellular constituents in proper proportions and at rates compatible with available nutrients. Some of this control can of course be accomplished by "short" feedback loops. For example, attaining the proper proportion of membrane phospholipids could easily be regulated by such short feedback loops. But how are overall rates of membrane synthesis determined and coordinated, for example, with rates of protein synthesis? Surely, these larger lines of production must be as closely regulated as, say, the decision to begin DNA replication. The growth effector pathways may therefore control such lines of production and be integrated by feedback control from critical substrates of these production lines. Perturbations of any one pathway, for example, by mutating or overexpressing critical controlling proteins, may therefore perturb the level of activity of the other pathways.

Mammalian Oncogenes

M. Wigler, O. Fasano, C. Birchmeier, D. Broek, D. Young, J. Nikawa, G. Colger, S. Efrat, D. Birnbaum, L. Edlund, G. Waitches

We have maintained our interest in the growth control of mammalian cells by studying mammalian oncogenes. Our studies on mammalian *ras* have taken clues from the study of *ras* in yeast. We have developed a sensitive in vivo bioassay for *ras* proteins: the induction of frog oocyte maturation. We have shown that *ras* proteins probably do not effect adenylate cyclase in the frog oocyte but rather exert strong effects through other pathways. In addition, we are studying two human oncogenes we have recently isolated: the *ros1* and *mas1* 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 (C. Birchmeier et al., submitted; D. Young et al., submitted).

MAMMALIAN *ras* GENES

Our studies of *Saccharomyces cerevisiae* prompted us to test whether *ras* proteins can effect adenylate cyclase in vertebrate cell systems. For this purpose, we used a frog oocyte microinjection system. This system was chosen because it was known that frog oocyte maturation is dependent on the activity of its cAMP effector pathway and because it is possible to perform biochemical experiments on small numbers of microinjected eggs. We have found that purified mammalian Ha-*ras* proteins, prepared from an *Escherichia coli* expression system (Gross et al., *Mol. Cell. Biol.* 5: 1015 [1985]) can induce immature oocytes to progress from prophase to metaphase (Birchmeier et al., *Cell* 43: 615 [1985]). The Ha-*ras*^{val12} is perhaps 100-fold more potent than the normal Ha-*ras*^{gly12} in inducing oocyte maturation. We have observed no significant changes in cAMP production in oocytes due to injection of mutant Ha-*ras* protein. Moreover, the *ras*-induced effects are only partly blocked by cholera toxin, an agent that totally blocks the maturation effects of progesterone by raising cAMP production. We con-

clude from this that the yeast *RAS* responsive systems are not necessarily functionally analogous to the vertebrate *ras* responsive systems. Nevertheless, it is clear that *ras*-induced frog oocyte maturation provides an excellent model system for testing ideas of *ras* protein function.

THE HUMAN *ros1* GENE

Last year, we described the isolation of two new human oncogenes called *mcf2* and *mcf3*, which were obtained using the DNA cotransfer and tumorigenicity assay (Fasano et al., *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 viral *v-ros* gene, which we have called *ros1*. It is likely that this rearrangement occurred during or after DNA transfer and was responsible for activating the oncogenic potential of *ros1*. This gene, like its *v-ros* counterpart, contains a transmembrane domain and a carboxyterminal 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 *ros1* gene, like the normal counterpart of the viral *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 *ros1* gene is expressed in a high proportion of human tumor cell lines and hence might be a useful tumor cell marker.

THE HUMAN *mas1* GENE

We have begun the characterization of another human oncogene that we have called *mas1*. This gene was isolated using the same methodology used for isolating the *mcf3* gene described above (D. Young et al., submitted). 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 *mas1* was activated in the tumor cells from which it ultimately derived. Unlike *mcf3*, the transforming *mas1* 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 *mas1* gene, cloned from a human placental cosmid library, is only weakly transforming.

Two aspects of the *mas1* gene make it of considerable interest as an oncogene. First, NIH-3T3 cells transformed with *mas1* are highly tumorigenic and grow to high saturation densities but appear to be morphologically indistinguishable from normal NIH-3T3 cells. Second, the protein encoded by *mas1* is very hydrophobic. In its hydropathy profile, it closely resembles the visual rhodopsins and is predicted to have seven transmembrane domains. In this respect, it is novel among cellular oncogenes. Our preferred hypothesis is that the *mas1* protein, like rhodopsin, is a signal-transducing receptor that activates a GTP-binding protein. An understanding of the mechanism whereby *mas1* transforms cells is likely to lead to a new insight into growth control mechanisms.

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BIOCHEMISTRY OF YEAST RAS PROTEINS

F. Tamanoi A. Fujiyama
 M. Rao
 N. Samiy

As indicated in last year's Annual Report, our interest is in characterizing the *RAS* proteins of the yeast *Saccharomyces cerevisiae*. During the past year, we have focused increasingly on events taking place within yeast cells. We have elucidated the pathway for the biosynthesis of the *RAS* proteins and have shown that the mature proteins are localized in plasma membranes.

MODIFICATION OF RAS PROTEINS

The indication that the *RAS* proteins are modified within yeast cells was obtained when we compared

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In Press, Submitted, and In Preparation

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the mobility in SDS-polyacrylamide gels of the proteins isolated from yeast cells with the mobility of the proteins produced in *Escherichia coli*. The proteins produced in yeast migrated slightly faster than the proteins produced in *E. coli*. The apparent molecular weights of *RAS1* proteins made in yeast and in *E. coli* were 36,000 and 37,000, respectively; likewise, the apparent molecular weights of *RAS2* proteins made in yeast and in *E. coli* were 40,000 and 41,000, respectively. In addition, *RAS* proteins synthesized in a rabbit reticulocyte cell-free translation system exhibited the same mobility as the proteins produced in *E. coli*.

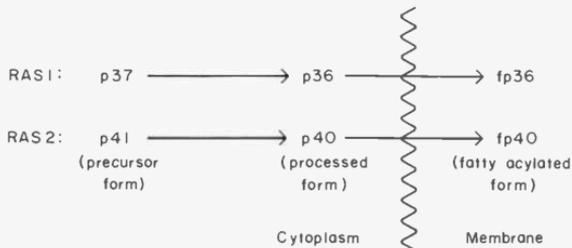


FIGURE 1 Proposed pathway for the biosynthesis of yeast *RAS* proteins.

The slow-migrating forms were found to be precursor forms. By very brief labeling with [³⁵S]methionine and fractionation into soluble and crude membrane fractions, we were able to detect the slow-migrating forms within yeast cells. They were present exclusively in the soluble fractions. The fast-migrating forms, on the other hand, were present in the crude membrane fractions as well as in the soluble fractions. Chase with cold methionine established that the slow-migrating forms were converted to the fast-migrating forms and that the fast-migrating forms accumulated in the crude membrane fractions.

Even though the fast-migrating forms were present in both crude membrane and soluble fractions, there was a physical difference between those in crude membranes and those in soluble fractions. The proteins present in crude membranes contained fatty acids as revealed by [³H]palmitic acid labeling. The label could be released from the protein by hydrolysis with methanolic KOH, indicating an alkali-labile ester bond between the fatty acid and the protein. We identified in the released material palmitic acid as well as myristic acid and lauric acid.

Thus, we have established that the yeast *RAS* proteins are first produced as precursor forms that are converted to fast-migrating forms and that they are further modified by fatty acylation. Our findings demonstrate that modification in addition to the fatty acylation is occurring on the *RAS* proteins. We are currently analyzing the exact nature of these modification events.

SUBCELLULAR LOCALIZATION OF *RAS* PROTEINS

To characterize further the *RAS* proteins within yeast cells, we have begun to analyze their subcellu-

lar localization. Our first attempt involved biochemical fractionation. Yeast cells were broken and then fractionated into soluble and crude membrane fractions by differential centrifugation. Crude membrane fractions were further separated into ER, plasma, and mitochondrial membranes by sucrose density gradient. We have shown that the majority of the *RAS* proteins migrate with the plasma membrane fraction. It is possible that a minor portion of the *RAS* proteins are also localized in other membranes. We intend to investigate further the localization of the *RAS* proteins by carrying out immunofluorescence of yeast cells. On the basis of these results, we have proposed the pathway for the biosynthesis of yeast *RAS* proteins (Fig. 1).

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MOLECULAR BIOLOGY OF MOUSE DEVELOPMENT

D. Hanahan S. Alpert J. Hager
 V. Bautch S. Grant
 T. Ashley M. Lacey
 S. Efrat S. Toda

The work of this group is focused on studies of the control of gene regulation and the consequences of oncogene expression in transgenic mice. Currently, our efforts are centered on targeted oncogenesis in pancreatic β cells. The 5'-flanking region of the rat insulin gene contains the major determinants of tissue specificity and regulation, given its observed ability to direct the expression of linked protein-coding information to β cells following the transfer of the recombinant genes into the mouse germ line, which is accomplished by DNA microinjection into fertilized one-cell mouse embryos. We are examining the regulatory characteristics of the transferred genes, as well as the ontogeny of tumor formation induced by targeted oncogene expression. Other studies are examining oncogenesis elicited by the bovine papilloma virus genome in transgenic mice, the structure of mouse DNA flanking transgene insertions, and the prospects for insertional mutagenesis by DNA microinjection or retrovirus infection.

Recombinant Insulin/Oncogenes in Transgenic Mice

D. Hanahan, S. Alpert, S. Efrat, J. Hager, M. Lacey

Hybrid oncogenes composed of regulatory information flanking the rat insulin (II) gene linked to protein-coding information for SV40 T antigen have been transferred into the mouse germ line (Hanahan, *Nature* 315: 115 [1985]). The rationale for employing such combinations is threefold: (1) to identify elements associated with the insulin gene that mediate its correct tissue-specific expression in the insulin-producing β cells of the endocrine pancreas, (2) to examine the consequences of oncogene expression in β cells, and (3) to address the prospect that oncogene expression can be employed to facilitate the establishment of β -cell lines.

Transgenic mice that harbor three different insulin/SV40 T antigen genes have been produced. One (called RIP1-Tag) carries 660 bp of DNA encom-

passing the insulin gene promoter aligned so as to transcribe the T-antigen gene. The second (called RIR-Tag) has the insulin promoter inverted with respect to the SV40 coding information. The third (RIP2-Tag) is a truncation of the first, which thereby includes only 410 bp of 5'-flanking information. All three recombinant genes elicit the heritable formation of solid β -cell tumors in transgenic mice that harbor them, suggesting that the tissue specificity element(s) reside within this region of the insulin gene. Expression of T antigen is detected only in insulin-producing β cells. The hybrid genes are expressing in virtually all β cells prior to tumor formation, which occurs in only a few of the several hundred islets of Langerhans that comprise the endocrine pancreas. This indicates that secondary events are necessary to convert a collection of islet cells expressing SV40 T antigen into a solid, well-vascularized tumor.

We are currently transferring a series of hybrid insulin/oncogenes into mice in order to compare and contrast the consequences of their expression in β cells. T antigen has two rather remarkable, and potentially distinguishable, consequences to mice which express it in their pancreatic β cells. The normal postnatal development of the islets is disrupted, and solid tumors arise in a few (but not all) of the islets expressing the oncogene. We hope to learn something about oncogenesis by examining the expression of other oncogenes, in view of the observations with T antigen. The hybrid oncogenes will include recombinants of the insulin promoter with *Ha-ras*, *v-fos*, *E1A*, *p53*, *c-myc*, *v-myc*, *erb-b*, *v-mos*, and the *trans*-activator genes of HTLV-II and HTLV-III. To date, transgenic mice harboring insulin-*Ha-ras*, *-E1A*, *-v-myc*, and *-v-fos* recombinant genes have been created, and their analysis has begun.

In another approach, we will examine the consequences of oncogene expression by performing complementation or cooperative experiments genetically. Mice harboring separate insulin-oncogenes (e.g., insulin-*myc* and insulin-*ras*) will be mated, and their progeny will be examined to deter-

mine the effects of coexpression at two oncogenes in the same cell.

Developmental Regulation of Insulin/SV40 T-antigen Hybrid Genes in Transgenic Mice

S. Alpert and D. Hanahan

Transgenic mice containing an insulin/SV40 T-antigen hybrid gene are being used to investigate the developmental control of insulin. In mammals, there are two developmental phases of insulin secretion. The first stage is a low level of insulin production detected at about day 14 of the 19 days of gestation in mice. This correlates with the early development of the pancreas. The second phase is a striking increase in insulin synthesis at approximately day 16 of embryogenesis; β cells are first apparent at this time. During the last 4 days of gestation, insulin synthesis remains high. The molecular mechanism controlling this developmental sequence has not yet been explained.

Our experimental approach is to compare the developmental expression of the endogenous insulin genes with that of the recombinant insulin gene. This hybrid gene contains 660 bp of the 5'-flanking region of the insulin gene joined to the coding region for the SV40 large T antigen. Analysis of transgenic mice carrying this gene has shown that the sequences required for tissue-specific regulation are present in the 5'-flanking region of the insulin gene (see above report by Hanahan et al.).

Immunohistology has been used to analyze mouse pancreata from embryonic day 16.5 and day 18.5. We have shown that T antigen, like insulin, is expressed in the developing islets at these stages. Further immunohistological analyses of the endocrine pancreas will be done to examine both T antigen and peptide hormone expression throughout prenatal and early postnatal development. If the sequences that elicit proper developmental expression of the insulin gene are contained in this recombinant gene, we would expect to see T antigen expressed from embryonic day 14, in the same temporal manner as insulin. Various constructions of the hybrid gene containing different pieces of the insulin gene and its flanking regions should allow us to identify the sequences required for the developmental regulation of insulin.

Immunohistological Analysis of Nesidioblastosis and Tumor Formation in the RIP1-Tag #2 Lineage

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

Certain endocrine cells, such as pancreatic β cells, normally have a limited potential for mitotic division. Persistent hypoglycemia in human infants has been attributed to nesidioblastosis, a disease characterized by multifocal abnormal islet-cell hyperplasia or abnormal islet-cell distribution. Little information is available on β -cell genesis. Theories on islet histogenesis include (1) division of existing cells, (2) differentiation of a stem-cell population, and (3) conversion of exocrine tissue. The RIP1-Tag #2 transgenic mice all exhibit β -cell hyperplasia and thus offer an ideal system to study β -cell neogenesis and tumorigenesis. We have recently begun to evaluate the process of β -cell proliferation using immunohistology and autoradiography.

Until 4 weeks of age, the RIP1-Tag #2 islets appear normal. Starting at 6 weeks of age, "holes" appear in the islets. Histological examination reveals a duct-like nature of these spaces, which is in accordance with the pattern of proliferation of islets in obese (ob/ob) mice (Larsson et al., *Lab. Invest.* 36(6): 593 [1977]). These duct-like structures proliferate as the animals age. Autoradiography clearly shows that a subset of the β cells are dividing.

By 13 weeks of age, most islets are enlarged and tumors have developed. Both islets and tumors contain a large number of rapidly proliferating insulin-producing cells; all of the insulin-producing cells contain T antigen. Additionally, some of the tumors contain a population of pancreatic polypeptide (PP)-producing cells. Tumors also contain cells expressing tyrosine hydroxylase (TH). Although TH appears during islet formation, it is rarely found in adult islets. Thus, some "dedifferentiation" is apparently occurring in the RIP1-Tag #2 tumor cells.

We are continuing this analysis to investigate the changes that occur between 6 and 13 weeks of age. These analyses will include experiments to investigate the possibility that these mice represent a mouse model for human nesidioblastosis. These investigations will characterize the events that occur when β cells are released from the normal divisional control in the RIP1-Tag #2 lineage. Although we

have already detected the presence of T antigen in ducts, double-label experiments with T antigen and insulin, mucine, and amylase may locate the production of new islets to β cells, ducts, or exocrine tissue.

Analysis of Expression of RIR-Tag Hybrid Genes

S. Elrat, D. Hanahan

Transgenic mice harboring RIR-Tag hybrid genes, in which an inverted rat insulin II control region is linked to the SV40 T-antigen structural gene, express large T protein specifically in the β cells of the endocrine pancreas. Their phenotype of hyperplastic islets, one of which develops to a solid, vascularized tumor of β cells at 4–6 months of age, is indistinguishable from that of mice in which the insulin control region is aligned to promote transcription of the T-antigen gene (RIP1-Tag). Northern blots of RNA from pancreatic tumors show comparable levels of T-antigen mRNAs in RIP1-Tag and in RIR-Tag mice. The reversed promoter generates mRNAs about 300 bases longer than the insulin-promoted version. Riboprobe protection and S1 mapping analyses show that transcription of these mRNAs is initiated at a specific site, mapping within the transcriptional enhancer region. Bidirectional transcription is observed with both hybrid gene orientations; in each case, one transcript represents the T-antigen gene and the other transcript represents the opposite strand extending off 5' to the T-antigen gene. Examination of the sequence of the minus strand, deduced from the published sequence of the rat insulin 5'-upstream region, reveals a putative TAATTA sequence 56 bp upstream of the initiation site, but no open reading frame. These studies involved analyses of two independent lineages of RIR-Tag transgenic mice, each of which shows the same phenotypic pattern. Hence, this phenotype likely reflects a property of the insulin promoter, rather than a position effect attributable to the site of integration.

These results demonstrate the existence of two promoters in the region extending to -540 upstream of the rat insulin gene cap site, operating on the two strands in opposite directions. Both obey correct cell-specific regulation in transgenic mice.

The existence of bidirectional transcription from the endogenous insulin genes, as well as the presence of a gene associated with the reversed promoter, is under investigation.

Bovine Papilloma Virus Genome in Transgenic Mice

M. Lacey, S. Alpert, D. Hanahan

A line of transgenic mice has been established that harbors the bovine papilloma virus (BPV) genome as a stable genetic element that is integrated into a mouse chromosome. BPV DNA was injected into fertilized mouse eggs as a linearized bacterial plasmid in which the BPV genome was reiterated as a partial tandem duplication. About five copies of this plasmid are integrated into high-molecular-weight DNA as a head-to-tail tandem array in the mice derived from the one live founder mouse that arose from the injected embryos.

The BPV genome is apparently dormant in young mice that are healthy and evidence no phenotype. However, beginning at approximately 8–9 months of age, these mice begin to develop benign skin tumors in various locations, primarily around the face and head, but also on the tail and at multiple locations on the skin of the body. These tumors are fibroepithelial in nature, being derived from dermal fibroblasts and epidermal epithelial cells, and thus are quite analogous to those produced *in vivo* (in cows), except that infectious BPV virus is *not* produced. The tumors all harbor episomal BPV DNAs, which apparently arise from the excision of the BPV genome from its stable integrated location in a mouse chromosome. Amplification of integrated BPV DNA is also observed in some tumors. There is a complete correspondence between tumor formation and the detection of BPV gene amplification (either integrated or as episomes). Episomal forms of BPV are not detected in normal tissues.

Tumors develop in virtually every transgenic mouse in this lineage, and multiple tumors usually arise on individual animals. In all cases, oncogenesis is slow, indicating that a secondary event is necessary to cause tumor formation. These mice can therefore be considered to be predisposed to developing cancer, which depends on the occurrence

of this other event. It is notable that normal mice virtually never develop fibroepithelial tumors, and as a consequence, these transgenic mice are unique in terms of their oncogenic phenotype.

Structural Analysis of an Integration Site in a Transgenic Mouse

S. Grant, D. Hanahan

A hybrid oncogene composed of the promoter element and 5'-flanking region of the chicken $\alpha 2(I)$ collagen gene linked to the protein-coding information for SV40 T antigen was injected into fertilized mouse embryos, which were implanted in foster female mice and allowed to develop. One transgenic mouse arose (M410) that carried 3–4 copies of the injected plasmid (pCOP3 Tag), presumably cointegrated at a single location. This mouse showed evidence of abnormal behavior at 2 weeks of age, which continued until its death at 7 weeks of age. This behavior was characterized by hyperactivity, frequent circling, and nodding of the head. When this mouse died, tissues were collected and homogenized. Protein-blotting analysis using monoclonal antibodies to SV40 T antigen demonstrated high-level expression of T antigen in the brain, and nowhere else. This result was surprising in view of the expected expression of type-I collagen in a variety of tissues such as skin, body wall, lung, and heart.

One explanation for this brain-specific expression is that the injected DNA integrated into a locus that allowed expression only in the brain. To test this hypothesis, we are retrieving mouse DNA that flanks the integrated plasmid array, using plasmid rescue. So far, we have rescued over 20 bona fide rescuants. Of these, one contains 2–3 kb of DNA that appears to be intercolated between two of the plasmids in the integrated array. The origin of this DNA is being examined for the possibility that it contains sequences from a brain-specific gene. So far, no flanking DNA has been isolated. We will investigate the nature of the integration to assess the extent of deletion and/or rearrangement. Flanking DNA will be used to probe RNA from the brain to determine whether this locus is expressed in the brain. The transgenic locus—composed of flanking DNA plus the plasmid—may be injected into embryos to produce transgenic mice harboring this

rearranged region, in an attempt to recapitulate the phenotype and thus evaluate its possible identity as a brain-specific locus, and the phenotype originally observed as a dominant consequence of this specific but inappropriate expression of the hybrid collagen-T-antigen gene.

Should this integration have disrupted such a brain-specific locus, the uninterrupted region will be isolated and studied to learn more about its ability to specify expression in the brain.

Integration of Retrovirus Sequences into the DNA of Early Mouse Embryos

V. Baultch, S. Toda

Identification of mutations that disrupt the normal program of embryogenesis is one way to study mammalian development. Insertional mutations also provide a molecular marker for the recovery of genes disrupted by the mutation. However, to be able to clone the preintegration site and analyze the disrupted gene, the integration event must preserve the linear order of cellular sequences in a recognizable way. Retrovirus genomes are particularly useful as potential agents of insertional mutation because retroviruses integrate with only a small (4–9 bp) duplication of cellular DNA. We are generating insertions in mice using a breeding scheme (Jenkins and Copeland, *Cell* 43: 811 [1985]) that allows for de novo integration of endogenous retrovirus genomes into the DNA of early embryos at a relatively high frequency. A subset of retrovirus genomes called ecotropic proviruses has been followed because most inbred strains of mice contain a few (0–6) copies that can be easily followed using molecular techniques.

Mice of the RF/J strain contain three ecotropic proviruses. Two of these, *Emv-16* and *Emv-17*, are genetically linked and have been implicated in integration events in early embryos. We have crossed male RF/J mice to females of two different strains (CBA/CaJ and SWR/J) that are negative for endogenous ecotropic proviruses. The F_1 progeny were backcrossed to the virus-negative strains. The proviral sequences of the N2 offspring were analyzed by hybridizing genomic DNAs prepared from tail clips to an ecotropic-virus-specific probe. N2 females carrying *Emv-16* and *Emv-17* were then back-

crossed to produce N3 progeny that were analyzed for newly integrated proviruses.

We have scored a total of 111 integration events. Analysis of the results led to the following conclusions: (1) New proviral integration events occur when *Emv-16* and *Emv-17* are present in female carriers (mothers) but not when present in male carriers (fathers). (2) Crossing *Emv-16* and *Emv-17* onto the SWR/J strain for three generations results in a higher frequency of integration events (0.134 per mouse) than the equivalent crosses onto the CBA/CaJ strain (0.000 per mouse). (3) Integration events appear to be distributed randomly with respect to breeding pairs and litters. (4) N4 female carriers of *Emv-16* and *Emv-17* that had sustained at least one new proviral integration produced more positive progeny (21–26%) than did unselected N2 and N3 female carriers (4–8%). These findings indicate that genetic factors contribute to this phenomenon and that it might be possible to select for increased efficiency of integrations. The results are consistent with a mechanism of reintegration involving infection of the egg or early embryo by maternal tissues shedding virus, although other possibilities exist.

These new loci have been transmitted through the germ line, and to date, we have established 40 lines with new proviral integrations. The average transmission frequency of the new loci varies with an average of approximately 25%. This suggests that many integration events may have occurred at the two-cell stage of development, concomitant with the onset of DNA synthesis. This hypothesis is supported by progeny testing of one mouse containing nine new proviral integrations. Analysis indicates that multiple integrations can occur in the same cell or in different cells of the same embryo; the combined transmission frequencies of the two different cell lineages approximate 50%, suggesting that each cell of the two-cell embryo sustained independent integrations.

Mouse lines containing these new proviral loci are being analyzed for insertional mutations. To date, no dominant phenotypes have been detected associated with the 40 loci carried in heterozygotes. We are currently generating homozygous mice carrying each locus in order to score for visible recessive phenotypes and for recessive lethal mutations. We will detect recessive lethal mutations by using molecular techniques to score a homozygous class of offspring, and the absence of this class will be indicative of such a mutation.

Analysis of DNA Microinjection into Mouse Embryos to Create Insertional Mutants

V. Bautch, D. Hanahan

It is known that insertion of DNA into the mouse germ line via microinjection can result in insertional mutations. Microinjected DNA is usually found as 5–50 copies in a head-to-tail tandem array at a single integration site, and the 5' and 3' ends of the array are sometimes missing sequences. Limited analyses by several investigators indicate that approximately 10% of transgenic mice carry a recessive-lethal mutation that cosegregates with the inserted DNA. However, the sequences flanking the integrated DNA have only been analyzed in one of these lines, and in this case, a deletion of approximately 1 kbp was associated with integration (Woychik et al., *Nature* 318: 36 [1985]). This parameter is important relative to insertional mutagenesis because only if integration of DNA preserves the linear order of cellular sequences or gives rise to small deletions or rearrangements (e.g., no larger than cosmid size) will it be possible to clone the preintegration site easily and to analyze the disrupted gene(s). Therefore, we plan to investigate the integrity of flanking sequences after the integration of microinjected DNA.

To this end, we have constructed a plasmid vector that can be used to rescue, by transformation of *Escherichia coli*, the inserted DNA and flanking cellular sequences. Although this vector is derived from pBR322, it has been altered so that deletion of some plasmid sequences from the end of a tandem array by "nibbling" prior to integration should not affect the ability to rescue this plasmid. It contains three different drug resistance genes, and each gene contains several restriction enzyme sites unique to the plasmid. In addition, a suppressor tRNA gene has been inserted that can complement bacteriophage λ mutations and provide an additional selection. A polylinker containing unique restriction sites has been inserted between two of the drug resistance markers in order to linearize the plasmid prior to microinjection. It is expected that the DNA will integrate in a head-to-tail tandem repeat so that the linear order after integration will be $-kan^R-ori-sup^F-amp^R-ter^R-$. The genomic DNA will be digested with a restriction enzyme whose site is in a drug resistance gene and religated. Any internal copies of the tandem DNA should

recircularize to yield plasmids resistant to all three drugs upon transformation; however, each end should contain some cellular DNA and also be uniquely sensitive to the drug whose resistance gene was originally cleaved. For example, digesting with an enzyme that cleaves the *tet^R* gene should yield a DNA fragment containing a truncated *tet^R* gene at one end and flanking cellular DNA at the other end. This fragment will ligate to form a plasmid with cellular DNA that is *amp^R*, *kan^R*, and *tet^R*. Moreover, in this scheme, a second rescue using an enzyme that cleaves the *kan^R* gene should yield a plasmid containing cellular DNA from the opposite end of the tandem array. In the event that plasmid sequences are altered by the integration so as to make plasmid rescue impossible, it should be possible to retrieve the flanking DNA by cloning into phage containing a nonsense mutation and selecting for the suppressor tRNA gene. Finally, if alteration of suppressor tRNA sequences makes phage rescue impossible, the flanking DNA can be retrieved by making a library of genomic DNA from the transgenic mouse and screening with the plasmid as a probe.

At this time, we have generated three lines of transgenic mice containing this vector. The lines are currently being bred, and preliminary results suggest that one of the lines may contain multiple chromosomal integration sites; separation of these loci in sublines should increase the total number of loci available for analysis. The flanking sequences will be cloned and their colinearity with the preintegration site will be assessed. We will also use breeding and molecular analysis to determine the efficiency of generating insertional mutants in mice using this technique.

Analysis of Transgenic Mouse Line RIR-Gag #2

M. Lacey, D. Hanahan, T. Ashley

Mouse 95 was one of two transgenic founder mice for the RIR-gag lineages. RIR-gag is a fusion of the rat insulin promoter region (700 bp) aligned in the reversed orientation with respect to the sequences encoding the gag proteins of Rous sarcoma virus. Mouse 95, a female, was mated to a C57/BL6 male and produced two litters. Dot-blot analysis of equal amounts of DNA extracted from the tails of the

progeny resulted in two groups of transgenic mice. The first group carried about 15 copies of the insert and was called RIR-Gag #1, and the second group showed more than 25 copies of the transgene and was labeled as RIR-Gag #2. The inheritance patterns of RIR-Gag #1 mice demonstrated that the injected DNA had inserted into the X chromosome of the founder and was therefore sex-linked. Females homozygous for the insert, as verified by outcrosses, were obtained and mated to hemizygous males to maintain the line.

The two RIR-Gag #2 mice, M140 (female) and M186 (male), were mated. In addition, M186 was crossed to a C57/BL6 female. Results of the dot-blot analysis of the progeny from the second cross included a transgenic male and a nontransgenic female among the progeny, suggesting that RIR-Gag #2 was not sex-linked. Therefore, we continued mating and analyzing mice to produce homozygous males and females. Homozygotes are postulated on the basis of a twofold stronger signal than heterozygotes on dot blots. Potential homozygotes are mated with nontransgenic mice. When all the progeny of this cross are transgenic, the postulated mouse is confirmed as a homozygote. By such analysis, females are readily obtained. The one potential male did not transmit to all progeny, and there was a high mortality among his progeny during the 3 weeks after birth.

In an attempt to clarify the inheritance patterns, all progeny from RIR-Gag #2 litters were pooled and the numbers of male, female, transgenic, and nontransgenic mice were compiled. Although the male:female ratio approached 1:1, the number of transgenic females was twice that of transgenic males. In crosses involving heterozygotes, RRG × C57/BL6 produced litters where 11% of the males (1/9) and 60% of the females (6/10) were transgenic. The reciprocal cross resulted in 45.4% of the males (5/11) and 80% of the females (8/10) being transgenic. Thus, females preferentially inherited the injected gene.

Since the RIR-Gag #2 line was derived from a mouse with a known insert in the X chromosome, it is possible that the observed preferential inheritance results from the incomplete segregation of two inserts, one of them RIR-Gag #1. Southern blotting analysis of 20 mice, including M95, M140, and M186, and an RIR-Gag #1 transgenic mouse, was done to detect mice with multiple insertions. The results confirmed that the injected DNA inserted in head-to-tail tandem repeats, since bands

of high intensity (primary bands) correspond to the predicted molecular weights of internal repeats for such a pattern of insertion. Secondary bands of lower intensity, which represent the flanks of the insert and some mouse DNA, were also apparent. A second Southern blot under conditions that should delineate the secondary bands should indicate the number and type of inserts in each mouse. An analysis of secondary bands (primary bands corresponding to internal repeats) should indicate the number and type of inserts in each mouse.

T. Ashley, a visiting scientist who has worked on meiotic synapsis in mice, reanalyzed the inheritance information previously compiled noting the parentage of specific crosses. Her analysis highlighted the unique quality of M186 (male); only progeny of this mouse generated the exceptions to the general pattern of X-linked inheritance. Mouse 140 (female) and those progeny that do not result from a cross with M186 demonstrate a typical pattern of

sex-linked inheritance. A repeat of the Southern blot analysis of recently established matings involving offspring of M186 should enable us to elaborate on our current theory that RIR-Gag #2 is a distal insert in the X chromosome and that the abnormalities observed in the progeny of M186 result from crossovers between the pairing region of the X and Y chromosomes.

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In Press, Submitted, and In Preparation

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GENETICS

A strong emphasis on genetics, and lately on molecular genetical studies, has continued at the Cold Spring Harbor Laboratory. For the last 10 years, the number of scientists working in this area has nearly quadrupled, with the future still calling for an increased commitment. In the following section, we describe recent results obtained within the past year. The areas emphasized are cell-cycle control, as well as the mating-type studies of both budding (*Saccharomyces cerevisiae*) and fission (*Schizosaccharomyces pombe*) yeasts. Among the prokaryotic systems, studies with the *Rhodospseudomonas capsulata*, *Clostridium pasteurianum*, and *Azotobacter vinelandii* have continued. Among the higher-plant systems, molecular characterization of the transposable elements of maize, as well as the biochemistry of polyamine biosynthesis in tobacco tissue culture, is being investigated vigorously.

YEAST GENETICS

A.J.S. Klar J. Ivy
J. Hicks C.I.P. Lin
 G.P. Livi

Molecular Cloning and Characterization of the *SUM1* Gene of *Saccharomyces cerevisiae*

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

The interconversion of mating type in the yeast *Saccharomyces cerevisiae* involves a genetic rearrangement in which cryptic copies of a or α information are transposed to and expressed at *MAT*. The mating-type information residing at the *HML* and *HMR* loci is negatively regulated by the action of at least four unlinked *MAR* (or *SIR*) loci; a mutation in any one of these loci can permit *HM* gene expression. The *MAR/SIR* gene products act in *trans* to prevent the transcription of *HML* and *HMR*, presumably by interacting with *cis*-acting regulatory sites (called E and I) that flank each locus (see Fig. 1).

We have recently identified a new locus that is somehow involved in the regulation of *HM* gene expression (Klar et al., *Genetics* 111: 745 [1985]). A cell with the genotype *HML α MAT α HMR α marl-1*

is unable to mate because of the simultaneous expression of both a and α information. An extragenic suppressor of the *marl-1* mutation was identified by isolating a mutant that exhibits an α mating type, due to the failure to express *HML* and *HMR*. A new locus, *SUM1* (*SU*ppressor of *Mar*), is defined by the mutation in this strain. The *sum1-1* mutation (1) is recessive, (2) is centromere unlinked, (3) does not correspond genetically to *MAT*, *HML*, or *HMR* or to any of the known *MAR/SIR* loci, (4) affects expression of both a and α information at the *HM* loci, (5) has no identifiable phenotype in a *Mar*⁺ strain, and (6) suppresses a *MAR1* deletion mutation as well as mutations in *SIR3* and *SIR4*, indicating that it is neither allele- nor locus-specific. Furthermore, direct transcript analysis has revealed that the *sum1-1* mutation results in a drastic reduction in the level of both *HML* and *HMR* transcripts but does not affect the level of *MAT* transcription. These data suggest a model in which the *MAR/SIR* gene products negatively regulate the *SUM1* locus, whose gene product is required for *HM* gene expression.

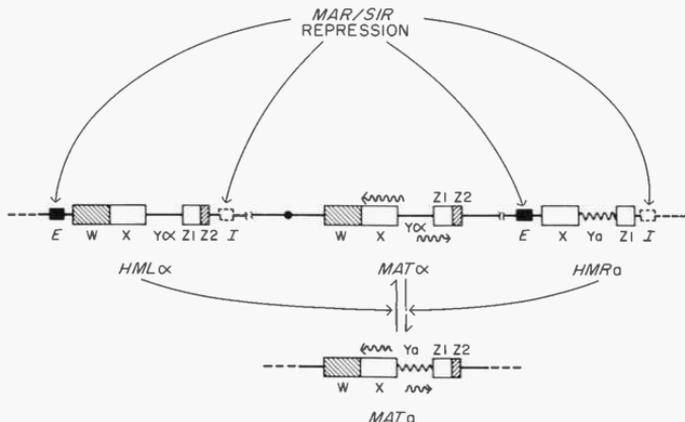


FIGURE 1 Structure of the mating-type genes on chromosome III. *HML* and *HMR* carry copies of mating-type information but are normally kept unexpressed by the action of the *MAR/SIR* gene products that presumably interact with the *cis*-acting *E* and *I* sites. Interconversion of *MAR* α and *MAT* α involves a unidirectional transposition-substitution event from *HML* or *HMR* to *MAT*.

We have cloned the *SUM1* gene by complementation. Plasmid DNA from a bank of *Sau3A* partially digested genomic *S. cerevisiae* DNA contained in the yeast-bacteria shuttle vector YEp13 was introduced by transformation into a strain with the genotype *HML* α *MAT* α *HMR* α *mar1-1* *sum1-1* *leu2* (α phenotype). *Leu*⁺ transformants were selected and screened for nonmating. Sterility was shown to cosegregate with *Leu*⁺, and the plasmids rescued from these strains were shown again to confer a nonmating phenotype when reintroduced into the original α strain. There is no apparent homology between these seven clones and other yeast sequences that, if present in high copy number, could cause sterility (e.g., *MAT* α , *HO*, anti-*sir*). Preliminary cross-hybridization studies indicate that among these clones there are at least three unique sequences able to complement *sum1-1*, one of which is represented by three overlapping DNAs. Work is in progress to distinguish the *SUM1* gene clone from other wild-type sequences that also complement the *sum1-1* mutation in vivo. RNA blot analysis will reveal any evidence of transcriptional regulation of *SUM1* by the *MAR/SIR* genes, and this will be combined with additional molecular and genetic experiments designed to challenge our current models for the control of *HM* gene expression.

Identification and Characterization of the *SUM2*, *SUM3*, *SUM4*, and *SUM5* Genes of *Saccharomyces cerevisiae*

C.I.P. Lin, J. Ivy, A.J.S. Klar

To study the possible interactions among the different *MAR/SIR* gene products and to search for new loci involved in regulating the expression of *HML* and *HMR*, extragenic suppressors of *mar2* mutations were isolated. Strain PL1 (*HML* α , *MAT* α , *HMR* α *mar2-1*) has a nonmating phenotype because the *mar2-1* mutation allows the simultaneous expression of both α and α information. Mutant strains were isolated that exhibit an α phenotype and therefore, presumably, fail to express the *HML* α and *HMR* α loci. The mutations responsible for the suppression of *mar2-1* in these strains define four complementation groups designated *sum2-1*, *sum3-1*, *sum4-1*, and *sum5-1*.

Genetic analysis of the four *mar2-1* suppressors show that they are recessive single gene mutations, unlinked to *MAT* or *MAR2*. As with *sum1-1*, the *mar2-1* suppressor mutations do not correspond to *SIR1*, *MAR1*, *MAR2*, or *SIR4*. The *mar2-1* suppressors also suppress a *mar2* deletion mutation, indicating that they are not translational suppress-

sors. Only *sum5-1*, however, is also able to suppress a *mar1* deletion.

Suppressors of *mar2-1* also differ from *sum1-1* in their ability to suppress the information at the *HM* loci. For example, *HMLa* is not suppressed by either *sum2-1*, *sum3-1*, or *sum4-1*, indicating that they, in fact, may be *a-specific* suppressors. In some experiments, *MATa* information in *HMLa* *MATa* *HMRa* *MAR* *sum* strains is also repressed, resulting in an *a* mating type. Further genetic and molecular analyses are under way to provide a clearer understanding of how these genes fit into the already complicated regulatory circuit that controls the expression of the silent mating-type genes of yeast.

Epigenetic Control of *MAT1* Switching in *Schizosaccharomyces pombe*

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

The most fascinating and central question in biology today is how different cell types are generated. The P (plus) and M (minus) mating types (cell types) of *Schizosaccharomyces pombe* have been shown to interchange by a transposition-substitution reaction in which copies of the donor loci (*MAT2-P* and *MAT3-M*) are transmitted to the expressed *MAT1* locus (see 1983 Annual Report). A fascinating property of this system is that between a pair of sister cells, only one member (in 92% of the cell divisions) generates a single switched cell in the next generation (Fig. 2) (Miyata and Miyata, *J. Gen. Appl. Microbiol.* 27: 365 [1981]). Our recent work has shown that cells that switch have inherited a *MAT* allele that carries the potential for switching; i.e., there seems to be semi-heritable epigenetic control for switching. This conclusion is based on the results described below.

We have shown that meiotic crosses involving strains deleted for *MAT2* and *MAT3* produce 20% of tetrads with the 1P:3M and 3P:1M types of *MAT1* gene conversions (see 1984 Annual Report). The observed high frequency of meiotic gene conversion is clearly a manifestation of the *MAT* switching system that normally occurs in mitotically dividing cells. We have used the high meiotic gene conversion system to ask whether the developmental potential to switch is asymmetrically segregated cytoplasmically or chromosomally. Should the observed pattern of switching in mitotically

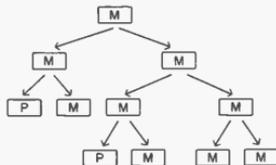


FIGURE 2 Miyata and Miyata (*J. Gen. Appl. Microbiol.* 27: 365 [1981]) mating-type switching pedigree analysis.

dividing cells be due to chromosomal “imprinting,” this event might be catalyzed by the *SWII* and/or *SWI3* and/or *SWI7* gene functions, since mutations in any one of these genes can result in a failure to create the DS cut (see 1983 Annual Report). To test this idea, we subjected a *MAT1-M swi3* × *MAT1-P SWI3* cross (both strains with deleted *MAT2* and *MAT3* cassettes) to meiotic tetrad analysis. It is important to realize that when *S. pombe* cells mate, the zygotic cells immediately undergo meiotic division. If the *SWI3* gene product is involved in chromosomal marking, only the *MAT1-P* allele would be expected to gene convert to the *MAT1-M* allele in this cross. Among the 103 four-spored tetrads analyzed, 16 were found to have a conversion event. A fascinating finding is that all of these conversions are of the 3M:1P type. Thus, only the *MAT1-P* allele, which was brought in from the *SWI3*⁺-containing nucleus, is capable of acting as a recipient for the meiotic gene conversion event. By a similar cross, but with the reversed *swi3* genotype, we have shown imprinting of the *MAT1-M* allele. Therefore, the potential to gene convert in meiosis must be imprinted at the template level and segregated in *cis* with the *MAT* allele. These results prompted us to suggest that only those cells that inherit an imprinted chromosome in mitotic cell lineages are competent to switch.

Is the DS break an imprinted event? The results involving *swi1* and *swi3* crosses are consistent with the idea that the allele that brings along the *MAT* DS break into meiosis acts as the recipient for meiotic gene conversion. However, the key question is why some chromosomes get the break and others do not. Results of the following experiment rule out the DS break as being the imprinted event. A *MAT1-M swi7* × *MAT1-P SWI7* cross (both deleted for *MAT2* and *MAT3*) generated conversions of either type with equal frequency, i.e., 1P:3M = 3P:1M. Should the DS break strictly con-

stitute an imprinted event, this cross also should have generated biased gene conversions as was observed for the *swi1* and *swi3* crosses. This expectation arises since *swi7* strains also fail to make the DS break. This result clearly demonstrates that the chromosomes grown in a *swi7* genotype are imprinted, even though they do not have the break. Thus, we propose that the *SWI1* and *SWI3* gene products epigenetically modify (imprint) the *MAT* DNA and that the *SWI7* gene product catalyzes a cut of the imprinted substrate.

These results clearly demonstrate that there is an epigenetic chromosomal basis for the asymmetric segregation of the switching potential. We should now ask how it is decided that only one among four progenies of a single cell switches in 92% of cell divisions. We imagine that the decision must have been made by the grandparent cell and that it takes two generations to materialize a switch. We propose that the *SWI1* and *SWI3* gene products alone, or in combination with other functions, catalyze a sequence-specific marking in the grandparent cell such that only one chain (e.g., the Watson Strand) is modified (Fig. 3). To maintain the asymmetry, we suggest that the modified Watson Strand serves as a template for a "modified" Crick Strand at the time of DNA replication in the grandparent cell. For example, a nicked template will allow the synthesis of a nicked new strand. The other unmodified Crick Strand of the grandparent cell serves as a template for an unmodified Watson Strand, the latter of which will be modified by the hypothesized *SWI* gene products. Thus, at the two-cell stage, one cell has both strands modified, and the sister cell, like the parental cell, is only modified on one strand. We further postulate that the cell containing both chains modified is imprinted to get cut and will produce a switched and an unswitched (or homologously switched) pair of cells. Once switched, the cycle will start again. This strand-segregation model (SS model) presumes that all of the cells have the identical cytoplasmic makeup, and the asymmetry in cell division is postulated to be due to the two chains of DNA being nonequivalent in acquiring the developmental potential. According to this model, any given cell division in any biological system will always generate two nonequivalent cells because one inherits the "old" Wat-

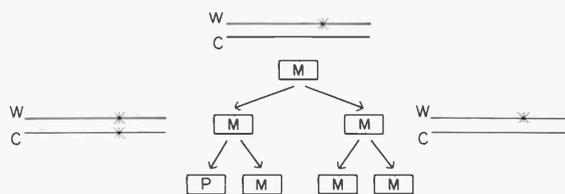


FIGURE 3 Strand segregation model.

son Strand and the other inherits the "old" Crick Strand.

We have designed a genetic test of the model that involves determining the switching pattern of two types of *MAT1* duplications. Two constructions, one containing directly oriented cassettes and the other containing inversely oriented cassettes, will be made. Our model predicts that a directly oriented duplication will allow switching of both cassettes in only one among four cells at the four-cell stage. In the oppositely oriented arrangement, however, each of two cells, one from each half of the pedigree, should switch single (but different) cassettes. Such studies are under way, and further experiments will be directed at determining the nature of the hypothesized DNA modification.

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CELL-CYCLE CONTROL IN YEAST

D. Beach M. McLeod L. Brizuela
J. Potashkin M. Stein
R. Booher S. Allen

During the winter and early spring of 1985, the laboratory previously occupied by Tom Broker and Louise Chow was completely renovated, and in May 1985, the research group headed by David Beach was able to move from Delbrück into the new laboratory in Demerec. This move reflects a shift in the emphasis of our work, away from purely genetic approaches to the cell cycle, toward a combination of genetic and biochemical studies for which the Demerec location is more suited. As in previous years, our research has centered on the fission yeast, *Schizosaccharomyces pombe*, which continues to be an ideal model organism for investigation of the control of mitotic cell division and meiosis.

Cell-cycle Control

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

Our work on the cell cycle of fission yeast during the past year has concentrated on the *cdc2⁺* gene and its product. The *cdc2⁺* gene is unusual because its activity is required at two independent stages of the cell cycle: in G_1 prior to DNA replication and in G_2 prior to mitosis.

The sequence of the *cdc2⁺* gene shares homologies with known protein kinases. Much of our efforts have been directed toward raising monoclonal antibodies against *cdc2⁺/lacZ* fusion protein in order to investigate whether *cdc2⁺* is indeed a protein kinase. We have also expressed the native *cdc2⁺* gene product at very high levels in *Escherichia coli*. This protein has turned out to be insoluble and we are presently attempting to resolubilize the protein in a biologically active form and to purify it.

A dominant allele of *cdc2⁺* that does not cause cell-cycle arrest but instead leads to advancement of cell-cycle events has been isolated and sequenced. This activated allele has a single mutation, *cdc2Asp146*, instead of the wild-type sequence, which has *Gly146*. The way in which this mutation affects the activity of the *cdc2⁺* gene product is unclear at present.

During the summer, an undergraduate research visitor, G. Costello, undertook experiments to investigate the behavior of fission yeast during entry into stationary phase. It was found that contrary to popular conception, fission yeast enters the stationary phase equally from the G_1 phase or from the G_2 phase of the cell cycle. The fraction of the culture entering $G_{0/1}$ or $G_{0/2}$ was highly dependent on the initial growth medium (Fig. 1).

$G_{0/1}$ and $G_{0/2}$ cells were shown to share several properties: (1) They are equally resistant to heat shock; (2) they have equal long-term viability; and (3) both require the *cdc2⁺* gene product for cell-cycle progression after restoration of fresh medium (Fig. 1, right). These experiments are inconsistent with the widely held view of the cell cycle in which it is presumed that cells enter stationary phase only from the G_1 phase of the cell cycle.

Control of Meiosis

M. McLeod, M. Stein, D. Beach

Our laboratory continues to be interested in understanding the molecular basis of the developmental switch that underlies the transition from vegetative growth to sexual differentiation. Ultimately, this process is controlled by the genes of the mating-type locus, since a diploid strain that is homozygous at the mating-type locus (e.g., *h⁺/h⁺*) merely enters stationary phase in response to starvation, whereas an otherwise identical strain that is heterozygous at the mating-type locus (*h⁺/h⁻*) undergoes meiosis and sporulation under the same conditions. The genes of the fission yeast mating-type locus are being studied by M. Kelly (Delbrück Laboratory). Our research has concentrated on investigating the pathway through which the mating-type genes exert their effect.

The *rnl1⁺* gene has a central role in the control of meiosis. Strains carrying temperature-sensitive alleles of *rnl1* undergo haploid sporulation at the restrictive temperature. We have proposed that dur-

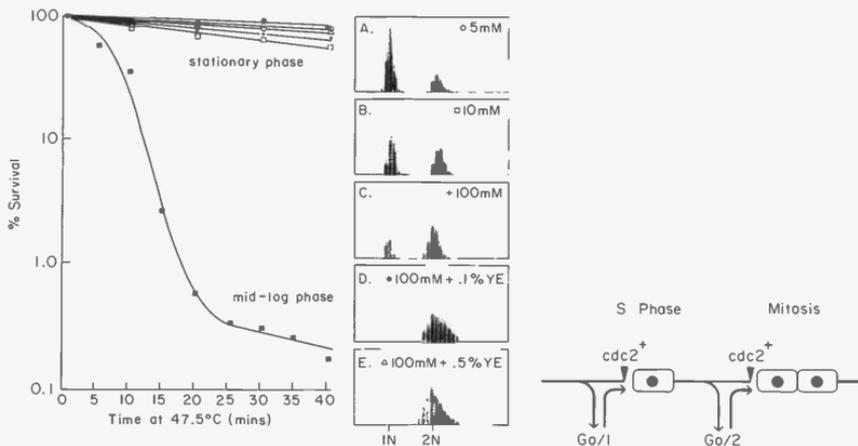


FIGURE 1 (Left) Heat-shock resistance of actively dividing cells (■) and those that have reached stationary phase in media containing either 5 mM NH₄Cl (○), 10 mM NH₄Cl (□), 100 mM NH₄Cl (+), 100 mM NH₄Cl and 0.1% yeast extract (●), or 100 mM NH₄Cl and 0.5% yeast extract (Δ). All media contained 2% glucose. (A–E) Frequency histograms of the number of cells in each medium that reach stationary phase with either a G₁ (1N) or G₂ (2N) DNA content. (Right) Diagrammatic illustration of the cell cycle of *S. pombe*. Cells enter stationary phase from either G₁ (G₀₁) or G₂ (G₀₂). Reentry into the cell-division cycle requires the *cdc2*⁺ gene product for both DNA synthesis and nuclear division.

ing normal sexual differentiation, the mating-type genes inhibit the activity of the *ran1*⁺ gene product, thereby provoking meiosis and sporulation.

The nucleotide sequence of the *ran1*⁺ gene has been determined. It shows extensive homology with genes known to encode protein kinases. The closest homolog of *ran1*⁺ is the *cdc2*⁺ gene of fission yeast. The predicted *ran1*⁺ and *cdc2*⁺ proteins share 20% amino acid homology. These data suggest that *ran1*⁺ encodes a protein kinase that is active during vegetative growth and is presumed to be inhibited during the initiation of meiosis. We are currently attempting to raise antibodies against a *ran1*⁺/*lacZ* fusion protein in order to test whether the *ran1*⁺ gene product is indeed a protein kinase.

In the meantime, an interesting connection has been drawn between the activity of the presumed *ran1*⁺ protein kinase and cAMP-dependent protein kinase. In the absence of the *ran1*⁺ gene product, cells cease vegetative growth and sporulate. However, this phenotype is completely suppressed by addition of either cAMP or caffeine (a phosphodiesterase inhibitor) to the culture medium. The *ran1*⁺ gene product is therefore not essential for growth if cAMP-dependent protein kinase is overstimulated. We have proposed that the presumed

ran1⁺ protein kinase shares certain key substrates with cAMP-dependent protein kinase.

We are particularly interested in the mechanism that controls the activity of the *ran1*⁺ product and have recently identified the product of the *mei3*⁺ gene as at least part of the pathway by which the activity of *ran1*⁺ is brought under the control of the mating-type locus. *mei3*⁺ mutant strains suffer no defect in vegetative growth and under appropriate conditions undergo sexual conjugation, but the resulting zygote arrests during meiosis before the initiation of premeiotic DNA replication. However, a *ran1(tsmei3)* double mutant sporulates at restrictive temperature, suggesting that *mei3*⁺ acts "upstream" of *ran1*⁺ in the meiotic pathway.

We have isolated and sequenced the *mei3*⁺ gene, which is predicted to encode a small basic protein of 148 amino acid residues. The *mei3*⁺ gene is not expressed in haploid strains under any conditions, but it is expressed in an *h*⁺/*h*⁻ diploid strain that is initiating meiosis as a result of nutritional starvation. The expression of *mei3*⁺ is therefore controlled by the genes of mating-type locus.

Although the *mei3*⁺ transcript is not normally expressed in haploid cells, we have been able to assess the consequence of such expression by driving the

mei3⁺ gene off the strong promoter of the alcohol dehydrogenase (ADH) gene. Expression of *mei3⁺* in a haploid cell is lethal. However, it appears that *mei3⁺* expression does not have a nonspecific cytotoxic action because strains carrying a mutation in the *mei2* gene survive overexpression of the *mei3⁺* gene. *mei2⁻* mutants have a phenotype very similar to that of *mei3⁻* mutants, except that the *ran1(ts)-mei2⁻* double mutant does not sporulate at the restrictive temperature and instead continues normal vegetative growth. In the absence of the *mei2⁻* gene product, *ran1⁺* is therefore a nonessential gene.

We suspect that overexpression of the *mei3⁺* gene, driven by the ADH promoter, results in inhibition of the activity of *ran1⁺* and therefore vegetative growth ceases, except in a *mei2⁻* strain in which *ran1⁺* is not essential for growth. A prediction of this model is that expression of *mei3⁺* in a wild-type cell should not merely cause cell death but should provoke sporulation similar to that observed in *ran1(ts)* mutants. Indeed, we have found that in the microcolonies, which develop after transformation of a haploid strain with a plasmid carrying *mei3⁺* driven by the ADH promoter, many cells contain spores. Thus, overexpression of *mei3⁺* phenocopies the *ran1(ts)* mutation. This suggests that the *mei3⁺* gene product inhibits the activity of the *ran1⁺* gene product. Whether the *mei3⁺* gene product interacts directly with the *ran1⁺* protein kinase will be a topic for future investigation.

Function of *ras* in Fission Yeast

D. Beach

I have undertaken a project in collaboration with S. Nadin-Davis (NRC Laboratory in Canada) to investigate the function of the *ras* gene in fission yeast. Dr. Nadin-Davis, who has spent several months at Cold Spring Harbor Laboratory during the year, had previously isolated the fission yeast *ras* gene. The gene is apparently unique, encodes a protein of 210 amino acid residues, and shares considerable homology with the *ras* genes of vertebrates.

We have constructed both null and activated (*rasvall2*) alleles of the *ras* gene and introduced them into yeast by "gene replacement." *ras* is not an essential gene in *Schizosaccharomyces pombe*. Strains lacking *ras* function grew quite normally; however, the process of sexual differentiation was disrupted. Haploids normally capable of sexual

conjugation were sterile and diploids were highly inhibited in meiosis and sporulation.

The *rasvall2* "activated" allele of the gene likewise had no effect on vegetative growth, no effect on long-term viability in stationary phase, and no effect on sporulation or meiosis. However, haploid strains carrying *rasvall2* were incapable of conjugation.

The research group of M. Wigler (Molecular Genetics of Eukaryotic Cells Section) has shown previously that in the unrelated budding yeast, *Saccharomyces cerevisiae*, *ras* controls the activity of adenylate cyclase. However, we have found that fission yeast strains carrying a null allele of *ras* do not lack cAMP and that the *rasvall2* mutation does not cause an increase in cAMP level. Indeed, the phenotype of *rasvall2* is quite different from that caused by increasing the intracellular level of cAMP. As in budding yeast, high levels of cAMP in fission yeast inhibit both entry into stationary phase and meiosis and sporulation.

These experiments suggest that the use to which the *ras* protein is put in different organisms may be entirely different. This is a little surprising, since we found that introduction of a mammalian *ras* protein into a *ras⁻* strain of fission yeast could partially restore *ras⁺* function. Although the *ras* protein is highly conserved and functions across an enormous evolutionary divide, the role of the *ras* protein in cellular function is apparently not highly conserved.

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PLANT GENETICS

S.L. Dellaporta	J. Chen	A.C. Hiatt	P. Hinton
J. Hicks	P. Chomet	B.A. Lowe	J. McIndoo
R.L. Malmberg	G. Giuliano	C. Mitchell	J. Wood
P.A. Scolnik		M. Alfenito	

Studies on maize controlling elements, particularly the *Ac/Ds* system, continue to add to our understanding of the mechanism of controlling element transposition and activation. We have concentrated our efforts on *phase changes* of *Ac* at the *waxy* locus. Phase changes are heritable, but reversible, events that are detected by the loss or gain of *Ac* genetic activity. As reported last year, we have been able to correlate changes in DNA methylation patterns of *Ac* with genetic activity. This methylation appears to be specific for *Ac* DNA sequences. Transposition studies of *Ac* from the *P* locus have been initiated to obtain molecular data on the mechanism and chromosomal pattern of *Ac* movement. Finally, we have continued to analyze the *R* locus of maize with molecular *R* probes obtained by transposon tagging with *Ac*.

Changes in Phase of *Ac*

P. Chomet, S.L. Dellaporta [in collaboration with S. Wessler, University of Georgia]

The *Ac* element at the *wx-m7* allele has the ability to undergo cyclical changes in genetic activity. This activity is assayed by the ability of *Ac* (1) to transpose autonomously from the *waxy* locus, (2) to *trans-activate* unlinked *Ds* elements, and (3) to contribute to *Ac* dosage effects; as the number of *Ac* elements increases, transposition is developmentally delayed. The *Ac* element at *wx-m7* can lose these functions during plant or kernel development and is believed to enter an inactive phase. Our studies have shown that a shift from an active phase to an inactive phase of *Ac* is associated with modification of *Ac* DNA sequences. This modification may involve cytosine methylation, as detected by restriction enzymes sensitive to this type of modification. The modification appears to be specific for the *Ac* element, since restriction sites flanking the *Ac* element in *Wx* DNA are unmodified (Fig. 1). At present, we do not know whether *Ac* modification is a cause or an effect of inactivation. We are deter-

mining whether any nucleotide changes are present in a genomic clone of the inactive element to help resolve this issue.

Molecular Analysis of the *R* Locus of Maize

S.L. Dellaporta, M. Alfenito [in collaboration with S. Wessler and I. Greenblatt, University of Connecticut, and J. Kermicle, University of Wisconsin]

The *R* locus of maize on chromosome 10 is one of several epistatic loci required for the production of anthocyanin pigments in plant and seed tissues. The pattern of plant pigmentation, i.e., which tissues will ultimately be pigmented in the plant and seed, is determined by various alleles of the *R* locus. Moreover, expression of other epistatic gene products in anthocyanin biosynthesis appears to require a functional *R* allele or certain *B* alleles (an unlinked locus) capable of substituting for *R*. For these reasons, *R* is thought to be a regulatory locus rather than a structural locus.

Last year we reported on the characterization of

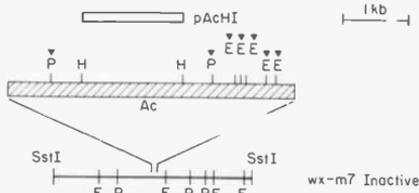


FIGURE 1 Restriction map of the cloned *wx-m7* inactive-phase allele. Restriction map of *PvuII* (P), *BstNI/EcoRII* (E), and *HindIII* (H) sites in the *Ac* and *Wx* sequence of the cloned *wx-m7* inactive allele. Hatched bars represent the *Ac* sequence and solid lines represent the flanking *Wx* sequence. The solid line below represents the *SsiI* fragment of *Wx* without the *Ac* sequence insertion. (▼) Sites that do not cleave in the genomic DNA sequence of the inactive *wx-m7* allele, yet are present in the cloned allele, indicating the presence of DNA modification at these sites.

an insertion of *Ac* at the *R* locus, isolated by I. Greenblatt. This past year, we have used *Ac* DNA probes to clone this mutant *R* allele and, with flanking probes, we have defined regions that are *R*-specific. We are presently characterizing the *R*:*standard* allele by Southern blot analysis and genomic cloning. This allele has been genetically characterized as a compound locus comprising a tandem chromosomal duplication (Fig. 2A). Each member carries an *R* component: The centromere-proximal region contains a plant (P) component that conditions pigmentation of tissues in the

plant, and the distal region contains a seed (S) component that conditions aleurone pigmentation in the kernel. Derivative *R-r* alleles obtained by displaced synapsis (Fig. 2B) and intralocus recombination have been recovered in J. Kermicle's laboratory that retain only one member of the duplication. *R-g* derivatives retain only S function and *r-r* derivatives retain only P function. We have examined the *R-r* and derivative alleles with an *R*-specific probe and have found homologous DNA fragments that correspond to the presence of S or P.

A third *R* component called leaf-color (*Lc*) has

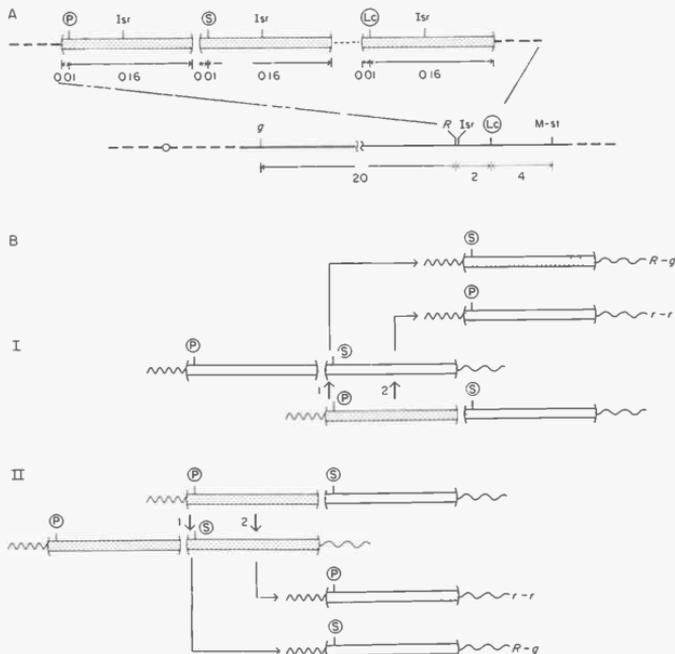


FIGURE 2 Genetic organization of the *R* locus. (A) Genetic map of the long arm of chromosome 10 of maize showing the genetic map positions of the *R* locus and flanking markers: (*g*) golden; (*Isr*) inhibitor of striate; (*Lc*) leaf color component; (*M-st*) modifier of stippled. The region that includes *R* is expanded to show the genetic fine-structure relationship of the P and S components of the *R-r*:*standard* allele. Each component is carried on a chromosome-duplicated segment (stippled area) and includes the linked marker *Isr* as part of the duplication. The position of the *R* component is calculated from the frequency of S and P derivatives obtained from *R-r* homozygotes (see Dooner and Kermicle, *Genetics* 67: 427 [1971], *Genetics* 82: 309 [1976]) derived by recombination events depicted in B. (B) Two possible synaptic configurations (I and II) of *R-r* homozygotes. Recombination in region 1 or 2 (arrows) will yield *R-g* (S) or *r-r* (P) derivatives, respectively.

also been examined and has been shown to correspond to a DNA fragment homologous to our *R* probe. These results suggest that these components, although functionally divergent, have retained significant DNA homology. They may have originated from a single progenitor *R* gene, and through a process of chromosomal duplication and DNA sequence divergence, these genes may have become independent tissue-specific regulators of anthocyanin production. We are also in the process of "walking" with each of these cloned component fragments to construct a physical map of the standard *R* locus. The reconstruction and characterization of a complete *R* locus will afford valuable insights into the mechanisms of plant gene regulation.

Transposition of *Ac* from the *P* Locus of Maize

J. Chen, S.L. Dellaporta [in collaboration with I. Greenblatt, University of Connecticut]

Maize offers an ideal system for studying somatic mutations such as transposition events, because somatic mutations can sometimes be recovered in subsequent progeny for genetic and molecular analyses. We have been investigating the transposition mechanism of *Ac* at the pericarp (*P*) locus that conditions red pigment formation in the somatic pericarp and cob tissues of the ear. Genetic studies on the transposition of *Ac* from the *P* locus indicate that *Ac* transposition may occur by excision of *Ac* from one chromatid, after replication of the *P* locus, to an unreplicated receptor site, usually on the same chromosome. Frequently observed "twinning" mutations on variegated pericarp ears (Fig. 3) can

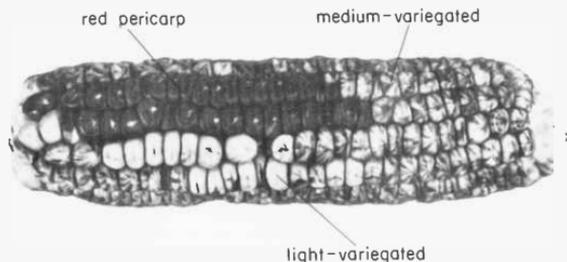
be explained by such a mechanism: subsequent replication of the receptor site along with the newly transposed *Ac* element would result in daughter cell lineages that differ in their genetic constitution at the *P* locus, yet both with a transposed *Ac* element at an identical chromosomal position.

We have initiated molecular studies of twinned mutations to test the current model for *Ac* transposition from the *P* locus. We have generated twinned mutations, and genomic DNAs have been isolated from offspring of each sector and subjected to genomic blot analysis. Analyses of *Ac* homologous restriction fragments have allowed us to identify the *Ac* element at the *P* locus and the transposed *Ac* element at the receptor site. We are currently cloning transposed *Ac* elements from this material to determine the molecular location of these elements. For molecular analysis of the donor element, we also have cloned the *P* locus using the *Ac* cloning strategy discussed in last year's Annual Report.

Cell and Developmental Genetics of Tobacco

A.C. Hiatt, R.L. Malmberg, B.A. Lowe, J. McIndoo

Mutagenesis and selection protocols for the study of plant development have recently been optimized for a variety of species. Tobacco is an especially useful plant for study because after mutagenesis it retains the ability to regenerate from cell culture into a whole plant. We have been interested in regenerating cells selected for resistance to inhibitors of polyamine synthesis. The regenerated variants have revealed numerous unusual developmental defects. Developmental defects in the flower are



common among plants that develop to maturity; however, arrested development at earlier stages is also common. The whole-plant characteristics of these polyamine synthesis variants were described in last year's Annual Report. In general, these variants have revealed that the precise regulation of intracellular polyamine titer may be a critical component in many developmental processes, including tobacco sexual development. A major drawback in the analysis of these variants has been the unavailability of meiotic genetic analysis because either the flowers are sterile or the plants do not develop to maturity. Therefore, our approach has been to explore the regulation of polyamine synthesis and degradation in both wild-type and selected mutant plants and to isolate genes that control the intracellular polyamine titer in the hope that reintroduction of these genes into the appropriate plant background will reveal something about the significance of polyamine metabolism during development.

REGULATION OF POLYAMINE BIOSYNTHESIS

Polyamine synthesis in plants is mediated by three enzymes that are all decarboxylases. Production of putrescine, the first polyamine, can come from decarboxylation of either arginine or ornithine, whereas conversion of putrescine to spermidine and then spermine depends on the decarboxylation of S-adenosylmethionine (Fig. 4). In cell cultures, these enzymes are regulated both at the level of synthesis and at the level of protein stability. Specific activities can vary by 40-fold, depending on the growth phase of the cells. The abundance of the enzymes is also developmentally regulated; where arginine decarboxylase (ArgDC) activities are high (in leaves and roots), ornithine decarboxylase (OrnDC) activities are virtually absent. OrnDC is only abundant in flowers. Addition of polyamines to cultures results in differential feedback regulation in that ArgDC activity is absent and OrnDC activity is unaffected. The kinetics of feedback regulation of ArgDC suggests that ArgDC synthesis is inhibited by exogenous polyamines. S-adenosylmethionine decarboxylase (SamDC) synthesis is also inhibited by elevated polyamine levels; however, addition of a spermidine analog, methylglyoxal-bis(guanylhydrazine) (MGBG), results in stabilization of the protein at the same time that the enzymatic activity is inhibited. Inhibition of OrnDC by difluoromethylornithine (DFMO) or

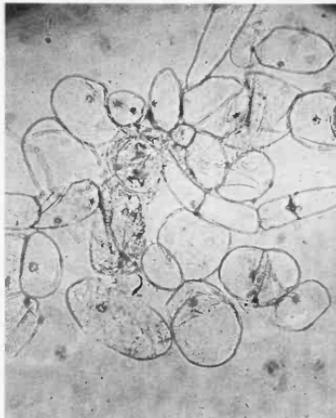
prolonged inhibition of SamDC by MGBG is lethal, whereas inhibition of ArgDC (the parallel pathway to putrescine) is not lethal under ordinary conditions.

The polyamine variants previously described are resistant to either MGBG or DFMO (Fig. 5). Most of these cell lines have not been extensively characterized biochemically but probably represent a variety of mechanisms of resistance (e.g., insensitive target enzyme, rapid detoxification, and slowed uptake of the inhibitor). They invariably have abnormal polyamine titers (either too high or too low). These observations and others have led us to conclude that regulation of the enzyme activities involved in polyamine metabolism occurs at multiple levels (e.g., protein synthesis, protein stability, and protein modification) and that coordinated control of synthesis and degradation of polyamines may be essential to a variety of developmental processes.

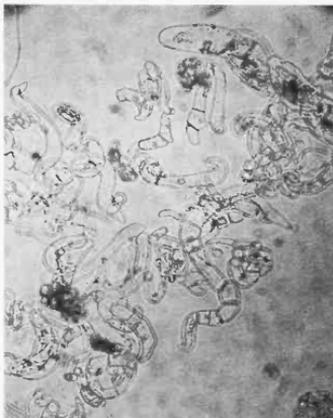
One variant resistant to DFMO has been examined in detail and has revealed multiple defects possibly due to aberrant coordinated regulation of enzyme levels. Resistance to DFMO in this variant (*Dfrr1*) is apparently due to a partially DFMO-insensitive allele of OrnDC. The overall OrnDC level in the absence of inhibitor, however, is less than 5% of the wild-type level, suggesting that both alleles are being affected. At the same time, there are very high levels of the parallel enzyme, ArgDC, which is elevated 15-fold. This variant also contains an excess of intracellular putrescine (>10 times the wild-type level) and secretes putrescine into the culture medium. Unfortunately, meiotic genetic analysis in this plant in its present state is not possible (Fig. 6). This type of analysis is essential to demonstrate that the variant does in fact contain a single mutation. We are currently trying techniques to fuse *Dfrr1* protoplasts (plant cells devoid of cell walls) to normal protoplasts in the hope that the resulting tetraploid cells will regenerate normally.

We have found that under ordinary culture conditions, ArgDC is not an essential enzymatic activity; however, it is regulated by a variety of cellular stresses, including low pH, osmotic shock, absence of potassium, and wilting. These types of stresses that induce ArgDC have been observed in shoots, whole plants, and cell cultures. We have found that during stress induction of ArgDC in culture, addition of its inhibitor (DFMA) is lethal, suggesting that the primary and essential function of ArgDC is to produce putrescine in response to these ionic stresses. We are currently exploring the physiologi-

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DFR



MGR



DFR + DFMO



FIGURE 5 Cells from wild-type or polyamine variant cultures. (DFR) DFMO-resistant cell line, (MGR) MGBG-resistant cell line, (285) wild-type.

cal effects of elevated putrescine both in wild-type cultures and in the polyamine variants.

MOLECULAR CLONING OF THE DECARBOXYLASE GENES

Our strategy for isolation of genes that control polyamine biosynthesis has relied heavily on our

understanding of the regulation of the enzymes developmentally as well as in culture. Isolation of a putative ArgDC cDNA has been accomplished by differential hybridization of potassium-stressed and normal oat-seedling RNAs to a potassium-stressed oat-seedling cDNA library. Stressed oat seedlings have very high levels of ArgDC compared with normal oat seedlings. The isolated popula-



FIGURE 6 Arrested regeneration of a tobacco cell line resistant to DFMO.

tion of cDNAs (representing potassium-regulated mRNAs) was then hybridized to RNA dot blots from cultures containing polyamines. In this way, a cDNA clone was identified that hybridized to an ionic stress-regulated RNA which was absent when polyamines were present in culture. Additional experiments have further suggested that the isolated clone contains an ArgDC cDNA. Using this cDNA, we have isolated genomic clones to study sequences responsible for its complex regulation. We have also purified the ArgDC protein and will soon obtain the amino acid sequence, which will enable the positive identification of the ArgDC gene.

We have also isolated putative OrnDC genomic clones by hybridization to a mouse OrnDC cDNA and are in the process of obtaining the amino acid sequence of purified tobacco SamDC to generate probes for gene isolation. Our primary goal will be to characterize these genes with an emphasis on understanding their complex regulation as well as the significance of this coordinated regulation during development. Our approach will be to reintroduce these genes into appropriate plants using recombinant *Agrobacterium* vectors. This will enable us to assess the developmental impact of additional copies of the genes and to explore the effects of aberrant regulation of the genes.

We have recently constructed and introduced

into tobacco the mouse OrnDC gene under the control of a constitutive plant promoter derived from the nopaline synthase gene of *Agrobacterium*. The mouse gene will enable us to distinguish between the endogenous and introduced transcripts and protein and to study the stability and developmental effects of a constitutively expressed foreign gene.

We have also begun seed mutagenesis and selection of *Arabidopsis thaliana* for resistance to MGBG. Unlike most other plants, *Arabidopsis* has a relatively small genome (~70,000 kb), which means that cloning by complementation using DNA from a resistant *Arabidopsis* plant will be technically feasible. This will eventually facilitate isolation of genes that control polyamine levels and have thus far not been characterized.

Finally, as an approach to studying the direct effects of polyamines on floral development, we have been using a recently described procedure that allows flowers to develop directly from thin cell layers in culture. This system is unique in that, unlike the situation in whole plants, the contents of the growth medium (e.g., added polyamine, enzyme inhibitors, etc.) will be taken up by the cells in culture and will directly influence floral development. Using this technique in conjunction with gene isolation and reintroduction, we hope to determine

how alterations in the regulation of polyamine metabolism can influence sexual development in plants.

Exploring the Systems of Communication between Nuclei and Chloroplasts in Plants

G. Giuliano, P. Hinton, P.A. Scolnik

There are three classes of pigments in plants: chlorophylls, carotenoids, and anthocyanins. Carotenoids are yellow or red hydrophobic pigments that are intrinsic components of the photosynthetic apparatus. Anthocyanins are water-soluble pigments ranging from pink to blue, and they are located in cytoplasmic vacuoles. Chlorophylls, carotenoids, and hydrophobic proteins are the basic components of the photosynthetic apparatus.

Carotenoids play an essential photoprotective role in all photosynthetic and many nonphotosynthetic organisms. This is necessary because light can react with chlorophyll or any other porphyrin and generate oxygen singlets, which through a free radical reaction can start a series of oxidations that result in the death of the organism. Carotenoids can prevent this reaction between chlorophyll and oxygen. These pigments are also synthesized in nonphotosynthetic tissues and are major contributors to the color of flowers and fruits.

Plants have a sensing mechanism by which they regulate the amount of chlorophyll that is formed when carotenoids are not present. At high light intensity, plants containing mutations in carotenoid biosynthesis do not accumulate chlorophyll (albino phenotype). However, at low light intensity, the risk of photooxidative reactions taking place is minimized and the plants induce chlorophyll biosynthesis and become pale green.

Ultrastructural analysis of the organelles formed under different light regimes in plant carotenoid mutants indicates that at high light intensity, proplastids, lacking lamellar structures, are formed, whereas chloroplasts with some membrane structures are accumulated at low light intensity. This conditional formation of photosynthetic membranes is not observed with other albino mutants.

Although the chloroplast contains its own genome, about 70% of the proteins are encoded by nuclear genes. We wanted to study the possibility that these genes are under a common transcrip-

tional control, which is dependent on the state of the chloroplast. The conditional formation of a photosynthetically active chloroplast in carotenoid mutants makes them an ideal system to study chloroplast-nuclei communication. We chose *Lycopersicon esculentum* (tomato) carotenoid mutants for the following reasons: (1) The tomato is easily cultivated under greenhouse or field conditions and is easily propagated by sexual or asexual means. (2) The cultivated tomato is self-pollinated, thus ensuring two important genetic properties: homozygosity and isogenicity. (3) Many genes affecting plant, flower, and fruit characteristics have been described and these lines are available for molecular studies. (4) The tomato has been used as a model system in biochemical and physiological studies, and the information obtained is now useful in designing experiments in molecular biology.

Most carotenoid mutants in tomato affect only the color of the fruit. However, the pathways of carotenoid biosynthesis in fruit and vegetative tissue seem to be identical. On the basis of this observation, we propose that there are at least two copies of each gene in the pathway affecting plant (P) and fruit (F) pigments. Since carotenoids are part of the photosynthetic apparatus, we expect the P components to be under light transcriptional control and the F system to be regulated by ethylene, the main fruit-ripening hormone.

There is one carotenoid mutation (*ghost*) that affects both vegetative and fruit tissues. This mutant was isolated in the 1950s by C. Rick at the University of California, Davis. Although it is generally mentioned as a mutant that would be interesting for the study of carotenoid biosynthesis, the fact that it is difficult to grow has prevented molecular biologists from using this system.

We obtained heterozygous seeds from the Tomato Cooperative and planted them in the greenhouse at Uplands Farm. Albino (*gh/gh*) and green (+/*gh* and +/+) plants segregated in the proportions expected for a Mendelian recessive gene. The albino seedlings become pale green under low light, indicating that this is indeed a carotenoid mutation. White plants are nonphotosynthetic, and, in general, they die within one month. However, in most *ghost* plants, we observed green clonal sectors that, according to the time of appearance in the plant, can extend from just a few cells to whole branches. The lifetime of the homozygous recessive plants is dependent on the total amount of green (photosynthetically competent) tissue present, indi-

cating that the death of the ghost plants is mainly due to the lack of nutrients and not to an intrinsic lethal effect of the mutation. Analysis of the pigment content in the green tissue revealed that both chlorophylls and carotenoids are present at wild-type levels, indicating that the ghost phenotype is somatically unstable.

We are interested in using ghost plants to study the systems of communication between nuclei and chloroplasts, the synthesis and assembly in the membrane of carotenoid pigments, and the molecular basis for the somatic instability described.

These two projects require the isolation of relatively large amounts of ghost tissue, which is difficult to achieve under normal conditions. On the basis of our observation that the green sectors can provide nutrients to the albino areas, we decided to use tissue culture to propagate selectively homozygous recessive plants containing green sectors. Seeds were germinated under sterile conditions and white plants were propagated by shoot culture. Green sectors were also observed under these conditions. By selectively cutting areas containing these sectors, we were able to obtain chimeric plantlets enriched on green tissue. These plants were then transferred to soil and grown in a growth chamber. Plants over 2 feet tall, containing approximately 60–70% white tissue, are now routinely obtained. Some branches in these plants are now flowering, and this should allow us to conduct crosses to determine whether the phenotypic reversion observed is due to a nuclear or a cytoplasmic event. In any case, using tissue culture, we have managed to obtain relatively large amounts of ghost tissue that can be used in molecular studies.

In our first experiments with these plants, we determined the levels of transcription of two nuclear genes coding for chloroplast proteins: the small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and the chlorophyll-*a/b*-binding protein. In both cases, high levels of transcription are only obtained in green tissue (Fig. 7). However, the hybridization signals obtained for two unrelated nuclear sequences are similar between green and white tissue (not shown), suggesting that the transcriptional turn-off affects mostly nuclear sequences coding for chloroplast proteins. We have also translated poly(A)⁺ RNA in a rabbit reticulocyte system, and the overall pattern obtained is very similar in the two types of tissue. Experiments are now being conducted to determine whether the differences observed are due to the existence of a



FIGURE 7 mRNAs for the genes coding for the Rubisco small subunit and chlorophyll-*a/b*-binding protein are not detected in white ghost tissue. Homozygous recessive plants were propagated through tissue culture, and white material and green material from the same plant were isolated. Poly(A)⁺ mRNA was extracted and analyzed in a Northern blot probing with cDNAs corresponding to both genes. (Lanes 1,3) Green tissue; (lanes 2,4) white tissue. Lanes 1 and 2 were probed with the Rubisco gene and lanes 3 and 4 were probed with chlorophyll *a/b* sequences.

regulatory mechanism by which the chloroplast and the nucleus communicate.

We have also examined by electron microscopy the cellular structure of both white and green tissues. In green tissue, normal chloroplasts are formed, and the general architecture of the cells is very similar to that of the wild-type material. In white tissue, however, the cellular vacuole is missing and proplastids are accumulated. Proplastids are organelles that have no photosynthetic membranes but can differentiate into chloroplasts if photosynthetic tissue is formed. Electron microscopy work was kindly arranged by I. Greenblatt (University of Connecticut).

From these studies, it is becoming increasingly clear that the *ghost* mutation arrests the development of chloroplasts by affecting either the synthesis or assembly of carotenoids, and this results in an albino phenotype and the turning off of, if not all, nuclear transcripts for chloroplast proteins. This mutation either reverts or is suppressed at relatively high frequency, and this results in the proplastids becoming fully developed chloroplasts and, in general, the white tissue becoming wild type. The molecular analysis of the *ghost* locus should provide a wealth of information about nuclear-cytoplasm interactions and the role of organelles in plant development.

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SITE-SPECIFIC MUTAGENESIS

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Protein Structure and Function

M. Zoller, K. Johnson, L. Levin, R. Kostriken

Our laboratory is interested in the manner in which proteins specifically interact with macromolecular substrates. The study of protein structure and function has been approached over the past 30 years using tools such as group-specific modification of amino acids, affinity-labeling, substrate analogs, limited proteolysis, synthetic proteins, nuclear magnetic resonance, and X-ray crystallography. Molecular biology has added a new dimension to the study of proteins by providing a means to synthesize and express a protein with any amino acid sequence desired. Proteins with primary sequences differing by only a single amino acid at a predetermined position can now be easily constructed by oligonucleotide-directed mutagenesis. Extremely sophisticated questions can now be asked about the structure and function of a protein with regard to the role of a particular amino acid, the location of a substrate-binding pocket, and the region of protein-protein interaction. In addition, these powerful techniques have spawned a renaissance in the field of protein crystallography and structure determination.

We are studying two types of protein-macromolecule interactions: (1) protein-protein interactions of yeast cAMP-dependent protein kinase and (2) protein-DNA interactions of yeast *HO* endonuclease. 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. In addition, we have begun to study one class of proteins (protein kinases) by site-directed mutagenesis.

Our approach is to construct site-directed amino acid substitutions within the protein and to study their effects in vitro using the *E. coli* expression systems and in vivo in yeast. Since none of the proteins we are currently studying have been crystallized, mutagenesis can at best yield general information, such as the location of domains for substrate binding or regions of protein-protein interactions. To obtain a more-detailed understanding of the structure of these proteins, we hope to produce enough pure protein to collaborate with the newly formed X-ray crystallography group.

Protein-Protein Interactions: cAMP-dependent Protein Kinase

M. Zoller, K. Johnson, L. Levin

cAMP-dependent protein kinase (cAMPdPK) has been shown to be part of a family of protein kinases that include cGMP-dependent protein kinase, protein kinase C, and the tyrosine-specific protein kinases of insulin and EGF receptors, and in a number of transforming viruses. cAMPdPK is ubiquitous in eukaryotes. Under conditions of low cAMP concentrations, cAMPdPK exists as an inactive holoenzyme, composed of two catalytic (C) subunits and two regulatory (R) subunits. When cAMP concentrations are increased by some stimulus, cAMP binds to the R subunits, thereby releasing two active C subunits that function to phosphorylate protein substrates on either serine or threonine residues. Substrates show a consensus of serine or threonine residues preceded by two basic residues (e.g., Arg-Arg-X-Ser-X). Phosphorylation serves to regulate the activity of enzymes in the cAMP control pathway. The best-studied system is the regulation of glycogen breakdown in muscle and liver. cAMPdPK activates the enzyme phosphorylase kinase, which in turn activates the enzyme phosphorylase and results in the breakdown of glycogen. cAMPdPK also phosphorylates glycogen synthetase, which results in a reduction of its activity. Little is known about the substrates in yeast.

The genes for the C and R subunits from yeast have been cloned and sequenced by T. Toda and S. Cameron of M. Wigler's group (Molecular Genetics of Eukaryotic Cells Section). There are three closely related genes for the C subunit and one for the R subunit (there may be another R subunit gene). Comparison of the predicted amino acid sequences for the yeast proteins and the mammalian proteins revealed a high degree of sequence similarity, as high as 75% in some regions. Biochemical experiments are under way to demonstrate that the yeast proteins are functionally homologous to the mammalian proteins.

CATALYTIC SUBUNIT (TPK1-3 GENES)

The C subunit carries out the specific phosphorylation of protein substrates. It is important to study the C subunit to learn how it interacts with its substrates, to study how it is regulated, and to un-

derstand the structural differences between the serine/threonine- and tyrosine-specific protein kinases. We hope to identify the specific contacts between the C and R units and use this information to develop an R subunit for a tyrosine kinase.

By using conventional protein-modification techniques, two regions of the primary sequence have been linked to functional domains. One is the ATP-binding site. Affinity-labeling studies by M. Zoller and S. Taylor identified a particular lysine residue in the mammalian protein that coordinates the terminal phosphate of ATP in the active site. The second region is thought to be involved in the binding of protein substrates. A particular cysteine residue was protected from alkylation in the presence of a peptide substrate analog. Both of these residues are conserved in the yeast *TPK* proteins.

A second method to identify functional domains of the protein has been through comparisons with other protein kinases such as cGMP-dependent protein kinase and the tyrosine-specific kinases from transforming viruses (e.g., *src*, *fps*, *erb-B*, *fes*, and *abl*). Such an analysis has revealed limited sequence homology throughout the protein. Thus, these kinases are related both structurally and functionally. The residues in common are thought to define the basic "kinase" activity, such as ATP and Mg²⁺ binding and transfer of the phosphoryl group to a specific substrate. Just as important are the regions of dissimilarity. One region must be involved in the means by which each of these kinases interacts with and discriminates between its substrates.

RESULTS

Expression of TPK1 (Yeast C Subunit) in E. coli. Using the T7 expression system, we produced *TPK1* in *E. coli*. In crude extracts, the *TPK1* protein comprised about 10% of the cellular protein; however, over 95% of the protein was insoluble. This is a common result of expression of heterologous proteins in *E. coli*. The protein is found in insoluble aggregates called inclusion bodies. Experiments aimed at solubilization of the protein revealed that only urea, guanidine, or SDS would release the protein in a soluble form. Triton and another detergent (LDAO) were ineffective in solubilizing *TPK1* protein, although these reagents released associated *E. coli* proteins. When the urea, guanidine, or SDS was dialyzed away, the protein precipitated. The characteristics of a protein responsible for insoluble

bility are not known but have been under intensive investigation by biotechnology companies.

TPK1 Encodes a Protein Kinase. The soluble fractions of *E. coli* extracts were assayed for protein kinase activity. Initially, we used a standard phosphotransferase assay in which the γ -phosphate from ATP is incorporated into histone. Extracts from cells containing the T7 vector alone without an insert showed a weak phosphotransferase activity. We then switched to a more specific assay, using a synthetic peptide as a substrate instead of histone. This lowered the background significantly. Only cells in which the *TPK1* gene was expressed exhibited phosphotransferase activity. No activity was observed in extracts from the control cells or from cells in which *BCY1* (R subunit) was produced (see below).

Additional evidence that *TPK1* was responsible for this activity came from analysis of a site-directed mutation we constructed in the ATP-binding site of *TPK1* (see below). This mutation has been shown to reduce the kinase activity of a number of viral oncogene tyrosine kinases. Extracts from cells containing the mutant protein showed insignificant phosphotransferase activity.

TPK1 Complexes with the Yeast R Subunit (BCY1) In Vitro. The purified cAMP-binding subunit (*BCY1*) was added to extracts containing *TPK1*. Addition of *BCY1* resulted in the inhibition of phosphotransferase activity. In the presence of cAMP, phosphotransferase activity was indistinguishable from *TPK1* activity alone. This result suggested that the *TPK1* protein was capable of forming a functional complex with *BCY1*.

Probing Functional Domains by Site-directed Mutagenesis: Lys-116 to Arg-116 Eliminates Catalytic Activity. We have begun to study the role of specific amino acids in several regions of the protein, and we are interested in amino acids that have been conserved throughout the kinase family. The first mutation we made was within a region thought to play a role in ATP binding in all kinases of this family.

Affinity-labeling experiments localized Lys-72 in the mammalian C subunit near the terminal phosphate of ATP. The analogous lysine (amino acid 116) of *TPK1* was changed to an arginine, thus maintaining the charge but changing the overall shape of the side chain at this position. As de-

scribed above, our initial experiments indicated that the protein was inactive, which is consistent with the results of similar experiments on the tyrosine kinases encoded by *v-fps* and *v-src*. We will now purify this protein and compare its ability to bind ATP and its ability to complex with the R subunit in vitro. In addition, we will assess whether this protein is functional in vivo.

FUTURE EXPERIMENTS

1. Purification of *TPK1*: Initially, we will try to purify the soluble *TPK1* produced in *E. coli*. This will not be as easy as the purification of *BCY1* (discussed below), but through the use of conventional column chromatographic procedures as well as ATP-affinity columns, purification should be feasible. In addition, we may be able to construct an affinity column using purified *BCY1*. *TPK1* could be efficiently bound to the column and specifically released by addition of cAMP to the column.
2. Production of antibodies to *TPK1*: The insoluble protein serves as an easy way to purify large quantities of protein to be used as antigen. We are using both gel-isolated protein and urea- or SDS-solubilized preparations from the insoluble fraction as antigen.
3. Production of *TPK2* and *TPK3* in *E. coli*: We are interested in determining the difference between the various *TPK* gene products. The three genes differ primarily at their amino terminus. Antibodies will be produced against *TPK2* and *TPK3* as well. It may be possible to obtain specific monoclonal antibodies for each of the proteins. These proteins may be expressed in a soluble form due to their structural differences.
4. Probing structure-function relationships: We will continue to locate important regions of the protein by making amino acid replacements by both site-directed and random approaches. Each protein will be analyzed in vitro as well as in vivo. Through such an approach, it may be possible to obtain temperature-sensitive proteins to be used in vivo. Site-directed mutations will be aimed at regions of sequence identity between the serine-specific kinases (e.g., cAMPdPK) and the tyrosine-specific kinases (e.g., *src*, *fps*, EGF receptor, and insulin receptor). We believe that these conserved regions define general kinase functions. Just as important are the regions of nonhomology between these two classes of ki-

nases. These regions are probably involved in the interaction with specific substrates and effector proteins. We will identify important amino acid residues by a strategy of random mutagenesis.

5. An R subunit for tyrosine kinases? Peptide substrate analogs of cAMPdPK have been synthesized that inhibit kinase activity. These bind very tightly to the C subunit. An analogous set of peptides have been synthesized for tyrosine kinases; however, these bind with low affinity. It may be possible to modify the R subunit from cAMPdPK so that it tightly binds to and inhibits tyrosine kinases. The R subunit contains a sequence that resembles substrates for cAMPdPK. By replacing these amino acids with analogs of tyrosine kinase substrate/inhibitors, the activity of these kinases may be regulated by cAMP.

REGULATORY SUBUNIT (*BCY1* GENE)

The function of the R subunit is to regulate (inhibit) the action of the C subunit. Upon binding cAMP, the C subunits are released to phosphorylate substrates. Once the cAMP concentration of the cell is reduced, the C and R subunits reassociate to form the inactive holoenzyme. The mechanism by which the C subunits are released and then recaptured is not known. Furthermore, cAMP binds to the R subunit with a K_d of approximately 10^{-7} M. It is not clear how cAMP is removed from the R subunit in order to reassociate with the C subunit. The R subunit in mammals exists as a dimer. Each subunit has two binding sites for cAMP, which can be distinguished using analogs of cAMP. The role of this apparent gene duplication is not known. Recently, R. Jungmann and co-workers reported that the purified R subunit from mammalian liver demonstrated topoisomerase activity. Perhaps the R subunit has some unknown function such as regulation of gene expression through this activity.

A gene from yeast encoding a protein with many regions of sequence identity with the R subunit of mammalian cAMPdPK was cloned and sequenced by T. Toda and S. Cameron as a gene that complemented the *bcy1* mutation. Comparison with the mammalian protein sequence identified it as the R subunit, similar to the mammalian "type II" R subunit. This was confirmed by comparison with peptide sequence data of the purified yeast R subunit in collaboration with J. Scott and E. Krebs (Howard Hughes Medical Research Institute, Seat-

tle, Washington). There may be a second R subunit gene in yeast, since some of the peptides from the yeast R subunit could not be positioned within the predicted *BCY1* sequence.

A general identification of the functional domains of the mammalian R subunit has been made through the use of affinity labeling, limited proteolysis, and sequence comparisons with CAP protein from *E. coli*. The major functional domains can be characterized as follows:

1. cAMP-binding domain(s): Comparison with the *E. coli* CAP protein has revealed regions of sequence identity. This identified amino acids that might be responsible for cAMP binding. In addition, in the eukaryotic cAMP-binding proteins, this putative binding region has been duplicated. Biochemical analysis supports this finding, since each monomer binds two molecules of cAMP.
2. C subunit-R subunit interaction sites: It is not known what parts of the C and R subunits interact to form the inactive holoenzyme. Somehow this tight interaction is broken upon binding cAMP.
3. R-R dimerization region: Limited proteolysis has revealed that the amino terminus is responsible for dimerization of the R subunits. An aminoterminal 16,000-dalton fragment of the mammalian protein still retains a dimeric form. Nothing is known about the specific amino acids responsible for this protein-protein interaction.
4. Phosphorylation site: The type II R subunit and the yeast (*BCY1*) R subunit contain a sequence that is recognized and phosphorylated by the C subunit. This region bears the substrate consensus sequence (Arg-Arg-X-Ser-X) for serine protein kinases. The role of this phosphorylation has never been clearly determined. This sequence may play a role in C-R interaction as described above in 2.
5. Topoisomerase activity: R. Jungmann and co-workers have presented data that the type II R subunit from bovine liver contains a topoisomerase activity that is dependent on phosphorylation. There is no direct evidence that the R subunits have a DNA-binding domain.

RESULTS

Expression of BCY1 in E. coli. Using the T7 expression system, we produced the *BCY1* protein in

E. coli. The protein was in the soluble fraction, in contrast to the C subunit (*TPK1*). The level of the protein in crude extracts was approximately 5% of the total protein.

Purification of BCY1 from *E. coli* Extracts. Using a standard procedure for purifying cAMP-binding proteins, we purified *BCY1* in a single step to about 90% purity by means of an agarose-cAMP affinity column. The protein was released from the column with cAMP. This procedure yielded approximately 1 mg of purified R subunit per liter of *E. coli*. It should be possible to obtain a slightly better yield of 2–3 mg/liter. To characterize the protein further, the bound cAMP was removed by rapid treatment with urea.

Characterization of Purified BCY1. Simply by binding the protein to the agarose-cAMP column demonstrated that the expressed protein exhibited a cAMP-binding function, as predicted. Using a cAMP-binding assay, we determined that the protein bound 2 moles of cAMP per monomer, with a K_d of 10^{-7} M. This is similar to the results obtained with the mammalian protein. Next, we showed that the purified protein functioned to inhibit the C subunit of bovine cAMPdPK in the absence of cAMP but not in its presence. The purified protein could be stoichiometrically phosphorylated by the bovine C subunit. This is found with the so-called “type II” R subunit in mammals as well. Finally, we demonstrated that the purified protein could inhibit the yeast C subunit (*TPK1*) from crude *E. coli* extracts. This suggested that *TPK1* and *BCY1* can interact at least in vitro, and probably in vivo as well.

FUTURE EXPERIMENTS

1. Topoisomerase activity: R. Jungmann and co-workers reported that the purified R subunit from mammalian liver demonstrated a topoisomerase activity. Furthermore, the activity required phosphorylation by the C subunit. We are interested in testing whether the yeast protein contains this activity as well.
2. Production of antibodies against the *BCY1* protein.
3. Probing structure-function relationships: We will begin to construct mutations within the *BCY1* protein and to test the effects both in vitro and in vivo.

4. Large-scale purification: We will try to purify large quantities of the protein to begin crystallization experiments.

Cloning the Type II R Subunit of the cAMP-Dependent Protein Kinase from Rat Skeletal Muscle

M. Zoller, D. Helfman [in collaboration with J. Scott and E.G. Krebs, Howard Hughes Medical Research Institute, Seattle, Washington]

We have cloned a cDNA that encodes the type II regulatory subunit from rat skeletal muscle. The cloning was done using a 47-nucleotide-long nondegenerate oligonucleotide probe based on the protein sequence from the bovine regulatory subunit and general codon bias of rat proteins. The use of long nondegenerate probes appears to reduce the isolation of “false positives” often found when using short degenerate oligomers.

Cloning and Sequencing of the *CDC25* Gene from *Saccharomyces cerevisiae*

L. Levin

The role of cAMP in controlling many cellular metabolic processes has been shown in both eukaryotes and prokaryotes. The apparent mediator of its effects, in eukaryotes, is the phosphorylation of cellular proteins by cAMP-dependent protein kinase (cAMPdPK). The requirement of cAMP for growth can be seen in the yeast *Saccharomyces cerevisiae*, where many cAMP-requiring mutants have been isolated.

The use of *S. cerevisiae* in understanding the specifics of cAMP control of growth has proved fruitful for two reasons. The first reason is the ability to isolate and utilize mutants in various steps along the pathway, and the second is the apparent similarity of those steps to what is known to exist in higher eukaryotes. The evidence for the relatedness of many cellular processes in yeast and in higher eukaryotes is extensive.

In yeast, cAMP has been postulated to be involved in the mitosis-meiosis decision. The cell-cycle mutations *cdc25* and *cdc35* have also been shown to affect the choice between mitosis and meiosis. Among the cell-cycle mutations affecting

"start," only these two have been shown to be able to undergo meiosis. In fact, homozygous diploids for either of these mutations sporulate at the restrictive temperature, even in rich media. The correlation of one of these two cell-cycle mutants with the cAMP pathway has already proved correct, since *CDC35* has been shown to encode the gene for adenylate cyclase.

In light of the apparent connection between *CDC25* and the cAMP regulatory pathway, we are attempting to determine the function of the *CDC25* gene. In conjunction with M. Wigler's laboratory, we isolated two distinct genes that were able to complement the temperature-sensitive *cdc25-1* allele. I isolated the gene encoding *CDC25* and T. Toda isolated *TPK1*, which showed protein sequence homology with the catalytic subunit of mammalian cAMPdPK. The ability of a catalytic-subunit-like gene to complement *cdc25-1* is further evidence for the association of this gene and the cAMP growth control pathway.

RESULTS

Cloning *CDC25*. The wild-type gene was isolated by complementation of the temperature-sensitive allele, *cdc25-1*, from a library of yeast DNA cloned into a 2I-base plasmid. The library was introduced into the *cdc25* strain by transformation of spheroplasts, and transformants were selected as leucine auxotrophs. Clones of cells containing the *CDC25* gene were selected by their ability to grow at the restrictive temperature of 37°C. Plasmid DNA was recovered from total yeast DNA by its ability to transform *Escherichia coli* to ampicillin resistance. Restriction enzyme analysis showed there to be two independent isolates of a single insert. It was shown to be allelic to the temperature-sensitive allele by integration mapping (work done by T. Toda). Subsequent subcloning experiments showed that a 2-kb *BglII-SphI* fragment from the original 6-kb fragment was able to rescue the temperature-sensitive phenotype.

Sequencing. A 2.6-kb *EcoRI-SphI* fragment that contained the 2-kb *BglII-SphI* complementing fragment was subcloned into the M13 vectors mp18 and mp19. Subfragments were also cloned into M13 using the *BglII*, *PstI*, *BamHI*, and *HindIII* restriction sites. The complete nucleotide sequence was determined, in both strands, by dideoxy sequencing. A large open reading frame extended through-

out the entire 2.6-kb subcloned region. Adjoining restriction fragments from the original 6-kb insert were subcloned and sequenced until the ends of the open reading frame were reached. At the 5' end, first the 1.6-kb *BglII-BglII* fragment was subcloned into M13. The nucleotide sequence still showed an open reading frame; therefore, the overlapping 1.5-kb *SalI-EcoRI* fragment was subcloned and partially sequenced. At the 3' end, the 1.3-kb *HindIII-SalI* fragment containing the vector-insert border was subcloned and partially sequenced. Synthetic oligonucleotide primers (usually 15 nucleotides long) were synthesized in order to complete the sequence through an entire M13 clone. In all, 29 primers were utilized. To determine the sequence of the second strand for the 5' end of the gene, unidirectional deletions were constructed in the M13 clones to allow sequencing through the entire clone without the aid of a synthetic primer. The open reading frame covered almost 5 kb and could code for a protein of 1589 amino acids with a predicted molecular weight of 179,121.

A computational matching program was used to compare the deduced amino acid sequence with data bases of known protein sequences for any possible homologies. No significant homologies were found. A hydrophobic profile was also constructed, with no obvious relationship to known protein structures found.

Characterization of Transcripts. The gene was shown to code for an RNA of approximately 5.3 kb by Northern blot analysis. Polyadenylated RNA was selected from wild-type strains of yeast containing YEp*CDC25.56* (one of the original complementing clones) or YEp13 (the vector without an insert) by passing total RNA over oligo(dT)-cellulose. The poly(A)⁺ RNA was separated on the basis of size on an agarose gel containing formaldehyde and blotted to nitrocellulose. The immobilized RNA was probed with nick-translated restriction fragments from the *CDC25* gene.

The 5' end of the putative transcript was mapped using both S1-nuclease and primer-extension analyses. S1 protection was carried out using a 430-bp *HinfI-SalI* fragment labeled at the *HinfI* site by phosphatase treatment, followed by kinasing with [γ -³²P]ATP. This probe was hybridized with either poly(A)-selected or total RNA isolated from the *cdc25ts* strain harboring the complementing plasmid. Subsequent digestion with S1 nuclease yielded three major protected fragments. The approximate

sizes of the protected fragments were 240, 260, and 270 nucleotides, corresponding to positions -130, -150, and -160 from the putative initiation codon.

Primer-extension analysis was done using two synthetic oligonucleotides of 24 and 25 nucleotides each. The oligonucleotides were labeled using T4 polynucleotide kinase and [γ - 32 P]ATP and annealed to the same RNA isolates as in the S1 experiments. Extension of the primers with AMV reverse transcriptase yielded cDNA that, when run alongside dideoxy-sequencing reactions using the same oligonucleotide, corresponded to the same positions as in the S1 data. Both experiments were repeated using RNA extracted from a number of *CDC25* strains of yeast, and although the signals were much weaker due to the presence of only the single genomic copy of the *CDC25* gene, the sizes of the cDNAs and protected fragments corresponded to those of the overexpressing strains.

FUTURE EXPERIMENTS

Our future experiments will follow a biochemical approach toward the understanding of the function of the *CDC25* gene product:

1. Subcellular localization: To determine the cellular localization of the gene products, indirect immunofluorescence will be used. A set of gene fusions using the *lacZ* gene will be constructed and introduced into yeast. Antibodies against the β -galactosidase portion will be used to visualize the fused gene in situ. Antibodies generated against the *CDC25* gene product itself will also be used to substantiate these results. They will be made by purifying the *CDC25* gene product from a strain of *E. coli* engineered to produce it and introducing this pure protein into mice to make monoclonal-antibody-producing cell lines.
2. Posttranslational modification: The *CDC25* gene product will be examined in vivo for any posttranslational processing, such as phosphorylation, glycosylation, and specific proteolytic cleavages. This will be done by immunoprecipitation of the protein from yeast cells labeled with either [32 P]phosphate or [35 S]methionine. Using two-dimensional gel electrophoresis, we will compare the protein produced in yeast with the protein synthesized in *E. coli*.
3. Complexes with other proteins: Immunoprecipitation experiments may help to identify

other proteins that interact directly with the *CDC25* gene product. Any other proteins that are immunoprecipitated with antibodies against the *CDC25* protein may be complexed with it in vivo.

4. Mammalian homolog: We will use the cloned *CDC25* gene as well as antibodies against the gene product to search for a mammalian equivalent to the *CDC25* gene product.
5. Genetic characterization: We will continue genetic studies in collaboration with M. Wigler's laboratory. Specifically, we will randomly mutagenize the *CDC25* gene, transfer the mutagenized gene into yeast, and screen for new temperature-sensitive mutants. This will help to localize functional domains within the *CDC25* protein itself and to identify other interacting proteins.

DNA-Protein Interactions

M. Zoller, K. Johnson, R. Kostriken

The *HO* endonuclease is a protein that is involved in the transposition of specific DNA sequences termed mating-type switching. This enzyme functions at a very early stage of the process by creating a break in the chromosome in the *MAT* gene. The specific phosphodiester bonds that are cleaved were identified by R. Kostriken and F. Heffron. Our laboratory has studied the interaction between the *HO* endonuclease and its recognition sequence by site-directed mutagenesis. Such an analysis identified a core of four nucleotides in this region that could not be altered for proper recognition by the *HO* endonuclease. In addition, we found seven other nucleotide positions that affected cleavage but did not abolish it.

Currently, our attention has turned toward an understanding of the protein. As in the study of cAMP-dependent protein kinase, we hope to obtain a three-dimensional structure for the *HO* endonuclease. Specifically, we would like to determine how this type of protein recognizes and cleaves DNA, what regions of the protein are important for binding and catalysis, and what regions (if any) play a role in the localization into the nucleus. In addition, we are interested in understanding how the mutants we previously described are discriminated from wild type. Our efforts this year

have been focused on overexpression of the protein in *Escherichia coli*. Although it has been possible to produce detectable levels of protein, the expressed protein represents less than 0.5% of the total cell protein. At present, we are searching for expression systems that result in increased yields. High expression of the *HO* endonuclease in *E. coli* may in fact be lethal.

Site-specific DNA Binding: Antennapedia Gene Product

R. Kostriken

Homeotic mutants, i.e., mutants in which one part of an organism is transformed into the semblance of another part, have been studied extensively in *Drosophila melanogaster*. Such mutations demonstrate that the organization of pattern can be altered by relatively minor genetic changes and that small changes in regulatory functions may be more important than small changes in structural components in the evolution of an organism. Homeotic mutants are thought to identify "master-switch" genes that control the selection of developmental pathways. An attractive hypothesis based on preliminary experiments in a number of laboratories is that the protein products of the homeotic genes function as modulators of gene transcription through sequence-specific interaction with DNA.

Four of the approximately 15 known *Drosophila* homeotic genes reside in the Antennapedia complex. The Antennapedia complex has been studied extensively using genetic and developmental methods. One of the genes in this cluster is Antennapedia (*Antp*), a homeotic gene in which dominant mutations have been observed to transform antennae into legs. Molecular analysis of cDNA clones has revealed that *Antp* shares homology with other homeotic genes, as well as a gene required for compartmentalization, engrailed, and a segmentation gene, fushi tarazu. DNA sequence analysis has shown that these genes share homology in a protein-coding region of 60 amino acids. The 60-amino-acid protein sequence is referred to as the homeo domain. The homeo domain is also encoded by genes found in higher vertebrates, including humans.

The homeo domain is very basic, which suggests that it may be involved in nucleic acid interactions.

Support for this idea comes from the homology of part of the homeo domain with the DNA-binding domains of certain bacterial proteins. The structures of three site-specific DNA-binding proteins have been determined; in each case, the part of the protein that contacts the DNA is composed of two α helices joined together by a sharp turn. Certain amino acids are required in particular framework positions in the structure. The homology between a 20-amino-acid part of the homeo domain and the bacterial helix-turn-helix motif is in the framework residues. Additional support for nucleic acid binding comes from immunolocalization studies of homeo-domain-containing proteins in embryos and imaginal disks which show that the antigen is located exclusively in the nuclei. Recently, it has been shown that a bacterially produced fusion protein between *E. coli* β -galactosidase and the homeo domain of engrailed exhibits site-specific DNA binding. Our efforts have focused on elucidating the mechanism whereby the *Antp* gene product acts to control tissue determination.

RESULTS

Expression of the Antp Protein in E. coli. We have constructed a bacterial expression vector that directs the synthesis of the *Antp* gene product in *E. coli*.

Purification of the Antp Protein. The *E. coli* *Antp*-expressing strain has proved to be an excellent source of the *Antp* protein, thereby enabling us to purify it and to study its biochemical properties in vitro.

Site-specific DNA Binding. The *Antp* protein exhibits a substantial affinity for double-stranded DNA, a characteristic shared by bacterial regulatory proteins known to act as transcriptional regulators. There is genetic evidence which suggests that the *Antp* gene product is capable of positively regulating its own expression. We have found high-affinity binding sites for the *Antp* protein 5' to the transcriptional start sites of two Antennapedia transcription units. We are presently characterizing these sites in order to determine if they play a part in the tissue-specific regulation of this gene.

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Cytochrome c_2 Is Not Essential for Photosynthetic Growth of *Rhodospseudomonas capsulata*

F. Daldal, S. Cheng

Cytochrome c_2 is an electron carrier common to the photosynthetic and respiratory machinery of *Rhodospseudomonas capsulata*. It is structurally homologous and functionally analogous to the cytochrome *c* of mitochondria and to the plastocyanin of chloroplasts. In bacterial photosynthesis, cytochrome c_2 is considered to be the primary electron donor to the reaction center. Equally, during respiration, it donates electrons to the cytochrome oxidase. Thus, this evolutionarily well-conserved heme protein interacts with the components of the photosynthetic reaction center, of the ubiquinol: cytochrome c_2 oxidoreductase, and of the cytochrome oxidase. These cytochromes have been well studied biochemically, spectroscopically, and immunologically, but their genetics is still scarce. Prior to this work, no mutant solely defective in cytochrome c_2 was available from a photosynthetic organism, and the *in vivo* role of c_2 in photosynthesis remained undefined.

To isolate a c_2^- mutant, we first cloned the structural gene of c_2 using synthetic mixed oligonucleotide probes and then determined its nucleotide sequence. The deduced amino acid sequence was found to be in perfect agreement with the known amino acid sequence obtained from the purified c_2 protein. Moreover, the nucleotide sequence showed the presence of a 21-amino-acid hydrophobic sequence, located at the aminoterminal part of c_2 and highly analogous to prokaryotic signal sequences. Thus, the c_2 protein is likely to be synthesized as a precursor which is processed during its secretion to the periplasm. We have also shown by appropriate genetic crosses that the cloned c_2 gene is expressed in *R. capsulata*. The control region of c_2 and the regulation of its expression are now under study using operon fusions between the c_2 and the β -galactosidase genes.

Insertion and insertion-deletion mutations in the c_2 gene were constructed *in vitro*. They were then used to replace the chromosomal wild-type allele via homologous recombination by genetic crosses mediated by the "gene-transfer agent." In these mutants, the disruption of the c_2 gene was confirmed by Southern analysis, and the absence of the c_2 gene product was proved by immunoprecipitation

and by reduced-oxidized difference spectrum analysis. c_2 mutants are stable and can grow well by photosynthesis. Thus, considering that during photosynthesis, cytochrome c_2 is the primary electron donor to the reaction center, these mutants show that in the absence of c_2 , *R. capsulata* must have alternative way(s) of reducing the oxidized photosynthetic reaction center.

Study of a c_2^- mutant (in collaboration with R. Prince, Exxon Corporation) by flash spectroscopy indicated that in the absence of c_2 , the electron donor to the reaction center is a membrane-bound c -type cytochrome with a redox mid-potential of approximately 350 mV. This electron donation is very rapid (<100 μ sec) but only equal to approximately one fifth of that observed in the wild type. Comparison of whole cells, spheroplasts, and chromatophores of the wild type and the c_2 mutants indicates that the rereduction of the reaction center may well be mediated by the cytochrome c_1 of the bc_1 complex. Even though the amount of the electron transfer between the bc_1 complex and the reaction center seems low in the absence of this cytochrome, the fact that c_2^- mutants can grow as well as a wild-type strain when high light intensity is available indicates perhaps that the photosynthetic machinery is highly overengineered.

Characterization of MT113, a Nonphotosynthetic Mutant of *Rhodopseudomonas capsulata* Lacking a Functional bc_1 Complex and c -type Cytochromes

E. Davidson, F. Daldal

MT113, a nonphotosynthetic mutant of *Rhodopseudomonas capsulata*, not mapping in the known cluster of photosynthetic-machinery-related genes, was isolated by Marrs et al. (*FEBS Lett.* 113: 289 [1980]) and was shown by spectroscopic analysis to lack cytochrome c_2 . With the later establishment that cytochrome c_1 , a component of the bc_1 complex, also absorbs at the same wavelength (550 nm) as c_2 , it became clear that MT113 also lacks cytochrome c_1 . Therefore, we decided to ascertain, in collaboration with R. Prince (Exxon Corporation) and B. Marrs (duPont Corporation), whether other

components of the bc_1 complex were also missing in this mutant.

Electron paramagnetic resonance (EPR) spectroscopy indicated that membranes derived from MT113 contained the Rieske Fe-S center at a much lower amount (<10%) than the wild-type membranes. This lack of Rieske Fe-S center cannot be attributed to a general defect in the synthesis of Fe-S clusters, since the same mutant contains an almost normal amount of total Fe-S clusters. Similarly, on the basis of EPR spectroscopy, an antimycin-sensitive signal characteristic of the anionic semiquinone Q_2^- was not detectable in MT113 membranes. Absorption spectroscopy showed that even though an almost normal amount of b -type cytochromes were present in this mutant, the antimycin-induced red shift of the cytochrome b_{560} of the bc_1 complex was absent.

Analysis of the MT113 membrane proteins by nondenaturing polyacrylamide gels (LDS-PAGE), coupled to a heme-specific staining procedure, indicated a general lack of c -type cytochromes. To determine whether the lack of a functional bc_1 complex was related to the absence of specific apoproteins corresponding to the bc_1 -complex components, cell extracts were submitted to Western analysis using antibodies raised against purified cytochrome c_2 of *R. capsulata* and cytochrome b , cytochrome c_1 , and Rieske Fe-S proteins of *Rhodopseudomonas sphaeroides*. The apoprotein of c_2 was not detected, but those of the bc_1 -complex components were present, although at a lower level, suggesting that MT113 was able to synthesize the analyzed bc_1 -complex components but was most likely not able to assemble them as a functional complex. Genetic analysis using the gene-transfer agent of *R. capsulata* and several plasmids carrying the structural genes for cytochrome c_2 and for bc_1 -complex components (Rieske Fe-S protein, cytochrome b , and cytochrome c_1) indicated that the single pleiotropic mutation in MT113 is not located in these genes. The nature of this mutation is still unknown, but a general lack of c -type (but not b -type) cytochromes in MT113 suggests that it could be related to the late steps of c -type cytochrome biosynthesis.

Interestingly, another c -type cytochrome, c' , as detected by CO difference spectroscopy, seems to be present at a low level. The possibility that this mutant synthesizes a b -type c' cytochrome with a noncovalently bound heme group is now under study.

Isolation of the Structural Genes for Rieske Fe-S Protein, Cytochrome *b*, and Cytochrome *c*₁, All Components of the *bc*₁ Oxidoreductase of *Rhodospseudomonas capsulata*

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

The ubiquinol:cytochrome *c*₂ oxidoreductase or the *bc*₁ complex is a membrane complex common to photosynthetic and respiratory electron-transfer chains of *Rhodospseudomonas capsulata*. It is made up of at least a Rieske Fe-S protein, a *b*-type cytochrome, and a *c*-type cytochrome. Models for electron-transfer pathways between the components of this complex (based on spectroscopic, biochemical, and immunological data) exist, but the assessment of these pathways by genetic approaches has not yet been carried out.

To study at the molecular level the electron pathways in and around the *bc*₁ complex, we sought to isolate the structural genes for the components of this complex. A nonphotosynthetic mutant with a lesion related to the *bc*₁ complex, isolated earlier by Marrs et al. (*Biochim. Biophys. Acta* 637: 96 [1981]), was used. A cosmid library of *R. capsulata*, kindly provided by P. Scolnik (this section), was introduced into this mutant, and colonies that can grow photosynthetically (Ps⁺) were selected. A plasmid, pR14A, carrying approximately 12 kb of *R. capsulata* DNA, consisting of five *Eco*RI fragments

of 7, 2.7, 1.5, 1.3, and 0.4 kb, was found to confer photosynthetic growth to R126 (Fig. 1).

Since the location of the mutation in R126 was not known, the presence of the structural genes for the *bc*₁-complex components on plasmid pR14A was assessed using as probes mixed synthetic oligonucleotides derived from the conserved amino acid sequences available from other organisms. The probe for Rieske Fe-S protein hybridized to the 1.3-kb *Eco*RI fragment and those for cytochromes *b* and *c*₁ recognized the adjacent 2.7-kb *Eco*RI fragment. Partial nucleotide sequence determination around the regions detected by hybridization proved that the structural genes for the Rieske Fe-S protein, cytochrome *b*, and cytochrome *c*₁ are present on pR14A. Moreover, it also indicated that these genes are clustered to form an operon in this organism, as also appears to be the case in *Rhodospseudomonas sphaeroides*.

To determine the role of the *bc*₁ complex, insertion mutations localized in the Rieske Fe-S protein, cytochrome *b*, and cytochrome *c*₁ structural genes were obtained, and deletions covering part or all of the *bc*₁ genes are being constructed (Fig. 1). These mutants were found to be impaired in photosynthesis, indicating that a functional *bc*₁ complex is absolutely essential for photosynthetic growth of *R. capsulata*. However, as expected, they were all able to grow by respiration. These results not only confirmed the presence of an alternate respiratory pathway independent of the *bc*₁ complex, but also

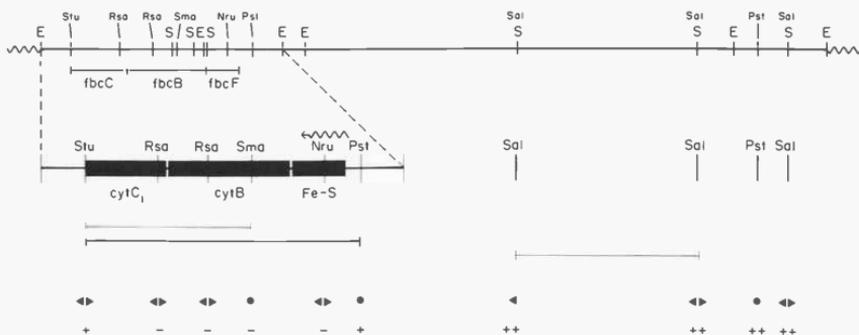


FIGURE 1 Restriction map of the plasmid pR14A carrying the *fbc* operon containing the *fbcF* (Rieske Fe-S protein), *fbcB* (cytochrome *b*), and *fbcC* (cytochrome *c*₁) structural genes. Zigzag arrow indicates direction of the transcription. Straight lines delineate the deletions, and arrowheads and dots represent the kanamycin cartridge insertions and their orientation in different genes. + and - indicate the photosynthetic growth phenotype of the obtained insertion mutants.

indicated that the branching point of these respiratory pathways must be before the *bc₁* complex, and thus, most likely at the quinone pool.

The presence of any other essential gene around the *bc₁* operon was assessed through the construction of several insertions localized on the available 12-kb cloned DNA. Mutants carrying these insertions were proficient in photosynthesis, indicating that they did not interrupt any essential genes. Furthermore, because of their close proximity to the *bc₁* operon, several of the insertions are very useful for mapping and construction of various mutations located in the *bc₁* structural genes. One of these insertions, located only a few hundred base pairs upstream of the Rieske Fe-S protein structural gene, the first in the *bc₁* cluster, is of special interest. This mutant grows very slowly by photosynthesis and by respiration, shows aberrant pigmentation, and reverts with very high frequency. The effect of this mutation is unknown, but it is apparently not related to the absence of a functional *bc₁* complex.

The localization of the mutation in R126, originally used for the isolation of the *bc₁* operon, is important because this mutant served as evidence that discrete catalytic sites for quinone processing were present in the *bc₁* complex. Various fragments of the *R. capsulata* DNA present in pR14A were introduced into this mutant, and it was found that those not carrying the *bc₁* operon were unable to rescue this mutation. For a more precise mapping, an insertion located at the last codon of the cytochrome *c₁* gene (and still P_s') was used. By appropriate genetic crosses, it was shown that the mutation *aer126* of the mutant R126 is linked to this insertion at a frequency of approximately 10%. This suggested that *aer126* might be located either in the carboxyterminal part of the Rieske Fe-S protein gene or in the aminoterminal part of the cytochrome *b* gene. The precise location and the nature of this mutation are now being determined by direct DNA sequencing after the cloning of the above-mentioned region from R126.

Primary Structure of the *fbc* Operon of *Rhodospseudomonas capsulata* Encoding the Rieske Fe-S, Cytochrome *b*, and Cytochrome *c₁* Proteins

E. Davidson, F. Daldal

The structural genes of the Rieske Fe-S, cytochrome *b*, and cytochrome *c₁* proteins, components of the *bc₁* complex of *Rhodospseudomonas capsulata*, are located on two adjacent *EcoRI* fragments with a total length of approximately 4.0 kb (Fig. 1). Nucleotide sequence determination of these fragments, which is now almost completed, suggests that they are part of an operon (*fbc*) made up of at least three genes arranged 5'→3' as follows: *fbcF* (Rieske Fe-S protein), *fbcB* (cytochrome *b* protein), and *fbcC* (cytochrome *c₁*). The intergenic regions between *fbcF* and *fbcB* and *fbcB* and *fbcC* are very short, 12 bp and 17 bp, respectively, and contain sequences similar to Shine-Dalgarno translation-initiation sites.

The deduced amino acid sequence from currently available nucleotide sequences shows that the Rieske Fe-S protein is 191 amino acids long. It contains a long carboxyterminal hydrophobic stretch that shows high homology with the Rieske protein of *Neurospora crassa*. The four cysteine residues present among the conserved amino acids of this region are thought to be involved in the binding of the 2Fe-2S clusters that form the prosthetic group of this protein. A comparison of hydropathy plots of the Rieske protein from both organisms suggests that they have an overall similarity in spatial folding.

The nucleotide sequences of the *fbcB* and *fbcC* genes are not yet complete; however, the available data show that the cytochrome *b* protein is over 400 amino acids long and has several regions of strong homology with the same protein from such diverse organisms as yeast, mouse, humans, and *Aspergillus nidulans*. There is less homology with cytochrome *b₆* and subunit IV (17-kD protein) from spinach chloroplasts. As with all of these organisms, *R. capsulata* has conserved its four histidine residues, which are thought to be ligands of the two heme groups bound to the cytochrome *b* protein.

Cytochrome *c₁* appears to be close to 300 amino acids long and features an aminoterminal well-conserved heme-binding sequence, and several short regions of homology with cytochrome *c₁* from yeast. No strong homology is observed between the bacterial cytochrome *c₁* and the cytochrome *f* of plant chloroplasts. A stretch of amino acids similar to prokaryotic signal sequences is also present at the beginning of the *fbcC* gene.

Once completed, the nucleotide sequence of the *fbc* operon will obviously provide important basic knowledge for future studies on the structure-

function relationship in the bc_1 complex of *R. capsulata*.

Spectroscopic Analysis of *Rhodopseudomonas capsulata* Reaction Centers

D.C. Youvan

In collaboration with R. Prince at Exxon Research and Engineering Company, we have successfully characterized wild-type reaction centers from *Rhodopseudomonas capsulata*, using several spectroscopic techniques (R. Prince and D.C. Youvan, in prep.). All of these measurements have previously been made in *Rhodopseudomonas sphaeroides*, and it has been our goal to repeat the measurements on *R. capsulata* reaction centers in preparation for the analysis of site-specific mutants.

Wild-type reaction centers were prepared by a highly efficient and economical procedure that we have developed in the last year for *R. capsulata*. LHII⁻ deletion backgrounds (Youvan et al., *Gene* 38: 19 [1985]) are used as a source of protein, since these strains may be grown photosynthetically in rich medium without reversion of the LHII⁻ lesion. Previously used strains such as MW442 (point mutation in LHII) rapidly revert under photosynthetic growth conditions, and the LHII antenna may contaminate the reaction center preparation. Chromatophores are prepared, solubilized in LDAO, and applied to a Bio-Gel DEAE-agarose column. This column material has a remarkably high binding capacity for reaction centers: a 5-liter preparation may be bound to a 20-ml column, yielding 30 mg of highly purified reaction centers. No contaminating proteins are observed in this one-step purification at moderate levels of protein loaded for SDS-PAGE. Typical A260:A800 ratios are 1.4. Some of this material was given to M. Michel, who is attempting crystallization. Other laboratories are also using our mutant and procedure for purposes of crystallography.

An optical absorption spectrum of wild-type *R. capsulata* reaction centers reveals three absorption peaks in the near-infrared very similar to the *R. sphaeroides* spectra. An actinic light source was used to bleach P870; the difference spectrum allows direct observation of the special pair chlorophylls. The dark spectrum is recovered after the light is

turned off. These data are almost identical to those from previous experiments conducted on *R. sphaeroides* reaction centers; however, our reaction centers contain wild-type levels of carotenoids.

The kinetics of electron transfer reactions leading to the reduction of Q_A and Q_B were inferred by measuring the kinetics of the back reactions leading to rereduction of the special pair. Rereduction was monitored by measuring the recovery rate of photobleaching at 600 nm. Our preparation of reaction centers shows rereduction of the special pair with a half-time of 80 msec following a 70% saturating single flash. Upon addition of 10 μ M ubiquinone-10, this half-time increases to about 1 second. Further addition of 1 mM ametryne (an atrazine derivative) results in the half-time returning to the 80-msec value. These values are very similar to what has been measured for *R. sphaeroides* and *Rhodopseudomonas viridis*.

We interpret the flash kinetic data as follows. The reaction centers as prepared contain Q_A but not Q_B , and thus electron transfer following a single-turnover flash of light results in the stable generation of oxidized bacteriochlorophyll dimer (special pair) and reduced Q_A^- . This state decays by direct electron transfer from the quinone to the dimer with a half-time of approximately 80 msec. Addition of ubiquinone-10, in this case about 3 molecules per reaction center, reconstitutes Q_B , so that electron transfer can now proceed from Q_A to Q_B . The back reaction from Q_B to the dimer is very much slower than from Q_A to the dimer, with a half-time of about 1 second. Addition of ametryne displaces Q_B , and the reaction centers then behave as they did before reconstitution of the Q_B activity. This series of measurements will be part of the routine analysis for reaction centers bearing site-specific mutations at the Q_A , Q_B , and Fe binding sites.

Wild-type reaction centers have been characterized by a series of cryogenic electron paramagnetic resonance (EPR) measurements that should also be useful in the routine characterization of site-specific mutants. A light-induced signal was observed in the $g = 2.0$ region, which is very similar to the signal observed in *R. sphaeroides* assigned to the photooxidized special pair B_2^+ . The *R. capsulata* peak appears to be slightly broader (~ 0.5 gauss) than the *R. sphaeroides* R26 peak. Illuminated reaction centers plus dithionite show a special-pair triplet signal near $g = 2.0$ that is very similar (but less intense) to the *R. sphaeroides* trip-

let. Again, the *R. capsulata* signal appears to be slightly broader, indicating that the unpaired electron is confined to a slightly different box than in *R. sphaeroides*. Under these conditions, a very strong signal is observed at $g = 1.82$ that has been assigned to the Q_A semiquinone anion, Q_A^- (see Fig. 2).

Clearly, the *R. capsulata* reaction centers are spectroscopically very similar to the much studied preparations from *R. sphaeroides*. This will allow the application of spectroscopic techniques to the question of what functional changes occur after site-specific mutagenesis of the quinone and Fe binding sites. This greatly facilitates the structure-function problem, since relatively simple spectroscopic analysis may be used to characterize mutants without resorting to X-ray crystallography. Our preliminary spectroscopic measurements strongly suggest that the vast spectroscopic literature on *R. sphaeroides* is directly applicable to *R. capsulata*.

Site-specific Mutagenesis of the Photosynthetic Apparatus

E.J. Bylina, S. Ismail, D.C. Youvan

In vitro mutagenesis has been used to study structure-function relationships of amino acid residues of the proteins involved in the light reactions of photosynthesis. In the light reactions, membrane proteins interact with prosthetic groups (bacteriochlorophyll, quinones, and carotenoids) to harvest light energy efficiently and to transduce this energy into a biologically useful reductant (for review, including genetics, see Youvan and Marrs, in *Molecular Biology of the Photosynthetic Apparatus*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York [1985]; Youvan and Marrs, *Sci. Am.* [1986] in press).

Unique restriction sites have been introduced into the plasmid-borne *puf* operon. This 3000-bp operon carries the structural genes encoding four membrane polypeptides: the photochemical core of the reaction center (L and M subunits) and light-harvesting I (LHI; α and β subunits). These restriction sites divide the operon into three domains: the LHI subunits, the L subunit, and the M subunit. Incorporation of restriction sites allows straightforward cloning of these genes into M13 vectors and

R. capsulata U34 Reaction Centers

A801 = 80
20 mW, 2.0 mT, 5K

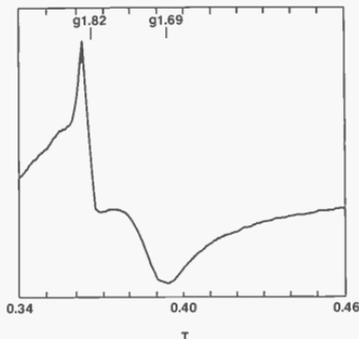


FIGURE 2 EPR signal from *R. capsulata* reaction centers showing the semiquinone anion radical Q_A^- . Using EPR and optical spectroscopy, single prosthetic groups within the 100-kD reaction center may be observed. Spectroscopy expedites the analysis of site-specific mutations that affect peptide-prosthetic group interactions, since much information can be obtained without resorting to X-ray crystallography.

simple shuttling of the mutagenized fragment back into the plasmid bearing the *puf* operon (pU21). This division of the *puf* operon is especially important for the two reaction center subunits. The L and M subunits are very homologous. Without separation of these two subunits, results from plaque hybridizations are difficult to interpret.

Oligonucleotide-directed in vitro mutagenesis of the LHI (B870) polypeptides of *Rhodospseudomonas capsulata* has been used to study bacteriochlorophyll binding (E.J. Bylina, in prep.). The putative bacteriochlorophyll binding sequence Ala-X-X-His has been extensively mutagenized in both subunits. Seven alanine mutations have been constructed in M13 vectors, shuttled back into pU21, and returned to *R. capsulata* deletion strains (Youvan et al., *Gene* 38: 19 [1985]) for phenotypic analysis. Six histidine mutations have also been analyzed in the deletion strains. In the future, similar experiments may be conducted on the LHI1 antenna (Youvan and Ismail, *Proc. Natl. Acad. Sci.* [1985] in press).

The results of our mutagenesis experiments are as follows: (1) Histidine is absolutely required for biogenesis of the LHI complex. Substitution at this position results in the loss of the LHI complex, a

reduced level of reaction centers in the membrane, and a decreased rate of growth under photosynthetic conditions. (2) Residues with small side chains can substitute for alanine. Replacement with glycine or cysteine results in a functional LHI complex, whereas a valine substitution results in a reduction of the LHI complex. Larger substitutions at alanine result in the same phenotype as seen for the histidine mutations. Similar results were obtained for a reaction center bacteriochlorophyll binding site. Mutagenesis of the histidine ligand to the bacteriochlorophyll dimer (special pair) from the L subunit (HL174) to leucine results in the complete loss of the reaction center from the membrane. However, this mutant does assemble LHI, breaking the previously observed pleiotropy between the reaction center and LHI. Hence, LHI biogenesis is not conditional on the insertion of the reaction center into the membrane. Chromatophores from this mutant serve as a pure source of the LHI antenna for spectroscopy and purification.

The availability of crystal structure data from reaction centers of *Rhodospseudomonas viridis* allows mutagenesis experiments to now be intelligently designed in our system. IL230, in the L subunit of the reaction center, is found in the Q_B pocket of the reaction center. A unique *Bam*HI restriction site has been introduced at this position. Using this *Bam*HI mutation as our "wild-type" template, we can enrich for mutations at this isoleucine site by cutting the M13 RF (replicative form) with the *Bam*HI enzyme after performing oligonucleotide-directed mutagenesis on the template. Any changes in the isoleucine codon will result in a *Bam*HI-resistant template, whereas wild-type templates remain *Bam*HI-sensitive. RFs linearized with *Bam*HI are taken up poorly by recipient cells. This method will be used at other residues that play im-

portant roles in the reaction centers as indicated from the crystal structure.

Cloning a Gene Coding for Phytoene Dehydrogenase in Photosynthetic Bacteria

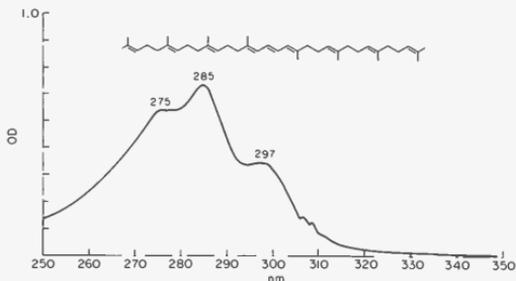
G. Giuliano, D. Pollock, P.A. Scolnik

We are interested in using carotenoid pigments as a model system for genetic studies in plants. The chemistry of these compounds has been very well studied during the last 30 years, but the fact that the biosynthetic enzymes are labile membrane-bound proteins has hampered the molecular studies. One possible approach toward solving this problem is to use a very simple system, such as photosynthetic bacteria, to identify the genes responsible for the biosynthesis of these pigments.

The first five steps of the carotenoid biosynthetic pathway are common among photosynthetic bacteria and plants, thus opening the way for using information obtained in this system for the study of higher plants. A key enzyme in the pathway is phytoene dehydrogenase (*Pdh*), which mediates the conversion of phytoene into colored carotenoids (see Fig. 6). To clone the gene responsible for this step, we analyzed several point mutations in the carotenoid biosynthetic pathway, and we identified two strains (BPY69 and BPY102) that accumulate phytoene (Fig. 3). A search for sequences that complement these mutations resulted in the isolation of *crtI*, the gene that presumably codes for *Pdh* (Fig. 4).

To characterize further the cluster of genes coding for carotenoid biosynthesis in this bacterium,

FIGURE 3 Isolation of two *R. capsulata* mutants defective in phytoene dehydrogenase. Strains BPY69 and BPY102 accumulate phytoene, which can be detected by its characteristic absorption spectrum, with maxima at 275, 285, and 297. The structure of the compound is shown at the top of the figure.



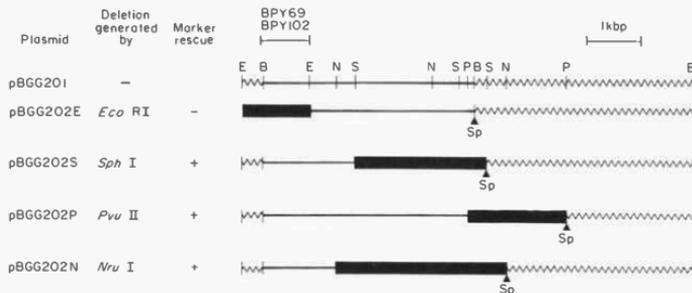


FIGURE 4 Mapping of the *crtI* gene. A 4.3-kbp *Bam*HI fragment, containing the gene, was cloned in pBR322. Deletions were constructed with the enzymes listed in the figure, and spectinomycin resistance gene cartridges were inserted at these sites. This allows the mobilization of these plasmids by conjugation and the mapping of the mutations. On the basis of this result, the gene is located between the left *Bam*HI site and the *Sph*I site.

we constructed several insertions with kanamycin and spectinomycin gene cartridges (Fig. 5). This technique allows for the fine structure mapping of sequences by insertional mutagenesis. We also analyzed the membrane proteins accumulated by the different mutants (Fig. 6). Mutations in the early steps of carotenoid biosynthesis result in the loss of several abundant membrane proteins. On the basis of these results, we postulate that carotenoids exist in the membrane in a stable form only if they are bound to structural proteins. The carotenoid and its binding protein(s) would stabilize each other; the loss or inactivation by mutation of either component would result in a pleiotropic effect in which both carotenoids and binding proteins are missing from the membrane.

This model has an immediate application on our plant projects, for it is very likely that carotenoid binding proteins also exist in plants. This implies that carotenoid mutants can be obtained by either

deficiencies in the biosynthetic enzymes or in the binding proteins, which is highly consistent with the genetic information available from several plant systems.

The Molybdenum-Pterin Binding Protein in *Clostridium pasteurianum*

S. Hinton, C. Slaughter, W. Eisner, B. Merritt, T. Fischer

The known metabolic function of molybdenum (Mo) is as a component of two distinct cofactors found in various redox enzymes. We have been studying Mo metabolism to understand how molybdate, MoO_4^{2-} , is processed for its eventual incorporation into molybdoenzymes. We have identified several molybdo proteins in *Clostridium pasteurianum* that have characteristics which suggest that they may play a role in Mo metabolism. The evi-

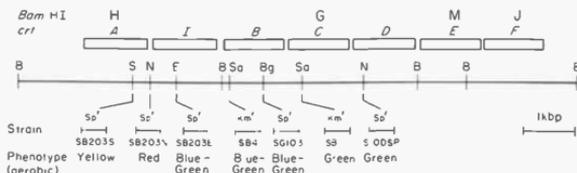


FIGURE 5 Mapping of the carotenoid biosynthetic cluster by site-directed gene cartridge mutagenesis. The phenotype of the insertions allows the mapping of the different genes.

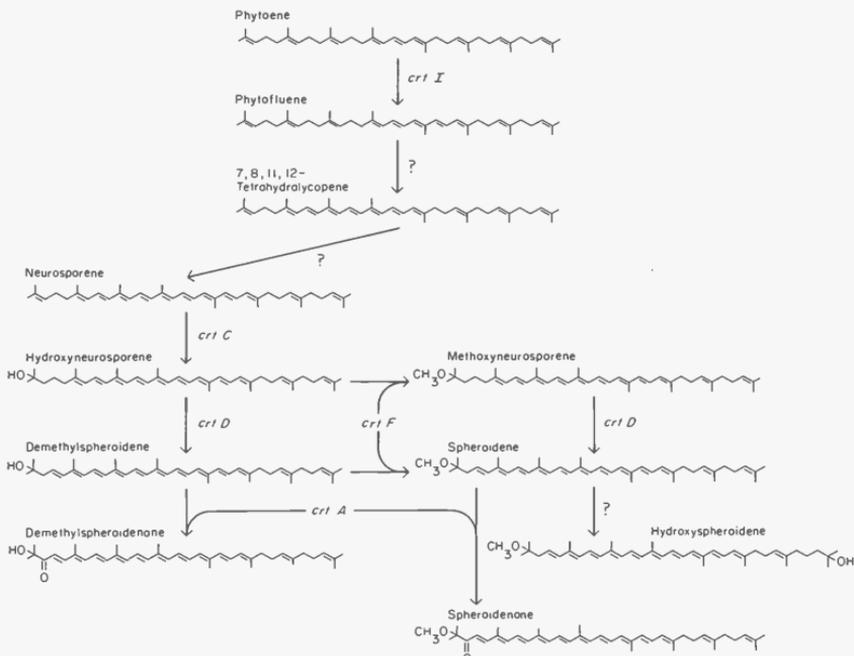


FIGURE 6 Biochemical-genetic map of the carotenoid biosynthetic pathway in *R. capsulata*. Question marks indicate genes that have not been identified.

dence prompted us to propose the hypothesis that during the biogenesis of Mo cofactors, precursors of the cofactor are protein-bound and therefore may be isolated and characterized.

The major Mo-binding protein (Mop) we identified in *C. pasteurianum* was isolated and biochemically characterized. The isolated protein contains approximately 1 Mo atom per molecule of 5700 molecular weight as determined by gel electrophoresis. Native Mop has a peak absorbancy at 293 nm, and the oxidized protein releases a pterin-like fluorescent chromophore. The purified chromophore was treated with alkaline permanganate, which oxidizes 6-alkylpterins to pterin-6-carboxylic acid. Pterin-6-COOH was isolated and identified from the oxidized mixture by reverse-phase high-performance liquid chromatography. Mop appears to bind a Mo atom and a pterin derivative, which are

the components of the Mo-cofactor (Mo-co). The molybdopterin species dissociated from Mop was not catalytically active in the biological Mo-co assay. Elemental analysis of the molybdopterin species showed that unlike the active cofactor, the pterin is not phosphorylated, suggesting that the inactive molybdopterin associated with Mop might be a precursor to Mo-co.

The *mop* gene cloned in *Escherichia coli* was detected by immunoscreening a genomic library of *C. pasteurianum*, and the protein from the clone cross-reacting with Mop antisera was shown to have the same electrophoretic mobility as purified protein. The identity of the gene was established by sequencing the insert of clostridial DNA, and the deduced amino acid sequence of an open reading frame proved to be identical to the first 12 residues of the purified protein. The DNA sequence flank-

ing the *mop* gene contains promoter-like consensus sequences observed in promoter regions in *E. coli*. The putative promoter and Shine-Dalgarno sequences before the initiation codon are probably responsible for the expression of Mop in *E. coli*.

The deduced amino acid sequence of Mop has been helpful in understanding the biochemical properties of the protein and in predicting a function. The amino acid composition shows that the protein is hydrophobic (50% of the residues are nonpolar), lacks aromatic and cysteine residues, and has a calculated molecular weight of 7038. The absence of aromatic amino acids in Mop suggests that the UV absorbance of Mop is attributed to the bound pterin chromophore. The aminoterminal region is rich in positively charged amino acids (30%), and the pattern and type of residues suggest that regions may form an α -helix-turn- α -helix structure observed in DNA-binding proteins. The aminoterminal amino acid sequence of Mop has 30% sequence homology with the DNA-binding region in the bacteriophage P22 repressor protein. Presently, we are investigating the DNA-binding properties of Mop because of the high degree of homology with DNA-binding proteins.

Aminoterminal amino acid sequence analysis of Mop revealed that there are two or more related proteins in the purified protein preparation, because there appear to be two amino acid residues at several positions. The results suggest that the *mop* gene has been duplicated and the Mop variants copurify. We have isolated and sequenced three distinct *mop* genes; the deduced amino acid sequence from the three genes accounts for all of the ambiguity observed in the amino acid sequence data and revealed two additional amino acid substitutions. A comparison of the DNA sequence of the *mop* genes (family) showed greater than 90% homology within the coding region. The DNA sequence flanking the *mop* genes is not conserved except within a 15-nucleotide sequence (*TGTATAATAATAAGT*), which is a direct inverted repeat spanning the putative TATAATA box.

In previous studies, we demonstrated that the level of molybdoproteins in *C. pasteurianum* responds to "Mo availability," and it seems reasonable that Mop may be a regulatory protein binding the anabolic source of Mo, allowing the cell to regulate molybdoenzyme synthesis according to the availability of an essential component, molybdenum.

Bacterioferritin from *Azotobacter vinelandii*

S. Hinton, C. Slaughter, W. Eisner, T. Fischer

Azotobacter vinelandii has the ability to accumulate iron at levels above its needs for growth. The iron appears to be stored in magnetically ordered aggregates, and the molecule harboring the iron, bacterioferritin, appears to be similar to ferritin in that it contains an average of 1600 Fe atoms in an oxide-hydroxide-phosphate core. However, bacterioferritin is a β -type cytochrome isolated from the soluble fraction of the cell lysate. It has been proposed that bacterioferritin may be an iron-storage protein.

We devised a large-scale purification scheme in which milligram quantities of bacterioferritin crystals are isolated in three steps. The aminoterminal amino acid sequence revealed high sequence homology with the *Escherichia coli* bacterioferritin, but there appears to be no homology with ferritin (horse spleen). Oligonucleotides were synthesized on the basis of the aminoterminal amino acid sequence of the protein, and Southern hybridization of total genomic DNA showed a 1.6-kb *XhoI-SalI* fragment that has homology with two distinct bacterioferritin oligonucleotide probes. The *XhoI-SalI* fragment has been cloned into M13 mp18 for restriction mapping, site-specific mutagenesis, and DNA sequencing analysis. Our goal is to understand the physiological role of bacterioferritin. Site-directed mutagenesis for structure-function studies may help determine the effect mutations have on iron metabolism.

Biogenesis of the Molybdenum Cofactor

S. Johann, S. Hinton

Our goal is to understand how the nutrient molybdate, MoO_4^{2-} , is biologically processed or chemically transformed into a biological catalyst. In *Klebsiella pneumoniae*, five gene products (*nifB*, *Q*, *N*, *E*, and *V*) are required for the synthesis, modification, and/or insertion of the unique iron-molybdenum cofactor of nitrogenase. In *Escherichia coli*, there are also five known gene products (*chlA*, *B*, *D*, *E* and *G*) thought to be involved in the biosynthesis of the molybdenum (Mo) cofactor.

Even though the two Mo cofactors are distinct, it has been proposed that the *chlD* gene product may be a common step early in their biogenesis.

We have isolated several clones that complemented each of the *chl* (Mu insertion) mutations, and Southern hybridization analysis showed that the clones have sequence homology with the chromosomal DNA flanking the Mu insertion in their respective *chl* mutant. DNA sequence analysis of the DNA fragment containing the *chlD* gene revealed an open reading frame that appears to code for a protein with a deduced molecular weight of approximately 34,000.

The *chlG* gene, which is thought to be involved in the insertion of Mo into the cofactor, has been cloned and is presently undergoing DNA sequence analysis. We are interested in looking at the deduced amino acid sequence of *chlD* and *chlG* gene products for structure-function studies of the *chl* gene products that appear to be directly involved in transforming molybdate into the molybdenum cofactors.

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Over the past year, we have continued our studies into the biology and biochemistry of cell transformation and differentiation. We have employed growth factors, oncogene proteins, monoclonal antibodies, heat shock, somatic-cell microinjection, two-dimensional gel electrophoresis, and molecular biology to further our analysis into these biological systems. Joining us this year as a Senior Staff Investigator was David Spector from Baylor, who uses electron microscopy to look at cell architecture, especially the nuclear structure. He brings a new expertise to our studies involving oncogenes and transformation. David Helfman and Bill Welch were promoted to Staff Investigator and Senior Staff Investigator, respectively, appointments that are well deserved. Below is an account of the work carried out over this past year.

MOLECULAR BIOLOGY OF THE CYTOSKELETON

D.M. Helfman S. Cheley Y. Yamawaki-Kataoka
E. Kuismanen L.A. Finn

The major emphasis of our research continues to be the molecular and cellular biology of the cytoskeleton. During the past year, we have concentrated our efforts on studying tropomyosins in nonmuscle cells (rat fibroblasts) and the genes that encode these proteins. Significant progress has been made in elucidating the structure of three of the five fibroblast tropomyosins. The complete structure of rat skeletal-muscle β -tropomyosin was also determined. Interestingly, we have found that rat embryonic fibroblast tropomyosin I and skeletal-muscle β -tropomyosin are expressed from a single gene via tissue-specific RNA splicing. In addition, we have isolated cDNA clones to a number of cellular proteins including the 90-kD heat-shock protein, nonerythroid spectrin, and the type II regulatory subunit of cAMP-dependent protein kinase. A brief description of these studies is given below.

Characterization of cDNA Clones Encoding Nonmuscle Tropomyosins

Y. Yamawaki-Kataoka, D.M. Helfman

To study the molecular basis for the five tropomyosin isoforms expressed in rat fibroblasts, we have

been isolating and characterizing cDNA clones encoding these proteins. Last year, we described the complete primary amino acid sequence of rat embryonic fibroblast tropomyosin I (TM-I) deduced from cDNA clones (Yamawaki-Kataoka and Helfman, *J. Biol. Chem.* 260: 14440 [1985]). These studies revealed that TM-I, like smooth muscle and skeletal muscle, contained 284 amino acids. However, comparison of the sequence of TM-I with that for skeletal-muscle tropomyosins indicated that these isoforms differ in only two regions: an internal region (amino acids 189–213) and a region at the carboxyterminal domain (amino acids 258–284). These two isoform-specific regions correspond to the troponin-T-binding domains of skeletal-muscle tropomyosin. Since nonmuscle cells do not contain a troponin complex, these different regions in TM-I, compared with those of the skeletal-muscle protein, likely provide specialized function(s) to the molecule. Using our cDNA clones to TM-I as a probe to analyze mRNAs expressed in various rat tissues, we identified a 1.1-kb transcript present in rat fibroblasts, stomach, and uterus, but a 1.3-kb transcript in skeletal muscle. These studies suggested a high degree of homology between the fibroblast and skeletal-muscle transcripts and prompted further studies on the skeletal-muscle

transcript. We subsequently isolated cDNA clones encoding the skeletal-muscle mRNA and corresponding genes that encode both the fibroblast and skeletal-muscle mRNAs. These studies showed that tissue-specific splicing and polyadenylation are responsible for the generation of nonmuscle and muscle isoforms from a single gene (see below).

This year, we have extended our studies to cDNA clones encoding rat embryonic fibroblast tropomyosin 2 (TM-2) and 4 (TM-4). Although analyses of these clones are still in progress, we have determined most of the amino acid sequence of TM-2 and the complete amino acid sequence of TM-4. It appears that TM-2 contains 284 amino acids, whereas TM-4 was found to contain only 247 amino acids. This is due to two 21-amino-acid deletions at positions 23-43 and 60-80 of the skeletal-muscle sequence, and a 5-residue extension at the amino terminus. These structural features of TM-4 correlate well with functional differences in the protein, compared with those of other nonmuscle tropomyosins. For example, it was recently reported that TM-4 shows less ability for head-to-tail polymerization and lower affinity to actin than the high-molecular-weight fibroblast tropomyosins (TM-1, TM-2, and TM-3) (Matsumura and Yamashiro-Matsumura, *J. Biol. Chem.* 260: 13851 [1985]). Comparison of the amino acid sequences of TM-2 and TM-4 with sequences of other nonmuscle and muscle (skeletal, cardiac, and smooth) tropomyosins shows them to be highly homologous. Nevertheless, structural differences do exist among the various isoforms. Interestingly, TM-1, TM-2, and TM-4 smooth-muscle and platelet tropomyosins were all found to be highly homologous to each other at their carboxyterminal domains, compared with skeletal and cardiac tropomyosins. These divergent regions likely represent functional domains of the protein.

The function(s) of each of the nonmuscle tropomyosins is presently unclear. Whether the alterations in patterns of tropomyosins expressed in fibroblasts following transformation are directly responsible for the changes in the organization of microfilaments and changes in cell shape are not known. Presently, we wish to ask questions about the function of the various isoforms expressed in fibroblasts. By using our cloned cDNA, we hope to express particular tropomyosin isoforms in transformed cells where the cellular gene product is repressed. Such studies may determine if expression of tropomyosin results in changes in microfilament

organization and cell shape. In addition, experiments are currently under way to study the molecular basis for the alterations of tropomyosin expression following transformation.

Characterization of cDNA Clones Encoding Muscle and Nonmuscle Tropomyosins: Evidence for Tissue-specific RNA Processing

S. Cheley, D.M. Helfman

Using the cDNA clones encoding rat embryonic fibroblast TM-1 as a probe to study mRNA in various rat tissues, we identified a 1.1-kb mRNA in rat fibroblasts and a 1.3-kb mRNA in skeletal muscle. These studies indicated a high degree of homology between the fibroblast and skeletal-muscle mRNAs and prompted structural studies on the skeletal-muscle transcript. To isolate cDNA clones encoding skeletal-muscle tropomyosins, a cDNA library was constructed in the plasmid expression vectors pUC8 and pUC9. The library was screened with ³²P-labeled DNA prepared from cDNA clones encoding rat embryonic fibroblast TM-1. A series of overlapping clones encoding rat skeletal-muscle β -tropomyosin were identified. DNA sequence analysis was performed in order to determine the amino acid sequence of the protein. One clone was found to contain the entire coding and 3'-untranslated regions as well as a portion of the 5'-untranslated region. The complete nucleotide sequence of the skeletal-muscle cDNA and the deduced amino acid sequence are shown in Figure 1. The amino acid sequence of the rat skeletal-muscle cDNA is identical with that of rabbit skeletal-muscle β -tropomyosin. Comparison of the rat skeletal-muscle β -tropomyosin-coding sequence with that of the rat embryonic

FIGURE 1 Nucleotide and derived amino acid sequence comparison of cDNA clones encoding rat skeletal-muscle β -tropomyosin (RSM) and rat embryonic fibroblast tropomyosin 1 (REF). The deduced amino acid sequences for the skeletal-muscle and fibroblast tropomyosins are shown above and below, respectively, the corresponding nucleotide sequences. Nucleotide sequences specifying the entire coding region of 284 amino acids as well as the 5'- and 3'-untranslated regions are shown. The polyadenylation signal AATAAA is underscored. Boxed areas indicate the isoform-specific coding sequences. Most of the fibroblast cDNA sequence is shown as dashed lines, indicating identity with the skeletal-muscle tropomyosin isoform.

fibroblast TM-I revealed some interesting structural features (Fig. 1). The skeletal-muscle tropomyosin was found to be identical with the fibroblast isoform except for two distinct regions: one internal region (amino acids 189–213) and one carboxyterminal region (amino acids 258–284). In this regard, biochemical studies from other laboratories have indicated the presence of two troponin-T-binding sites on skeletal-muscle tropomyosin. One troponin-T-binding site is located at the carboxyl terminus of the tropomyosin molecule, and the second site is located near Cys-190. It is apparent from the sequence comparison presented in Figure 1 that these troponin-T-binding domains correspond to those regions where the sequences differ between the fibroblast and skeletal-muscle isoforms. Thus, these isoform-specific regions appear to delineate functional domains of the protein. The skeletal-muscle cDNA was found to have a relatively long 3'-untranslated region (233 nucleotides), compared with that from fibroblasts (73 nucleotides). These differences in the 3'-untranslated region explain the size difference observed between the skeletal-muscle mRNA and the fibroblast mRNA.

Since muscle and nonmuscle cells contain different tropomyosin isoforms, we investigated the tissue specificity of the skeletal-muscle cDNA clone. Northern transfer analysis of various rat tissue RNAs using our skeletal-muscle cDNA as a probe revealed the existence of two hybridizing classes of mRNA: a 1.3-kb mRNA species present in adult skeletal muscle and a 1.1-kb mRNA expressed in smooth muscle (stomach, uterus, and vas deferens) and nonmuscle cells (embryonic fibroblasts). Cardiac muscle, on the other hand, contains neither mRNA species. This is consistent with previous reports which indicate that rat cardiac muscle contains a single isoform of tropomyosin that is likely encoded by the same gene that encodes rat skeletal-muscle α -tropomyosin. To determine which sequences encoding amino acids 189–213 in skeletal-muscle or fibroblast RNA were expressed in various smooth-muscle cells, S1-nuclease analysis was performed. The results indicated that identical tropomyosin isoforms are expressed in fibroblast and smooth-muscle cells. In no case have we detected mRNAs containing a combination of the "fibroblast-specific" sequences with the "skeletal-muscle-specific" sequences. This suggests that co-expression of specific isotype-switch regions (amino acids 189–213 and 258–284) is essential to the function of these tropomyosin isoforms. In agreement with

Northern blot analyses, no S1-nuclease-protected fragment was detected in cardiac muscle. This further confirms that rat cardiac tissue does not express the β isoform of tropomyosin.

Since the nucleotide sequence of the rat skeletal-muscle cDNA was found to be identical with the sequence of the rat embryonic fibroblast TM-I cDNA, except for the two isoform-specific regions and the 3'-untranslated sequences, the data strongly suggest that both mRNAs arise from a common gene via alternative RNA processing. In this regard, we have analyzed rat tropomyosin genomic clones and have determined that both mRNAs are expressed from a single gene via tissue-specific processing (see below).

Nonmuscle and Muscle Tropomyosin Isoforms Are Generated from a Single Gene by Alternative RNA Splicing and Polyadenylation

D.M. Helfman, S. Cheley, E. Kuismanen, L. Finn, Y. Yamawaki-Kataoka

Two possibilities exist to explain the expression of the fibroblast and skeletal-muscle mRNAs described in the previous section. Either a single gene codes for these two isoforms via tissue-specific RNA processing or both fibroblast and skeletal-muscle tropomyosins are encoded by two genes that are highly conserved. The cDNA clones we isolated to rat embryonic fibroblast TM-I and skeletal-muscle β -tropomyosin were used to assess the number and structure of the corresponding genes. Analysis of genomic clones indicated that there are two separate loci in the rat genome that contain sequences complementary to the fibroblast and skeletal-muscle cDNA clones. One locus contains a functional gene and the other represents a processed pseudogene.

Analysis of genomic clones representing the functional gene indicated that one clone (λ 17) contained sequences to both the 5'- and 3'-untranslated sequences of the fibroblast and skeletal-muscle cDNAs. Since this clone appeared to contain sequences to the entire functional gene, including the 5'- and 3'-flanking regions, λ 17 was selected for detailed analysis. By using restriction enzyme analysis, we constructed a complete restriction map of the DNA (Fig. 2). Positions of the coding regions were determined by Southern blot analysis using

32 P-labeled cDNA clones as probes and then by direct DNA sequence analysis. As shown in Figure 2, the gene is divided into 11 exons and spans approximately 10 kb. As predicted from the cDNA analysis, common exons were found for amino acids 1-188 (exons 1 through 5) and for amino acids 214-257 (exons 8 and 9). Exon 6 was found to contain sequences for amino acids 189-213 in fibroblasts, whereas exon 7 was found to contain sequences for amino acids 189-213 in skeletal-muscle tropomyosin. In addition, exon 10 was found to encode sequences to the carboxyterminal region of skeletal-muscle tropomyosin (amino acids 258-284), including the 3'-untranslated sequences through the polyadenylation site. Likewise, exon 11 was found to encode sequences to the carboxyterminal region of fibroblast tropomyosin (amino acids 258-284), including the 3'-untranslated sequences through the polyadenylation site. The organization of this gene indicates that a combination of both alternative RNA splicing and polyadenylation is responsible for the generation of the tissue-specific isoforms. Southern blot analysis of high-molecular-weight DNAs from a variety of cells and tissues (fibroblast, liver, heart, skeletal muscle, brain, and stomach) reveals no rearrangement of the DNA at this level of detection, suggesting that the mRNAs are not generated by DNA rearrangements.

To determine if all mRNAs expressed from this gene are transcribed from the same promoter, primer-extension and S1-nuclease experiments were carried out. These studies demonstrated that all mRNAs (fibroblast, stomach, uterus, vas deferens, and skeletal muscle) contain identical 5'-untranslated regions and involve the use of the same promoter. Thus, the two different species of mRNA

generated from this gene are processed from a transcript that has identical 5' ends. Furthermore, the first exon contains the entire 5'-untranslated sequences in addition to the first 38 amino acids of the protein.

The structural features of the tropomyosin gene raise a number of questions concerning the mechanisms involved in the tissue-specific expression of this gene. Figure 3 represents a schematic model for the generation of protein isoforms from the tropomyosin gene. Although capping of the primary transcript is known to occur very early after transcription begins, the temporal order of polyadenylation and splicing remains to be determined. In the case of the tropomyosin gene where both alternative splicing and polyadenylation are involved, regulation could occur at both the levels of splice site selection and polyadenylation. The mechanism(s) responsible for generating the two tropomyosin isoforms must be under regulatory control. In no case have we detected both the 1.1-kb and 1.3-kb mRNAs in the same cell type, nor have we observed mRNAs containing a combination of the fibroblast-specific exon and a skeletal-muscle-specific exon, i.e., exon 6 with exon 10 or exon 7 with exon 11. These results clearly indicate that utilization of tissue-specific exons does not occur in a random manner but is under fine control. The tissue specificity of the RNA processing events implies developmental regulation of these processes. Understanding the mechanism(s) by which different mRNAs arise from the tropomyosin gene has important implications for understanding muscle gene regulation and the determination of muscle type (e.g., smooth vs. skeletal muscle). In addition, the mechanisms by which different proteins are

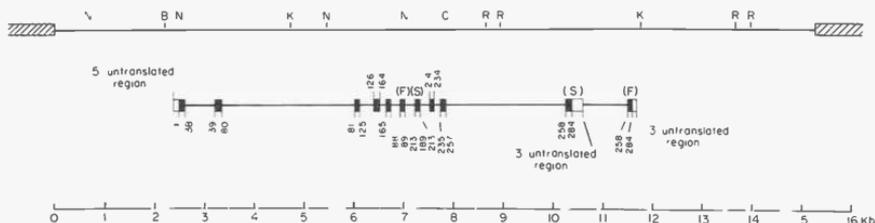


FIGURE 2 Physical map of the tropomyosin gene contained in λ clone 17. Boxes represent exons, and lines represent introns. The amino acids encoded by each exon were determined by sequence analysis and are indicated. Positions of the fibroblast and skeletal-muscle-specific exons encoding amino acids 189-213 and 258-284 are indicated by F and S, respectively. All other exons are common to the fibroblast and skeletal-muscle mRNAs. The restriction sites for *Bam*HI (B), *Cla*I (C), *Eco*RI (R), *Kpn*I (K), and *Nco*I (N) are indicated.

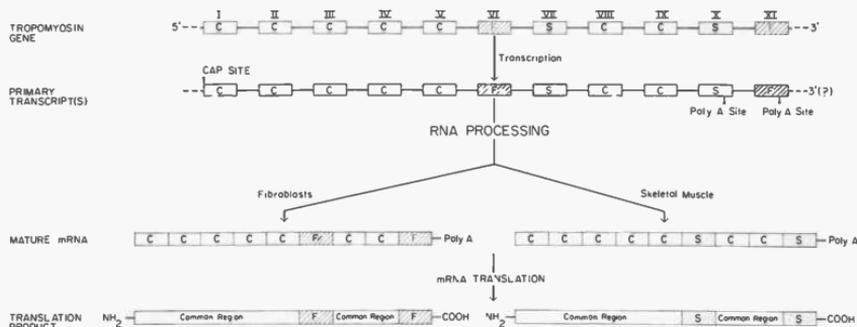


FIGURE 3 A model describing the tissue-specific expression of the tropomyosin gene and generation of different protein isoforms. Boxed areas represent exons and solid lines represent introns, but are not drawn to scale. The 5' and 3' regions of the gene are indicated as dashed lines because the structural and functional borders of the gene remain to be determined. The CAP site and polyadenylation signals are indicated. Exons common to all mRNAs generated from this gene are indicated by a C, and fibroblast and skeletal-muscle-specific exons are indicated by an F and S, respectively.

turned on and off during myogenesis are not known. When myoblasts fuse to form myotubes, there is cessation of cell division together with repression of the fibroblast TM-1 synthesis, followed by fusion of the myoblasts with induction of skeletal-muscle β -tropomyosin. Understanding the various levels of control involved in tropomyosin gene expression should provide a fundamental understanding of how various tissues are developmentally regulated. Work is currently under way to study the molecular mechanisms responsible for the tissue-specific expression of this gene.

Identification of cDNA Clones Encoding the 90-kD Stress Protein

D.M. Helfman, J.R. Feramisco [in collaboration with M. Grazia-Catelli and N. Binart, Inserm Lab, Bicetre, France]

Steroid hormone receptors found in the cytosol of target cells have an approximate 8S sedimentation coefficient corresponding to an oligomeric structure with a molecular mass of 250–300 kD. This oligomeric complex contains a nonhormone-binding 90-kD component. The 90-kD protein is more abundant (approximately 100 times) than the steroid-hormone-binding subunits and is present in all

tissues of the chick. Recently, it was reported that the 90-kD protein is identical with the 90-kD heat-shock protein (hsp 90) or stress protein (Grazia-Catelli et al., *EMBO J.* 4: 3131 [1985]). No function is known for hsp 90, a protein widely conserved among species, whose expression is regulated during development and a variety of stress conditions. Only the yeast hsp 90 cDNA and the *Drosophila* hsp 83 gene have been cloned. Comparison of hsp 90 cDNA and deduced amino acid sequences between different species, as well as gene structure and regulatory elements, may help in further analysis of the role of hsp 90 in cell function. To isolate cDNA clones for the 90-kD heat-shock protein, a cDNA expression library prepared from smooth muscle was screened using both polyclonal and monoclonal antibodies. One colony was identified that reacted with both antibodies. Plasmid 9.11 (~1100-bp insert) was found to hybrid-select mRNA for the 90-kD heat-shock protein. Northern blot analysis revealed that RNA isolated from various tissues contains a single mRNA of approximately 3 kb. Heat-shock treatment of fibroblasts resulted in increased steady-state levels of a 3-kb mRNA in both poly(A)⁺ and poly(A)⁻ RNA fractions. Southern blot analysis of chick genomic DNA indicated that the cDNA hybridizes to a single copy sequence. Sequence homology shows that the cDNA clone displays a high degree of homology with the 5' portion of the yeast 90-kD heat-shock protein.

Identification of cDNA Clones Encoding Nonerythroid Spectrin

D.M. Helfman [in collaboration with C.S. Birkenmeier, D.M. Bodine, and J.E. Barker, The Jackson Laboratory, Bar Harbor Maine; E.A. Repasky, Roswell Park Memorial Institute, Buffalo, New York; S.H. Hughes, Frederick Cancer Research Facility, Frederick, Maryland]

Spectrin is a membrane structural protein that is localized on the inner surface of the cell membrane. Originally, it was thought to be unique to erythrocytes but has since been identified in nonerythroid tissues. Biochemical and immunological studies demonstrate the existence of multiple forms of spectrin with tissue-specific distribution and differential expression during development. Spectrin proteins are heterodimeric, with α and β subunits ranging in size from 220 kD to 265 kD. The β subunits are heterogeneous, with three avian and two mammalian tissue-specific β subunits known thus far. It appears that a common α -subunit occurs in all avian tissues, whereas in mammals, the erythroid α -subunit is distinct from the nonerythroid α -subunit. The avian α -subunit is immunologically closely related to the mammalian nonerythroid α -subunit but only weakly related to the mammalian erythroid α -subunit. The existence of multiple forms of spectrin with tissue-specific patterns of expression poses interesting questions about the number and structure of the genes that encode the spectrin proteins.

A cDNA clone for nonerythroid α -spectrin was identified by direct immunological screening of a chicken smooth-muscle cDNA library. Northern blots of poly(A)⁺ RNA from various tissues of chicken and mouse show that the cDNA hybridizes to an 8-kb mRNA. The cDNA hybridized to a single-copy sequence on Southern blots of chicken genomic DNA. The complete nucleic acid sequence of the clone has a single open reading frame of 1419 bp. The derived amino acid sequence is organized into two partial and three complete 106-amino-acid repeats that show homology with the repeats described for human erythroid α -spectrin and β -spectrin. Immunological and biochemical data indicate that chicken nonerythroid and human erythroid α -spectrin are two of the more widely diverged members of the spectrin family. In this respect, the degree of homology found between them was unexpected. The data suggest a common evolutionary origin for these two α -spectrin molecules.

Isolation of cDNA Clones Encoding the Type II Regulatory Subunit of cAMP-dependent Protein Kinase

D.M. Helfman, M. Zoller [in collaboration with J. Scott and E.G. Krebs, Howard Hughes Medical Institute, University of Washington, Seattle, Washington]

cAMP is known to regulate numerous cell functions and biological events. Most, if not all, of the actions of cAMP in eukaryotic cells are thought to be mediated through the action of cAMP-dependent protein kinase. cAMP-dependent protein kinase is composed of two regulatory subunits and two catalytic subunits. The regulatory subunits bind two molecules of cAMP per monomer, and the binding of cAMP leads to dissociation of the complex, with release of the catalytic subunits, which are now active. Two major classes of cAMP-dependent protein kinase are present in most cells, termed type I and type II. The two isoforms contain identical catalytic subunits but different regulatory subunits (termed R^I and R^{II}). The isolation of cDNA clones encoding the type I regulatory subunit of bovine cAMP-dependent protein kinase has been reported (Lee et al., *Proc. Natl. Acad. Sci.* 80: 3608 [1983]). To analyze further the molecular basis for the two regulatory subunits and to study their expression, we have isolated cDNA clones encoding the type II regulatory subunit. Briefly, a 100,000-member cDNA library was prepared from adult rat skeletal muscle. The library was screened with a 47-nucleotide-long oligonucleotide probe derived from the amino acid sequence of mouse and bovine R^{II}. Positive colonies were isolated, and the cDNA inserts were sequenced. One clone was found to contain a sequence for R^{II} from approximately amino acid 30 through the 3'-untranslated region. The clone will be used to examine the structure of the R^{II} subunit and to study the organization and expression of the genes encoding this protein.

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BIOCHEMISTRY OF CYTOSKELETAL PROTEINS

F. Matsumura S. Yamashiro-Matsumura

We have previously reported that changes in the expression of the multiple isoforms of tropomyosin (TM) are consistently found in a variety of transformed cells, including virus-transformed rat cells, NIH-3T3 cells transformed with DNA transfection, BALB/3T3 cells transformed with UV irradiation and chemical carcinogen, and chemically transformed human fibroblasts (Matsumura et al., *J. Biol. Chem.* **258**: 13954 [1983]). During the past year, we have concentrated our studies on the molecular functions of the changes in TM expression that are involved in the reorganization of microfilaments upon cell transformation. In one approach, we examined whether these TM isoforms differentially modulate the actin-binding activity of two new actin-binding proteins ($M_r = 55,000$ and $83,000$), whose functions are suggested to organize microfilament bundles of stress fibers by their intercellular localization (Yamashiro-Matsumura and Matsumura, *J. Biol. Chem.* **260**: 5087 [1985]). In addition, we have continued to produce monoclonal antibodies to explore the organization of the multiple TM isoforms in microfilaments.

Modulations of the Interactions between Actin and Actin-binding 55-kD and 83-kD Proteins by the Multiple Isoforms of Tropomyosin

F. Matsumura, S. Yamashiro-Matsumura

The 55-kD protein is a monomeric globular protein with a native molecular weight of 57,000. The binding of the protein to actin is saturated at an approximate stoichiometry of four actin monomers to one 55-kD molecule. The protein makes F-actin aggregate side-by-side into bundles as described for other F-actin-binding proteins such as fimbrin ($M_r = 68,000$) and fascin ($M_r = 58,000$). Biochemical, morphological, and immunological characterization, however, revealed that the 55-kD protein is a new actin-binding protein (Yamashiro-Matsumura and Matsumura, *J. Biol. Chem.* **260**: 5087 [1985]). The protein is localized in microspikes as well as in stress fibers.

None of the TM isoforms of cultured rat cells significantly alter the F-actin-binding activity of the 55-kD protein, whereas skeletal-muscle TM inhibits the bundling activity to about 50%. This is consistent with the results that cultured rat cell TMs do not affect actin binding of the 55-kD protein, whereas skeletal-muscle TM inhibits binding of the 55-kD protein to actin by 50%. Actin binding of each TM isoform, in contrast, is affected by the 55-kD protein, depending on the TM isoforms. Most TMs (80%) with low relative molecular weights (32,400 [TM-4] and 32,000 [TM-5]) are dissociated from actin by the 55-kD protein, whereas only 20% of the TMs with high relative molecular weights (40,000 [TM-1] and 36,500 [TM-2]) are dissociated from actin. Immunofluorescence has shown that although TM was localized in stress fibers, the 55-

kD protein is found in microspikes as well as stress fibers, both of which are known to contain bundles of microfilaments. Therefore, we suggest that the 55-kD protein, together with the multiple TM isoforms, may regulate the formation of two types of actin-filament bundles: bundles containing TM and bundles without TM (Matsumura and Yamashiro-Matsumura, *J. Biol. Chem.* [1986] in press).

The 83-kD protein has previously been identified as one of the microfilament-associated proteins (Matsumura et al., *J. Biol. Chem.* 258: 6636 [1983]). Actin binding of the 83-kD protein was saturated at an approximate polypeptide ratio of five to six actins to one 83-kD protein. The 83-kD protein shares some physicochemical properties with TM. Both proteins are heat-stable (unlike other actin-binding proteins) and are rod-like proteins as determined by their Stokes' radii and sedimentation coefficients. Unlike TM, however, the 83-kD protein is a basic protein. The amino acid composition is considerably different from that of typical TM, especially showing the presence of many proline residues.

The 83-kD protein also binds to calmodulin in a calcium-dependent manner. In the presence of micromolar calcium, calmodulin binds to the 83-kD protein, and the resulting 83-kD protein cannot bind to actin. In contrast, in the absence of Ca^{++} , calmodulin cannot bind to the 83-kD protein, which allows the 83-kD protein to bind to actin. These results therefore suggest that the 83-kD protein is similar to nonmuscle caldesmon, recently purified from bovine adrenal modulla (Sobue et al., *Proc. Natl. Acad. Sci.* 82: 5052 [1985]). The 83-kD protein makes F-actin aggregate into bundles above the approximate polypeptide ratio of one 83-kD protein to eight actins when examined by electron microscopy. The actin-bundling by the 83-kD protein is also regulated by calmodulin and micromolar concentrations of Ca^{++} (S. Yamashiro-Matsumura and F. Matsumura, in prep.).

We have investigated whether the 83-kD protein has any effects on the interaction between actin and the multiple isoforms of TM. We have recently reported that low-*M_r* TMs have less affinity to actin than high-*M_r* TMs (Matsumura and Yamashiro-Matsumura, *J. Biol. Chem.* 260: 13851 [1985]). The 83-kD protein, however, stimulates the actin binding of low-*M_r* TMs up to four times higher than that for the control. This stimulating effect is not observed for high-*M_r* TMs. Because the actin binding of the 83-kD protein can be regulated in a calcium-dependent manner by calmodulin, the 83-

kD protein and calmodulin can also regulate the actin binding of low-*M_r* TMs in a calcium-dependent manner. In the absence of Ca^{++} , calmodulin does not bind to the 83-kD protein; thus, the 83-kD protein can bind to actin and stimulate actin binding of low-*M_r* TMs. In the presence of Ca^{++} , however, calmodulin binds to the 83-kD protein and the resulting 83-kD protein cannot bind to actin. Thus, low-*M_r* TMs bind to actin to a lesser extent. Immunofluorescence has shown that the 83-kD protein is present in both stress fibers and membrane ruffles. Furthermore, the staining patterns of stress fibers with anti-83-kD protein antibodies were periodic and very similar to those with anti-TM antibodies. These observations suggest that the 83-kD protein may change the organization of stress fibers through the regulation of the binding of TMs to stress fibers.

Organization of the Multiple Isoforms of Tropomyosin in the Microfilaments as Examined by Monoclonal Antibodies

F. Matsumura, S. Yamashiro-Matsumura

To explore the organization of the multiple isoforms of tropomyosin (TM) in the microfilament, we have attempted to produce specific monoclonal antibodies and examined whether these TM isoforms bind to microfilaments randomly or selectively. Among many hybridoma clones to TM, we have found two interesting monoclonal antibodies, IV15 and IV20. Both ELISA and immunoprecipitation showed that IV20 monoclonal antibody recognized TM-1 strongly and TM-2 and TM-3 weakly but did not react with TM-4 or TM-5. On the contrary, IV15 reacted with all five TM isoforms. Using these antibodies, we have isolated TM-containing microfilaments. SDS-gel analyses showed that microfilaments isolated by either IV15 or IV20 contained all five TM isoforms in spite of the specificity of IV20. Densitometry, however, revealed some differences in TM compositions. The ratios of TM-1:TM-2:TM-3 (high-*M_r* TMs) appeared to be the same for both microfilaments isolated by IV15 and IV20 monoclonal antibodies. However, the relative contents of TM-4+TM-5 (low-*M_r* TMs) in the microfilaments isolated by IV20 were found to be half of those isolated by IV15. These results suggest that high- and low-*M_r* TMs co-localize in the same

microfilaments, although some (~20%) of the microfilaments enrich low- M_r TMs.

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CELL BIOLOGY OF ONCOGENE PROTEINS

J.R. Feramisco	D. Bar-Sagi	K. Riabowal	G. Binns
W.J. Welch	T. Kamata	N.F. Sullivan	C. Hallaran
	J.C. Lamb	M.W. Wooten	N. Kronenberg

Microinjection of the *ras* Oncogene Protein into PC-12 Cells Induces Morphological Differentiation

D. Bar-Sagi, J.R. Feramisco

The activation of *ras* genes has been implicated in transformation in vitro and in tumorigenesis in vivo, but the role of these genes in the sequential events leading to the acquisition of the transformed phenotype is unclear. The *ras*-encoded proteins in mammalian cells are approximately 21,000 daltons (p21), bind guanine nucleotides, and are localized to the inner face of the plasma membrane. Oncogenic *ras* proteins differ from their normal homologs by a single amino acid substitution, usually at position 12 or 61. These mutations do not affect the localization or the nucleotide-binding properties of *ras* proteins. However, normal p21, encoded by the Ha-*ras* gene, has been shown to possess an intrinsic GTPase activity that is significantly impaired in the mutated oncogenic protein. By drawing an analogy between the *ras* proteins and the regulatory proteins (G proteins) of the adenylate cyclase system, it has been proposed that the deficiency in GTPase activ-

ity of the *ras* oncogene protein could result in the derangement of normal regulatory mechanisms that control cell proliferation.

Recent studies have indicated a role for the *ras* proteins in growth-factor-mediated control of cell proliferation. However, growth factors do not affect solely the proliferative capacity of cells. For example, in rat pheochromocytoma cells (PC-12), nerve growth factor (NGF) promotes cell differentiation rather than cell proliferation. We have undertaken studies to explore the possible participation of the *ras* proteins in this particular growth-factor-mediated signaling pathway.

PC-12 cells grow indefinitely in culture as round, chromaffin-like cells. When NGF is added, the cells differentiate into sympathetic neuron-like cells and stop growing. In the absence of NGF, microinjection of the human Ha-*ras* oncogene protein into PC-12 cells results in morphological differentiation similar to that induced by NGF (Fig. 1). These results indicate that *ras* proteins may exert different biological effects in different cell types, perhaps by utilizing a common molecular mechanism involving the coupling of growth factors to intracellular signals.



FIGURE 1 A PC-12 cell was injected with the *ras* oncogene protein and stained by immunofluorescence for the injected protein 20 hr later.

Microinjection of the *ras* Oncogene Protein Induces DNA Synthesis in Nonestablished Cells: Potentiation of Its Action by Growth Factors and Adenovirus E1A Protein

N.F. Sullivan, J.R. Feramisco [in collaboration with R.W. Sweet, B.Q. Ferguson, and M. Rosenberg, Smith Kline and Beckman, Pennsylvania]

In some cell lines cultured *in vitro*, growth factors

present in serum can interact with specific cellular receptors apparently to activate both physiological and biochemical changes that result in DNA replication and cell division. A number of studies have associated growth-factor-mediated pathways with transformation. For example, both a cellular growth-factor and a growth-factor-receptor gene have been found to be homologous to oncogenes of known acutely transforming retroviruses. In addition, growth factors can induce the expression of

oncogenes in either a cell-cycle-dependent or -independent manner. Furthermore, transforming growth factors can be secreted by cells transformed with the viral *Ki-ras* gene.

We are studying the role of proteins encoded by the *Ha-ras* protein in the control of cellular proliferation. These oncogenes have been associated with many types of human tumors and may contribute at least partially to the malignant phenotype. In human cells, the *ras* gene family consists of *Ha-ras*, *Ki-ras*, and *N-ras*, each of which appears to have an oncogenic form (activated, henceforth *ras* oncogene protein) and a protooncogenic form (wild-type, henceforth *ras* protooncogene protein). These *ras* proteins bind guanine nucleotides and display GTPase activity *in vitro*. Moreover, the *ras* oncogene protein displays less GTPase activity than the *ras* protooncogene protein. These results have suggested similarities between *ras* and the G protein of the β -adrenergic/adenylate cyclase system. In fact, in yeast, the *ras* gene products seem to regulate the adenylate cyclase system. In the *ras* family, the transforming lesion can be due to either point mutations that alter the protein sequence or increased levels of expression. In particular, protooncogenic *ras* becomes amplified as a consequence of passage of primary cells in culture. How these alterations affect the process of growth and division in animal cells is unclear, although microinjection of *ras* oncogene protein into established cells results in both DNA synthesis and cell division. In contrast, *ras* protooncogene protein showed no effect upon cell growth.

We now report on the microinjection of purified *ras* oncogene protein into both early- and late-passage nonestablished rat embryo fibroblasts (REF). These cells exhibit a markedly different response to *ras* oncogene protein than established cells, and their response changes significantly with continued passage; i.e., late-passage but not early-passage cells proliferate in response to the *ras* oncogene protein. We show that co-microinjection of *ras* with the human adenovirus serotype-C 13S mRNA gene product enables early-passage cells to respond more like late-passage cells by proliferating. Moreover, we have found that conditioned medium from late-passage rat embryo fibroblasts supports growth of 49F cells in soft agar while early-passage cells do not and that this medium potentiates the effects of the microinjected *ras* protein (Fig. 2).

Rapid Induction of Cell-surface Activities in Quiescent Fibroblasts following Microinjection of *Ha-ras* Proteins

D. Bar-Sagi, J.R. Feramisco

We have studied the early morphological and biochemical events that occur in response to microinjection of human *Ha-ras* proteins into quiescent rat embryo fibroblasts. Within 30 minutes to 1 hour after microinjection of the *Ha-ras* proteins, the injected cells show a marked increase in surface ruffles. The increase in ruffling activity is accompanied by an increase in fluid-phase pinocytosis, as measured by the uptake of fluorescein-conjugated dextran (Fig. 3). We have shown previously that the *Ha-ras* oncogene protein stimulates cell proliferation when microinjected into quiescent fibroblasts, whereas the protooncogenic protein is not mitogenic in this assay. The rapid enhancement of membrane ruffling and pinocytosis is induced by both the protooncogenic form and the oncogenic form of the *Ha-ras* protein. However, the effects produced by the oncogenic protein persist for more than 20 hours after microinjection, whereas the effects of the protooncogenic protein are short-lived, being restricted to a 3-hour interval after injection. The stimulatory effect of *ras* proteins on ruffling and pinocytosis is dependent on the amount of injected protein, requires extracellular calcium, and is independent of ongoing protein synthesis. Analysis of the phospholipid composition of ^{32}P -labeled cells reveals an apparent increase of 1.5–2-fold in levels of lysophosphatidyl choline and lysophosphatidyl ethanolamine by 1 hour after injection of the *ras* oncogene protein. These rapid changes in cell-membrane activities induced by *ras* proteins are the earliest *ras*-mediated effects detected to date and, as such, may represent primary events in the mechanism of action of *ras* proteins.

Production of Monoclonal Antibodies to the *fos* Oncogene

K. Riabowol, W.J. Welch, N. Sullivan, N. Kronenberg, G. Blöse, J.R. Feramisco [in collaboration with B. Vostalke and E. Ziff, New York University Medical Center]

Viral *fos* is the transforming gene of two murine retroviruses that induce osteosarcomas and trans-

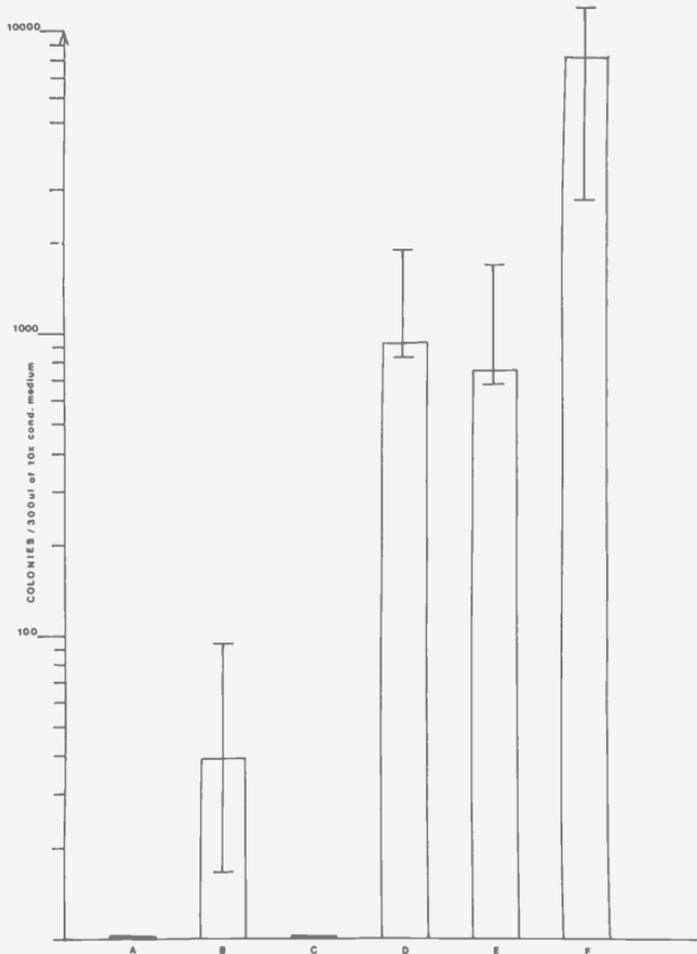


FIGURE 2 Conditioned medium from 1°REF (A), passaged REF (B), 1°REF to which fresh serum was added (C), REF52 cells (D), REF52 cells to which serum was added (E), or *ras*-transformed NRK cells (F) was incubated with 49F NRK cells in soft agar.

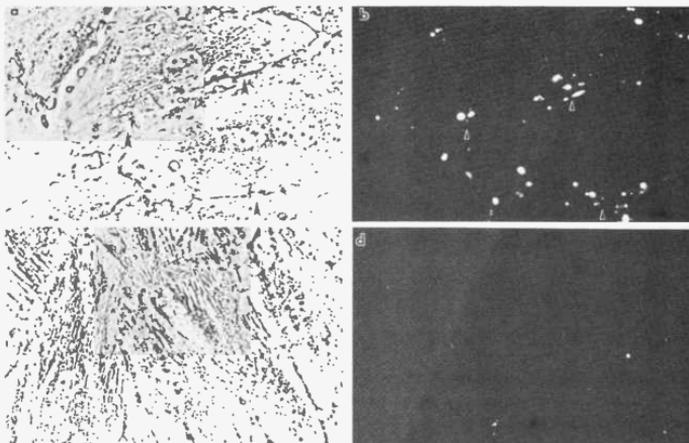


FIGURE 3 Confluent REF52 cells were microinjected with the *ras* oncogene protein (a) or with control protein (c) and incubated with FITC-dextran for 10 min. Note the increased uptake of the fluorescent marker (b, which is the same field as a) by the *ras*-injected cells compared with the control injected cell (d, which is the same field as c). Arrowheads mark the areas of ruffling and pinocytosis.

form fibroblasts *in vitro*. Transcriptional induction of *c-fos* mRNA occurs within 5 minutes of mitogen stimulation and reaches a level at least tenfold higher than in unstimulated cells. Although sequences necessary for (1) the transforming ability of *v-fos* and (2) the mitogen regulation of *c-fos* mRNA have been identified by mutational analysis, little is known about the function of the nuclear-localized *fos* gene product p55.

To address questions of function and synergism with other oncogenes, we are preparing monoclonal antibodies directed against a *trp-fos* fusion protein produced in bacteria (supplied by B. Vosatke and E. Ziff). Several hybridomas positive to the fusion protein (but not to *trp* alone) recognize a protein with a relative molecular weight of 55,000, by both immunoblot and immunoprecipitation assays, and selectively stain the nuclei of cells when used in immunofluorescence analysis. We are now in the process of both characterizing these antibodies and developing a system for the bacterial production of p55^{*trp-fos*} for use in generating antibodies directed solely against *fos* p55.

Cell Biology of cAMP-dependent Protein Kinase

J.C. Lamb, M.W. Wooten, W.J. Welch, J.R. Feramisco
[in collaboration with Dave Glass, Emory University]

Over a number of years, studies of the phosphoprotein substrates for cAMP-dependent protein kinase (A-kinase) have identified a number of substrates for this kinase. The nature of the substrates has led to the implication of the kinase in a number of cellular events including cell division and cellular morphology. To test these hypotheses, we have initiated living-cell assays for A-kinase in which the purified catalytically active protein is directly injected into cells, and the biological effects are assessed. Using this assay, we have found dramatic effects on the morphology of the cells and the state of phosphorylation of certain proteins within the cell.

Using a concentration that produces reversible changes in cellular morphology, we have injected small numbers of cells with the protein kinase or

control substances. Following pulse labeling with ^{32}P , we have identified a number of the protein substrates for the kinase in cells as well as temporal variation in the kinase-substrate specificity. We report that there are apparently fewer substrates for the kinase *in vivo* than have been found *in vitro*, and we have tentatively identified a number of these to be major cytoskeletal proteins (Fig. 4).

A number of recent reports have suggested that changes in the phosphorylation state of vimentin may be regulatory in the control of intermediate filament (IF) assembly. Similar implications have previously been made with respect to the assembly of microfilament (MF) networks involving myosin light-chain kinase (MLCK). We have therefore extended our present studies to determine if there is any significance in the specific phosphorylation of these proteins by A-kinase. Using double-label immunofluorescence with antibodies specific for vimentin (V9), tubulin (DMA 1A), and phalloidin (a stain specific for F-actin), we have investigated whether there are any changes in the integrity of the IF, microtubulin (MT), and MF networks as a result of injecting the catalytic subunit. We have been able to identify a direct temporal correlation between the changes in the distribution of actin MF

and vimentin IF with the changes in substrate phosphorylation following injection (Fig. 5). After injection of the kinase, significant changes in both MF and IF networks occur, including the complete dissolution of the MF network and the bundling of the IF. Concomitant with these changes, cellular morphology is altered and the cells become rounded. Additionally, we have noted a number of other effects resulting from injection, including the partial condensation of the chromatin and rearrangement of the nuclear envelope. We have shown that these effects are complete by 90 minutes and stable for at least 24 hours. By 36 hours, the effects have completely reversed; i.e., the distribution of both MF and IF networks, as well as cellular morphology, resembles that in uninjected cells. We can elicit similar changes in the distribution of the IF and MF networks with agents that increase the level of cAMP, although to a lesser extent than following injection of high levels of the active protein kinase.

The significance of these changes awaits further characterization. The interrelationship of the cAMP-dependent kinase and calmodulin and a number of other kinases *in vitro* is intriguing. Reports have suggested A-kinase, calmodulin, and cGMP-dependent protein kinase effect phosphorylation changes

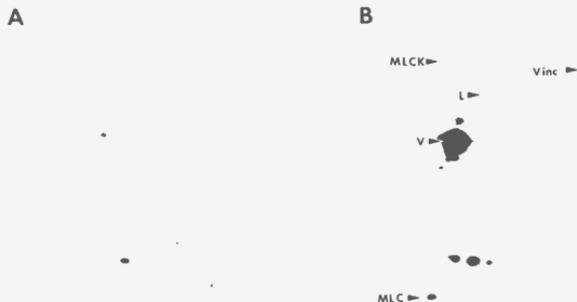


FIGURE 4 Two-dimensional analysis of the phosphoprotein patterns in rat embryo fibroblasts following microinjection of A-kinase. REF52 cells (~150 cells) growing on 2×4 -mm glass chips were microinjected with either injection buffer (100 mM K^+ glutamate, 40 mM K^+ citrate, 1 mM MgSO_4 at pH 7.30) (A) or 50 $\mu\text{g}/\text{ml}$ A-kinase in injection buffer (B). After injection (30 min), the cells were labeled for 30 min with 100 μCi ^{32}P in phosphate-free Dulbecco's modified Eagle's medium (DMEM) containing no serum. Following the labeling period, the cells were solubilized in two-dimensional gel electrophoresis sample buffer and then analyzed by isoelectric focusing (pH 5–7 ampholines), followed by electrophoresis on 12.5% SDS-acrylamide gels. Autoradiograms of the gels are shown with the acidic end to the left. (V) Vimentin; (MLCK) myosin light-chain kinase; (MLC) myosin light-chain; (L) nuclear lamins; (Vinc) vinculin.

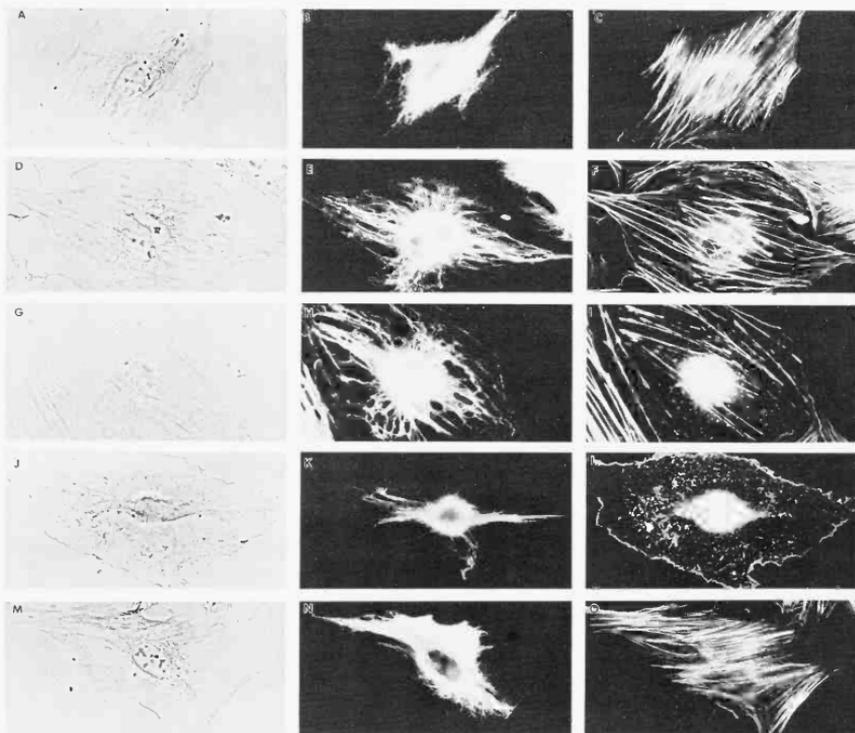


FIGURE 5 Effects of microinjection of the catalytic subunit of A-kinase on the distribution of microfilaments or intermediate filaments. Rat embryo fibroblasts grown on glass coverslips were microinjected with 50 $\mu\text{g}/\text{ml}$ of the catalytic subunit of A-kinase in injection buffer. The cells were fixed after different incubation periods following injection and stained by double-label fluorescence with an antibody specific for vimentin (B,E,H,K,N) and FITC-phalloidin (a stain specific for F-actin) (C,F,I,L,O). Phase micrographs (A,D,G,J,M) correspond to the double-label fluorescent micrographs for each row. (A,B,C) Cells injected with buffer and incubated for 90 min postinjection; (D,E,F) cells injected with A-kinase and further incubated for 30 min postinjection; (G,H,I) cells injected with kinase and further incubated for 60 min postinjection; (J,K,L) cells injected with the kinase and further incubated for 90 min postinjection; (M,N,O) cells injected with the kinase and incubated for 36 hr postinjection.

that occur when cells enter mitosis, and one possibility that we are currently investigating is that these changes reflect those involved in the induction of cell division. The changes in vimentin structure clearly resemble those seen when cells proceed into mitosis, and a similar case applies to the changes in cellular morphology, the condensation of chromatin, and the redistribution of actin. An-

other and not mutually exclusive possibility is that the changes represent alterations in the interaction between IF and MT, and actin and its associated proteins. As it is our aim to deduce precisely within living cells the biological and biochemical role of cAMP-dependent protein kinase in these physiological functions, this assay should prove useful in this goal.

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MAMMALIAN STRESS RESPONSE

W.J. Welch	J. Lamb	G. Blose
J.R. Feramisco	M. Wooten	N. Kronenberg
	J. Suhan	L. Miranda

During the past year, substantial progress has been realized with regard to our understanding of the physiological changes occurring in mammalian cells confronted with environmental trauma. Our studies, utilizing classical methods in cell biology, protein biochemistry, and immunology, have begun to provide a solid foundation for future experiments designed to address how cells both recognize abrupt changes in their local environment and trigger subsequent events at both the transcriptional and translational levels, leading to the increased production of the stress proteins. In addition, considerable efforts are being directed toward determining the biochemical function(s) of the individual stress proteins. As discussed below, these efforts have now begun to provide us with a better understanding of some of the properties of the stress proteins in both the normal cell and the stressed cell. Finally, we plan to accelerate our efforts in determining the molecular basis underlying the known hypersensitivity of various transformed cells and/or human tumors to physiological stress. We hope that these latter studies will eventually prove useful in exploiting the use of various forms

of physiological stress in the successful clinical treatment of human tumors.

The 70-kD Family of Stress Proteins Bind ATP

W.J. Welch, J.R. Feramisco

During the course of studies designed to assess various in vitro properties of both the constitutively expressed 73-kD stress protein and the highly stress-inducible 72-kD protein, we discovered that these proteins exhibit avid binding to various nucleotides, with the highest binding being observed for ATP. This observation has now proved most useful with regard to the rapid isolation of the 72/73-kD stress proteins from mammalian cells and has provided us with a new outlook regarding the possible biochemical function of these proteins. As shown in Figure 1, we have developed a simple two-step purification scheme of the 70-kD proteins utilizing ion-exchange chromatography on DEAE-cellulose, followed by affinity chromatography on

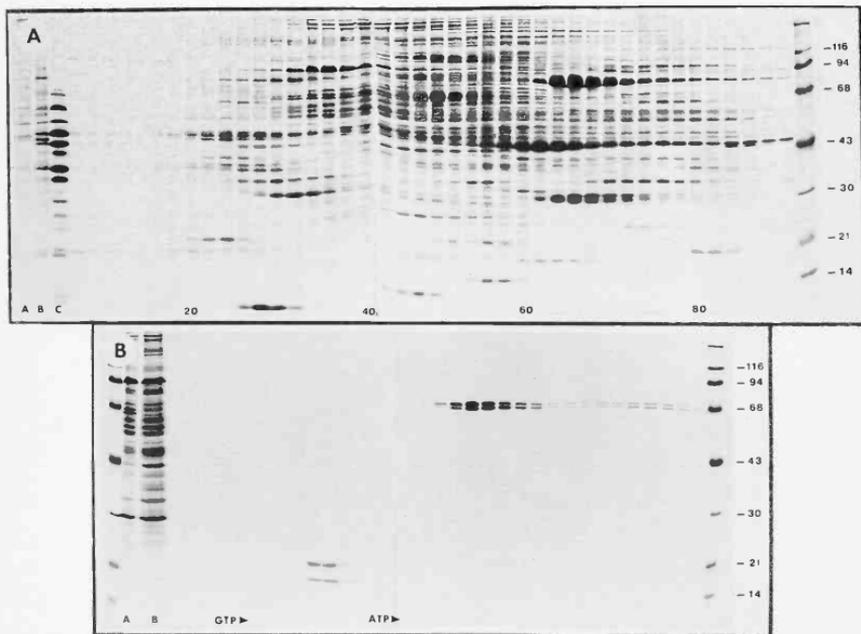


FIGURE 1 Rapid purification of the 72/73-kD stress proteins using affinity chromatography on ATP-agarose columns. HeLa cells grown in suspension were heat-shock treated at 42°C for 3 hr and then returned to 37°C for 5 hr. The cells were collected, washed with phosphate-buffered saline, and hypotonically lysed by Dounce homogenization. The lysed cells were centrifuged at 12,000g, and the supernatant was applied to a DE-52 ion exchange column. The column was then washed, and the proteins were eluted with a linear gradient of increasing NaCl. Fractions 38–54 were pooled and applied directly to an agarose column containing covalently linked ATP, and the column was then washed extensively with numerous cycles of low and high salt. The column was first developed with 1 mM GTP followed by 10 mM ATP. (A) Coomassie-blue-stained gel of the proteins eluting from the DE-52 column; (B) proteins eluted from the ATP-agarose column. Note that the 72/73-kD proteins eluted in an equimolar fashion and appear homogeneous.

agarose columns containing covalently linked ATP (Welch and Feramisco, *Mol. Cell. Biol.* 5: 1229 [1985a]). Using our new procedure, we found that it is relatively easy to purify milligram quantities of the 72-kD and 73-kD proteins in a rather short period of time. The proteins so isolated appear “native,” since they assume their proper locale within the cell following their reintroduction into cells via microinjection. In addition, using such microinjection techniques, we are continuing to examine the autoregulatory properties of the 70-kD stress proteins, as well as examining their putative role in the protection of cells from environmental stress. In addition to these *in vivo* studies, we are developing

in vitro assays aimed at dissecting the biochemical function of the individual 72-kD and 73-kD stress proteins. It is anticipated that this combined cellular and biochemical approach will soon provide us with the function of these proteins in both the normal cell and the stressed cell.

A Family of ATP-binding Stress Proteins

W.J. Welch

During the course of our purification studies of the

70-kD stress proteins, we discovered two other polypeptides that similarly displayed an affinity for ATP *in vitro* (Welch and Feramisco, *Mol. Cell. Biol.* 5: 1229 [1985a]). One of these, a 75-kD protein, is not synthesized at elevated levels in the stressed cell and therefore by definition does not constitute a classical stress protein. The 75-kD protein, however, does appear to be related to the 72/73-kD stress proteins by virtue of its similar isoelectric point and its proteolytic peptide pattern. Therefore, we are continuing to characterize the 75-kD protein in more detail and are trying to determine its exact relationship to that of the 72/73-kD stress proteins.

A second protein found to exhibit avid binding to ATP *in vitro* is an 80-kD polypeptide. We have previously shown that this protein is present primarily in the particulate fraction of cells (specifically the endoplasmic reticulum) and is one member of a group of proteins referred to as the "glucose-regulated" proteins (GRPs) (Welch et al., *J. Biol. Chem.* 258: 7102 [1983]). The GRPs comprise a class of proteins whose synthesis in cells is markedly affected by extracellular levels of glucose and/or calcium. For example, depriving cells of either extracellular glucose or calcium or treatment of the cells with calcium ionophores results in induction of the 80-kD as well as a 100-kD protein and a concomitant repressed synthesis of the 90-kD stress protein. In many cases, the GRPs appear to be regulated in an inverse fashion with respect to that of the stress proteins. For example, other investigators have reported an induction of the GRPs following anaerobic treatment, and following release back to aerobic conditions, both an induction of the stress proteins and a concomitant repression of GRP expression occur. These and other observations then indicate a close relationship between the regulation of synthesis of these two groups of proteins and perhaps a role for the GRPs and the stress proteins in certain metabolic pathways. A further similarity is our observation and that of J. Rothman's group at Stanford University that the 80-kD glucose-regulated protein is immunologically related to both the 72-kD and 73-kD stress proteins. Hence, it would appear that there exists a family of structurally related proteins in eukaryotic cells, all of which (1) show increased synthesis in response to different environmental traumas, (2) exhibit binding to ATP, but (3) have different intracellular locales and therefore probably perform different functions within the cell. Using immunological and biochemical techniques, we are investigating whether the

80-, 75-, 73-, and 72-kD all possess a common peptide domain that is involved in the binding of ATP.

Clathrin Vesicle Uncoating ATPase Is a Member of the 70-kD Stress Protein Family

W.J. Welch [in collaboration with T. Chappell, D. Schlossman, and J. Rothman, Stanford University]

Studies over the past several years in J. Rothman's laboratory at Stanford University have focused on the mechanism of protein trafficking in eukaryotic cells, with a particular emphasis on the role of clathrin-coated vesicles. Their efforts have resulted in the identification and purification of a 70-kD protein that releases clathrin triskelions from coated vesicles in an ATP-dependent manner (Schlossman et al., *J. Cell Biol.* 99: 723 [1984]). We noted that the purification scheme of this uncoating ATPase, and its apparent molecular mass, isoelectric point, and ability to bind ATP *in vitro* all were similar to, if not identical with, the properties we had determined for the mammalian 70-kD stress proteins. In a collaborative effort, therefore, we investigated whether the bovine brain uncoating ATPase was a member of the mammalian 70-kD stress protein family. Using two-dimensional gel analysis and comparative one-dimensional peptide-mapping procedures, we found that uncoating ATPase was indeed similar to, if not identical with, the constitutively expressed and abundant 73-kD stress protein (Chappell et al., *Cell* [1986] in press). Although this result provides the first indication of the function of one of the 70-kD stress proteins, the teleological ramifications of our observation remain somewhat obscure; i.e., how do we explain why a protein involved in the uncoating of clathrin-coated vesicles is also required in the response of cells to environmental stress? While further work will be necessary to answer this question, we do know already that physiological stress, particularly heat-shock treatment, results in a significant disruption of intracellular membranous organelles, including the Golgi complex and endoplasmic reticulum (Welch and Suhan, *J. Cell Biol.* 101: 1198 [1985]). Hence, during recovery from heat-shock treatment, the cells must rebuild these cytoplasmic organelles; and it is quite possible that clathrin-coated vesicles, which function in part in membrane biogenesis events, are

required in this rebuilding process. Indeed, using indirect immunofluorescence analysis with a monoclonal antibody specific for clathrin, we have observed a significant increase in the number of clathrin-coated vesicles throughout the perinuclear region during the recovery period following heat-shock treatment (W.J. Welch, unpubl.). Such clathrin-coated vesicle activity may then represent repair and/or rebuilding of the Golgi and endoplasmic reticulum, and the increased levels of the 73-kD stress protein in the recovering cells may facilitate this repair process.

Co-localization of the Major Stress-induced 72-kD Protein with Ribosomes

W.J. Welch, J.P. Suhan

Because it is the most highly induced stress protein, we have continued to pay special attention to the properties of the 72-kD protein with the aim, of course, to determine its function in the stressed cell. Our recent studies have centered on the fate of the 72-kD stress protein in cells recovering from physiological stress as induced by (1) heat-shock treatment, (2) exposure to heavy metals, and (3) exposure to amino acid analogs. Using metabolic pulse-labeling, we have found that the elevated synthesis of the 72-kD protein and that of the other stress proteins continue for as long as 12 hours following removal of the particular stress stimulus. Only after 12–24 hours does the synthesis of the stress proteins begin to subside and the cells begin to restore their normal patterns of protein synthesis. Interestingly, we always observe that repression of 72-kD protein synthesis precedes that of the other stress proteins. We think it possible then that regulation of the 72-kD protein synthesis is integral to the regulation of synthesis of the other stress proteins. Further studies in which the levels of the 72-kD protein are being manipulated in the recovering cells via microinjection of the purified protein or its corresponding antibody are in progress to address the possible role of the 72-kD protein in the down-regulation of either its own synthesis or the synthesis of the other stress proteins.

Using our battery of antibodies specific to the 72-kD protein, we have also been examining its intracellular fate in cells recovering from physiologi-

cal stress. After induction of the stress response, we find the majority of the 72-kD protein localized within the nucleus and nucleolus of the stressed cells (Welch and Feramisco, *J. Biol. Chem.* 259: 4501 [1984]). Using light and electron microscopy, we have observed that in those cells exhibiting a nucleolar locale of the 72-kD protein, the individual nucleoli are significantly altered. Specifically, we find that the nucleoli display a relaxed condensation state and an accompanying disruption and aggregation of its granular components (which consist primarily of pre-ribosomal particles). Using immunoelectron microscopy, we now know that the majority of the intranucleolar 72-kD protein is localized within this disrupted granular region. At later times following removal of the stress stimulus, the location of the 72-kD protein in the cell slowly changes; e.g., 4–8 hours after return of the shocked cells to normal growth conditions, the amount of nuclear/nucleolar 72-kD protein begins to decrease and there is a corresponding increase in the level of cytoplasmic 72-kD protein. The cytoplasmic 72-kD protein is found in both the perinuclear region and along the border of the cells, right underneath the plasma membrane where there also appear to be unusual phase-dense aggregates. Using immunological methods and electron microscopy, we now know that these phase-dense structures are composed of aggregated ribosomes, many of which also contain significant amounts of the 72-kD stress protein (Fig. 2) (W.J. Welch and J.P. Suhan, in prep.).

Thus, one major property of the 72-kD stress protein is its co-localization with pre-ribosomes (i.e., those within the granular region of the nucleolus) or cytoplasmic ribosomes. In addition, we speculate that these nucleolar and cytoplasmic ribosomes have become altered (i.e., perhaps denatured) as a result of the physiological stress. Therefore, it is possible that the 72-kD stress protein somehow functions in binding to denatured ribosomes in the stressed cell. Such a function could perhaps explain why such high levels of the protein are synthesized in response to stress (i.e., serving a stoichiometric rather than enzymatic function). In addition, because the 72-kD protein is an avid binder of ATP, we speculate that the interaction of the 72-kD protein with these ribosomes may also involve an ATP hydrolysis event, perhaps analogous to the situation in which the related 73-kD stress protein hydrolyzes ATP during its course of uncoating clathrin-coated vesicles.

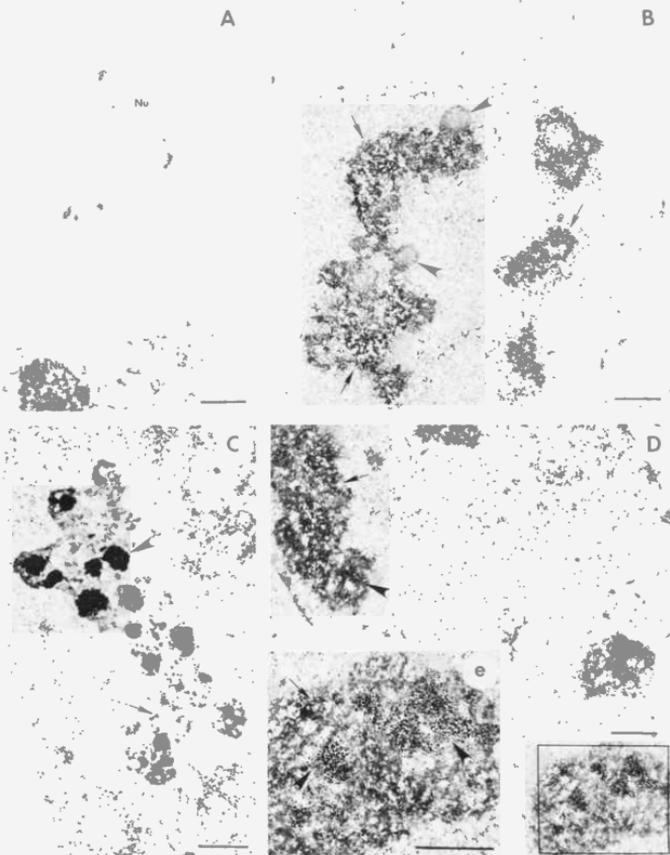


FIGURE 2 Distribution of the 72-kD stress protein within the granular region of the nucleolus. REF52 cells were heat-shock treated for 1 hr at 43°C and then returned to 37°C for 1 hr. The cells were fixed, permeabilized, and analyzed by either (1) silver staining to detect fibrillar centers involved in ribosomal RNA synthesis, (2) immunostaining with a mouse monoclonal antibody specific for the 72-kD protein, followed by peroxidase-conjugated goat anti-mouse antibody, or (3) combined silver staining and immunostaining. (A) Nucleoli of cells stained with preimmune serum; (B) nucleoli of cells stained with the anti-72-kD serum; (C) nucleoli stained with silver to indicate fibrillar centers; (D) combined immunostaining and silver staining; (e) a higher magnification of one of the nucleoli shown in D. Arrowheads in B–e indicate the silver staining fibrillar centers, and arrows indicate the granular region containing the 72-kD stress protein. Bars, 1 μ m.

Calcium, C-Kinase, and the Stress Response

M.W. Wooten, J.R. Feramisco, W.J. Welch

One major effort of the Cell Biology group has centered on determining the role of the calcium-sensitive/phospholipid-dependent protein kinase (C-kinase) in cell proliferation. Other laboratories have demonstrated that treatment of cells with tumor-promoting agents, such as the phorbol ester (PMA), or with various calcium ionophores results in an activation of C-kinase. Our laboratory had found previously that exposure of rat fibroblasts to PMA resulted in a marked increased phosphorylation of the 28-kD stress protein (Welch, *J. Biol. Chem.* 260: 3058 [1985]). In addition, we also observed that treatment of cells with a calcium ionophore also resulted in an increased phosphorylation of the 28-kD protein, as well as affecting the synthesis of three of the other stress proteins (Welch, *J. Biol. Chem.* 260: 3058 [1985]; Welch et al., *J. Biol. Chem.* 258: 7102 [1983]). Hence, because of these seemingly overlapping phenomena, we decided to examine whether changes in protein kinase C activity accompanied the induction of the stress response.

Biochemical analysis of rat fibroblasts following a 3-hour heat-shock treatment indicated that indeed there were changes occurring with respect to protein kinase C activity. First, we observed an enhanced activity of the enzyme (as assayed by phosphorylation of exogenous substrate, lysine-rich histone) following heat-shock treatment and that such activity quickly returned to basal levels following return of the cells to normal growth conditions. Second, we observed increases in the levels of phosphatidylinositol-4-phosphate and phosphatidylinositol-4,5-bisphosphate (PIP₂). PIP₂ serves as a substrate for phospholipase C, which generates diacylglycerol, the activator of PKC, and inositol-1,4,5-triphosphate (IP₃). Utilizing [³H]inositol labeling, we have observed a persistent elevation of IP₃ in stressed cells and a return to normal levels upon reversal of the cells to normal conditions. Finally, we have found a rapid but transient increase in the uptake of extracellular calcium into the cells following a short exposure to heat-shock treatment (M.W. Wooten et al., in prep.). Although still preliminary, we think these results are suggestive of there being an involvement of calcium and protein kinase C in the early stages of the stress response.

Further studies are in progress to test this hypothesis. In addition, using the purified C-kinase protein both in *in vitro* assays and in microinjection studies, we are investigating whether phosphorylation of the 28-kD stress protein is mediated by C-kinase itself or perhaps another kinase, itself activated by a C-kinase-mediated phosphorylation event.

The Stress Response in Normal and Transformed Human and Rodent Cells

W.J. Welch, J.R. Feramisco

Although it has been observed for many years that transformed cells *in vitro* or tumors *in vivo* are generally very hypersensitive to heat-shock treatment, the molecular basis underlying this hypersensitivity remains obscure. Consequently, we have initiated efforts to determine the basis for the high sensitivity of transformed cells to physiological stress and to develop experimental protocols utilizing physiological stress that may prove useful in the treatment of tumors *in vivo*. Due to a shortage of manpower, these studies have progressed rather slowly, but we anticipate a more intensive effort over the next few years.

We have elected to study the effects of physiological stress in transformed cells utilizing two experimental systems: (1) normal and transformed rat embryo fibroblasts and (2) various human tumors grown *in vitro*. For the former, we have been examining both normal rat embryo fibroblasts (REF52) and REF52 cells stably transformed by either SV40 (WT6 cells) or adenovirus (Ad5d4A cells). As illustrated in Figure 3, a 3-hour heat-shock treatment of cells resulted in a less than 10% cell killing of the normal REF52 cells but a greater than 95% cell killing of either of the two transformants. Even more dramatic differences in the survival curves are observed when sodium arsenite is used as the elicitor of the stress response. As described in last year's Annual Report, the survival curves can also be greatly affected by simply manipulating the metabolic state of the cells prior to stressing the cells. For example, removal of extracellular glucose or calcium, lowering the pH of the growth medium, or interfering with mitochondrial function can greatly enhance the cell killing resulting from the heat-shock treatment. These

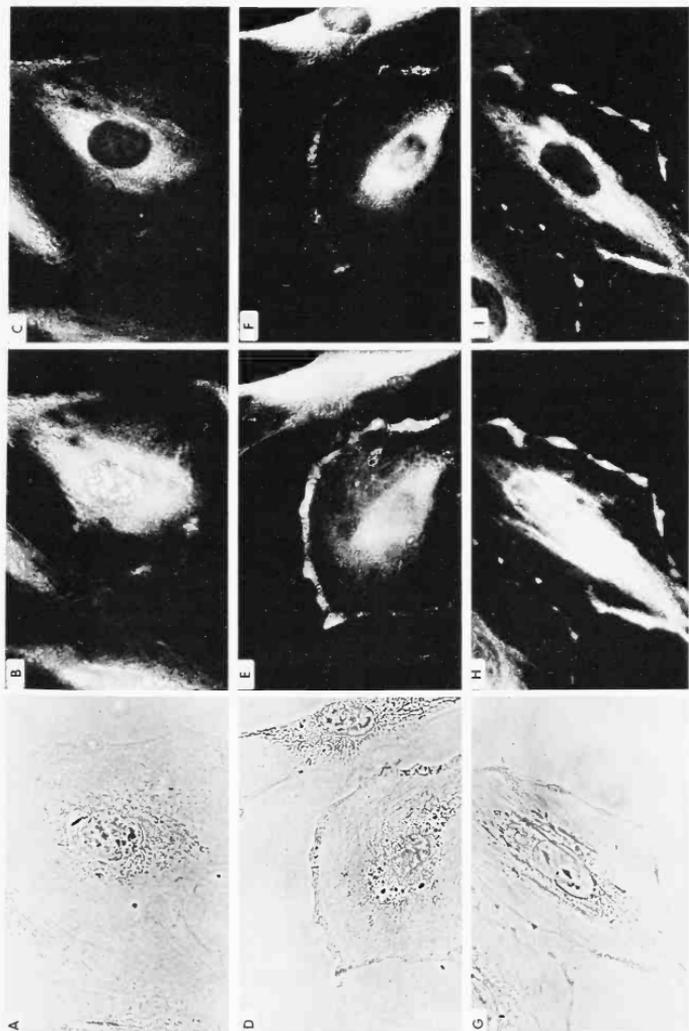


FIGURE 3 The 72-kD stress protein co-localizes with cytoplasmic ribosomes in cells recovering from physiological stress. REF52 cells growing on glass coverslips were subjected to either heat shock treatment (3 hr at 42°C), exposure to Azc (5 mM, 10 hr at 37°C), or exposure to sodium arsenite (60 μ M, 1.5 hr at 37°C). The cells were then returned to normal growth conditions and allowed to recover for 4 hr (heat shock) or 8 hr (Azc and sodium arsenite). The cells were then fixed, permeabilized, and analyzed by double-label indirect immunofluorescence using a rabbit polyclonal antibody specific for the 72-kD protein and a human autoantibody specific for eukaryotic ribosomes. Primary antibodies were visualized using a mixture of fluorescein-conjugated goat anti-rabbit antibody and rhodamine-conjugated rabbit anti-human antibody. (A-DG) Phase micrographs; (B,E,H) fluorescent micrographs of 72-kD staining; (C,F,I) fluorescent micrographs of ribosomal staining. (A-C) Cells 4 hr after heat shock reversal; (D-F) cells 8 hr after Azc reversal; (G-I) cells 8 hr after sodium arsenite reversal.

types of studies, which again reinforce our ideas regarding the importance of the stress proteins in certain metabolic pathways (i.e., sensitivity to glucose levels, calcium, etc.), will hopefully provide new insight regarding the mechanism responsible for the hypersensitivity of the transformed cells to physiological stress. We hope that these types of *in vitro* studies may someday prove useful in clinical studies utilizing physiological stress in the successful treatment of human tumors.

We have also been examining the expression of the 72-kD stress protein in various normal and transformed human and rodent cell lines. These studies were prompted in part from the previous observations of other investigators showing that the adenovirus E1A gene product (13S) stimulated the expression of the 72-kD protein in various human cell lines (Nevins, *Cell* 29: 913 [1983]; R. Morimoto, pers. comm.). Indeed, we have found that 293 cells, which constitutively express the E1A and E1B gene products, synthesize extremely high levels of the 72-kD proteins even at their normal physiological growth temperature. In addition, analysis of a number of other human tumor cell lines, many of which contain the oncogenic form of the *ras* protein (provided by M. Wigler, Molecular Genetics of Eukaryotic Cells Section), reveals that they also constitutively produce modest levels of the 72-kD protein (W.J. Welch, unpubl.). This constitutive expression of the 72-kD protein is also observed in normal human foreskin fibroblasts but at a somewhat lower level than that observed for the various transformed human cell lines.

We should also point out that the constitutive expression of the 72-kD protein in human cells appears to be cell-cycle-regulated as well as stimulated by the addition to cells of fresh serum (R. Morimoto, pers. comm.). Hence, it will be important to determine the relevance of this constitutive 72-kD protein expression apparently unique to different human cell lines and to assess the effects of transformation on 72-kD protein expression.

The expression of the 72-kD protein in normal and transformed rodent cell lines is very much different from that observed for the various human cell lines. At physiological growth temperatures, there is little or no constitutive expression of the 72-kD protein in either normal or transformed rat cells. Moreover, even in rat cells stably transformed by the adenovirus E1A gene, induction of the 72-kD protein is not observed. Thus, although E1A appears to promote expression of the 72-kD protein

in human cells, it appears to have little or no effect on 72-kD protein expression in the rodent cells. Again, it should be emphasized, however, that in the normal human cell lines (but not the rodent cells), there already exists a low level expression of the 72-kD protein. Hence, E1A could conceivably act in some manner to amplify this constitutive expression of the 72-kD protein, but it would have no effect in the rodent cells where there does not already occur a basal level of the 72-kD protein expression. Suffice it to say that at the present time we do not understand the basis for this differential expression of the 72-kD protein in both normal and transformed human and rodent cell lines and that much continued effort will be required to sort out this confusing issue.

Polyclonal and Monoclonal Antibody Production

W.J. Welch, J.R. Feramisco, N. Kronenberg, G. Blöse

Over the past year, we have succeeded in producing a number of different monoclonal antibodies to some of the stress proteins as well as to the *ras* oncogene protein. In the case of the stress proteins, we have approximately ten different monoclonal antibodies specific for the 72-kD stress protein. We have also produced a polyclonal and monoclonal antibody that appears to recognize the related family of ATP-binding proteins, namely, the 72-, 73-, and 80-kD proteins. Current effort is being directed at obtaining antibodies specific to the small 28-kD stress protein, the 80-kD glucose-regulated protein, and the 90-kD stress protein.

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QUEST LABORATORY

J.I. Garrels	C. Chang	J. Kos	J. Ross
B.R. Franza, Jr.	M. Hannaford	P. Mailloux	H. Sacco
M.E. Lambert	D. Harty	P. Myers	S. Scheib

We have continued and expanded our efforts to apply quantitative electrophoresis technologies (QUEST) to the development of novel and widely useful protein databases. Standardized two-dimensional gel electrophoresis, followed by detailed computer analysis, allows us to quantify and compare the changes of several thousand proteins in each of our experimental systems. Databases, containing baseline characterizations and regulatory information for each detected protein, are now being assembled to include the results of hundreds of separate experiments. Our primary goal has been to develop protein databases for cancer research. These databases, which are a proving ground for the standardized electrophoresis and image analysis procedures we have developed over the past several years, have already begun to reveal interesting regulatory data about many proteins never before described.

The past year has been a year of scaling up. We have received funding from the National Institutes of Health to become a Biotechnology Resource Facility for the purpose of large-scale database development and dissemination. The first year of the

new program has been devoted largely to equipment acquisition, software conversion and enhancement, and, in the last few months, hiring of the resource staff. New quarters in the Grace Auditorium are nearly ready for our occupancy. A detailed description of the activities of the resource facility is presented below.

A new postdoctoral fellow, Michael Lambert, has joined the laboratory to pursue studies of chemical carcinogenesis and responses to DNA damage. Michael comes from the laboratory of I.B. Weinstein at Columbia University and brings a wide knowledge of systems of experimental carcinogenesis. He will study the effects of DNA-damaging agents in detail using the REF52 family of normal and transformed cell lines and is developing epithelial cell systems in which induced responses to DNA damage can be studied in the context of multistep, multifactor transformation.

Our efforts to develop the REF52 family of cell lines as a controlled experimental system for cancer research and for protein database development have accelerated. Led by Robert Franza, and assisted by several collaborators, REF52 cells trans-

formed by cloned oncogenes have been studied at the biochemical level and at the level of tumorigenicity. Even prior to generating the capacity for routine analysis of all the electrophoretic data, interesting findings have already been made about the differential responses of these lines to external stimuli.

A Resource Facility for Two-dimensional Gel Protein Databases

J.I. Garrels, B.R. Franza, Jr.

A Biotechnology Resource facility has been established for the purpose of developing and disseminating databases derived from quantitative analysis of two-dimensional gels. The intent of the Resource Award is to foster the development of several pioneering databases of great scientific utility. Electrophoresis and computer methods worked out over the past few years will be applied to the routine analysis of two-dimensional gel data from many experiments involving the REF52 family, mouse embryo cells, and yeast. The construction of large, internally consistent, and easily accessible databases in these areas is the major challenge of the new facility.

SCOPE OF THE RESOURCE ACTIVITIES

The core research component of the Resource Facility will build the nucleus of each database through a series of baseline experiments. These experiments are necessary for the proper interpretation of all subsequent experiments. The core experiments include extensive controls for the reproducibility of the electrophoresis and analysis procedures, controls for the reproducibility of cell-labeling under normal culture conditions, and a set of basic experimental characterizations of each cell system under study. Other experiments needed for the nucleus of each database are (1) behavior of cells throughout the range of normal culture conditions; (2) variability between clones or strains; (3) responses to physiological stresses such as heat, anoxia, or oxidative stress; and (4) responses to defined growth factors or hormones. Additional studies will investigate intrinsic parameters of each protein that may help to reveal its identity. Labeling the cells with several different amino acid precursors,

one at a time, can reveal the relative amino acid ratios in each detected protein, and these can be compared to the amino acid compositions of known proteins. Incorporation of radiolabeled phosphate, sugars, or lipids can reveal which proteins are posttranslationally modified. Pulse-chase kinetics can reveal which proteins are stable and which are rapidly turning over. Subcellular fractionations can serve to classify proteins by localization in nuclei, membranes, mitochondria, etc. Such characterizations provide fundamental knowledge for each detected protein and are necessary for proper interpretation of experiments directed at specific biological questions.

Another core activity of no less importance is the identification of the known proteins on our two-dimensional maps. Hundreds of specific antibodies and purified proteins are available which, through immunoprecipitation, immunoblotting, and direct comigration, can be used to identify a particular spot on our two-dimensional maps. Each time a spot is identified, its name, source of antibodies, and literature citations can be added to the database. Approximately half the time of our core research staff will be devoted to protein identifications.

The facility will engage in in-house and collaborative experiments in each database area to add new information from the leading edge of research in each field. Many of our collaborators in the transformation field are mentioned in our current and previous reports. The mouse and yeast databases depend primarily on collaborations. In the mouse field, prior work with L. Silver and colleagues is now being extended through the active interest of D. Hanahan and D. Solter (Wistar Institute). For the yeast database, D. Beach and C. McLaughlin (University of California, Irvine) will carry out most of the initial experiments. As each database grows and matures, other collaborations will allow in-depth work in specific subfields of mutual interest.

The databases would be of little value to the scientific community if they were not widely accessible. Our resource has provisions for service use and for dissemination of databases to other laboratories. Outside researchers with projects directly relating to our databases can have gels run in our laboratory and can use our facilities for analysis. In addition, interested researchers can visit our laboratory to use the databases, and database materials (tapes, maps, spot graphs) can be sent to other

laboratories. Several of our resource technicians will be trained to help the service users and to respond to outside requests for information.

PHYSICAL FACILITIES

The two-dimensional gel resource facilities will be housed, in early 1986, on the lower floor of the new Grace Auditorium. A large computer room, conference room, reception area, offices, and workstation rooms have been designed specifically for our purpose. The workstations are MASSCOMP 32-bit computers with integrated graphics and array processors. Each workstation will have over 400 megabytes of disk storage and all are connected via Ethernet to each other and to the other laboratory computers. A rotating drum scanner is presently available, and a fast laser scanner will be purchased in 1986.

RESOURCE STAFF

In addition to J. Garrels and R. Franza, the staff consists of Cecile Chang (operations supervisor), Jim Kos (programmer/analyst), and Mary Hananof (office administrator). Heidi Sacco will become database manager in 1986, Susan Scheib and Janet Ross will assist with database development and analysis, and two additional core research technicians will be hired. The gel laboratory (P. Myers, D. Harty, and P. Mailloux) is formally a separate facility funded by user charges, but much of its capacity is reserved for users of the Biotechnology Resource. Users of the facility will communicate with Cecile on matters of sample preparation and electrophoresis and with Cecile or Heidi on details of analysis. Both Heidi and Jim Kos will help users to learn to use the workstations.

EQUIPMENT ACQUISITION AND SOFTWARE CONVERSION

Most of the first-year budget has been for equipment acquisition and for software conversion. After careful survey of available equipment, the MASSCOMP workstations were chosen because of the cost/performance ratio and because they support UNIX and a standard assortment of peripherals. Being compatible with Ethernet, the MASSCOMP machines can easily communicate with the PDP-11, SUN, and VAX computers that may exist at Cold Spring Harbor Laboratory now

or in the future. Our facility will contain four MASSCOMP workstations, each with an internal array processor and graphics processor. Each workstation, without its array processor, has about the power of a VAX-11/750, but with the array processor, it is at least tenfold faster for our application.

Software conversion has been a major undertaking that has occupied much of the first year. The original software was written in Fortran under RSX-11M for a PDP-11 with small address space. It was necessarily split into many separate data and program units. The new software is written in C language under the UNIX operating system operating in 16 megabytes of virtual memory space. Obviously, in the course of conversion, substantial condensation and enhancement could be effected. Among the enhancements are real-time synthetic image generation (images are created from spot lists as they are displayed), true two-dimensional gaussian fitting, and fast multipass matching using the array processor. The entire program now is an integrated unit so that any operations of spot-fitting, editing, matching, or data analysis can be performed and repeated by the user at will. An entire experiment, involving as many as 20-30 gels, can be loaded into the system for quantitation and matching as a unit. Through these enhancements of function and performance, the old system, which served as proof of principle, has been converted into a routine production system.

Inducible Cellular Responses to DNA Damage in Mammalian Cells

M.E. Lambert, J.I. Garrels

We have begun to study the ordered sequence of cellular responses elicited in mammalian cells after exposure to different DNA-damaging agents, including known human chemical carcinogens and radiation. As an approach, we are examining changes in the patterns of cellular protein expression in both rat fibroblasts and epithelial cells by use of the QUEST system. Our objective is to identify specific markers for inducible responses to different classes of DNA-damaging agents and to study the involvement of these proteins in tissue-specific repair processes and in cellular transformation.

In preliminary experiments (in collaboration

with I.B. Weinstein, Columbia University), we have found that single low-dose exposures of REF52 cells to either of two direct-acting chemical carcinogens, benzo(a)pyrene-*trans* 7,8-dihydrodiol, 9,10-epoxide (anti) (BPDE) or *N*-acetoxy-2-acetyl-aminofluorene (NAAF), induce the rapid appearance of a common major protein species of 30K and an IEF of pH 5.0. This newly identified protein, which we call carcinogen inducible protein 1 (CIN 1), is a specific marker for treatment with these agents and is not induced by growth arrest alone. Studies are under way to characterize the subcellular localization, rate of synthesis and half-life, posttranslational modifications (including poly(ADP) ribosylation), and transcriptional regulation of both CIN 1 and other, less abundant, cellular proteins induced by these two chemical carcinogens.

We are extending our survey of inducible responses to DNA damage in REF52 cells to other agents and regimens, including exposure to ionizing radiation (in collaboration with C. Borek, Columbia University), UV light, bleomycin, hydrogen peroxide, and microinjection of carcinogen-modified DNA substrates. Using antibodies to known DNA replication and repair enzymes, including DNA polymerase alpha, topoisomerase 1 and 2, and thymidine kinase, we are focusing, in particular, on changes in nuclear proteins during the acute phase of cellular responses to DNA damage.

We are also exploring the involvement of inducible cellular responses to DNA damage in multistep, multifactor cellular transformation. In one study (in collaboration with N. Sullivan), we are examining changes in cellular protein expression during immortalization and transformation of nonestablished rat kidney epithelial cells in culture by either chemical carcinogens or viral and cellular oncogenes. Chemical carcinogens, such as *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), induce a high frequency of both preneoplastic and neoplastic changes in rat epithelial cells. The molecular basis of these high-frequency transitions is not known but may involve the induction of a eukaryotic "SOS"-like response. In our study, we are developing both cell-type-specific and stage-specific cellular protein markers on the basis of a systematic comparison of both partial and full transformants derived independently by either treatment with MNNG or transfection of cloned oncogenes. Such markers may also be of value in the early detection of human cancers of epithelial origin.

Finally, we are utilizing the QUEST system to

study the synergy between specific chemical carcinogens, such as methyl methane sulfonate (MMS) and the cold-sensitive host-range mutant of human adenovirus type 5, Hrl1, in transformation of the rat embryo fibroblast line CREF (in collaboration with P. Fisher, Columbia University). We have designed protein-labeling experiments to distinguish between the individual effects of treatment with MMS (or infection with Hrl1) and the synergistic effects that lead to full transformation, which result from combined treatment with both carcinogen and virus. Using QUEST, we can now ask whether the molecular and cellular bases of this synergy may lie in part in the ability of MMS to induce the altered expression of specific cellular proteins that enhance the transforming potential of Hrl1.

Oncogenic Transformation Discriminated from In Vitro Morphologic Transformation

B.R. Franza, J.I. Garrels, C. Chang, J. Ross [in collaboration with S. Blose, Protein Databases Inc., Huntington, New York; B.R. Bloom and R. Goldschmidt, Albert Einstein College of Medicine, New York; and H.E. Ruley, Massachusetts Institute of Technology]

We have derived an interesting family of cells by transfecting various oncogenes into the rat cell line, REF52. Unlike many rodent cell lines, the REF52 line is not oncogenically transformed by activated *ras* genes. Although REF52 is established, i.e., it grows continuously in tissue culture when supplied with serum growth factors, it still requires as yet undefined biochemical activities provided by adenovirus 5 early region 1A gene (Ad5E1A) products that appear to collaborate with activated *ras* gene products in generating in vitro growth characteristics classically referred to as transformed. As a result of these unique responses to different classes of oncogenes, we have been able to isolate lines that constitutively express either the Ad5E1A products or the products of the activated *ras* genes or both.

Early in this project, several of the lines that coexpressed both the Ad5E1A and T24 Ha-*ras*-1 oncogenes were screened for oncogenesis in syngeneic F344 rats. Of interest was the fact that although certain clones always generated tumors, other clones never generated detectable tumors. Of additional interest was the fact that using techniques that allow for detection of cell-surface proteins, one of the clones that induced tumors had

several labeled polypeptides significantly different from those of a clone that did not yield tumors.

At this point in the project, we were fortunate to be able to form a collaboration with colleagues at the Albert Einstein College of Medicine. We specifically designed experiments to explore whether the tumorigenicity of the two transformed clones could be related to their immunogenicity. Since there is considerable evidence that natural killer (NK) cells have the ability to recognize and lyse many virus-infected target cells and tumor cell lines, we were intrigued by the possibility that one of the transformed cell lines that failed to grow in vivo may be susceptible to attack by NK cells and that the clone that is able to grow may be resistant to immunological attack.

Results of *in vivo* studies in which each clone is transplanted subcutaneously into syngeneic F344 rats indicated consistently that one clone (IA7) failed to produce tumors even at inocula of 10^6 cells. However, when the animals were treated with anti-asialo-GM1 antibodies known to react with and inactivate NK cells *in vivo* and *in vitro*, two thirds of the animals transplanted with clone IA7 now exhibited tumors at 10 days: all tumors regressed by day 17. In contrast, all animals transplanted with clone IA9, irrespective of being injected with anti-asialo-GM1, exhibited tumor formation by day 6. In addition, all clone IA9 tumors regressed by day 14. These results suggested that failure of clone IA7 to generate tumors might be the consequence of rejection via an NK cell mechanism.

Consequently, these experiments were repeated in athymic nude rats that lack the ability to generate cytotoxic T lymphocytes or produce lymphokines such as γ -interferon. Once again, clone IA7 cells failed to produce tumors unless the animals were inoculated with anti-asialo-GM1 antibodies. Clone IA9 cells did not require inoculation with the anti-asialo-GM1 antibodies to produce tumors. In all cases when tumors did form, they did not regress as they did in the normal F344 rats.

Provisional interpretation of these experiments would be that NK cells, which can be blocked by anti-asialo-GM1 antibodies, are able to recognize some determinant present on clone IA7 cells and lacking on clone IA9 cells and thereby initiate early rejection of the IA7 cells. Regression occurs in intact rats but not in athymic rats, suggesting that in addition to an early rejection by NK cells, there is sensitization by both clones leading to the development of cytotoxic T lymphocytes that appear to be

able to recognize determinants on both tumor lines—a process that does not occur in athymic animals.

Preliminary *in vitro* experiments are now under way to examine the susceptibility of the different clones to killing by NK cells and cytotoxic T cells. In addition, tumor explants are being grown in tissue culture to enable comparative studies between the transplanted clone and the resulting tumor-derived cells. Efforts are also under way to determine if metastases have formed and if so, attempts will be made to expand these cells in culture.

From our earliest studies with REF52, we were encouraged by the fact that expression of a single oncogene did not result in oncogenic transformation, yielding an *in vitro* system for the study of multistep acquisition of an oncogenic potential. Equally encouraging is the fact that even the coexpression of two oncogenes is not sufficient for oncogenic transformation as noted in this report. We now have a family of cells, derived from a very stable parental line, that exhibit a range of growth and oncogenic properties allowing for a number of experimental strategies that take advantage of the ability to compare such a related series of cell lines differentially—a process largely dependent on the analytical and database capabilities of the QUEST computerized system for two-dimensional gel analysis.

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Franza, B.R., Jr., K. Maruyama, J.I. Garrels, and H.E. Ruley. 1986. In vitro establishment is not a sufficient prerequisite for transformation by activated *ras* oncogenes. *Cell* 44: (in press).

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Lambert, M.E., J.I. Garrels, J. McDonald, and I.B. Weinstein. 1986. Inducible cellular responses to DNA damage in mammalian cells. In *Mechanisms of antimutagenesis and anticarcinogenesis* (ed. Shankel et al.). Plenum Press, New York. (In press.)

Since the middle of the last century, neurologists have considered that specialized neural functions of the human organism were represented in discrete areas of the brain. In experimental animals, different functions have been localized to specific areas using a variety of techniques. How does the brain achieve and maintain the parcellation of discrete functions to specific areas? How can different areas be identified and studied? One possible explanation of functional differences among neurons holds that the neurons themselves are molecularly identical, but differences in interconnections alone determine functional divisions. Alternatively, neurons may be molecularly heterogeneous in a pattern that might reflect functional subdivisions of the brain. The experiments of the neurobiology group have shown that intrinsic molecular differences exist among neurons that appear identical by morphological criteria. These molecular differences can be used to study the development and the functional organization of the brain in experimental animals and potentially in humans. Our studies in the last year have extended our earlier work identifying neural cell types and have provided important new information about the organization of the human brain.

Characterization of Cellular Diversity in the Mammalian Nervous System with Monoclonal Antibodies

S. Hockfield, B. Friedman, S. Zaremba, C. Bautista, E. Waldvogel

ORGANIZATION OF THE CORTEX IS REVEALED WITH MONOCLONAL ANTIBODY CAT-301

Functionally Related Areas of Monkey Cortex Are Antigenically Related. Visual stimuli are processed as parallel channels of component information. In simplified terms, motion, color, and form are each carried separately through a series of multiple relays in the brain. In the visual cortex, neurons can be identified that respond selectively to one of these features of visual information. In the monkey, at least ten different areas process visual stimuli, each performing a characteristic set of computations on incoming information. Some areas, or subdivisions of an area, are apparently dedicated to one information channel. Monoclonal antibody Cat-301 recognizes neurons in several, but not all, visual cortical areas. Our analyses using double-labeling techniques and correlating antibody-staining patterns with electrophysiologically generated maps of cortical function suggest that Cat-301 selectively recognizes neurons involved in

processing the motion component of visual stimuli. Five areas that constitute consecutive relays in the motion channel contain Cat-301-positive neurons. Differences in staining characteristics (number and location of antibody-positive neurons) allow us to map the boundaries of each area accurately.

In each area that contains Cat-301-positive neurons, only a subset of neurons are recognized by the antibody. The pattern of antibody-positive neurons reveals distinctive features of the organization of each area. For example, in the primary visual cortical area, V1, Cat-301-positive neurons line up in rows that reflect the alternating input from right and left eyes (the ocular dominance organization). In the secondary visual area, V2, Cat-301-positive neurons form strips that correspond to the subdivision of V2 that relays motion information to the next cortical area, MT (the middle temporal area). A subset of the neurons in MT are Cat-301-positive neurons, as are neurons in the area to which it projects, MST (the medial superior temporal area). Functionally distinct neuronal subsets in MT and MST have not yet been identified, but staining with Cat-301 suggests that functional subdivisions may exist within these higher-order processing areas.

Now that we have shown that the general maps of "motion" areas and Cat-301-positive areas overlap, we would next like to know if individual neu-

rons of the motion channel are Cat-301-positive. Our current experiments combine a metabolic tracer of neuronal activity (2-deoxyglucose) with Cat-301 immunohistochemistry to resolve at the level of single neurons whether motion stimuli selectively activate the population of neurons identified with Cat-301.

Cat-301 Demonstrates the Functional Organization of the Human Cortex. Our studies of the organization of the cortex have relied on a large body of data generated from investigations in experimental animals. The organization of the human cortex has been more difficult to determine, as the experimental manipulations used to demonstrate selective cortical involvement in specific tasks often require invasive techniques. Descriptions of human cortical organization have relied on analogies in subtle features of the cellular organization between human and experimental animals and on studies of individuals with cortical injuries. Recent advances in tissue visualization methods, the PET and CAT scanners, have demonstrated localization of cortical function, but these techniques generate a relatively low resolution picture and do not provide information at the level of individual nerve cells.

The conservation of antigens through evolution potentially allows studies in experimental animals to be extended to the human nervous system. Previously, we showed that Cat-301 recognizes an antigen in the human cortex that is related to the antigen in monkey and cat. Recently, we have used Cat-301 to study the organization of the human visual cortex. In the first visual cortical area, V1, the human cortex displays a periodicity with Cat-301 similar to that found in the monkey. We know from our earlier studies that Cat-301-positive neurons mark the center of each ocular dominance stripe. The width of an ocular dominance stripe in monkey is 0.5 mm. We have now shown with Cat-301 that the human visual cortical area V1 is also organized in periodic stripes and that these stripes are 1 mm in width. In the monkey, the end of the area containing the stripes marks the boundary between area V1 and the adjacent area V2. Similarly, the border of human V1 is marked by the end of the region containing the 1-mm-wide Cat-301 stripes.

The organization of V2 in monkey has been studied with electrophysiological, histochemical, and mapping techniques (as described above), but it has not been mapped in human. As in the monkey area

V2, in human area V2, Cat-301 shows that a subset of neurons line up to form thick strips, oriented perpendicular to the border between V2 and V1. These strips are therefore homologous to the thick strips in monkeys which contain neurons that participate in the motion channel. We next plan to localize areas MT and MST in the human. From these results, it is clear that reagents like Cat-301 can be enormously useful in dissecting functionally significant areas of the human brain.

Expression of Cat-301 Is Controlled by Early Visual Experience. Early experience can permanently alter the structure and function of the mammalian brain. In the visual system, deprivation from birth through a "critical period" of development profoundly alters the shape and physiology of neurons in central visual areas. How such changes are mediated is not well understood. We have found that visual deprivation results in a loss of the Cat-301 antigen from areas that would normally receive the deprived input. The loss is not tied simply to a reduction in neuronal activity, as visual deprivation in an adult does not affect antigen expression. This suggests that the phenotypic changes in neurons consequent to early experience are reflected by the expression of particular molecules. We are now identifying other experience-dependent molecules in order to obtain a description of the molecular events associated with late developmental events. One potential use of such markers will be to describe developmentally significant periods in human neural development.

The Antigen Recognized by Cat-301 Is a Protein. Do all Cat-301-positive neurons express the same antigen or do different groups of neurons express distinct molecular species that share only the epitope recognized by Cat-301? Do Cat-301-negative neurons express a molecule related to the Cat-301 antigen? To address these questions, we are characterizing and purifying the antigen recognized by Cat-301. As the subcellular localization of the antigen is consistent throughout the nervous system and in all species, it is likely that related molecules are expressed by all Cat-301-positive neurons. Our ultrastructural studies suggest that Cat-301 may recognize some component of the extracellular matrix. Biochemical studies have shown that the antigen is soluble in deoxycholate and that an acidic species is specifically eluted from a Cat-301 cyanogen-bromide-activated Sepharose column, charac-

teristics that might be consistent with a component of the extracellular matrix. Immunoreactivity is lost after digestion with several proteases, suggesting that the antigen contains protein. On Western blots, the eluted fractions from a Cat-301 affinity column show 30-kD and 20-kD immunoreactive bands. Our current efforts are to purify enough of the antigen to obtain a partial protein sequence to then use in conjunction with the monoclonal antibody in an effort to clone the molecule.

GROWING NEURONS NAVIGATE ON NONNEURONAL CELLS AND ON PREVIOUSLY ESTABLISHED AXON TRACTS

How neurons find the appropriate routes to grow along to make correct connections has been a major focus of our work over the past 4 years. In cell culture, axons have been shown to prefer some substrates to others and to grow readily on other axons or on nonneuronal cells. Studies of axon growth substrates *in vivo* have been very difficult due to the greater complexity of the intact nervous system. One of our goals has been to obtain markers for cell types present early in development that could be used to follow the events in axon outgrowth.

Surface differences have been postulated to mediate route and target specificity of axons. In our early experiments in the leech, we showed that growing axons carry antigenically distinct surface glycoproteins. In the adult, axons that express a common surface molecule travel together in bundles, suggesting that these surface molecules might provide routing cues. While axon-axon interactions might guide axons into a particular route, the first axons grow into an environment devoid of other neuronal elements. Even so, these first axons grow along defined routes. What guides the first axons?

We now have evidence that nonneuronal cells, specifically a cell called the Schwann cell, may be involved in the earliest stages of axon growth. This work contradicts the classic notion that early vertebrate axons are naked and blaze their own trail to their targets. One of the monoclonal antibodies we generated to the developing nervous system, Rat-401, recognizes an antigen expressed by a population of cells that delineate early nerve routes. These cells precede and predict axon routes into the periphery. The Rat-401-positive cell is temporally and spatially suited to serve as a substrate for new axon growth, and early axons are found juxtaposed to its membrane both in tissue culture and *in vivo*.

We have now shown that these cells are not a transient embryonic cell type, but persist into adulthood. This conclusion is based on the observation that Rat-401 stains morphologically recognizable Schwann cells in developing and in mature nerves. In the adult, the Schwann cell is intimately associated with mature axons, it forms the myelin sheath that accelerates nerve conduction, and, in addition, it has been implicated in supporting nerve regeneration.

Because of the difficulty of definitively discriminating Schwann cells from other cell types *in vivo*, cultures of Schwann cells and of mixed populations of Schwann cells and fibroblasts were stained to show that Rat-401 specifically recognizes Schwann cells. This result suggests that the early Rat-401-positive cell is actually a Schwann cell precursor and that this nonneuronal, axon-associated Schwann cell guides initial axonal outgrowth. Rat-401, then, provides a marker for Schwann cells throughout their life history, prior to axon ensheathment and during and after myelination of axons.

Two classes of Schwann cells have previously been described based on morphological criteria: Schwann cells that form myelin sheaths around axons and Schwann cells that are intimately associated with axons but do not form myelin. Our recent experiments suggest that Rat-401 recognizes one of the two classes of Schwann cells, those that form myelin. Neither the unmyelinated axons of the sympathetic trunk nor the unmyelinated axons of the olfactory nerve have associated Rat-401-positive cells, in contrast to the prevalent Rat-401-positive cells found in most axon tracts that contain large proportions of myelinated axons. Our immunohistochemical and immunohistochemical studies have shown that Rat-401 recognizes a species distinct from structural components of myelin.

Previous work suggested that the expression of myelin by a Schwann cell is signaled entirely from the axon and that nonmyelinating and myelinating Schwann cells are interconvertible members of one cell class. The observation that different classes of Schwann cells express different molecules suggests that there are two molecularly distinct classes of Schwann cells. To establish definitively whether the antigenic dichotomy between Schwann cells holds, we are currently examining cultures of Schwann cells prepared from sciatic nerve (a source of myelin-producing Schwann cells) and from the sympathetic trunk (a source of nonmyelinating

Schwann cells). One of our current goals is to identify further antigens expressed in subsets of Schwann cells. These markers will allow us to follow the genesis and differentiation of Schwann cells and to examine the role of each class in axon route formation and axonal myelination.

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COLD SPRING HARBOR MEETINGS



Recto: Meeting participants enjoy informal discussions and quiet vistas on the lawn behind Blackford Hall.

50TH COLD SPRING HARBOR SYMPOSIUM ON QUANTITATIVE BIOLOGY

Molecular Biology of Development

May 29 – June 5

254 participants

Developmental biologists are an audacious breed. Their ambition is to provide an intellectually satisfying account of the forces that guide the development of multicellular organisms around the circle from fertilized egg to embryo to adult to gamete. These processes, however, lie close to the border beyond which genetics may not be feasible nor necessarily useful; furthermore, meaningful biochemical studies pose extraordinary challenges because of the small amounts of material that are available and the general rapidity of events in the early embryo. In the face of these difficulties it is not surprising that for many years developmental biology was essentially a branch of anatomy, in that it was exclusively descriptive in nature. Accurate and elegant though these descriptions were, they led to an understanding of developmental processes less frequently than to an amazement at their beauty.

In recent years, however, developmental biology has undergone a dramatic change and has matured from a descriptive to an analytical science. This change stems almost entirely from two technical advances—the ability to use molecular cloning to isolate and characterize wild-type and mutant versions of genes that control or are expressed at specific developmental stages and, second, the ability to generate transgenic organisms in which the expression of the introduced gene(s) is correct both spatially and temporally. In consequence, the developmental biologist has the capacity not only to describe but also now to analyze and influence the events that guide a fertilized egg to its destiny. Our choice of the topic molecular embryology for this year's Symposium—the 50th—celebrates this new-found freedom.



The traditional introduction, given on the first evening by John Gurdon, was an appropriate mixture of philosophy, history, and foresight. We then heard talks from Seymour Benzer, Sydney Brenner, and Anne McLaren who reminisced about earlier Cold Spring Harbor Symposia and spoke about aspects of developmental biology of particular interest to them. The 50th Symposium therefore started on a high intellectual plane that was sustained through the 110 formal presentations given during the next 6 days. Despite this length, most of the 254 people attending the Symposium stayed for the entire meeting. The final talk was given by Gerry Rubin who provided an excellent summary of the highlights of the meeting.

This meeting was supported in part by the National Science Foundation, Department of Energy, and the National Cancer Institute, National Institutes of Health.

Welcoming remarks: J.D. Watson

Introduction: J. Gurdon, Cambridge University, England

SESSION I DEVELOPMENTAL PERSPECTIVES

Chairperson: S. Benzer, California Institute of Technology, Pasadena

Benzer, S., California Institute of Technology, Pasadena: A fly's eye view of development.

Brenner, S., Medical Research Council, Cambridge, England: Lineages in development.

McLaren, A., Medical Research Council, London, England: Gametogenesis and embryogenesis.

SESSION 2 NUCLEAR/CYTOPLASMIC INTERACTIONS IN EARLY DEVELOPMENT

Chairperson: J. Gurdon, Cambridge University, England

Wassarman, P., Bleil, J., Florman, H., Greve, J., Roller, R., Salzmann, G., Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: The mouse egg's receptor for sperm—What is it and how does it work?

Rebagliati, M., Weeks, D., Perry, H., Melton, D., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Maternal mRNA localization in frog and ascidian eggs.

Dawid, I.B., Sargent, T.D., Jamrich, M., NICHD, National Institutes of Health, Bethesda, Maryland: Gene expression in *Xenopus* embryogenesis.

Wylie, C.C., Snape, A., Heasman, J., Dept. of Anatomy, St. George's Hospital Medical School, London, England: Germ plasma is not an early determinant for the germ line in *Xenopus* embryos.

Solter, D., McGrath, J., Wistar Institute, Philadelphia, Pennsylvania: Nuclear transfer in mouse embryos—Activation of embryonic genome.

Schierenberg, E., Max-Planck-Institute, Gottingen, Federal Republic of Germany: Cytoplasmic determination of embryonic development in the nematode *Caenorhabditis elegans*.

Hirsh, D., Kempthues, K., Stinchcomb, D., Jefferson, R., Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: Genes affecting early development in *Caenorhabditis elegans*.

Miller, K., Carr, T., Walter, M., Alberts, B., Dept. of Biochemistry and Biophysics, University of California, San Francisco: Cytoplasmic organization in the early *Drosophila* embryo.

SESSION 3 LINEAGE AND SEGMENTATION/PATTERN FORMATION

Chairperson: D. Hogness, Stanford University Medical Center, California

Horvitz, R., Ambros, V., Desai, C., Ellis, H., Finney, M., Fixsen, W., Greenwald, I., Ruvkun, G., Sternberg, P., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Genes that affect the development of the nematode *Caenorhabditis elegans*.

Gergen, J.P., Coulter, D., Wieschaus, E., Dept. of Biology,

Princeton University, New Jersey: Cell identities and the detailed segment pattern.

Lawrence, P.A., Laboratory of Molecular Biology, Cambridge, England: Development of the *Drosophila* mesoderm.

Gelbart, W.M., Hoffman, F.M., Irish, V.F., Posakony, L., St.

Johnston, D., Segal, D., Spencer, F., Irick, H., Blackman, R., Grimaila, R., Figueroa, P., Taylor, M., van Doren, M., Kerkhof, L., Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: Structure and expression of the *decapentaplegic* gene complex in *Drosophila*.

Frei, E.,¹ Baumgartner, S.,¹ Edström, J.-E.,² Noll, M.,¹
¹Dept. of Cell Biology, Biocenter, University of Basel, Switzerland; ²EMBL, Heidelberg, Federal Republic of Germany: Isolation and identification of the *extra sex combs* gene of *Drosophila*.

Howard, K.R., Ingham, P.W., Pinchin, S.M., Rushlow, C.A., Howe, K.R., Ish-Horowicz, D., Imperial Cancer Research Fund, London, England: Molecular and genetic analysis of the *hairy* locus in *Drosophila*.

Nusslein-Volhard, C., Freidrich Miescher Laboratory, Tubingen, Federal Republic of Germany: Genes affecting the segmental subdivision of the *Drosophila* embryo.

Flytzanis, C.F., Hough-Evans, B.R., Katula, K.S., Franks, R.R., Britten, R.J., Davidson, E.H., California Institute of Technology, Pasadena: Initial specification of cell lineage in the sea urchin embryo studied by gene transfer.

Grant, S.R., Woodward, H.D., Farach, M.C., Lennarz, W.J., University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston: Developmental expression of cell-surface (glyco) proteins involved in gastrulation and spicule formation in sea urchin embryos.



D. Hogness

SESSION 4 HOMEOTIC MUTANTS

Chairperson: E.B. Lewis, California Institute of Technology, Pasadena

Lewis, E.B., California Institute of Technology, Pasadena: Cis-regulation of the bithorax gene complex.

Sánchez-Herrero, E., Casanova, J., Morata, G., Universidad Autónoma de Madrid, Spain: Anatomy and function of the bithorax complex of *Drosophila*.

Bender, W.,¹ Karch, F.,¹ Weiffenbach, B.,¹ Lewis, E.B.,²
¹Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts; ²Division of Biology, California Institute of Technology, Pasadena: Domains of polarity in the bithorax complex in *Drosophila*.

Hogness, D.S., Beachy, P.A., Lipshitz, H., Peattie, D., Paro, R., Gavis, E., Harte, P., Dept. of Biochemistry, Stanford University School of Medicine, California: Regulation and products of the *Ubx* domain of the bithorax complex of *Drosophila*.

Akam, M.E.,¹ Martínez-Arias, A.,² ¹Dept. of Genetics, ²MRC Laboratory of Molecular Biology, Cambridge, England: Expression of the *Ultrabithorax* domain of the bithorax complex within the *Drosophila* embryo.

Ingham, P.W., Imperial Cancer Research Fund, London, England: The regulation of antennapedia and bithorax complex expression in *Drosophila melanogaster*.

Harding, K., Wedden, C., Doyle, H., Levine, M., Columbia University, New York, New York: Spatial regulation of homeotic gene expression in *Drosophila*.

Steward, R., Ambrosio, L., Schedl, P., Dept. of Biology, Princeton University, New Jersey: Molecular analysis of *dorsal*, a gene essential for polarity in the *Drosophila* embryo.

Ali, Z., Coleman, K., Drees, B., Gustavson, E., Karr, T., Kauer, L., Poole, S., Soeller, W., Weir, M., Kornberg, T., Dept. of Biochemistry and Biophysics, University of California, San Francisco: Studies of the *Drosophila engrailed* locus—A gene controlling segment and compartment formation.



D. Baltimore, R. Jaenisch

SESSION 5 INDUCED DEVELOPMENTAL DEFECTS

Chairperson: M. Lyon, Medical Research Council, Oxon, England

Jaeinisch, R.,^{1,2} Harbers, K.,² Jahner, D.,² Kratochwil, K.,³ Löhler, J.,² ¹Whitehead Institute, Cambridge, Massachusetts; ²Heinrich-Pette-Institut, Hamburg, Federal Republic of Germany; ³Osterreichische Akademie de Wissenschaften, Salzburg, Austria: Retroviruses and mouse development.

Mintz, B., Covarrubias, L., Nishida, Y., Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania: Early developmental mutations caused by rearrangements accompanying DNA insertions in the mouse genome.

Lacy, E., Signorelli, K., Mark, W., Memorial Sloan-Kettering Cancer Center, New York, New York: A recessive lethal mutation in a transgenic mouse line.

Leder, P., Woychik, R., Stewart, T., Dept. of Genetics, Harvard Medical School, Boston, Massachusetts: A limb deforming mutation produced by insertional mutagenesis in a transgenic mouse.

Hanahan, D., Alpert, S., Cold Spring Harbor Laboratory, New York: Perturbation by oncogene expression in the development of the endocrine pancreas.

Weintraub, H.,¹ Izant, J.G.,¹ Harland, R.,² Roberts, J.,¹ ¹Dept. of Genetics, Fred Hutchinson Cancer Research Center, Seattle, Washington; ²Virus Laboratory, University of California, Berkeley: Constitutive and conditional suppression of exogenous and endogenous genes by antisense RNA.

Rosenberg, U.B., Preiss, A., Seifert, E., Jackle, H., Knipfle, D.C., Max-Planck Institut für Entwicklungsbiologie, Tübingen, Federal Republic of Germany: Production of phenocopies by Kruppel antisense RNA injection into *Drosophila* embryos.

Malmberg, R.L., McIndoo, J., Hiatt, A.C., Lowe, B., Cold Spring Harbor Laboratory, New York: Polyamine mutants and developmental switches in the tobacco flower.

SESSION 6 CELLULAR DIFFERENTIATION

Chairperson: D. Kaiser, Stanford University, California

Lodish, H.F., Cappello, J., Cohen, S., Handelsman, K., Whitehead Institute, Cambridge, Massachusetts: Gene expression during early differentiation of *Dictyostelium discoideum*.

Loomis, W.F., Dept. of Biology, University of California, San Diego: Regulation of cell-type specific differentiation in *Dictyostelium*.

Blaschke, A., Prause, I., MacWilliams, H., Zoologisches Institut der Ludwig-Maximilians-Universität, München, Federal Republic of Germany: Feedback regulation of cell-type proportioning in *Dictyostelium*.

Devreotes, P., Klein, P., Theibert, A., Knox, B., Fontana, D., Sherring, J., Dept. of Biological Chemistry, Johns Hopkins University Medical School, Baltimore, Maryland: Reversible cyclic AMP-induced cyclic AMP receptor modification as a possible adaptation mechanism in *Dictyostelium*.

Brydoff, B., Crowley, T., Datta, S., Gomer, R., Mann, S., Mehdy, M., Nellen, W., Raymond, C., Silan, C., Sivertsen, A., Firtel, R.A., Dept. of Biology, University of California, San Diego, La Jolla: Regulation of cell-type differentiation in *Dictyostelium*.

Gerisch, G., Max-Planck-Institute für Biochemie, Martins-

S. Brenner, F. Jacob,
and S. Benzer



ried, Federal Republic of Germany: Bypassing steps in the control of early *Dictyostelium* development by sequential mutagenesis.

Kaiser, D., Kroos, L., Kuspa, A., Dept. of Biochemistry, Stanford University, California: Cell interactions govern the temporal pattern of *Mycococcus* development.

Shapiro, L., Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Temporal and spatial regulation of flagellar (*fla*) and chemotaxis (*che*) gene products in *Caulobacter*.

Edelman, G.M., Rockefeller University, New York, New York: Cell adhesion molecules in induction and morphogenesis.



J.D. Watson

SESSION 7 HOMEBOXES

Chairperson: W. Gehring, University of Basel, Switzerland

Gehring, W.J., Biocenter, University of Basel, Switzerland: Homeotic genes and the control of development.

Scott, M.P., Birmingham, J., Carroll, S., Laughon, A., Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: A protein encoded by the *antennapedia* gene—Features common to several homeotic proteins in *Drosophila*.

McGinnis, W., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: The conservation of the homeo box in genes of *Drosophila* and in the genomes of other metazoans.

De Robertis, E.M., Fritz, A., Götz, J.M., Salo, E., Smith, G.D., Mattaj, I.W., Wright, C., Zeller, R., Martin, G., Biocenter, University of Basel, Switzerland: The *Xenopus* homeo boxes.

Ruddle, F.H.,¹ Hart, C.P.,¹ Awgulewitsch, A.,¹ McGinnis, W.,² ¹Dept. of Biology; ²Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut:

Organization and expression of homeo box sequences in the mouse (*Mus musculus*).

Colberg-Poley, A.M., Voss, S.D., Chowdhury, K., Dony, C., Gruss, P., Center for Molecular Biology, University of Heidelberg, Federal Republic of Germany: Analysis of murine genes containing homeo boxes and their expression in murine development.

Hauser, C.A.,¹ Joyner, A.L.,² Martin, G.M.,² Tjian, R.,¹ ¹Dept. of Biochemistry, University of California, Berkeley; ²Dept. of Anatomy, School of Medicine, University of California, San Francisco: Structure and expression of human and mouse genes containing homeo box sequences.

Joyner, A.L.,¹ Kornberg, T.,² Martin, G.M.,¹ Depts. of ¹Anatomy, ²Biochemistry and Biophysics, University of California, San Francisco: Isolation of a mouse sequence containing an engrailed-like homeo box and studies of its expression in embryos and teratocarcinoma cells.

SESSION 8 CONTROL OF GENE EXPRESSION

Chairperson: D. Brown, Carnegie Institution of Washington, Baltimore, Maryland

Losick, R., Zuber, P., Igo, M., Youngman, P., Perkins, J., Sandman, K., Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: Complex regulation of developmental promoters in a simple organism.

Struhl, K., Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Yeast promoters.

Ashburner, M.,¹ Savakis, C.,² ¹Dept. of Genetics, University of Cambridge, England; ²Institute of Molecular Biology and Biotechnology, Crete, Greece: The developmental regulation of the *Adh* gene of *Drosophila melanogaster*.

Posakony, J.W., Fischer, J.A., Corbin, V., Maniatis, T., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Analysis of the larval to adult switch in *Drosophila* alcohol dehydrogenase (*Adh*) gene expression.

Wesink, P., Brandeis University, Waltham, Massachusetts: Independent control elements that determine yolk protein gene expression in alternative tissues.

Orr-Weaver, T., deCicco, D., Kalfayan, L., Kelley, R., Levine, J., Parks, S., Wakimoto, B., Spradling, A., Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: Amplification and expression of *Drosophila* chorion genes.

Kalatos, F.C., Mitsialis, S.A., Delidakis, C., Spoerel, N., Lingappa, J., Mariani, B., Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: Regulated expression and amplification of chorion genes.

Brown, D.D., Schlissel, M.S., Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: Discrimination between the 5S RNA genes in *Xenopus*.

Ausubel, F., Buikema, W., Lang-Unnasch, N., Marvel, D., Dept. of Genetics, Harvard Medical School and Dept. of Molecular Biology, Massachusetts General Hospital, Boston, Massachusetts: Symbiotic nitrogen fixation—Developmental genetics of nodule formation.

SESSION 9 SEX DETERMINATION

Chairperson: D. Botstein, Massachusetts Institute of Technology, Cambridge

Wilson, K., Johnson, A., Fields, S., Mitchell, A., Herskowitz, I., Dept. of Biochemistry and Biophysics, University of California, San Francisco: A master regulatory locus determines yeast cell type.

Klar, A.J.S., Cold Spring Harbor Laboratory, New York: Developmental potential for fission yeast mating-type switching is chromosomally inherited.

Wood, W.B.,¹ Strome, S.,² Meneely, P.,³ Schedin, P.,¹ Scholey, S.,¹ Donahue, L.,¹ Quarantillo, B.,¹ Trent, C.,¹ ¹Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder; ²Dept. of Biology, Indiana University, Bloomington; ³Fred Hutchinson Cancer Research Center, Seattle, Washington: Control of early developmental decisions in embryos of *Caenorhabditis elegans*.

Hodgkin, J., Doniach, T., Shen, M., MRC Laboratory of Molecular Biology, Cambridge, England: Genes that determine sex in the nematode *C. elegans*—Variations on a theme.

Maine, E., Salz, H., Cronmiller, C., Schedl, P., Cline, T., Dept. of Biology, Princeton University, New Jersey: Molecular analysis of *sex-lethal*, a major sex-determining gene in *Drosophila*.

Belote, J., McKeown, M., Baker, B., Dept. of Biology, University of California, San Diego, La Jolla: Sex determination in *Drosophila melanogaster*.

Nöthiger, R., Steinmann-Zwicky, M., Zoological Institute, University of Zurich, Switzerland: Sex determination—Apparent variety in hiding a common principle.

SESSION 10 CELL-CYCLE EFFECTS

Chairperson: M. Birnstiel, Institute for Molecular Biology, University of Zurich, Switzerland

Botstein, D., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: The yeast cell cycle and cytoskeleton as models for the genetic analysis of development.

Reed, S., de Barros Lopes, M., Ferguson, J., Hadwiger, J., Ho, J.-Y., Horwitz, R., Jones, C., Lörincz, A., Mendenhall, M., Peterson, T., Richardson, S., Wittenberg, C., Dept. of Biological Sciences, University of California, Santa Barbara: Genetic and molecular analysis of division control in yeast.

Wigler, M., Kataoka, T., Toda, T., Birchmeier, C., Broek, D., Powers, S.W., Cold Spring Harbor Laboratory, New York: Function of *RAS* genes.

Breeden, L., Miller, A., Nasmyth, K., MRC Laboratory of Molecular Biology, Cambridge, England: Cell-type-specific and cell-cycle-regulated transcription of the yeast *HO* gene.

Beach, D., Cold Spring Harbor Laboratory, New York: *ran1* controls the transition from mitotic division to meiosis in fission yeast.

Newport, J., Dept. of Biology, University of California, San Diego, La Jolla: Regulation of the cell cycle during early embryonic development in *Xenopus laevis*.

Laskey, R.A.,¹ Mills, A.D.,¹ Dingwall, C.,¹ Dillworth, S.M.,¹ Kleinschmidt, J.,² ¹CRC Molecular Embryology Group, Dept. of Zoology, University of Cambridge, England; ²Institut für Zell und Tumorbologie, Deutsches Krebsforschungszentrum, Heidelberg, Federal Republic of Germany: Chromosome replication in early *Xenopus* embryos.

Birnstiel, M.L., Schümperli, D., Busslinger, M., Institut für Molekularbiologie II der Universität, Zürich, Switzerland: Regulation of histone gene expression in cell cycle and development.

SESSION 11 TISSUE SPECIFICITY/POSITION EFFECTS

Chairperson: N. Federoff, Carnegie Institution of Washington, Baltimore, Maryland

Federoff, N., Banks, J., Kelly, S., Carnegie Institution of Washington, Dept. of Embryology, Baltimore, Maryland: Transposable controlling elements in maize.

Emmons, S.W., Ruan, K., Yesner, L., Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Tissue-specific regulation of the Tc1 transposable element in *C. elegans*.

Rubin, G.M., Hazelrigg, T., Laski, F., Laverty, T., Levis, R., Rio, D., Spencer, F., Zuker, C., Dept. of Biochemistry, University of California, Berkeley: Germ-line specificity of P element transposition and some novel patterns of expression of transduced copies of the *white* gene.

Bingham, P., Zachar, Z., Chapman, C., Dept. of Biochemis-

try, State University of New York, Stony Brook: *Cis-* and *Trans-*acting regulators of developmentally programmed transcription in *Drosophila*.

Meyerowitz, E., Crosby, M., Garfinkel, M., Martin, C., Mathers, P., Raghavan, K.V., Division of Biology, California Institute of Technology, Pasadena: *cis-* and *trans-*acting regulators of the *Drosophila* 68C *glue* gene cluster.

Costantini, F., Magram, J., Chada, K., Dept. of Human Genetics and Development, College of Physicians & Surgeons, Columbia University, New York, New York: Developmental regulation of cloned globin genes in transgenic mice.

Krumlauf, R.,¹ Hammer, R.E.,² Brinster, R.,² Chapman, V.,³



D. Baltimore, F. Jacob, W. Gilbert, L. Shapiro, and D. Hogness

Tilghman, S.M.,¹ ¹Institute for Cancer Research, Philadelphia, Pennsylvania; ²University of Pennsylvania School of Veterinary Medicine, Philadelphia; ³Roswell Park Memorial Institute, Buffalo, New York: Developmental regulation of α -fetoprotein genes in transgenic mice.

Van Dyke, T., Finlay, C., Teresky, A.J., Levine, A.J., Dept. of Molecular Biology, Princeton University, New Jersey: Characterization of several lines of mice transmitting the SV40 early-region genes in the germ line.

SESSION 12 EXPRESSION OF GENES INTRODUCED INTO TRANSGENIC ORGANISMS

Chairperson: A. McLaren, Medical Research Council, London, England

Hammer, R.E.,¹ Palmiter, R.D.,² Brinster, R.L.,¹ ¹School of Veterinary Medicine, University of Pennsylvania, Philadelphia; ²Dept. of Biochemistry, University of Washington, Seattle: Expression of bovine and human growth hormone fusion genes in transgenic mice.

Evans, R.M.,¹ Wineberger, C.,¹ Hollenberg, S.,^{1,2} Swanson, L.,¹ Nelson, C.,^{2,3} Rosenfeld, M.G.,³ ¹Gene Expression Laboratory, Salk Institute; ²Dept. of Biology, ³School of Medicine, University of California, San Diego: Inducible and developmental control of neuroendocrine genes.

Ornitz, D.M.,¹ Palmiter, R.C.,¹ Hammer, R.E.,² Brinster, R.L.,² ¹Dept. of Biochemistry, University of Washington, Seattle; ²School of Veterinary Medicine, University of Pennsylvania, Philadelphia: The elastase-I promoter region directs expression of human growth hormone and SV40 T antigen to pancreatic acinar cells in transgenic mice.

Westphal, H., Overbeek, P.A., Khillan, J.S., Chepelinsky, A.B., Schmidt, A., Mahon, K.A., Piatigorsky, J., de Crombrughe, B., NCI, National Institutes of Health, Bethesda, Maryland: Promoter sequences of murine α -A-crystallin, murine α 2(I)-collagen, or avian sarcoma virus genes linked to the bacterial chloramphenicol acetyl transferase (CAT) gene direct tissue-specific patterns of CAT expression in transgenic mice.

Baltimore, D.,¹ Grosschedl, R.,¹ Weaver, D.,¹ Imanishi-Kari, T.,² Costantini, F.,³ ¹Whitehead Institute for Biomedical Research, ²Center for Cancer Research, Cambridge, Massachusetts; ³Columbia University, New York, New York: Control of immunoglobulin (Ig) heavy-chain gene expression and rearrangement.

Evans, M., Dept. of Genetics, University of Cambridge, England: Transformation of EK cell lines and formation of Chimeric mice.

Schell, J., Max-Planck Institut für Zuchtungsforchung, Cologne, Federal Republic of Germany: Transfer and regulation of expression of chimeric genes in plants.

Horsch, R., Fraley, R., Rogers, S., Corporate Research Laboratories, Monsanto Co., St. Louis, Missouri: Transgenic plants.



A. McLaren

SESSION 13 PLURIPOTENT CELLS/ONCOGENES

Chairperson: F. Jacob, Institut Pasteur, Paris, France

Wagner, E.,¹ Rüther, U.,¹ Stewart, C.,¹ Muller, R.,¹ Keller, G.,² ¹European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany; ²Basel Institute for Immunology, Switzerland: Expression of foreign genes in murine stem cells and mice.

Gorman, C., Cooper, A., Reith, A., Watson, C., Lane, D., Rigby, P., Dept. of Biochemistry, Imperial College of Science and Technology, London, England: Gene expression in the murine teratocarcinoma cells and embryos.

Verma, I.M., Salk Institute, San Diego, California: Proto-oncogenes—Role in development, growth, and differentiation.

Mulligan, R., Massachusetts Institute of Technology, Cambridge: Tissue specific expression of genes introduced into mammalian cells via retrovirus vectors.

Bishop, J.M.,¹ Katzen, A.,² Kornberg, T.,² Montarras, D.,¹ Simon, M.,² ¹Hooper Research Foundation; ²Dept. of Biochemistry and Biophysics, University of California, San Francisco: Proto-oncogenes in *Drosophila melanogaster*.

Nicolas, J.F., Rubenstein, J.L.R., Jacob, F., Institut Pasteur, Paris, France: Recombinant retroviruses with an internal promoter are efficient vectors to transduce and express genes in multipotential embryonic cells.

SESSION 14 DEVELOPMENTAL NEUROBIOLOGY

Chairperson: S. Brenner, Medical Research Council, Cambridge, England

Yedvobnick, B., Muskavitch, M.A.T., Wharton, K.A., Grimwade, B.G., Markopoulou, K., Schlessinger-Bryant, R., Artavanis-Tsakonas, S., Dept. of Biology, Yale University, New Haven, Connecticut: Molecular genetics of *Drosophila* neurogenesis.

Anderson, D.J., Stein, R., Axel, R., Columbia University College of Physicians & Surgeons, New York, New York: Molecular probes for the development and plasticity of neural crest derivatives.

Young, M.W.,¹ Jackson, F.R.,¹ Shin, H.-S.,² Bargiello, T.A.,¹ ¹Rockefeller University; ²Memorial Sloan-Kettering Cancer Center, New York, New York: A biological clock in *Drosophila*.

Goodman, C.S., Dept. of Biological Sciences, Stanford University, California: Cell recognition during neuronal development.

Summary: G. Rubin, University of California, Berkeley

MEETINGS

Molecular Biology of Hepatitis B Viruses

May 2-5

ARRANGED BY

Harold Varmus, University of California, San Francisco

Jesse Summers, Institute for Cancer Research, Philadelphia, Pennsylvania

171 participants

The 1985 meeting on the Molecular Biology of Hepatitis B viruses was attended by more than 170 conferees. The international flavor of the meeting was evident from the attendance of 63 scientists from overseas. Sessions for oral presentation were divided into broad topics covering the replication cycle of hepatitis B viruses; the gene products; pathogenesis, tropism, immune response, and host range; expression of viral genes; and hepatocellular carcinoma.

The meeting produced for the first time a synthesis of the major steps and details of the replication cycle of hepadnaviruses as determined by comparisons of the four members of the hepadnavirus family. Significant new information concerning the reverse transcription pathway for production of virion DNA, the maintenance of the intracellular transcriptional template, and the behavior of viral gene products expressed in heterologous cells was presented. Integration of hepadnavirus DNA into the genome of hepatocellular tumors obtained from all over the world was extensively described and compared in an ongoing attempt to fathom the possible role of viral information in the origin of these cancers. It was clear that the application of molecular genetic techniques and the increasing use of animal models to study hepadnavirus infections have moved this important area of biomedical research onto a new stratum of understanding.

This meeting was supported in part by the National Science Foundation, Cold Spring Harbor Laboratory Cancer Center Grant and 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 Cancer Institute.

SESSION 1 THE REPLICATION CYCLE OF HEPATITIS B VIRUSES

Chairperson: **W. Robinson**, Stanford University School of Medicine, Stanford, California

Büscher, M., Reiser, W., Will, H., Schaller, H., Dept. of Microbiology, ZMBH, Heidelberg, Federal Republic of Germany: Transcripts and the putative pregenome of DHBV—Implications for reverse transcription.

Will, H., Reiser, W., Büscher, M., Pfaff, E., Sprengel, R., Cattaneo, R., Schaller, H., Dept. of Microbiology, ZMBH, Heidelberg, Federal Republic of Germany: Structure of transcripts and of the DNA minus strand of human HBV—Implications for transcription, translation, replication, and integration.

Enders, G.H.,¹ Ganem, D.,^{2,3} Varmus, H.,^{1,3} Depts. of ¹Biochemistry and Biophysics; ²Microbiology and Immu-

nology; ³Medicine, University of California, San Francisco: Definition of the two major classes of viral transcripts in GSHV-infected livers.

Möröy, T.,¹ Etienne, J.,¹ Trépo, C.,² Tiollais, P.,¹ Buendia, M.A.,¹ ¹INSERM, Institut Pasteur, Paris; ²UER Alexis Carrel, Lyon, France: Transcription of WHV in chronically infected liver.

Roggendorf, M., Summers, J., Fox Chase Cancer Center, Philadelphia, Pennsylvania: Studies of the replication of WHV in chronically infected liver cells.

Lien, J.-M., Petcu, D.J., Molnar-Kimber, K.L., Aldrich, C.E., Mason, W.S., Institute for Cancer Research, Philadelphia,



J. Summers, H. Varmus, J. Gerin

Pennsylvania: RNA is covalently linked to the 5' end of the plus strand of DHBV DNA.

Newbold, J. E., Dept. of Microbiology and Immunology, University of North Carolina School of Medicine, Chapel Hill:

Analysis of DHBV liver core particles in buoyant CsCl density gradients.

Tuttleman, J., Pugh, J., Mason, W., Summers, J., Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania: An *in vitro* experimental infection of primary duck hepatocyte cultures with DHBV.

Tuttleman, J., Pourcel, C., Summers, J., Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania: Mechanism of synthesis of covalently closed circular DNA of DHBV.

Seeger, C.,¹ Ganem, D.,^{1,2} Varmus, H. E.,¹ Depts. of ¹Microbiology and Immunology, ²Medicine, University of California, San Francisco: A genetic approach to hepadnavirus replication—*In vitro* mutagenesis of the GSHV genome.

Volovitch, M.,¹ Mazzolini, L.,² Yot, P.,² ¹Institut Curie, Paris; ²Groupeement Scientifique Microbiologie, Toulouse, France: CA MV reverse transcription step in viroplasm—Analysis of nucleic acids and DNA polymerase activity involved in asymmetric replication.

Hohn, T.,¹ Pietrzak, M.,¹ Fütterer, J.,¹ Pfeiffer, P.,² ¹Friedrich Miescher-Institut, Basel, Switzerland; ²Institut de Biologie Moléculaire du CNRS, Strasbourg, France: The reverse transcriptase of cauliflower mosaic virus, a dsDNA plant virus.

SESSION 2 PRODUCTS OF HEPATITIS B VIRUS STRUCTURAL GENES

Chairperson: W. Gerlich, Hygiene Institute, Göttingen, Federal Republic of Germany

Sallfeld, J.,¹ Pfaff, E.,¹ Will, H.,¹ Leban, J.,² Schaller, H.,¹ ¹Dept. of Microbiology, ZMBH, University of Heidelberg, Federal Republic of Germany; ²Biogen S.A., Geneva, Switzerland: HBV antigens—Mapping in the genome, determinants, and mechanisms of biosynthesis.

Offensperger, W.,¹ Wahl, S.,¹ Price, P.,¹ Christman, J. K.,¹ Acs, G.,¹ Neurath, A. R.,² Strick, N.,² Kent, S. B. H.,³ ¹Mount Sinai School of Medicine, New York; ²New York Blood Center, New York; ³California Institute of Technol-

ogy, Pasadena: Cloning and expression of the pre-S1 region of HBV in *E. coli*.

Neurath, A. R.,¹ Kent, S. B. H.,² Strick, N.,¹ Sproul, P.,¹ ¹New York Blood Center, New York; ²Division of Biology, California Institute of Technology, Pasadena: Direct involvement of pre-S gene-coded protein domains in attachment of HBV to liver cells.

Persing, D. H., Varmus, H. E., Ganem, D., Dept. of Biochemistry and Biophysics, University of California, San Francisco: Biosynthesis and functions of presurface proteins of mammalian hepadnaviruses.

Wong, D. T.,¹ Prince, A.,² Sninsky, J. J.,¹ Nath, N.,³ ¹Dept. of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York; ²New York Blood Center, New York; ³American Red Cross Blood Laboratories, Bethesda, Maryland: HBV polypeptides encoded by the entire pre-S ORF—Identification and possible clinical significance.

Schaeffer, E.,¹ Snyder, R.,² Sninsky, J. J.,¹ ¹Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York; ²Penrose Research Laboratory, Zoological Society of Philadelphia, Pennsylvania: Identification of novel WHV polypeptides containing pre-S encoded amino acids.

Moriarty, A.,¹ Alexander, H.,¹ Mitamura, K.,² Thornton, G.,³ Lerner, R.,¹ ¹Dept. of Molecular Biology, Scripps Clinic, La Jolla, California; ²Dept. of Medicine, University of Tsukuba, Japan; ³Johnson and Johnson, Biotechnology Center, San Diego, California: The screening of HBV serum samples for X antigen and studies on the molecular nature of p28.



A. Prince, T. Hohn

Kay, A.,¹ Mandart, E.,¹ Trepo, C.,² Galibert, F.,¹
¹Laboratoire d'Hématologie Expérimentale, Hôpital Saint-Louis;
²INSERM, Faculté de Médecine Alexis Carrel, Lyon, France: The HBV X gene expression in *E. coli* is recognized by sera from hepatitis patients.

Uy, A., Bruss, V., Köchel, H., Thomssen, R., Gerlich, W.H.,
Dept. of Medical Microbiology, University of Göttingen, Federal Republic of Germany: Effect of the precore sequence on functions of the hepatitis B core protein.

Ou, J.,¹ Laub, O.,² Rutter, W.J.,¹ ¹Hormone Research Lab-

oratory, University of California, San Francisco: ²Dept. of Virology, Weizmann Institute of Science, Rehovot, Israel: Expression of HBcAg and HBsAg in mammalian cells.

Hirschman, S.Z., Tackney, C., Dept. of Medicine, Mount Sinai School of Medicine, New York, New York: Viral RNA transcripts in mouse cells synthesizing HBsAg following transfection with DNA encompassing the HBcAg gene.

Hirschman, S.Z., Tackney, C., Dept. of Medicine, Mount Sinai School of Medicine, New York, New York: Synthesis of HBsAg by mouse cells transfected with HBcAg DNA.

SESSION 3 MECHANISMS OF PATHOGENESIS, TROPISM, IMMUNE RESPONSE, HOST RANGE

Chairperson: J.L. Gerin, Georgetown University, Rockville, Maryland

Bonino, F., Rosina, F., Rizzi, R., Chiaberge, E., Actis, G.C., Rizzetto, M., Verme, G., Dept. of Gastroenterology, Molinette Hospital, Turin, Italy: Chronic anti-HBe hepatitis and serum HBV-DNA—A separate clinical entity.

Gerin, J.,¹ Tennant, B.,² Popper, H.,³ Tyeryar, F.,⁴ Purcell, R.,⁴ ¹Georgetown University, Rockville, Maryland; ²Cornell University, Ithaca, New York; ³Mount Sinai School of Medicine, New York, New York; ⁴NIAID, National Institutes of Health, Bethesda, Maryland: Chronic hepatitis and hepatocellular carcinoma in woodchucks following experimental WHV infection.

Millman, I., Venkateswaran, P., Halbherr, T., Feitelson, M., Fox Chase Cancer Center, Philadelphia, Pennsylvania: Experimental induction in woodchucks of chronic infections with WHV.

Seeger, C.,¹ Ganem, D.,^{1,2} Varmus, H.E.,¹ Depts. of ¹Microbiology and Immunology, ²Medicine, University of California, San Francisco: In vitro recombinants between GSHV and WHV are viable and offer an approach to determinants for species specificity and pathogenicity.

Korba, B.E., Gerin, J.L., Georgetown University Medical Center, Rockville, Maryland: HBV in peripheral blood lymphocytes—A comparison between chronically infected patients and animal models.

Pasquinelli, C., Lauré, F., Romet-Lemonne, J.L., Degos, F., Tiollais, P., Bréchet, C., INSERM, Institut Pasteur, Paris, France: HBV DNA blood in mononuclear cells—An early, frequent, and stable event which reflects the liver cells' status.

Zeldis, J.B.,¹ Mugishima, H.,² Steinberg, H.,¹ Neir, E.,³ Gale, R.P.,² ¹Beth Israel Hospital, Harvard Medical School, Boston, Massachusetts; ²University of California Medical School, Los Angeles; ³Los Angeles Children's Hospital, University of Southern California School of Medicine: Myeloid stem cell inhibition by HBV.

Celis, E., Kato, I., Miller, R.W., Chang, T.W., Centocor Inc., Malvern, Pennsylvania: Regulation of the human immune response to HBsAg—Factors influencing antigen capture by monocytes and stimulation of helper T cells.

Milich, D.R.,¹ Thornton, G.B.,² Neurath, A.R.,³ Kent, S.B.,⁴ Michel, M.,⁵ Tiollais, P.,⁵ Chisari, F.V.,¹ ¹Scripps Clinic and Research Foundation, La Jolla, California; ²Johnson and Johnson, Biotechnology Center, San Diego, California; ³New York Blood Center, New York; ⁴California Institute of Technology, Pasadena; ⁵Institut Pasteur, Paris, France: Circumvention of nonresponse to HBsAg by immunization with pre-S containing particles.

SESSION 4 MECHANISMS OF EXPRESSION OF VIRAL GENES

Chairperson: P. Tiollais, Institut Pasteur, Paris, France

Shaul, Y.,¹ Rutter, W.J.,² Laub, O.,³ Depts. of ¹Virology, ²Genetics, Weizmann Institute of Science, Rehovot, Israel; ³Hormone Research Institute, University of California, San Francisco: The human HBV contains an enhancer element.

Eflassi, E., Haseltine, W., Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Localization of enhancer elements in HBV genome—Comparison of enhancer activity in human hepatoma, lymphoid, and myeloblastoid cell lines.

Tur-Kaspa, R., Burk, R.D., Shafritz, D.A., Liver Research Center, Albert Einstein College of Medicine, Bronx, New York: Glucocorticoids stimulate HBV enhancer activity.

Negro, F., Chiaberge, E., Isabella, L., Actis, G.C., Bonino, F., Dept. of Gastroenterology, Molinette Hospital, Turin, Italy: Simultaneous detection of intrahepatic HBV-DNA and HBcAg.



P. Tiollais



K. Matsubara, P. Hofschneider

Price, P., Sells, M.A., Zelent, A., Christman, J.K., Acs, G., Mount Sinai School of Medicine, New York, New York: Characterization of RNA transcribed from the "short"

strand of HBV DNA integrated into the genome of mouse 3T3 cells.

Eble, B.,¹ Lingappa, V.,^{2,3} Ganem, D.,^{1,3} Depts. of ¹Microbiology and Immunology, ²Physiology, ³Medicine, University of California, San Francisco: The transmembrane translocation of HBsAg.

Kasambalides, E.J., Gilja, B.K., Gerber, M.A., Dept. of Pathology, Mount Sinai School of Medicine and City Hospital Center, Elmhurst, New York: Expression of receptors for polymerized human serum albumin (pHSA-R) by HBsAg in vitro.

Chisari, F.V.,^{1,2} Milich, D.R.,¹ Moriarty, A.,¹ Filippi, P.,¹ Palmer, R.,³ Pinkert, C.,⁴ Brinster, R.,⁴ ¹Scripps Clinic and Research Foundation, La Jolla, California; ²Institut Pasteur, Paris, France; ³University of Washington, Seattle; ⁴University of Pennsylvania, Philadelphia: Expression of HBV gene products in transgenic mice.

Pourcel, C., Farza, H., Morello, D., Hadchouel, M., Babinet, C., INSERM, Institut Pasteur, Paris, France: Expression of HBsAg in transgenic mice.

SESSION 5 POSTER SESSION

Hartley, J.L., Berninger, M., Jessee, J.A., Bloom, F.R., BRL/GIBCO Division of Life Technologies, Inc., Gaithersburg, Maryland: Nonradioactive detection of HBV-DNA in serum. Romet-Lemonne, J.L.,^{1,2} Thornton, G.B.,³ Dubois, F.,¹ Essex, M.,² Milich, D.R.,⁴ Chisari, F.V.,⁴ ¹Faculté de Médecine, Tours, France; ²Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts; ³Johnson and Johnson Biotechnology Center, San Diego, California; ⁴Scripps Clinic and Research Foundation, La Jolla, California: Early detection of antibodies to pre-S antigen in the preclinical phase of hepatitis B.

Lok, A.S.F.,¹ Lai, C.L.,¹ Wu, P.C.,¹ Wong, V.,¹ Yeoh, E.K.,² Lin, H.J.,¹ ¹Depts. of Medicine, Pathology, Obstetrics, and Clinical Biochemistry Unit, University of Hong Kong; ²Queen Elizabeth Hospital, Republic of China: Intrafamilial spread of HBV infection in Chinese.

Müller, R., Brunkhorst, R., Klein, H., Schmidt, F.W., Zentrum Innere Medizin, Medizinische Hochschule Hannover, Federal Republic of Germany: Antiviral treatment with interferon-gamma in chronic active hepatitis B.

Pontisso, P., Bortolotti, F., Fattovich, G., Diiodati, G., Schiavon, E., Alberti, A., Realdi, G., Istituto di Clinica Medica, University of Padua, Italy: Relevance of serum HBV-DNA in acute and chronic hepatitis type B.

Jilg, W., Delhous, C., Deinhardt, F., Max von Pettenkofer-Institut, Munich, Federal Republic of Germany: Antibodies to protein subunits of HBsAg in sera of persons vaccinated against hepatitis B and individuals after natural infection.

McGlynn, K., London, W.T., Lustbader, E., McMahon, B., Heyward, W., Fox Chase Cancer Center, Philadelphia, Pennsylvania, and Centers for Disease Control, Indian Health Service, Anchorage, Alaska: Prevention of chronic liver disease and liver cancer—HBV replication and tuberculin reactivity.

Mondelli, M., Chermello, L., Bortolotti, F., Schiavon, E., Alberti, A., Rondanelli, E.G., Realdi, G., Dept. of Infectious

Diseases, University of Pavia; Istituto di Clinica Medica, University of Padua, Italy: Enhanced natural killer cell activity in the early phase of acute HBV infection.

Lai, C.L.,¹ Gregory, P.B.,² Wu, P.C.,¹ Wong, K.P.,¹ Lok, A.S.F.,¹ Ng, M.M.T.,¹ ¹University of Hong Kong, Republic of China; ²Stanford University, California: Primary liver cell cancer in men and women.

Kodama, K., Ogasawara, N., Gerin, J., Yoshikawa, H., Murakami, S., Cancer Research Institute, Kanazawa University, Japan, and Dept. of Microbiology, School of Medicine, Georgetown University, Rockville, Maryland: Nucleotide sequence of a cloned WHV genome—Evolutionary relationships between hepadnaviruses.

Tagawa, M., Omata, M., Okuda, K., Dept. of Medicine, Chiba University School of Medicine, Japan: Early events during the initiation of DHBV infection.

Chow, T.-Y., Chen, C.-S., Institute of Botany, Academia Sinica, Taiwan, Republic of China: Expression of HBsAg with presequence in *E. coli*.

Chow, T.-Y., Chen, C.-S., Chen, R.-Y., Cheng, C.-T., Chang, J.-H., Institute of Botany, Academia Sinica, Taiwan, Republic of China: Cloning of HBV genome and expression of HBsAg with presequences in *E. coli*.

Michel, M.L.,^{1,2} Pontisso, P.,³ Sobczak, E.,¹ Malpié, Y.,¹ Strecek, R.,¹ Tiollais, P.,² ¹Groupement de Génie Génétique; ²INSERM, Institut Pasteur, Paris, France; ³Instituto di Clinica Medica, University of Padua, Italy: Synthesis of HBsAg particles containing the pre-S region expression product.

Elfassi, E.,¹ Romet-Lemonne, J.L.,¹ Dienstag, J.,² Haseltine, W.,¹ ¹Dana-Farber Cancer Institute, Harvard Medical School; ²Massachusetts General Hospital, Boston: Molecular cloning and expression of the X gene in *E. coli*.

Meyers, M.L.,¹ Trepo, L.V.,² Nath, N.,³ Sninsky, J.J.,^{1,4} Depts. of ¹Molecular Biology, ⁴Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York; ²Institut National de la Santé et de la Recherche

Medicale, Lyon, France; ³American Red Cross Blood Laboratories, Bethesda, Maryland: HBV polypeptide X—Expression in *E. coli* and identification of specific antibodies in sera from HBV-infected humans.

Feitelson, M., Fox Chase Cancer Center, Philadelphia, Pennsylvania: Products of the X gene in HBV and related viruses.

Minns, L.,¹ Mayer, K.,¹ Fox, L.,² Sarin, V.,² Depts. of ¹Hepatitis Research and Development, ²Molecular Biology, Abbott Laboratories, North Chicago, Illinois: Pre-S proteins of HBsAg contain epitopes recognized by AU-SAB positive human sera.

Salanoubat, M.,¹ Sonigo, P.,¹ Courroucé, A.M.,² Wain-Hobson, S.,¹ Tiollais, P.,¹ INSERM, Institut Pasteur; ²Ets Cabanel, Paris, France: Molecular basis of HBsAg subtypes.

Siddiqui, A., Jameel, S., Gholson, C., Mapoles, J., Dept. of Microbiology, University of Colorado Medical School, Denver: Expression of HBsAg gene in mammalian cells.

Delpyroux, F., Chenciner, N., Lim, A., Malpière, Y., Streeck, R.E., Groupement de Génie Génétique, Institut Pasteur, Paris, France: Effect of insertions in the HBsAg gene on secretion and antigenicity of 22-nm particles.

Chenciner, N.,¹ Delpyroux, F.,¹ Lim, A.,¹ Lambert, M.,¹ Malpière, Y.,¹ Streeck, R.E.,¹ Houssais, J.F.,² ¹Groupement de Génie Génétique, Institut Pasteur, Paris; ²C.N.R.S. et institut Curie, Orsay, France: Effect of cell fusion with primary adult hepatocytes on the expression of the HBsAg.

Tackney, C., Hirschman, S.Z., Dept. of Medicine, Mount Sinai Hospital of Medicine, New York, New York: Production of hepatitis B antigens by fragments of viral DNA packaged in pseudovirions of Moloney leukemia virus.

Yoakum, G.H.,¹ Korba, B.E.,² Wilson, V.L.,¹ Boumpas, D.T.,¹ ¹NCI, National Institutes of Health, Bethesda; ²Georgetown University School of Medicine, Rockville, Maryland: Studies of HBV core gene—Expression and cytopathology.

Ma, X.-K., Ding, G., Zho, P., Huang, Y., Shi, C., Lu, H., Zhu, Y., Laboratory of Molecular Genetics, Institute of Molecular Genetics, Institute of Basic Medical Sciences, Beijing, Republic of China: Properties of HBeAg synthesized in *E. coli* and its conversion to e antigen.

Sureau, C.,¹ Roingeard, P.H.,² Romet-Lemonne, J.L.,¹ Essex, M.,¹ ¹Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts; ²Dept. Biologie Humaine, Faculté de Médecine, Tours, France: Expression of a nuclear-associated antigen in human hepatoma cell line Hep G2 after transfection with circular HBV-DNA.

Sastry, K.J.,¹ Radhakrishna, G.,² Chan, T.-S.,² ¹Dept. of Pathology, M.D. Anderson Hospital and Tumor Institute,



J. Zeldis, J. Gerin

Houston; ²Dept. of Microbiology, University of Texas Medical Branch, Galveston: Oncogene expression in a human hepatocellular carcinoma cell line, HCC36.

Chan, T.-S.,¹ Lee, C.-S.,² Radhakrishna, G.,¹ ¹Dept. of Microbiology, University of Texas Medical Branch, Galveston; ²Department of Surgery, National Taiwan University College of Medicine, Republic of China: A newly established hepatocellular carcinoma cell line (HCC36) carrying integrated HBV.

Freitag von Loringhoven, A.,¹ Koch, S.,¹ Hofschneider, P.H.,¹ Koshy, R.,² ¹Max-Planck-Institute, Martinsried, Federal Republic of Germany; ²Dept. of Medicine, State University of New York, Stony Brook: Hybrid host-virus mRNAs augment HBV gene expression.

Su, T.,¹ Chang, C.,¹ Chou, C.,¹ Ting, L.,¹ Han, S.,¹ Lui, W.,² Peng, F.,² Depts. of ¹Medical Research, ²General Surgery, Veterans General Hospital, Taiwan, Republic of China: Study of HBV-DNA and RNA in hepatoma.

Unoura, M., Kobayashi, K., Fukuoka, K., Matsushita, F., Morimoto, H., Ohshima, T., Kaneko, S., Morioka, K., Ohno, A., Hattori, N., Murakami, S., Yoshikawa, H., Cancer Research Institute, First Dept. of Internal Medicine, Kanazawa University, Japan: Establishment of a cell line of tissue culture of a woodchuck hepatocellular carcinoma.

Bréchet, C., Tiollais, P., Dejean, A., INSERM, Institut Pasteur, Paris, France: Analysis of HBV integration site in cellular DNA of two different liver tumors.

Chen, J.-Y.,¹ Harrison, T.J.,¹ Lee, C.S.,² Chen, D.S.,² Zuckerman, A.J.,¹ ¹Dept. of Medical Microbiology, London School of Hygiene and Tropical Medicine, England; ²College of Medicine, National Taiwan University, Taipei, Republic of China: Oncogenesis of hepatitis B virus—A study of thirty Taiwanese hepatomas.

SESSION 6 HEPATOMA AND INTEGRATION OF VIRAL DNA

Chairperson: K. Matsubara, Osaka University, Suita City, Japan

Su, C.Y., Rogler, C.E., Liver Research Center, Albert Einstein College of Medicine, Bronx, New York: Structure of cloned WHV integrations from chronically infected woodchuck liver.

Yaginuma, K.,¹ Kobayashi, H.,¹ Kobayashi, M.,¹ Morishima, T.,² Matsuyama, K.,³ Koike, K.,¹ ¹Dept. of Gene Re-

search, Cancer Institute, Tokyo; ²Dept. of Pediatrics, Nagoya University; ³Nagoya First Red Cross Hospital, Japan: Analysis of integrated HBV sequences in human hepatoma and chronic active hepatitis tissues.

Hino, O.,^{1,3} Shows, T.B.,² Rogler, C.E.,¹ ¹Liver Research Center, Albert Einstein College of Medicine, Bronx, New



I. Millman, B. Tennant

York; ²Dept. of Human Genetics, Roswell Park Memorial Institute, Buffalo, New York; ³Dept. of Pathology, Cancer Institute, Tokyo, Japan: Structure and chromosomal localization of HBV integrations from hepatocellular carcinomas.

Kaneko, S., Kodama, K., Ohshima, T., Unoura, M., Kobayashi, K., Hattori, N., Yoshikawa, H., Murakami, S., Cancer Research Institute, First Dept. of Internal Medicine, Medical School, Kanazawa University, Japan: Stable integration of WHV in established cells of tissue culture from a woodchuck primary hepatocellular carcinoma and structure of the integrated viral DNA.

Fung, Y.K.,¹ Lai, C.L.,² Lok, A.,² Todd, D.,² Varmus, H.E.,¹
¹Dept. of Microbiology and Immunology, University of Cal-

ifornia, San Francisco; ²Dept. of Medicine, Queen Mary Hospital, University of Hong Kong, Republic of China: Analysis of HBV-associated human hepatocellular carcinomas for oncogene expression and structural rearrangement.

Shih, C.,¹ Zeldis, J.,¹ Burke, K.,¹ Chou, M.J.,² Yang, C.S.,² Lee, C.S.,² Isselbacher, K.,¹ Wands, J.,¹ Goodman, H.M.,¹ ¹Massachusetts General Hospital, Boston; ²National Taiwan University, Republic of China: Site-specific recombination of HBV in human hepatoma cells.

Matsubara, K., Nagaya, A., Fukushige, S., Fujiyama, A., Tsurimoto, T., Chisaka, O., Shinya, T., Nakamura, T., Institute for Molecular and Cellular Biology, Osaka University, Japan: Studies with junctions between integrated HBV-DNA and host chromosomal DNA.

Koike, K., Yaginuma, K., Iwami, M., Imazeki, F., Kobayashi, M., Dept. of Gene Research, Cancer Institute, Tokyo, Japan: HBV integration and expression of the *c-myc* gene in human hepatoma cells.

Chang, C., Chou, C.-K., Ting, L.-P., Su, C.-S., Han, S.-H., Hu, C.-P., Graduate Institute of Microbiology and Immunology, National Yang Ming Medical College, and Dept. of Medical Research, Veterans General Hospital, Taiwan, Republic of China: The cellular and antigenic properties of human hepatoma cell lines.

Roingeard, P., Romel-Lemonne, J.L., Barin, F., Goudeau, A., Lesage, G., Faculté de Médecine, Tours, France: Hepatoma patients have antibodies directed toward an 18-kD cytoplasmic protein present in human hepatoma cell lines, PLC/PRF/5 and Hep 3 B.

Chromosome Structure and Expression

May 8 – May 12

ARRANGED BY

Michael Grunstein, University of California, Los Angeles

Gary Felsenfeld, National Institutes of Health, Bethesda, Maryland

146 participants

More than 50 abstracts were presented at the Spring 1985 meeting on Chromosome Structure and Expression. These presentations were organized into eight sessions including such topics as DNA Structure, Chromatin Structure, Chromosome Structure and Genetic Approaches, and Nuclear Structures.

Genetic information is carried in DNA, but the expression of that information depends on a variety of factors, many yet unknown. Discussed at the meeting was the manner in which DNA conformation and structural proteins that contribute to chromosome structure influence gene expression. The meeting also described the interactions that take place between chromosomes and the nuclear matrix, interactions that affect chromosome shape and function. These problems brought together investigators working in areas as diverse as X-ray crystallography, cytology, classical biochemistry, and genetics.

This meeting was supported in part by the National Science Foundation.

SESSION 1 DNA STRUCTURES

Chairperson: J. Wang, Harvard University, Cambridge, Massachusetts

Wang, J.C.,¹ Snyder, L.,² Goto, T.,¹ Giaever, G.,¹ ¹Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts; ²Dept. of Microbiology, Michigan State University, East Lansing, Michigan: On the degree of supercoiling of intracellular DNA.

Azorin, F., Fenton, M., Rich, A., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Z-DNA binding proteins in SV40 minichromosomes.

Jovin, T.M., Robert-Nicoud, M., Arndt-Jovin, D., MPI for Biophysical Chemistry, Goettingen, Federal Republic of Germany: Z-DNA in chromatin during growth and differentiation.

Bird, A.P., Taggart, M., Frommer, M., Miller, O.J., Macleod, D., MRC Mammalian Genome Unit, Edinburgh, Scotland: Islands of nonmethylated, CpG-rich DNA in the vertebrate genome.

SESSION 2 DNA UNDER STRESS

Chairperson: M. Gellert, National Institutes of Health, Bethesda, Maryland

Menzel, R., Gellert, M., NIADDK, National Institutes of Health, Bethesda, Maryland: Supercoiling-sensitive promoters in *E. coli*.

Bonven, B.J., Gocke, E., Westergaard, O., Dept. of Molecular Biology and Plant Physiology, University of Aarhus, Denmark: Is rRNA synthesis regulated by a sequence-specific topoisomerase I activity associated with DNase I hypersensitive sites?

Villeponteau, B., Martinson, H.G., Dept. of Chemistry and Biochemistry, University of California, Los Angeles: Torsional stress, topoisomerases and active chromatin.

Sternglanz, R., DiNardo, S., Thrash, C., Voelkel-Meiman, K., Brill, S.J., State University of New York, Stony Brook: Roles of yeast DNA topoisomerases I and II in DNA replication and transcription.

Georgiev, G.P.,¹ Luchnik, A.N.,² Bakayev, V.V.,¹ Zbarsky, I.B.,² ¹Institute of Molecular Biology; ²Institute of Developmental Biology, Union of Soviet Socialist Republics Academy of Sciences, Moscow: Torsional strain in transcriptionally active chromatin.

Ruberti, I., Worcel, A., University of Rochester, New York: Analysis of the process of chromatin assembly in vitro.

SESSION 3 CHROMATIN STRUCTURE

Chairperson: J. Thomas, University of Cambridge, England

Allfrey, V.G., Johnson, E.M., Sterner, R., Hoffman, H.P., Rockefeller University, New York, New York: Changes in nucleosome structure and composition during transcription of ribosomal and β -globin genes.

Bradbury, E.M., Dept. of Biological Chemistry, School of Medicine, University of California, Davis: Histone modifications, H1⁹, and chromatin structure.

Lambert, S.F., Thomas, J.O., Dept. of Biochemistry, University of Cambridge, England: Core histone-DNA interactions in chromatin—A chemical approach.

Sen, D., Crothers, D.M., Dept. of Chemistry, Yale University, New Haven, Connecticut: Chromatin structure and con-

densation determined by photochemical dichroism and rotational relaxation.

van Holde, K.E.,¹ Yager, T.,² Ausio, J.,¹ ¹Dept. of Biochemistry and Biophysics, Oregon State University, Corvallis; ²Institute of Molecular Biology, University of Oregon, Eugene: Salt-dependent conformational changes and dissociation of hypo- and hyperacetylated nucleosomes.

Olins, A.L., Biology Division of the Oak Ridge National Laboratory, University of Tennessee, Oak Ridge: Chromatin structure—Three-dimensional reconstruction using electron microscope tomography (EMT).

SESSION 4 ACTIVE AND INACTIVE CHROMATIN

Chairperson: R. Simpson, National Institutes of Health, Bethesda, Maryland

Simpson, R.T., Thoma, F., FitzGerald, P.C., Clarke, M., NIADDK, National Institutes of Health, Bethesda, Maryland: Mechanisms of nucleosome positioning.

Caplin, A., Kimura, T., Allan, J., Gould, H.J., Dept. of Biophysics, King's College, London, England: Proteins required to establish and maintain the open conformation of the chicken adult β -globin gene in erythroid chromatin.

Mirzabekov, A., Bavykin, S., Usachenko, S., Schick, V., Belyavsky, A., Lishanskaya, A., Undritsov, I., Institute of Molecular Biology, Union of Soviet Socialist Republics Acad-

emy of Sciences, Moscow: The structure of transcriptionally active and inactive chromatin.

Zachau, H.G., Bauer, H.G., Falkner, F.G., Klöbeck, H.G., Lötscher, E., Pech, M., Pohlentz, H.D., Straubinger, B., Institut für Physiologische Chemie, Physikalische Biochemie und Zellbiologie der Universität, München, Federal Republic of Germany: Immunoglobulin light-chain genes.

Udvardy, A., Schedl, P., Dept. of Biology, Princeton University, New Jersey: Chromatin structure of the 87A7 heat-shock locus of *Drosophila melanogaster*.

Szent-Gyorgyi, C., Gross, D.S., Finkelstein, D.B., Garrard, W.T., Dept. of Biochemistry, University of Texas Health Science Center, Dallas: Chromatin structure of the yeast *HSP82* gene—Mapping 12 kb of the locus with single nucleotide resolution of the promoter region.

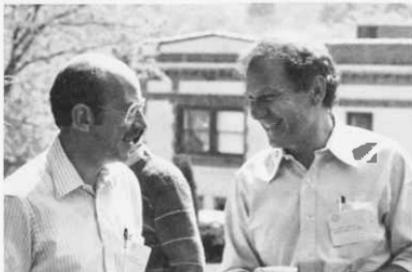
Wang, X.-F., McDougall, S., Mercola, M., Calame, K., Dept. of Chemistry, University of California School of Medicine, Los Angeles: Ability of the immunoglobulin heavy chain enhancer to activate multiple promoters and to activate promoters located 17 kb away.

SESSION 5 SITE-SPECIFIC FACTORS

Chairperson: K.R. Yamamoto, University of California, San Francisco

Zaret, K.S., Yamamoto, K.R., Dept. of Biochemistry and Biophysics, University of California, San Francisco: Formation of a DNase I hypersensitive structure appears essential for glucocorticoid-dependent enhancer function.

Berk, A.J., Gaynor, R.B., Yoshinaga, S.K., Dean, N., Feld-



G. Felsenfeld, M. Grunstein

man, L.T., Molecular Biology Institute, University of California, Los Angeles: Evidence that adenovirus E1A functions stimulate transcription of class III genes by increasing the activity of transcription factor I1IC.

Wu, C., NCI, National Institutes of Health, Bethesda, Maryland: Protein-binding sites in chromatin and regulation of heat-shock genes.

Felsenfeld, G., Emerson, B., Jackson, D., Lewis, C., NIAADD, National Institutes of Health, Bethesda, Maryland: Chromatin structure near an expressed gene.

Zenke, M., Wildeman, A., Matthes, H., Schatz, C., Grundström, T., Winterth, M., Takahashi, K., Chambon, P., CNRS, INSERM, Strasbourg, France: Functional anatomy of the simian virus 40 enhancer.

Learned, R.M., Tjian, R., Dept. of Biochemistry, University of California, Berkeley: Transcription and binding properties of A factor that confers promoter specificity to RNA polymerase I.

Hennighausen, L., Siebenlist, U., Davis, L., Danner, D., Chung, J., Leder, P., Dept. of Genetics, Harvard Medical School, Boston, Massachusetts: Sequence-specific protein-DNA interaction—Impact on gene expression.

SESSION 6 CHROMOSOME STRUCTURE AND GENETIC APPROACHES I

Chairperson: E. Blackburn, University of California, Berkeley

Blackburn, E., Budarf, M., Challoner, P., Cherry, M., Greider, C., Henderson, E., Larson, D., Ryan, T., Shampay, J., Spangler, E., Dept. of Molecular Biology, University of California, Berkeley: Structure and formation of telomeric DNA in lower eukaryotes.

Varshavsky, A., Javaherian, K., McCartney, J., Pan, R., Snapka, R., Solomon, M., Strauss, F., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Studies on chromatin.

Grunstein, M., Schuster, T., Han, M., Kayne, P., Choe, J., Molecular Biology Institute, University of California, Los Angeles: Histone function and evolution.

Wagner, D., Carson, M., Koshland, D., Hartwell, H., Dept. of Genetics, University of Washington, Seattle: Genes involved in mitotic chromosome transmission.

Elgin, S.C.R., Amero, S.A., Cartwright, I.L., Dietrich, V., Eisenberg, J.C., Flick, J.T., James, T.C., Siegfried, E., Thomas, G.H., Zhu, J., Washington University, St. Louis, Missouri: DNA and chromatin structural patterns related to gene expression in *Drosophila*.

Cantor, C.R., Morse, R., Benezra, R., Axel, R., College of Physicians and Surgeons, Columbia University, New York, New York: Aspects of nucleosome organization and topology relevant to gene expression.

Allis, C.D.,¹ Horowitz, S.,² Gorovsky, M.A.,² ¹Dept. of Biochemistry, Baylor College of Medicine, Houston, Texas; ²Dept. of Biology, University of Rochester, New York: Histone variants and histone genes in *Tetrahymena*.

SESSION 7 CHROMOSOME STRUCTURE AND GENETIC APPROACHES II

Chairperson: W. Fangman, University of Washington, Seattle

Hill, A., Bloom, K., University of North Carolina, Chapel Hill: Structural requirements for a yeast centromere.

Fangman, W.L., McCarroll, R.M., Brewer, B.J., Zweifel, S.G.,

Dept. of Genetics, University of Washington, Seattle: ARS elements and chromosome replication in yeast.

Murray, A.W., Schultes, N.P., Dawson, D., Szostak, J.W.,

Dept. of Molecular Biology, Massachusetts General Hospital, Boston: Behavior of artificial chromosomes in yeast. Gottschling, D.E.,¹ Zakian, V.A.,¹ Cech, T.R.,² ¹Fred Hutchinson Cancer Research Center, Seattle, Washington; ²Dept. of Chemistry, University of Colorado, Boulder: Telosome—A DNA-protein complex containing the molecular ends of *Oxytricha* macronuclear DNA.

Moreland, R.,¹ Straussberg, R.,² Garcia, R.,¹ Hereford, L.,¹ ¹Dana Farber Cancer Institute, Boston, Massachusetts; ²Genex Corporation, Gaithersburg, Maryland: Sequences required for nuclear localization of yeast H2B-1.

Struhl, K., Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Relationships between chromatin structure and gene expression in yeast.

Beckendorf, S.K., Shermoen, A.W., McNabb, S., Heemskerck, J., Jongens, T., Dept. of Molecular Biology, University of California, Berkeley: The use of gene transfer experiments to study expression of the SGS-4 glue protein gene.

SESSION 8 NUCLEAR STRUCTURES

Chairperson: U.K. Laemmli, University of Geneva, Switzerland

Gasser, S.M., Mirkovitch, J. Laemmli, U.K., Depts. of Molecular Biology and Biochemistry, University of Geneva, Switzerland: Higher-order chromatin loops—Evidence for cell-cycle and differentiation-dependent changes.

McKeon, F.D.,¹ Kirschner, M.W.,¹ Sanchez-Pescador, R.,² Caput, D.,² ¹Dept. of Biochemistry and Biophysics, University of California, San Francisco; ²Chiron Corporation, Emeryville, California: Molecular cloning and expression of human nuclear lamin cDNAs using human autoantibodies.

Pienta, K., Coffey, D., Johns Hopkins University School of Medicine, Baltimore, Maryland: The nuclear matrix—An organizing structure for the interphase nucleus and chromosome.

Fey, E.G., Penman, S., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: The nuclear matrix—intermediate filament scaffold—Protein and RNA components revealed by sequential fractionation and resinless TEM sections.

Olins, D.E., Biology Division of the Oak Ridge National Laboratory, University of Tennessee, Oak Ridge: Analysis of

macronuclear structure in the protozoan *Euplotes eurystomus*.

Mathog, D., Hochstrasser, M., Gruenbaum, Y., Agard, D., Sedat, J., Dept. of Biochemistry and Biophysics, University of California, San Francisco: The three-dimensional nuclear arrangement of *Drosophila* polytene interphase chromosomes.

Belmont, A., Sedat, J., Agard, D., Dept. of Biochemistry, University of California, San Francisco: The three-dimensional structure of mitotic chromosomes.

Additional Abstracts

Vanderbilt, J.N., Brown, J.W., Anderson, J.N., Dept. of Biological Sciences, Purdue University, West Lafayette, Indiana: Analysis of HMG proteins 1, 2a, and 2b.

Villeponteau, B., Martinson, H., Dept. of Chemistry and Biochemistry, University of California, Los Angeles: Topoisomerases bind to enhancer-like DNA sequences in the histone gene cluster of *Drosophila*.

C. elegans

May 15 – May 19

ARRANGED BY

H. Robert Horvitz, Massachusetts Institute of Technology, Cambridge
Robert H. Waterston, Washington University School of Medicine, St. Louis, Missouri
Scott Emmons, Albert Einstein College of Medicine, Bronx, New York
Donna Albertson, Medical Research Council, Cambridge, England

238 participants

The 1985 Cold Spring Harbor *C. elegans* meeting allowed the assembly of essentially all researchers who study this microscopic roundworm. Both communication and spirit were outstanding, and recent successes in molecular biology provided the basis for considerable excitement. For example, methods are now available for the molecular cloning of the many genes known from genetic stud-



ies to affect development and/or behavior. In addition, about 65% of the genome has been "fingerprinted," and the definition of a complete physical map seems to be a realistic short-term goal. Molecular studies of morphogenesis were described that should now lead to an understanding of muscle thick-filament assembly and of the process that generates an elongated worm from an ellipsoidal embryo. Many similarities between *C. elegans* and higher animals were reported at both the phenomenological and molecular levels; the DNA sequence homology between *lin-12*, a developmental control gene implicated in cell interactions, and the precursor for mammalian epidermal growth factor was particularly striking. Detailed genetic pathways were described that control sex determination, cell lineage, and neurogenesis. A newly developed technique for mosaic analysis has allowed the site of action of particular genes to be identified at the level of resolution of single cells. Studies of the nervous system have moved from anatomical description to functional analysis. In addition, specific and interesting neuroanatomical lesions were described in certain behaviorally uncoordinated mutants. Many other biological problems were discussed, including aging, neuronal degeneration, programmed cell death, collagen, and nonmuscle motility.

This meeting was supported in part by the National Science Foundation, and the following divisions of the National Institutes of Health: National Institute on Aging, and Division of Research Resources.

SESSION 1 GENOME ORGANIZATION AND MOLECULAR BIOLOGY

Chairperson: D. Hirsh, University of Colorado, Boulder, Colorado

Coulson, A., Sulston, J., MRC Laboratory of Molecular Biology, Cambridge, England: Physical map of the *C. elegans* genome.

Albertson, D.G., MRC Laboratory of Molecular Biology, Cambridge, England: Mapping genes by in situ hybridization using biotin-labeled probes.

Beckenbach, K.A., Baillie, D.L., Dept. of Biological Sciences, Simon Fraser University, Burnaby, Canada: Molecular studies in the region around *unc-22*.

Ruvkun, G.,¹ Ambros, V.,² Horvitz, R.,¹ ¹Dept. of Biology, Massachusetts Institute of Technology; ²Dept. of Cellular and Developmental Biology, Harvard University, Cambridge: Molecular genetic analysis of the *lin-14* locus.

Howell, A.M., Rose, A.M., Dept. of Medical Genetics, University of British Columbia, Vancouver, Canada: Identification of essential genes in the *sDp2* region of LG I.

Mello, C.,¹ Roberts, J.,² Hirsh, D.,² Stinchcomb, D.,¹ ¹Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts; ²Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: *C. elegans* DNA that directs segregation in *S. cerevisiae*.

Felsenstein, K.M., Emmons, S.W., Albert Einstein College of Medicine, Bronx, New York: Organizational and functional analysis of interspersed repetitive DNA sequences.

Ward, S., Burke, D.J., Hogan, E., Dept. of Embryology, Car-

negie Institution of Washington, Baltimore, Maryland: The organization of expressed MSP genes.
Roberts, S., Sanicola, M., Saltzberg, L., Emmons, S., Childs, G., Depts. of Genetics and Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Genomic or-

ganization and sequence analysis of *C. elegans* histone genes.
Rogalski, T.M., Bullerjahn, A.M., Riddle, D.L., Division of Biological Sciences, University of Missouri, Columbia: RNA polymerase II genetics.

SESSION 2 GAMETOGENESIS AND EMBRYOGENESIS

Chairperson: S. Strome, Indiana University, Bloomington, Indiana

Roberts, T.M., Pavaiko, F.M., Dept. of Biological Science, Florida State University, Tallahassee: Membrane and cytoplasmic proteins are transported in the same organelle complex during nematode spermatogenesis.
Sepsewot, S.,¹ Johnson, C.D.,² ¹Dept. of Biology, University of Wisconsin, Stevens Point; ²Dept. of Zoology, University of Wisconsin, Madison: Monoclonal antibodies against sperm-activating factor in *Ascaris*.
Schierenberg, E., Max-Planck-Institute, Göttingen, Federal Republic of Germany: Determination of early cleavage pattern in embryos of *C. elegans*.
Edgar, L.G., McGhee, J.D., Dept. of Medical Biochemistry, University of Calgary, Alberta, Canada: Embryonic expression of a gut-specific esterase in *C. elegans*.
Kempthues, K.,¹ Priess, J.,² ¹Section of Genetics and De-

velopment, Cornell University; ²MRC Laboratory of Molecular Biology, Cambridge, England: Maternal-effect lethal mutations that affect cleavage spindle orientation, timing of cell division, and cytoplasmic segregation.
Priess, J., MRC Laboratory of Molecular Biology, Cambridge, England: e2072—An embryonic determinant?
Durbin, R.M., Priess, J., Thomson, J.N., MRC Laboratory of Molecular Biology, Cambridge, England: Early development of the ventral nerve cord.
Manser, J., Wood, W.B., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Mutants defective in embryonic cell migrations.
Fixsen, W., Horvitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Characterization of two loci that affect nuclear migrations during development.

SESSION 3 POSTER SESSION I

Aamodt, E., Culotti, J., Dept. of Biochemistry, Molecular Biology, and Cellular Biology, Northwestern University, Evanston, Illinois: Isolation and partial characterization of microtubules and microtubule-associated proteins from *C. elegans*.
Albert, P.S., Riddle, D.L., Division of Biological Sciences, University of Missouri, Columbia: Genetics, development, and ultrastructure of dauer-like larvae.
Ardizzi, J.P., Epstein, H.F., Dept. of Neurology, Baylor College of Medicine, Houston, Texas: In situ localization of MHC isoforms in adults and larvae of *C. elegans*.
Austin, J., Kimble, J., University of Wisconsin, Madison: *gpl-1*, a gene central to germ-line development.
Avery, L., Horvitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Factors that affect the rate of pharyngeal pumping.
Barstead, R., Schriefer, L., Moerman, D., Benian, G., Francis, R., Waterston, R., Dept. of Genetics, Washington University, St. Louis, Missouri: A new expression vector—Libraries and *unc-22*.
Barton, M.K., Schedl, T.B., Kimble, J.E., University of Wisconsin, Madison: Characterization of *fem-3* semidominant alleles.
Bennett, K.L., Ward, S., Carnegie Institution of Washington, Baltimore, Maryland: Cloning and analysis of the germ-line-specific gene coding for the MSP from *A. lumbricoideis*.
Bhatt, H., Hedgecock, E., Roche Institute of Molecular Biology, Nutley, New Jersey: Culturing embryonic cells.
Bird, D.M., Riddle, D.L., Division of Biological Sciences, University of Missouri, Columbia: Molecular cloning of the *ama-1 IV* gene of *C. elegans*.

Burr, A.H., Dept. of Biological Sciences, Simon Fraser University, Burnaby, Canada: Light and temperature changes modulate reversal frequency.
Cassada, R., Biology Institute, University of Freiburg, Federal Republic of Germany: Attempts to approach embryonic/cytoplasmic actin genetically.
Chen, V.,¹ Hieb, W.,² Depts. of ¹Biophysical Science, ²Biological Sciences, State University of New York, Buffalo: Laser diffraction spectra of *T. acetii* and *C. elegans*.
Ciokey, G., Zigmond, D., Bashor, O.J., Jacobson, L., Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: *daf-4* mutants—Three characters in search of an order.



D. Hirsch, D. Albertson



R. Horvitz, J. Sulston, A. Coulson

- Conklin, D., Hawdon, J., Jacobson, L., Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: A screen for mutants deficient in protein degradation.
- Cummins, C., Anderson, P., Dept. of Genetics, University of Wisconsin, Madison: MLC genes of *C. elegans*.
- Curry, A.M., Waterston, R.H., Dept. of Genetics, Washington University, St. Louis, Missouri: Gene interactions in thick-filament formation.
- Denich, K., Otsuka, A., Dept. of Genetics, University of Cal-

- ifornia, Berkeley: Cloning using DNA restriction fragment polymorphisms.
- Donati, L.A., Wild, G.C., Baillie, D.L., Dept. of Biological Sciences, Simon Fraser University, Burnaby, Canada: Genetic analysis of the *unc-22* to *dyf-4* region in *C. elegans*.
- Etebar, S., Johnson, T., Dept. of Molecular Biology and Biochemistry, University of California, Irvine: Effects of ionizing irradiation on life span.
- Fiebach, B., Cassada, R., Biology Institute, University of Freiburg, Federal Republic of Germany: Embryonic lethal mutants with a paternal-lethality effect.
- Finney, M., Horvitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Progress in cloning *unc-86*.
- Fodor, A.,¹ Maheshwari, D.K.,² Deak, P.,¹ Institute of Genetics, Hungarian Academy of Sciences, Szeged, Hungary; ²Dept. of Botany, D.A.V. College, Muzaffarnagar, India: DEO mutagenesis in *C. briggsae* G16 strain.
- Foltz, N., Thimann Laboratories, University of California, Santa Cruz: Equilibrium sex ratio in cultures of *C. elegans* (N2).
- Francis, R., Waterston, R.H., Dept. of Genetics, Washington University School of Medicine, St. Louis, Missouri: More monoclonals to muscle and hypodermal proteins.
- Friedman, D., Johnson, T., Dept. of Molecular Biology and Biochemistry, University of California, Irvine: Genetic analysis of a long-lived mutant.

SESSION 4 MUSCLE BIOLOGY

Chairperson: S. Ward, Carnegie Institution of Washington, Baltimore, Maryland

- Epstein, H.F., Traeger, L.A., Ortiz, I., Dept. of Neurology, Baylor College of Medicine, Houston, Texas: Roles of myosin isoenzymes in assembly.
- Miller, D.M.,¹ Stockdate, F.,² Karn, J.,³ ¹Dept. of Zoology, North Carolina State University, Raleigh; ²Stanford University School of Medicine, California; ³MRC Laboratory of Molecular Biology, Cambridge, England: Immunological identification of *C. elegans* MHC genes.
- Otsuka, A., Dept. of Genetics, University of California, Berkeley: *sup-3* directly controls the accumulation of a Body-wall MHC in *C. elegans*.
- Herman, R.,¹ Kari, C.,¹ Johnson, C.,² ¹Dept. of Genetics and Cell Biology, University of Minnesota, St. Paul; ²Dept. of Zoology, University of Wisconsin, Madison: Analysis of expression of *ace-1+* and *mec-4(e1611)* in genetic mosaics.
- Park, E., Horvitz, R., Dept. of Biology, Massachusetts Insti-

- tute of Technology, Cambridge: Dominant behavioral and morphological mutations of *C. elegans*.
- Bejsovec, A., Anderson, P., Dept. of Genetics, University of Wisconsin, Madison: New types of muscle-defective mutants.
- Moerman, D.G., Benian, G.M., Rioux, S.D., Waterston, R.H., Dept. of Genetics, Washington University, St. Louis, Missouri: *unc-22*—Cloning by Tc1-transposon tagging and structure of the gene.
- Yarborough, P.,¹ Hayden, M.,¹ Dunn, L.,¹ Vermersch, P.,³ Klass, M.,² Hecht, R.,¹ Depts. of ¹Biochemical Sciences, ²Biology, University of Houston, Texas; ³Dept. of Biochemistry, Rice University, Houston, Texas: Isolation of a GADPH gene from the nematode, *C. elegans*.
- Krause, M., Hirsh, D., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Actin gene transcription in *C. elegans*.

SESSION 5 POSTEMBRYONIC DEVELOPMENT AND AGING

Chairperson: J. Kimble, University of Wisconsin, Madison, Wisconsin

- Kenyon, C., MRC Laboratory of Molecular Biology, Cambridge, England: *mab-5* activity is cell-autonomous.
- Sternberg, P.,^{1,2} Ferguson, C.,¹ Horvitz, R.,¹ ¹Dept. of Biology, Massachusetts Institute of Technology, Cambridge; ²Dept. of Biochemistry and Biophysics, University of California, San Francisco: Control of vulval cell lineages.

- Greenwald, I., MRC Laboratory of Molecular Biology, Cambridge, England: Molecular cloning of the *lin-12* locus of *C. elegans*.
- Johnson, T., Dept. of Molecular Biology and Biochemistry, University of California, Irvine: Life spans of induced mutants.

Ellis, H., Horvitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Mutants abnormal in programmed cell deaths.

Kusch, M., Edgar, R.S., Thimann Laboratory, University of California, Santa Cruz: *Squat-1*—A member of a gene family affecting cuticle morphology in *C. elegans*.

Wadsworth, W.G., Riddle, D.L., Division of Biological Sciences, University of Missouri, Columbia: Developmental regulation of energy metabolism and the discovery of a novel metabolite.

Ambros, V., Liu, A., Dept. of Cellular and Developmental Bi-

ology, Harvard University, Cambridge, Massachusetts: Heterochronic genes and dauerlarva development.

Politz, S.M.¹, Edgar, R.S.² ¹School of Applied Biology, Georgia Institute of Technology, Atlanta; ²Thimann Laboratories, University of California, Santa Cruz: Adult-specific surface antigens in *C. elegans*.

Stock Center Report

Edgley, M.L., Riddle, D.L., Division of Biological Sciences, University of Missouri, Columbia: *Caenorhabditis* genetics center.

SESSION 6 POSTER SESSION II

Goldstein, P., Dept. of Biological Sciences, University of Texas, El Paso: Synaptonemal complex analysis of translocation (mnT6) and duplication mutants (mnDp1) of *C. elegans*.

Gremke, L., Cutotti, J., Interdepartmental Program in Neuroscience, Northwestern University, Evanston, Illinois: The tubulin genes in *C. elegans*.

Harris, L.J., Rose, A.M., Dept. of Medical Genetics, University of British Columbia, Vancouver, Canada: Tc1 elements in the Bristol strain.

Hartman, P.¹, Simpson, V.², Johnson, T.² ¹Dept. of Biology, Texas Christian University, Fort Worth; ²Dept. of Molecular Biology and Biochemistry, University of California, Irvine: Life spans of recombinant inbreds do not correlate with sensitivities to three DNA-damaging agents.

Heine, U., Blumenthal, T., Dept. of Biology, Indiana University, Bloomington: Characterization of regions of the *C. elegans* X-chromosome containing vitellogenin genes.

Hovelone, J., Hartman, P., Dept. of Biology, Texas Christian University, Fort Worth: Isolation and characterization of the major deoxyribonuclease of *C. elegans*.

Hedgecock, E.,¹ Fire, A.,² ¹Roche Institute of Molecular Biology, Nutley, New Jersey; ²Medical Research Council, Cambridge, England: Using *clr-1* (*e1745ts*) for Nomarski microscopy.

Heschl, M.F.P., Kaan, P., Baillie, D.L., Dept. of Biological Sciences, Simon Fraser University, Burnaby, Canada: Isolation of lethals around the *unc-60* and *dpy-11* genes that affect the homozygous F1, F2, and F3 generations.

Hodgkin, J., MRC Laboratory of Molecular Biology, Cambridge, England: New amber suppressors.

Honda, S., Epstein, H.F., Dept. of Neurology, Baylor College of Medicine, Houston, Texas: Runoff transcription in isolated nuclei and its application in studying gene activity in mutants.

Hyman, A.A., MRC Laboratory of Molecular Biology, Cambridge, England: The role of structural molecules in early asymmetric divisions of the embryo.

Ishii, N., Suzuki, K., Dept. of Molecular Biology, School of Medicine Tokai University, Kanagawa, Japan: Sensitivity of *C. elegans* and *R. tokai* embryos to X-rays.

Kaan, P., Banfield, D., Bird, S., Byman, A., Hole, M., Johnson, R., Khosla, M., Luinenburg, I., McKim, K., Peters, K., Turner, L., Vahidi, H., Baillie, D.L., Dept. of Biological Sciences, Simon Fraser University, Burnaby, Canada: Relative frequencies of lethal and maternal-effect mutations balanced by *eT1* (*III*, *V*).

Kagawa, H., Karn, J., Brenner, S., MRC Laboratory of Molecular Biology, Cambridge, England: Cloning of paramyosin gene—*unc-15* of *C. elegans*.

Kaminuma, T., Minamikawa, R., Takano-Ohmuro, H., Suzuki, I., Tokyo Metropolitan Institute of Medical Science, Bunkyo-Ku, Japan: 3-D Reconstruction of spatiotemporal series of optical pictures.

Khosla, M., Devlin, R.H., Nelson, D.W., Honda, B.N., Dept. of Biological Sciences, Simon Fraser University, Burnaby, Canada: Transcription in vitro using cell-free extracts from *C. elegans*.

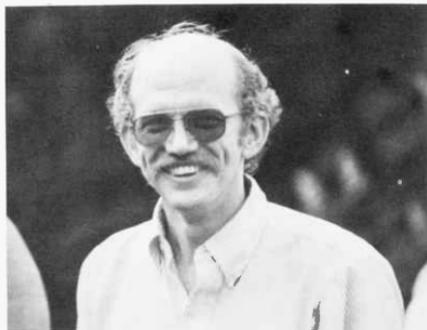
Kim, J.S., Rose, A.M., Dept. of Medical Genetics, University of British Columbia, Vancouver, Canada: Radiation-induced map expansion.

Lewis, J.A., Hodgkiss, T., Prenger, J., Malisch, T., Dept. of Biological Sciences, University of Missouri, Columbia: Spontaneous levamisole-resistant mutants of the Bergerac strain of *C. elegans*.

Link, C., Graf, J., Wood, W.B., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Looking for transposons in *P. redivivus*.

Maryama, I., Miller, E. III, Brenner, S., MRC Laboratory of Molecular Biology, Cambridge, England: Cloning the *eD1* gap.

Mawji, N.R., Rose, A.M., Dept. of Medical Genetics, University of British Columbia, Vancouver, Canada: Identification



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of coding elements in the *sP1 (dpy-14 unc-13)* region of LG I.

McGhee, J.D., Ferrari, D., Birchall, J., Cottrell, D., Dept. of Medical Biochemistry, University of Calgary, Canada: Purification and characterization of an esterase specific to the gut of *C. elegans*.

McIntire, S., Horvitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Immunocytochemical reactivity of *C. elegans* neurons with antisera against known neurotransmitters.

McNeil, K., Rose, A., Dept. of Medical Genetics, University of British Columbia, Vancouver, Canada: Mitochondrial DNA from *C. elegans*.

Meheus, L., Vanfleteren, J.R., Laboratory of Zoology, State University of Ghent, Belgium: Chromosomal proteins and aging of *C. elegans*.

Meneely, P.M.,¹ Wood, W.B.,² ¹Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, Washington; ²Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Dosage compensation mutations.

Miller, D.M.,¹ Maruyama, I.,² ¹Dept. of Zoology, North Carolina State University, Raleigh; ²MRC Laboratory of Molecular Biology, Cambridge, England: *sup-3* is closely linked to an MHC gene.

Morgan, P.,¹ Cascorbi, H.,¹ Sedensky, M.,² Meneely, P.,³ ¹Dept. of Anesthesia, University Hospital of Cleveland, Ohio; ²University of Washington; ³Fred Hutchinson Cancer Research Center, Seattle, Washington: An uncoordinated mutant hypersensitive to human anesthetics.

Moritz, K., Jansen, P., Seidl, C., Teschke, C., Zoologisches Institut, Ludwig Maximilian University of Munich, Federal Republic of Germany: Blastomere determination and chromatin diminution in *Ascaris*.

Munakata, N., Morohoshi, F., Radiobiology Division, National Cancer Center Research Institute, Tokyo, Japan: DNA glycosylase activities in *C. elegans*.

Nelson, D.W., Honda, B.M., Dept. of Biological Sciences, Simon Fraser University, Burnaby, Canada: Characterization and mapping of *C. elegans* 5S RNA genes.

SESSION 7 SEX DETERMINATION AND DOSAGE COMPENSATION

Chairperson: W. Wood, University of Colorado, Boulder, Colorado

Hodgkin, J., Doniach, T., Shen, M., MRC Laboratory of Molecular Biology, Cambridge, England: Major and minor interactions in sex determination.

Kimble, J., Schedl, T., Barton, K., Rosenquist, T., Ahringer, J., University of Wisconsin, Madison: Genes controlling the sperm-oocyte decision.

Doniach, T., MRC Laboratory of Molecular Biology, Cambridge, England: Hermaphroditism, the dotted line, oocytes and sperm.

De Long, L., Plenefisch, J., Meyer, B.J., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Toward a genetic understanding of dosage compensation.

Donahue, L., Quarantillo, B., Wood, W.B., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Molecular analysis of dosage compensation.

lar, Cellular, and Developmental Biology, University of Colorado, Boulder: Molecular analysis of dosage compensation.

Meyer, B.J., Champness, W.C., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: The nematode compensates for differences in X-chromosome dosage.

Schedl, P., Wood, W.B., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Intestinal mosaicism for expression of vitellogenin genes in *C. elegans* intersexes.

Blumenthal, T., Spieth, J., Cane, J., Bektesh, S., Dept. of Biology, Indiana University, Bloomington: The vitellogenin genes and promoters.

SESSION 8 NEUROBIOLOGY

Chairperson: A. Stretton, University of Wisconsin, Madison, Wisconsin

Angstadt, J.D.,¹ Stretton, A.O.W.,² ¹Neuroscience Training Program, ²Dept. of Zoology, University of Wisconsin, Madison: Rhythmic membrane potential oscillations in motoneurons of *Ascaris*.

Johnson, C.D., Stretton, A.O.W., Dept. of Zoology, University of Wisconsin, Madison: GABA and 5HT immunoreactive neurons in *Ascaris*.

Lewis, J.A., Elmer, J.S., Skimming, J., McLafferty, S., Fleming, J., Prenger, J., Hodgkiss, T., McGee, T., Dept. of Biological Sciences, University of Missouri, Columbia: Properties and purification of the nematode levamisole receptor.

Rand, J., Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: Genetic analysis of *cha-11unc-17*.

White, J.G., Southgate, E., Thomson, J.N., Brenner, S., MRC Laboratory of Molecular Biology, Cambridge, England: Friends and neighbors in the *C. elegans* nervous system.

Siddiqui, S., Culotti, J., Depts. of Chemistry, and Neurobiology, Physiology, Northwestern University, Evanston, Illinois: Axonal growth mutants of *C. elegans* studied immunocytochemically.

Hedgecock, E.,¹ Culotti, J.,² Thomson, N.,³ Perkins, L.,² ¹Rochester Institute of Molecular Biology, Nutley, New Jersey; ²Northwestern University, Evanston, Illinois; ³Medical Research Council, Cambridge, England: Axonal guidance mutants.

Challie, M., Dept. of Biological Sciences, Columbia University, New York, New York: *deg-1* mutants.

Desai, C., Horvitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Mutations that impair the functioning of the HSN neurons.

- Nelson, G.,¹ Hammen, R.,² Benton, E.,² ¹Jet Propulsion Laboratory, California Institute of Technology, Pasadena; ²Dept. of Physics, University of San Francisco, California: Effect of heavy ion irradiation on *C. elegans*.
- Park, E., Edwards, K., Horvitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Molecular genetics of a region of *C. elegans* LG II.
- Pavalko, F.M., Roberts, T.M., Dept. of Biological Science, Florida State University, Tallahassee: Membrane movement and *C. elegans* sperm motility.
- Perkins, L.,¹ Hedgecock, E.,² Thomson, J.N.,³ Culotti, J.,¹ ¹Northwestern University, Evanston, Illinois; ²Dept. of Cell Biology, Roche Institute, Nutley, New Jersey; ³MRC Laboratory of Molecular Biology, Cambridge, England: Mutant sensory cilia in *C. elegans*.
- Powers, T.O., Platzer, E.G., Hyman, B.C., Depts. of Nematology and Biology, University of California, Riverside: Remarkable mitochondrial genome structure of a non-free-living nematode.
- Prasad, S., Baillie, D.L., Simon Fraser University, Burnaby, Canada: Investigation of genomic and cDNA sequences of *C. elegans* having homology with *Drosophila* "tropomyosin" sequences.
- Prenger, J., Tollefson, A., Bullerjahn, A., Golomb, M., Division of Biological Sciences, University of Missouri, Columbia: Monoclonal antibodies against RNA polymerase II from *C. elegans*.
- Priest, J., MRC Laboratory of Molecular Biology, Cambridge, England: Morphogenesis in *C. elegans* embryos.
- Ratray, B., Rose, A., Dept. of Medical Genetics, University of British Columbia, Vancouver, Canada: Characterization of *rec-1*. II. Mutation rate and competitive ability.
- Reilly, E., Dean, E., Chalfie, M., Dept. of Biological Science, Columbia University, New York, New York: Benomyl-resistant mutants of *C. elegans*.
- Rioux, S.D., Moerman, D.G., Waterston, R.H., Dept. of Genetics, Washington University, St. Louis, Missouri: Dominant suppressors of *unc-52*.
- Rosenbluth, R.E., Turner, L.M., Baillie, D.L., Dept. of Biological Sciences, Simon Fraser University, Burnaby, Canada: Deficiency mapping and crossover suppression on LGV (left) of *C. elegans*.
- Ruan, K.-S., Emmons, S.W., Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Extrachromosomal copies of transposon *Tc1* in *C. elegans*.
- Salvato, M., Suisson, J., Albertson, D., MRC Laboratory of Molecular Biology, Cambridge, England: *C. elegans* gene for calmodulin.
- Sanicola, M., Roberts, S., Childs, G., Emmons, S.W., Depts. of Molecular Biology and Genetics, Albert Einstein College of Medicine, Bronx, New York: Expression of the *C. elegans* histone gene family.
- Shakes, D., Ward, S., Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: Effect of calmodulin inhibitors on spermiogenesis in *C. elegans*.
- Shen, M., Kenyon, C., Hodgkin, J., MRC Laboratory of Molecular Biology, Cambridge, England: A new sex determination mutant, "yolk-maker."
- Siddiqui, S.,¹ Kamimura, T.,² Takano-Ohmuro, H.,² Miwa, J.,³ Tabuse, Y.,³ ¹Dept. of Biochemistry and Neurobiol-



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- ogy, Physiology, Northwestern University, Evanston, Illinois; ²Tokyo Metropolitan Institute of Medical Sciences; ³NEC Corporation, Fundamental Research Laboratories, Kamagawa, Japan: Nerve-specific monoclonal antibodies raised against *C. elegans*.
- Siddiqui, S., Culotti, J., Depts. of Biochemistry and Neurobiology, Physiology, Northwestern University, Evanston, Illinois: *C. elegans* shares neural antigenic determinants with other invertebrates and vertebrates.
- Sigurdson, C., Herman, R., Horton, C., Kari, C., Pratt, S., Dept. of Genetics and Cell Biology, University of Minnesota, St. Paul: An X-autosome fusion chromosome of *C. elegans*.
- Simpson, V.,¹ Johnson, T.,¹ Klass, M.,² ¹Dept. of Molecular Biology and Biochemistry, University of California, Irvine; ²Dept. of Biology, University of Houston, Texas: Absence of 5-methylcytosine in DNA at any age.
- Sithigorngul, P., Johnson, C.D., Stretton, A.O.W., Dept. of Zoology, University of Wisconsin, Madison: Monoclonal antibodies to neural antigens in *Ascaris* generated after specific immunosuppression.
- Snutch, T.S.,¹ Beckenbach, K.A.,¹ Baillie, D.L.,¹ Mawji, N.R.,² Rose, A.M.,² ¹Dept. of Biological Sciences, Simon Fraser University, Burnaby; ²Dept. of Medical Genetics, University of British Columbia, Vancouver, Canada: Formation of closed, circular *Tc1* is not dependent on *Tc1* copy number.
- Stern, B., Russell, R., Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: Purification and cellular localization of class C acetylcholinesterase (coming attraction-class D).
- Strome, S.,¹ Kempheus, K.,² Priest, J.,³ Morten, D.,² ¹Dept. of Biology, Indiana University, Bloomington; ²Dept. of Genetics and Development, Cornell University, Ithaca, New York; ³MRC Laboratory of Molecular Biology, Cambridge, England: A grandchildless mutant that exhibits altered P-granule distribution.
- Strome, S.,¹ Scholey, S.,² Wood, W.B.,² ¹Dept. of Biology, Indiana University, Bloomington; ²Dept. of Biology, University of Colorado, Boulder: Biochemical characterization of P-granule antigens in *C. elegans*.
- Thomas, J., Horvitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Extragenic suppressors of a *lin-12* dominant allele.

- Trent, C., Wood, W.B., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Dipoxybutane as a mutagen in *C. elegans*.
- Trent, C., Wood, W.B., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Isolation of cloned sequences in the vicinity of *her-1 V*.
- Uchida, K., Otsuka, A., Dept. of Genetics, University of California, Berkeley: Immunocompetition experiments utilizing two-dimensional protein gel blots.
- Veretto, F., Otsuka, A., Dept. of Genetics, University of California, Berkeley: Phosphotyrosine is present in acid hydrolysates of *C. elegans*.
- Villeneuve, A.M., Meyer, B.J., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: *egl-16*—A maternal-effect sex-determining gene.
- Walthall, B., Challie, M., Dept. of Biological Science, Columbia University, New York, New York: Postembryonic development of the touch sensory neurons in *C. elegans*.
- Ward, S.,¹ Roberts, T.,² Strome, S.,³ Pavalko, F.,² ¹Carnegie Institution of Washington, Baltimore, Maryland; ²Dept. of Biological Science, Florida State University, Tallahassee; ³Dept. of Biology, Indiana University, Bloomington: Monoclonal antibodies that recognize a polypeptide epitope shared by multiple sperm-specific proteins.
- Williams, S.A.,^{1,2} DeSimone, S.M.,¹ McReynolds, L.A.,² ¹Smith College, Northampton; ²New England Biological Laboratories, Beverly, Massachusetts: Cloning and sequence analysis of repeat family DNA from the human parasitic nematodes *B. malayi* and *B. pahangi*.
- Wolinsky, E., Horvitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Pharmacological and genetic studies of *C. elegans* egg laying.
- Yuan, J., Ellis, H., Horvitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Studies of the cross-reactivity with *C. elegans* of monoclonal antibodies directed against the *Drosophila* nervous system.
- Zucker, E., Blumenthal, T., Dept. of Biology, Indiana University, Bloomington: The vitellogenin genes of *C. briggsae*.

SESSION 10 TRANSPOSITION AND TRANSFORMATION

Chairperson: B. Meyer, Massachusetts Institute of Technology, Cambridge, Massachusetts

- Stinchcomb, D.,¹ Shaw, J.,² Hirsh, D.,² ¹Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts; ²Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Extrachromosomal DNA transformation of *C. elegans*.
- Shaw, J.E.,¹ Stinchcomb, D.T.,² Hirsh, D.,¹ Wood, W.B.,¹ ¹Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder; ²Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: Transformation of *C. elegans* with linear DNA.
- Jefferson, R.A., Burgess, S.M., Wolf, N., Hirsh, D., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Expression of chimeric genes in *C. elegans*.
- Klass, M.R., Dept. of Biology, University of Houston, Texas: Expression of an MSP- β glucuronidase fusion gene in transformed worms.
- Fire, A., MRC Laboratory of Molecular Biology, Cambridge, England: Transient expression of microinjected *sup-7* DNA and a stable transgenic suppressor line.
- Emmons, S.W., Yesner, L., Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Tissue-specific regulation of the *Tc1* transposable element in *C. elegans*.
- Benian, G.M., Mori, I., Moerman, D.G., Waterston, R.H., Dept. of Genetics, Washington University, St. Louis, Missouri: Some properties of *Tc1* transposition in *C. elegans*.
- Eide, D., Anderson, P., Dept. of Genetics, University of Wisconsin, Madison: Transposition and excision of *Tc1*.
- Anderson, P., Saari, B., Collins, J., Dept. of Genetics, University of Wisconsin, Madison: Elevated *Tc1* activity in mutant (*mut*) strains.

RNA Tumor Viruses

May 21 – May 26

ARRANGED BY

Joan Brugge, State University of New York, Stony Brook
Doug Lowy, National Institutes of Health, Bethesda, Maryland

413 participants

This year's RNA Tumor Virus meeting continued to attract a large, diverse group of investigators. The field has generated enormous interest because it represents an area where many basic findings continue to have direct relevance to clinical

medicine. Abstracts on viral and cellular oncogenes accounted for about one-half the presentations. These studies included comparisons between viral oncogenes and their cellular homologs, regulation of oncogene expression, identification of new oncogenes, mechanisms of oncogene activation, functional and biochemical activities of oncogene products, and interaction of oncogene products with other macromolecules. Among significant findings reported, the *fms* proto-oncogene was identified as the macrophage CSF-1 receptor, a new *erbA*-containing retroviruses that induced erythroblastosis in only one strain of chickens were described, a viral *ras* oncogene was found to induce differentiation (instead of proliferation) of a rat neuroectocrine cell line, and the viral *mos* gene product was shown to have serine kinase activity.

The meeting also gave considerable attention to retroviruses that do not contain oncogenes. There were sessions devoted to the human retroviruses associated with T-cell lymphomas and the acquired immune deficiency syndrome and related animal models, to oncogenesis by animal viruses lacking oncogenes, to viral biology and genetics, to viral proteins and replication, to control of transcription by *cis*- and *trans*-acting factors, and to retroviral vectors. These sessions included reports of avian retroviruses making the *gag-pol* fusion proteins by ribosomal frameshifting, molecular studies of the *cis* and *trans* control elements of HTLV transcription, aspects of the mechanism by which the *pol* endonuclease mediates viral LTR cleavage, and new viral integration sites associated with disease.

This meeting was supported in part by the Cold Spring Harbor Laboratory Cancer Center Grant from the National Cancer Institute, National Institutes of Health.

SESSION 1 ONCOGENES 1: *ERB*, *FMS*, *SIS*, *ETS*

Chairpersons: C. Sherr, St. Jude Children's Research Hospital, Memphis, Tennessee
H. Robinson, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts

Rettenmier, C.W., Rousset, M.F., Sherr, C.J., Dept. of Tumor Cell Biology, St. Jude Children's Research Hospital, Memphis, Tennessee: Products of the *v-fms/c-fms* oncogene.

Woolford, J., Rothwell, V., Rohrschneider, L., Fred Hutchinson Cancer Research Center, Seattle, Washington: Identification of a human cell protein homologous to the *v-fms* gene product.

Kung, H.J.,¹ Nilsen, T.W.,¹ Maroney, P.A.,¹ Goodwin, R.,¹ Rottman, F.M.,¹ Crittenden, L.,² Callaghan, T.,¹ Raines, M.,¹ ¹Dept. of Molecular Biology and Microbiology, Case Western Reserve University, Cleveland, Ohio; ²USDA, Regional Poultry Research Laboratory, East Lansing, Michigan: Novel findings in *c-erb-B* activation and EGF-receptor truncation in ALV-induced erythroblastosis.

Beug, H.,¹ Hayman, M.J.,² Raines, M.A.,³ Kung, H.-J.,³ Vennström, B.,¹ ¹EMBL, Heidelberg, Federal Republic of Germany; ²ICRF, St. Bartholomew's Hospital, London, England; ³Dept. of Molecular Biology, Case Western Reserve University, Cleveland, Ohio: *c-erb-B* transducing retroviruses isolated from ALV-induced erythroleukemia transform erythroblasts, but not fibroblasts.

Robinson, H.L., Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Susceptibility to RSV-1-induced erythroblastosis is a dominant trait.

Ng, M., Privalsky, M.L., Dept. of Bacteriology, University of

California, Davis: Use of site-directed mutagenesis to define functional domains in the AEV *erb-B* protein.

Ishii, S.,¹ Xu, Y.-H.,¹ Stratton, R.H.,² Roe, B.A.,² Merlino, G.T.,¹ Pastan, I.,¹ ¹NCI, National Institutes of Health, Be-



J. Brugge



C. Sherr, G. Vande Woude

SESSION 2 HUMAN RETROVIRUSES AND ANIMAL MODELS

Chairpersons: F. Barré-Sinoussi, Institut Pasteur, Paris, France
M. Popovic, NCI National Institutes of Health, Bethesda, Maryland

Alizon, M.,¹ Sonigo, P.,² Danos, O.,¹ Cole, S.,³ Montagnier, L.,¹ Wain-Hobson, S.,² CNRS,² INSERM,³ Groupement de Genie Genetique, Institut Pasteur, Paris, France: Molecular characterization of the AIDS virus LAV.
Clavel, F., Krust, B., Barré-Sinoussi, F., Chermann, J.C., Montagnier, L., Institut Pasteur, Paris, France: The major envelope glycoprotein of LAV—Identification and maturation processing.
Sargadharan, M.,¹ Veronese, F.,¹ Rahman, R.,¹ Devico, A.,¹ Bruch, L.,¹ Chang, N.,² Ghayeb, J.,¹ Gallo, R.,¹ NCI, National Institutes of Health, Bethesda, Maryland; ²Centocor, Inc., Malvern, Pennsylvania: Structural characteristics of HTLV-III gag- and env-*for*-encoded proteins.
Ratner, L.,¹ Starcich, B.,¹ Fisher, A.,¹ Collalti, E.,¹ Haseltine, W.,² Pearson, M.L.,³ Livak, K.,³ Reddy, E.P.,⁴ Josephs, S.F.,¹ Gallo, R.C.,¹ Wong-Staal, F.,¹ NCI, National Institutes of Health, Bethesda, Maryland; ²Dana-Farber Cancer Institute, Boston, Massachusetts; ³E.I. du Pont de Nemours, Wilmington, Delaware; ⁴Roche Institute, Nutley, New Jersey: Characterization of structure-function relations of proteins encoded by the genome of the AIDS virus, HTLV-III.
Fisher, A.G., Collalti, E., Wong-Staal, F., Gallo, R.C., NCI, National Institutes of Health, Bethesda, Maryland: Characterization of an infectious molecular clone of HTLV-III.
Henderson, L.E., Sowder, R., Smythers, G., Oroszlan, S., NCI-Frederick Cancer Research Facility, Frederick, Maryland: gag proteins of D-type retroviruses and lentiviruses.
Montelaro, R., Salinovich, L., Payne, S., Issel, C., Depts. of Biochemistry, Veterinary Science, Veterinary Microbiology and Parasitology, Louisiana State University, Baton Rouge: EIAV—Antigenic variation and retrovirus persistence.
Starcich, B., Hahn, B.H., Josephs, S., Shaw, G.M., Gallo, R.C., Wong-Staal, F., NCI, National Institutes of Health, Bethesda, Maryland: Comparison of the envelope genes of different HTLV-III isolates.
Goudsmit, J.,¹ Krone, W.,¹ Smit, L.,¹ Bakker, M.,¹ Cou-

thesda, Maryland; ²University of Oklahoma, Norman; Characterization of the promoter region of the EGF receptor gene.
Birnbaum, D.,¹ Birchmeier, C.,¹ Fasano, O.,¹ Fogh, J.,² Wigler, M.,¹ Cold Spring Harbor Laboratory, New York; ²Sloan-Kettering Memorial Institute, Rye, New York: New human transforming genes detected by a tumorigenicity assay.
Giese, N., King, C.R., Robbins, K.C., Aaronson, S.A., NCI, National Institutes of Health, Bethesda, Maryland: In vitro mutagenesis of *v-sis* identifies regions that are necessary for cellular transformation.
Hannink, M., Donoghue, D.J., Dept. of Chemistry, University of California, San Diego, La Jolla: Mutants affecting the secretion and biological activity of the *v-sis* gene product.
Chen, J.H., Dept. of Tumor Cell Biology, St. Jude Children's Research Hospital, Memphis, Tennessee: Gene products of E26 oncogene, *myb*-ets.

linho, R.,² Breederveld, C.,¹ Danner, S.,¹ Lange, J.,¹ Reiss, P.,³ van't Wout, J.,³ van der Noordaa, J.,¹ Internal Medicine, Academic Medical Centre UVA, Amsterdam; ²Municipal Health Service, Amsterdam; ³Academic Hospital ULV, Leiden, The Netherlands: Sero-epidemiology of HTLV-III infection in The Netherlands.
Harper, M.E.,¹ Marselle, L.M.,² Gallo, R.C.,¹ Wong-Staal, F.,¹ NCI, National Institutes of Health, Bethesda; ²Biotech Research Laboratories, Rockville, Maryland: Detection of rare HTLV-III-infected cells in primary tissue from AIDS patients by in situ hybridization.
Chayt, K.J.,¹ Harper, M.E.,¹ Biberfeld, P.,² Wong-Staal, F.,¹ Gallo, R.C.,¹ NCI, National Institutes of Health, Bethesda, Maryland; ²Dept. of Pathology, Karolinska Institute, Stockholm, Sweden: HTLV-III-infected lymphocytes are rare and preferentially located in follicles in AIDS and PGL lymph nodes.
Klatzmann, D.,¹ Gruet, J.,² Champagne, E.,¹ Gluckman, J.C.,¹ Alizon, M.,² Montagnier, L.,² UER Pitié-Salpêtrière, Paris; ²Institut Pasteur, Paris, France: Different susceptibility to LAV infection of subclones of a tumoral T-cell line.
Popovic, M.,¹ Read-Connole, E.,¹ Neuland, C.,² Stockbauer, P.,¹ Gartner, S.,¹ Mann, D.,¹ NCI, National Institutes of Health, Bethesda; ²Uniformed Services University of Health Sciences, Bethesda, Maryland: A receptor of HTLV-III—Identification of epitopes on OKT4 antigen bearing molecule utilized by the virus in "early" infection.
Daugherty, D.,¹ Bess, S.,¹ Rabson, A.,¹ Theodore, T.,¹ Rutledge, R.,¹ Willey, R.,¹ Powell, D.,¹ Gold, J.,² Folks, T.,¹ Martin, M.,¹ NIAID, National Institutes of Health, Bethesda, Maryland; ²Memorial Sloan-Kettering Cancer Center, New York, New York: Characterization of the AIDS retrovirus in cytopathically infected T-cells.
Kanki, P.J.,^{1,2} King, N.W., Jr.,² Letvin, N.L.,² Daniel, M.D.,² Hunt, R.D.,² Essex, M.,¹ Desrosiers, R.C.,² Dept. of Cancer Biology, Harvard School of Public Health, Boston,

Massachusetts; ²New England Regional Primate Research Center, Southborough, Massachusetts: Identification and serologic characterization of a macaque T-lymphotropic retrovirus closely related to HTLV-III.

- Mullins, J.I.,¹ Hoover, E.A.,² Overbaugh, J.,¹ Chen, C.S.,¹
¹Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts; ²Dept. of Pathology, Colorado State University College of Veterinary Medicine and Biomedical Sciences, Fort Collins: Disease-specific replication of FeLV variant integrated viral DNA in experimentally induced feline AIDS.
- Hoover, E.A.,¹ Mullins, J.I.,² ¹Colorado State University, Fort Collins; ²Harvard School of Public Health, Boston, Massachusetts: An experimental model of AIDS in cats.
- Poiesz, B.,^{1,2} Han, T.,³ Lehr, B.,¹ Ehrlich, G.,¹ Tomar, R.,¹
¹State University Upstate Medical Center, Syracuse;

²Veterans Administration Medical Center, Syracuse; ³Roswell Park Memorial Institute, Buffalo, New York: Transient HTLV-I-associated immature and mature T-cell lymphocytosis.

- Jarrett, R., Mitsuya, H., Yarchoan, R., Broder, S., Mann, D., Gallo, R.C., Reitz, M.S., NCI, National Institutes of Health, Bethesda, Maryland: Configuration and expression of T-cell receptor genes in cells infected by HTLV-I.
- Rasheed, S.,¹ Norman, G.L.,¹ Gill, P.,² Meyer, P.,¹ Levine, A.M.,² ¹Depts. of Pathology and Medicine, University of Southern California School of Medicine; ²Kenneth Norris, Jr. Cancer Hospital and Research Institute and Los Angeles County-University of Southern California Medical Center: Isolation of retroviruses for malignant B-cell lymphoma in homosexual men.

SESSION 3 POSTER SESSION I

- Hoover, E.A.,¹ Mullins, J.I.,² Gaspar, P.W.,¹ ¹Colorado State University, Fort Collins; ²Harvard School of Public Health, Boston, Massachusetts: Molecularly cloned feline retroviruses induce virus-specific diseases.
- Riedel, N.,¹ Binari, R.C., Jr.,¹ Steillard, V.,¹ Mullins, J.I.,¹ Hoover, E.A.,² ¹Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts; ²Dept. of Pathology, Colorado State University, College of Veterinary Medicine and Biomedical Sciences, Fort Collins: Nucleotide sequence of the envelope gene and LTR of an aneuploid FeLV.
- Beltz, G.A., Storey, J.R., Cambridge BioScience Corporation, Hopkinton, Massachusetts: Isolation and characterization of FeLV subgroup A molecular clones.
- Wright, S.E.,^{1,2} Bennett, D.D.,¹ ¹Viral Oncology Laboratory, Veterans Administration Medical Center, ²Depts. of Medicine and Cellular, Viral and Molecular Biology, University of Utah School of Medicine, Salt Lake City: The subgroup determinant of the envelope of avian RNA tumor viruses does not enhance lymphomagenesis.
- Angel, J.M., Bedigian, H.G., Jackson Laboratory, Bar Harbor, Maine: Ecotropic virus involvement in spontaneous B-cell lymphomas of CWD/AgI mice.
- Rassart, E., Ming, S., Nelbach, L., Jolicoeur P., Clinical Research Institute of Montreal, Canada: Molecular cloning and biological characterization of lymphotropic nonfibrotropic leukemogenic radiation leukemia viruses.
- Fenyő, E.M., Asjö, B., Dept. of Virology, Karolinska Institutet, Stockholm, Sweden: Clonal isolates of Mo-MLV induce lymphomas that involve different subtypes of T cells.
- Bilello, J.A.,^{1,3,4} Hoffman, P.M.,^{1,2,3} Max, S.R.,² ¹Research Service, Veterans Administration Medical Center; Depts. of ²Neurology, ³Microbiology, ⁴Oncology Program, University of Maryland, Baltimore: Alterations in PC-12 cell function following infection with a ts mutant of Mo-MLV.
- Li, Y.,¹ Koka, P.,¹ Holland, C.A.,² Hopkins, N.,¹ Hartley, J.W.,³ ¹Center for Cancer Research, Massachusetts Institute of Technology, Cambridge; ²University of Massachusetts Medical School, Worcester; ³NCI, National Institutes of Health, Bethesda, Maryland: LTRs as determinants of disease specificity, tissue tropism, and MCF generation in nondefective MLVs.

- Silbon, M., Hayes, S.F., Chesebro, B., National Institutes of Health, Rocky Mountain Laboratories, Hamilton, Montana: Evidence for two different mechanisms of anemia induction following inoculation of newborn mice with F-MLV.
- Robert-Lezennes, J.,¹ Moreau-Gachelin, F.,¹ Wendling, F.,² Larsen, C.J.,³ ¹INSERM, Faculté Lariboisière-St. Louis, Paris; ²INSERM, Institut Curie, Orsay; ³INSERM, Hôpital St. Louis, Paris, France: Isolation of a new defective acute leukemogenic murine retrovirus—Biochemical characterization.
- Moreau-Gachelin, F.,¹ d'Auriol, L.,² Tambourin, P.,³ Galibert, F.,² Robert-Lezennes, J.,¹ Tavanti, A.,¹ ¹INSERM,



E. Gilboa



D. Lowy

Faculté de Médecine, Paris; ²Laboratoire d'Hématologie Expérimentale e, Hôpital Saint-Louis, Paris; ³INSERM, Institut Curie, Orsay, France: Detection of integrated SFFV proviruses in Friend tumor cells by hybridization with a synthetic octadecanucleotide.

Wending, F.,¹ Charon, M.,¹ Varlet, P.,¹ Martial, M.A.,² Tambourin, P.,¹ ¹INSERM, Institut Curie, Orsay; ²INSERM, Hôpital Cochin, Paris, France: Isolation of a new defective acute leukemogenic murine retrovirus—Physiopathological studies.

van der Feltz, M.J.M.,¹ Krandendonk-Odijk, M.E.,¹ Klaassen, P.,¹ de Both, N.J.,¹ Dept. of Pathology, Erasmus University, Rotterdam, The Netherlands: Viral integration in Rauscher virus-induced leukemic cell lines.

Ruscetti, S.,¹ Wolff, L.,¹ NCI, National Institutes of Health, Bethesda, Maryland: Biological and biochemical differences between SFFV₆ and SFFV_A localized to a region containing the 3' end of the *env* gene.

Pinter, A.,¹ Honnen, W.,¹ Broxmeyer, H.,² ¹Dept. of Viral Oncology, Memorial Sloan-Kettering Cancer Center, New York, New York; ²Dept. of Medicine, Indiana University School of Medicine, Indianapolis: Characterization of biochemical and biological properties of the secreted SFFV *env* gene product, gp65.

Li, J.-P., Bestwick, R., Kabat, D., Dept. of Biochemistry, Oregon Health Sciences University, Portland: Roles of the membrane glycoprotein and LTR in Friend viral erythroleukemia—Nucleotide sequences of nonleukemogenic mutant and spontaneous revertant viruses.

Geib, R.,¹ Anand, R.,² ¹Indiana University School of Medicine, Terre Haute Center for Medical Education; ²Centers for Disease Control, Division of Viral Diseases, Atlanta, Georgia: Comparison of Fv-2⁺ adapted strains of Friend erythroleukemia virus.

Kozak, C.A.,¹ NCI, National Institutes of Health, Bethesda, Maryland: Analysis of wild mouse populations for the Fv-1 and Fv-2 MLV restriction loci.

Silver, J.,¹ Kozak, C.,¹ Buckler, C.,¹ NCI, National Institutes of Health, Bethesda, Maryland: A new common integration region, *Fvi-1*, in Fr-MLV-induced leukemias.

Economou-Pachnis, A.,¹ Tschlis, P.N.,¹ Fox Chase Cancer Center, Philadelphia, Pennsylvania: Insertion of an *Alt* site in the human homologue of the *Mvi-2* locus in a human B-cell lymphoma.

Tschlis, P.N.,¹ Schaffer, B.,¹ Fox Chase Cancer Center, Philadelphia, Pennsylvania: Two independent insertions of line sequences in the J region of the Igh locus in the rat.

Villemur, R.,¹ Moczak, Y.,¹ Kozak, C.,² Rassart, E.,¹ Jolicoeur, P.,¹ ¹Clinical Research Institute of Montreal and Université de Montreal, Canada; ²NCI, National Institutes of Health, Bethesda, Maryland: Frequent rearrangement of a unique cellular sequence (*Gin-1*) in Gross passage MLV-induced mouse thymomas.

Gurgo, C.,¹ Franchini, G.,² Tajana, G.F.,³ Montagnani, S.,³ Bridges, S.,¹ ¹CEOS, Naples; ²NCI, National Institutes of Health, Bethesda, Maryland; ³University of Naples, Italy: Expression of c-onc genes and viral mRNA in AKR T-cell lymphomas.

Shackelford, G.M.,¹ Fung, Y.-K.T.,¹ Sanders, G.,¹ Varmus, H.E.,¹ Dept. of Microbiology and Immunology, University of California, San Francisco: Molecular cloning sequence analysis of *int-1* cDNA.

Garcia, M.,¹ Vessaz, A.,¹ Wellinger, R.,¹ Marcoli, R.,¹ Diggetmann, H.,¹ Swiss Institute for Experimental Cancer Research, Bern, Switzerland: The role of MMTV in the formation of kidney adenocarcinomas in BALB/cJf/Cd mice.

Redmond, S.M.S.,¹ Hynes, N.E.,¹ Ludwig Institute for Cancer Research Inselpital, Bern, Switzerland: Activated oncogenes in tumors induced by MMTV.

Gray, D.A.,¹ McGrath, C.M.,² Jones, R.F.,² Morris, V.L.,¹ ¹Dept. of Microbiology and Immunology, University of Western Ontario, Canada; ²Dept. of Tumor Biology, Michigan Cancer Foundation, Detroit: A common MMTV proviral integration site in precancerous mammary hyperplasias.

Hsu, L.,¹ Dudley, J.,¹ Dept. of Microbiology, University of Texas, Austin: Amplified MMTV proviruses in B6 T-cell lymphomas are closely linked to the transcribed repetitive sequence L1.

Khan, A.S.,¹ Laigret, F.,¹ Bouloukos, K.,¹ Repaske, R.,¹ Rabson, A.,¹ NCI, National Institutes of Health, Bethesda, Maryland: Identification and characterization of novel MCF-related mRNAs expressed in AKR mouse tissues.

Evans, L.H.,¹ NIAID, National Institutes of Health, Rocky Mountain Laboratories, Hamilton, Montana: Ecotropic and recombinant virus production in preleukemic AKR/J mice.

Wolfe, J.H.,¹ Blank, K.J.,² ¹Memorial Sloan-Kettering Cancer Center, New York, New York; ²University of Pennsylvania School of Medicine, Philadelphia: H-2-linked genes control retrovirus replication in cultured tumor cell lines.

Quérat, G.,¹ Filippi, P.,¹ Barban, V.,¹ Sauze, N.,¹ Vigne, R.,¹ Laboratoire de Virologie, Faculté de Médecine, Marseille, France: *Visna* lentivirus—Study on peculiar features of its lytic cycle.

Vigne, R.,¹ Quérat, G.,¹ Barban, V.,¹ Sauze, N.,¹ Verwoerd, D.W.,² ¹Laboratoire de Virologie, Faculté de Médecine, Marseille, France; ²Dept. of Agriculture, Onderstepoort, Republic of South Africa: Genetic heterogeneity among lentiviruses—Isolation of a new virus associated with a lung adenocarcinoma of sheep.

Pyper, J.M.,¹ Clements, J.E.,¹ Gonda, M.A.,² ¹Dept. of Neurology, Johns Hopkins University School of Medicine, Baltimore; ²NCI-Frederick Cancer Research Facility, Frederick, Maryland: Sequence homology between the cloned DNAs of CAEV and visna virus, two neurotropic retroviruses.

Carpenter, S., Sevoian, M., Dept. of Veterinary and Animal Sciences, University of Massachusetts, Amherst: The detection of cross-neutralizing antibodies in horses infected with EIAV.

Venkatessan, S., Daugherty, D., Benn, S., Boulukos, K., Martin, M., Folks, T., Rabson, A., NCI, National Institutes of Health, Bethesda, Maryland: Analysis of RNA transcripts of the AIDS retrovirus.

Barré-Sinoussi, F., Rey, F., Clavel, F., Montagnier, L., Chermann, J.C., Institut Pasteur, Paris, France: Identification of the gag gene products of LAV.

Dina, D.,¹ Steimer, K.,¹ Van Nest, G.,¹ Barr, P.,¹ Levy, J.,² Luciw, P.,¹ ¹Chiron Research Laboratories, Chiron Corporation, Emeryville; ²Dept. of Medicine, Cancer Research Institute, University of California, San Francisco: Molecular characterization of AIDS-related retroviruses.

Heidecker, G.,¹ Marx, P.A.,² Bryant, M.,² Gardner, M.B.,² Barker, C.,³ Hunter, E.,³ ¹Dept. of Pathology, Medical School, ²California Primate Research Center, University of California, Davis; ³Dept. of Microbiology, University of Alabama, Birmingham: Nucleotide sequence of a biologically active cloned SAIDS virus.

Wood, C., Anderson, P.A., Devare, S., Depts. of Cancer Research and Molecular Biology, Abbott Laboratories, North Chicago, Illinois: Molecular studies of HTLV-III.

Sodroski, J.,¹ Rosen, C.,¹ Goh, W.C.,¹ Ratner, L.,² Wong-Staal, F.,² Gallo, R.,² Haseltine, W.,¹ ¹Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts; ²NCI, National Institutes of Health, Bethesda, Maryland: HTLV-I, -II, and -III—Genomic determinants of transcriptional *trans*-activation.

Pavliakis, G.N., Felber, B., Paskalis, H., Kleinman-Ewing, C.,



P. Jolicoeur

NCI-Frederick Cancer Research Facility, Frederick, Maryland: The pX protein of HTLV-I is an activator of its LTRs.

Rice, N.R., Simek, S., Stephens, R.M., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Expression of the bovine leukemia virus "pX" region.

Shimotohno, K., Miwa, M., Sugimura, T., National Cancer Center, Research Institute, Tokyo, Japan: Structure of a putative pX protein of HTLV-I.

Rosen, C.A., Sodroski, J.G., Haseltine, W.A., Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Activation of enhancer sequences in the HTLV-II and BLV LTR by viral associated *trans*-acting factors.

Sagata, N., Tsuzuku-Kawamura, J., Ikawa, Y., Institute of Physical and Chemical Research, Wako, Japan: Identification and characterization of the pX_{BL} product of BLV.

SESSION 4 ONCOGENES II: RAS, MOS, MIL/RAF, REL

Chairpersons: G. Vande Woude, NCI-Frederick Cancer Research Facility, Frederick, Maryland
T. Shih, NCI-Frederick Cancer Research Facility, Frederick, Maryland

DeGudicibus, S.J., Smith, M.R., Mulcahy, L.S., Stacey, D.W., Roche Institute of Molecular Biology, Nutley, New Jersey: The requirement for cellular *ras* proteins during tumor cell proliferation.

Smith, M.R., DeGudicibus, S.J., Mulcahy, L.S., Stacey, D.W., Roche Institute of Molecular Biology, Nutley, New Jersey: The requirement for *c-ras* activity during transformation of NIH-3T3 cells by a second oncogene.

Willumsen, B.M.,¹ Tambourin, P.E.,² Lowy, D.R.,² ¹University Microbiology Institute, Copenhagen, Denmark; ²NCI, National Institutes of Health, Bethesda, Maryland: Ha-MSV p21 protein—Biologic significance of its activating mutations and flexibility in its major variable region.

Hattori, S., Ullsh, L.S., Clanton, D.J., Chen, Z.-q., Shih, T.Y., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Probing the active center of p21 *ras* proteins—I. Inhibition of p21 biochemical activities by a monoclonal antibody.

Chen, Z.-q.,¹ Ullsh, L.S.,¹ DuBois, G.,² Shih, T.Y.,¹ ¹NCI, National Institutes of Health; ²Program Resources, Inc., NCI, Frederick Cancer Research Facility, Frederick, Maryland: Posttranslational processing of p21 *ras* proteins in-

volves palmitation of the carboxyterminal tetrapeptide containing cysteine-186.

Lacal, J.C., Aaronson, S.A., NCI, National Institutes of Health, Bethesda, Maryland: Localization of a new domain relevant to the normal function of p21 *ras* proto-oncogene product.

Finkel, T.,¹ Der, C.,² Cooper, G.,² ¹Harvard University, Cambridge; ²Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Structural and functional characterization of codon 61 Ha-*ras* mutants.

Kasid, A., Dickson, R., Huff, K., Bates, S., Lowy, D., Lippman, M., Gelmann, E., NCI, National Institutes of Health, Bethesda, Maryland: v-Ha-*ras* transfection mimics and bypasses estrogen-induced tumor phenotype of a human breast cancer line.

Rein, A.,¹ Orison, B.M.,² Ozato, K.,² Cavanaugh, D.,¹ ¹NCI-Frederick Cancer Research Facility; ²NIHCD, NCI, National Institutes of Health, Bethesda, Maryland: Infection and transformation of cells from midgestation mouse embryos by murine retroviruses.

Noda, M.,^{1,2} Ko, M.,² Takano, T.,² Amano, T.,³ Ikawa, Y.,¹ ¹Laboratory of Molecular Oncology, Institute of Physical

and Chemical Research, Wako: ²Dept. of Microbiology, Keio University, Tokyo; ³Mitsubishi-Kasei Institute of Life Sciences, Tokyo, Japan: Ki- and Ha-MSV arrest growth and induce neurite extension of a NGF-responsive cell line, PC12.

Donoghue, D.J., Bold, R.J., Hannink, M., Dept. of Chemistry, University of California, San Diego, La Jolla: Site-directed and deletion mutants of the HT1MSV *mos* gene—Evidence for an ATP-binding site.

Seth, A., Vande Woude, G., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Nucleotide sequence and the biochemical activities of the HT1MSV *mos* gene.

Probst, F., Vande Woude, G.F., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Detection of transcripts homologous to the *c-mos* proto-oncogene in mouse tissues.

Maxwell, S., Arlinghaus, R., Dept. of Molecular Biology, Scripps Clinic and Research Foundation, La Jolla, California: Serine kinase activity associated with Mo-MSV-124-encoded *p37^{mos}*.

Béchéade, C., Calothy, G.,¹ Pessac, B.,¹ Martin, P.,² Coll, J.,² Denhez, F.,² Saule, S.,² Ghysdael, J.,² Stehelin, D.,² ¹Institut Curie, Orsay; ²INSERM, Institut Pasteur, Lille, France: The *mil* and *myc* oncogenes of chicken MH2 are biologically active and cooperate for chicken neuroretina cell transformation.

Rapp, U.R.,¹ Cleveland, J.L.,¹ Varesio, L.,¹ Jansen, H.W.,² Bister, K.,² Frederickson, T.N.,³ Morse, H.C. III,³ ¹NCI-Frederick Cancer Research Facility, Frederick, Maryland; ²Max-Planck Institut, Federal Republic of Germany; ³NIH, NCI, National Institutes of Health, Bethesda, Maryland: Carcinoma induction in mice by a *rat/myc* recombinant murine retrovirus.

Goldsborough, M.D.,¹ Gunnell, M.A.,¹ Cleveland, J.L.,¹ Bonner, T.I.,² Schultz, A.M.,² Sutrave, P.,¹ Rapp, U.R.,¹ ¹NCI, Frederick; ²National Institutes of Health, Bethesda; ³Litton Biometrics, Inc., Frederick, Maryland: Delta *raf*, a new *raf*-related gene.

Mark, G.,¹ Shows, T.,² Seeley, T.,¹ ¹NCI, National Institutes of Health, Bethesda, Maryland; ²Roswell Park Memorial Institute, Buffalo, New York: *c-pps*, a new *raf*-related sequence in man.

Simek, S., Stephens, R.M., Rice, N.R., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Cellular localization of the *v-rel* protein in an avian transformed lymphoid cell line.

Marshall, C.J.,¹ Toksoz, D.,¹ Verlaan-de Vries, M.,² Bos, J.L.,² ¹Institute of Cancer Research, London, England; ²Dept. of Medical Biochemistry, State University of Leiden, The Netherlands: Activation of N-ras in acute myeloid leukemias by codon 13 mutations.

SESSION 5 VIRAL PROTEINS AND REPLICATION

Chairpersons: E. Hunter, Cold Spring Harbor Laboratory, New York
J. Coffin, Tufts University Medical School, Boston, Massachusetts

Jacks, T., Varmus, H., Depts. of Biochemistry and Microbiology, University of California, San Francisco: In vitro synthesis of RSV *gag-pol* fusion proteins—Evidence for ribosomal frameshifting.



J. Coffin

Fu, X.,¹ Phillips, N.,¹ Jentoft, J.,¹ Tuazon, P.,² Traugh, J.,² Leis, J.,¹ ¹Case Western Reserve University School of Medicine, Cleveland, Ohio; ²University of California, Riverside: Site-specific phosphorylation of avian retrovirus pp12 regulates binding to viral RNA and changes the conformation of the protein.

Yoshinaka, Y., Copeland, T.D., Smythers, G.W., Oroszlan, S., NCI, Frederick Cancer Research Facility, Frederick, Maryland: BLV protease—Purification, chemical analysis and in vitro processing of *gag*-precursor polyprotein.

Yoshinaka, Y., Smythers, G.W., Katoh, I., Oroszlan, S., NCI-Frederick Cancer Research Facility, Frederick, Maryland: NH₂-terminal amino acid analysis of the endonuclease and organization of Mo-MLV genome.

Elder, J.H.,¹ McGee, J.,¹ Munson, M.,¹ Houghten, R.,¹ Kloetzer, W.,² Naso, R.,² ¹Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla; ²Johnson and Johnson Biotechnology Center, Inc., La Jolla, California: Neutralization of FeLV by anti-synthetic peptide antibodies to gp70 and p15E.

Elder, J.H., McGee, J., Alexander, S., Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California: Studies on the role of carbohydrate in the immune response to viral glycoproteins of feline and murine retroviruses.

Davis, G.L., Perez, L., Hunter, E., Dept. of Microbiology, University of Alabama, Birmingham: Site-directed point mutations in the carboxyterminal coding sequences of the RSV *env* gene affect surface expression.

- Dorner, A.J., Coffin, J.M., Tufts University School of Medicine, Boston, Massachusetts: Avian retroviruses with extended or restricted host range.
- Handelin, B., Machida, C., Bestwick, R., Kabat, D., Dept. of Biochemistry, Oregon Health Sciences University, Portland: Synthesis of functional receptors for ecotropic MLVs in hamster cells transfected with mouse DNA.
- Hackett, P.B., Petersen, R.B., Hensel, C.H., Dept. of Genetics and Cell Biology, University of Minnesota, St. Paul: Synthesis of a heptapeptide encoded in the leader RNA of RSV may be important in the retrovirus life cycle.
- Roth, M.J., Tanese, N., Goff, S.P., Dept. of Biochemistry and Molecular Biophysics, Columbia University College of Physicians & Surgeons, New York, New York: Expression of portions of the *pol* gene of Mo-MLV in *E. coli* and characterization of the gene products.
- Duyk, G.,¹ Longiaru, M.,² Cobrinik, D.,² Kowal, R.,¹ Katz, R.,² Skalka, A.,² Leis, J.,¹ ¹Case Western Reserve University School of Medicine, Cleveland, Ohio; ²Hoffmann-La Roche, Inc., Nutley, New Jersey: Circles with two tandem LTRs are specifically cleaved by the ASLV *pol* gene-associated endonuclease—Nucleotide sequences required for site-specific cleavage.
- Grandgenett, D.P., Vora, A.C., St. Louis University, Missouri: Generation of a 4-bp staggered cut at the RSV LTR circle junction by the viral pp32 DNA endonuclease.
- Colicelli, J., Goff, S.P., Dept. of Biochemistry and Molecular Biophysics, Columbia University, New York, New York: Analysis of mutants and pseudorevertants of Mo-MLV with alterations at the viral integration site.
- Olsen, J., Swanson, R., Dept. of Biochemistry and Cancer Research Center, University of North Carolina, Chapel Hill: Use of a retrovirus shuttle vector to dissect molecular forms of unintegrated viral DNA.
- and Parasitology, Louisiana State University Medical Center, New Orleans: Inhibition of cleavage of Mo-MLV *gag* and *env* precursor polyproteins by cerulenin.
- Luftig, R.B., Ikuta, K., Depts. of Microbiology, Immunology, and Parasitology, Louisiana State University Medical Center, New Orleans: Differences in the degree of heterogeneity between intracellular and structural Mo-MLV virus p30.
- Bova, C., Swanson, R., Dept. of Biochemistry, University of North Carolina, Chapel Hill: Avian retrovirus *env* genes—Nucleotide sequence and molecular recombinants define host-range determinants.
- Friedrich, R., Institute of Immunobiology, University of Freiburg, Federal Republic of Germany: Similarity between retroviral envelope genes and cellular sequences.
- Schmidt, D.M., Sidhu, N.K., Cianciolo, G.J., Snyderman, R., Howard Hughes Memorial Institute, Duke University Medical Center, Durham, North Carolina: Expression in *E. coli* of the major hydrophilic portion of the immunosuppressive retroviral protein p15E.
- Katoh, I.,¹ Yoshinaka, Y.,¹ Rein, A.,¹ Shibuya, M.,² Odaka, T.,² Oroszlan, S.,¹ ¹NCI-Frederick Cancer Research Facility, Frederick, Maryland; ²Dept. of Genetics, University of Tokyo, Japan: MLV mutants defective in assembly and/or mutation.
- Crawford, S., Goff, S.P., Dept. of Biochemistry and Molecular Biophysics, Columbia University, New York, New York: Homologous recombination between a deletion mutant of Mo-MLV lacking the *pol* protease and host sequences can restore the missing viral function.
- Tanese, N., Goff, S.P., Dept. of Biochemistry and Molecular Biophysics, Columbia University, New York, New York: Construction and analysis of linker insertion mutations affecting reverse transcriptase Mo-MLV.
- Hagino-Yamagishi, K., Donehower, L.A., Varmus, H.E., Dept. of Microbiology and Immunology, University of California, San Francisco: Sequence analysis of proviruses generated by an integration-deficient *pol* mutant of Mo-MLV.
- Panganiban, A., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Characterization of the SNV *att* site and the viral integrase.
- Alexander, F.,¹ Soltis, D.,¹ Crowl, R.,¹ Leis, J.,² Danho, W.,¹ Poonian, D.,¹ and Parasitology, Louisiana State University Medical Center, New Orleans: Inhibition of cleavage of Mo-MLV *gag* and *env* precursor polyproteins by cerulenin.
- Luftig, R.B., Ikuta, K., Depts. of Microbiology, Immunology, and Parasitology, Louisiana State University Medical Center, New Orleans: Differences in the degree of heterogeneity between intracellular and structural Mo-MLV virus p30.
- Bova, C., Swanson, R., Dept. of Biochemistry, University of North Carolina, Chapel Hill: Avian retrovirus *env* genes—Nucleotide sequence and molecular recombinants define host-range determinants.
- Friedrich, R., Institute of Immunobiology, University of Freiburg, Federal Republic of Germany: Similarity between retroviral envelope genes and cellular sequences.
- Schmidt, D.M., Sidhu, N.K., Cianciolo, G.J., Snyderman, R., Howard Hughes Memorial Institute, Duke University Medical Center, Durham, North Carolina: Expression in *E. coli* of the major hydrophilic portion of the immunosuppressive retroviral protein p15E.
- Katoh, I.,¹ Yoshinaka, Y.,¹ Rein, A.,¹ Shibuya, M.,² Odaka, T.,² Oroszlan, S.,¹ ¹NCI-Frederick Cancer Research Facility, Frederick, Maryland; ²Dept. of Genetics, University of Tokyo, Japan: MLV mutants defective in assembly and/or mutation.
- Crawford, S., Goff, S.P., Dept. of Biochemistry and Molecular Biophysics, Columbia University, New York, New York: Homologous recombination between a deletion mutant of Mo-MLV lacking the *pol* protease and host sequences can restore the missing viral function.
- Tanese, N., Goff, S.P., Dept. of Biochemistry and Molecular Biophysics, Columbia University, New York, New York: Construction and analysis of linker insertion mutations affecting reverse transcriptase Mo-MLV.
- Hagino-Yamagishi, K., Donehower, L.A., Varmus, H.E., Dept. of Microbiology and Immunology, University of California, San Francisco: Sequence analysis of proviruses generated by an integration-deficient *pol* mutant of Mo-MLV.
- Panganiban, A., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Characterization of the SNV *att* site and the viral integrase.
- Alexander, F.,¹ Soltis, D.,¹ Crowl, R.,¹ Leis, J.,² Danho, W.,¹ Poonian, D.,¹

SESSION 6 POSTER SESSION II

- Dunwiddie, C., Resnick, R., Mickelson, D., Boyce-Jacino, M., Faras, A., Dept. of Microbiology, University of Minnesota, Minneapolis: Structural analysis of *gag*-, *pol*-, and *env*-related sequences in ALV-lacking chick embryo fibroblasts.
- Chambers, J., Chen, P.-J., Taylor, J.M., Institute for Cancer Research, Philadelphia, Pennsylvania: Endogenous retrovirus-related elements of the Japanese quail—Association with major repetitive element family.
- Soe, L.H.,¹ Shimizu, R.,² Landolph, J.R.,^{1,2} Roy-Burman, P.,^{1,3} Depts. of ¹Pathology, ²Microbiology, ³Biochemistry, University of Southern California School of Medicine, Los Angeles: Structural and functional analysis of four classes of endogenous FeLV elements.
- Hutchison, K.W.,¹ Eicher, E.M.,² ¹Dept. of Biochemistry, University of Maine, Orono; ²Jackson Laboratory, Bar Harbor, Maine: Molecular analysis of a retrovirus amplified on the murine Y chromosome.
- Pampeno, C., Meruelo, D., Dept. of Pathology, New York University Medical Center, New York, New York: Isolation of an endogenous retroviral sequence from the *TL* region of the murine major histocompatibility complex.
- Horak, I., Wirth, T., Schmidt, M., Baumruker, T., Kröger, B., Institut für Virologie und Immunobiologie, Universität Würzburg, Federal Republic of Germany: Structure and possible function of a new family of murine retrovirus-related sequences (LTR-IS/MuRRS).
- O'Neill, R.R., Khan, A.S., Hoggan, M.D., Kozak, C.A., Martin, M.A., Repaske, R., NIAID, NCI, National Institutes of Health, Bethesda, Maryland: Specific hybridization probes demonstrate more MCF than xenotropic MLV *env*-related sequences in laboratory mouse DNAs.
- Stoye, J.P., Coffin, J.M., Tufts University School of Medicine, Boston, Massachusetts: Structural and functional analyses of endogenous murine retroviruses.
- Jacquemin, P.C., Pasteur Institute, Brussels, Belgium: Expression of antigenic determinants of endogenous human reverse transcriptase on the surface of some human hematopoietic cells.
- Löwer, J., Ferdinand, F.-J., Wondrak, E., Löwer, R., Kurth, R., Paul Ehrlich-Institut, Frankfurt, Federal Republic of Germany: Isolation of human endogenous retroviruses.
- Ikuta, K., Luftig, R.B., Depts. of Microbiology, Immunology,

- M., Skalka, A.M.,¹ Hoffmann-La Roche, Inc., Nutley, New Jersey; ²Case Western Reserve University, Cleveland, Ohio: Synthesis of *alv* pol gene products in bacteria—Evidence for carboxyterminal processing.
- Hu, S.C.,¹ Court, D.,² Zweig, M.,³ Levin, J.G.,¹ NICHID, NCI, National Institutes of Health, ³PRI, Frederick, Maryland: Expression of MLV reverse transcriptase and endonuclease in *E. coli*.
- Hippenmeyer, P.,¹ Quinn, T.,² Grandgenett, D.P.,² St. Louis University; ²Monsanto Company, St. Louis, Missouri: Structure-functional relationships of the avian retrovirus reverse transcriptase and endonuclease domains.
- Hafenrichter, R.,¹ Weiland, F.,¹ Schneider, J.,² Thiel, H.-J.,¹ Federal Research Centre for Virus Diseases of Animals, Tübingen; ²Deutsches Primatenzentrum, Abteilung Virologie und Immunologie, Göttingen, Federal Republic of Germany: Friend leukemia virus-associated proteases(s)—In vitro activity and localization in the virion.
- Dutta, A., Wang, L.-H., Hanafusa, T., Hanafusa, H.-B., Rockefeller University, New York, New York: Partial nucleotide sequence of RSV-29 suggests that the original RSV was replication-defective.
- Ikawa, S., Yamagishi, K., Hihara, H., Yamamoto, T., Kawai, S., Toyoshima, K., Institute of Medical Science, University of Tokyo, Japan: Retroviral transduction of the proto-sr gene.
- Chang, L.-J.,¹ Cooling, L.,² Turek, L.P.,² Stoltzfus, C.M.,¹ Depts. of ¹Microbiology, ²Pathology, University of Iowa, Iowa City: Expression of intronless RSV DNA constructs and specific inhibition by anti-sense RNA.
- Darlix, J.-L.,^{1,2} Spahr, P.-F.,¹ Dept. of Molecular Biology, University of Geneva, Switzerland; ²Université Paul Sabatier, CNRS, Toulouse, France: Characterization of RNA sequences and structures regulating stability and expression of RSV RNA.
- Kane, S.E., Arrigo, S.J., Beemon, K., Dept. of Biology, Johns Hopkins University, Baltimore, Maryland: Localization of 5^m-methyladenosine residues in RSV RNA.
- Park, H., Kaji, A., Dept. of Microbiology, University of Pennsylvania School of Medicine, Philadelphia: Reduced amount of subgenomic RSV RNA and production of defective virus by the RSV-infected, terminally differentiated chicken myotubes.
- Jhappan, C.,¹ Blair, D.,² Vande Woude, G.F.,³ Robins, T.,³ Dept. of Biochemistry, Georgetown University, Washington, D.C.; ²Laboratory of Molecular Oncology, ³LBL-Basic Research Program, NCI-Frederick Cancer Research Facility, Frederick, Maryland: Generation of transforming virus from cells with integrated copies of a plasmid containing *mos* and a 3' LTR.
- Horowitz, T.S., Radcliffe, G., Shank, P.R., Division of Biology and Medicine, Brown University, Providence, Rhode Island: Correlations between chromatin conformation and ASV proviral instability in quail cells.
- Hirano, A.,¹ Vogt, P.K.,² Wong, T.C.,¹ Dept. of Microbiology and Immunology, University of Washington School of Medicine, Seattle; ²Dept. of Microbiology, University of Southern California School of Medicine, Los Angeles: Cloned DNA of defective ASV mutant LA46 encodes the cis-acting temperature-sensitive defect in replication.
- Racevskis, J., Dept. of Viral Oncology, Memorial Sloan-Kettering Cancer Center, New York, New York: Expression of the protein product of the MMTV LTR gene in phorbol ester-treated mouse T-cell leukemia cells.
- Barker, C.S.,¹ Tainsky, M.,² Engler, J.,¹ Hunter, E.,¹ Depts. of Microbiology and Biochemistry, University of Alabama, Birmingham; ²NCI-Frederick Cancer Research Facility, Frederick, Maryland: Biological and molecular analysis of MPMV proviral DNA clones.
- Lee, W.T.L., Prakash, O., Klein, D., Sarker, N.H., Sloan-Kettering Memorial Cancer Center, Rye, New York: The structure of the LTR of a newly integrated MMTV provirus in a murine T-cell leukemia.
- Panet, A., Bar Shira, A., Kronman, H., Honigman, A., Hebrew University-Hadassan Medical School, Jerusalem, Israel: Analysis of the signal in the LTR DNA which generates a uniform 3' end viral RNA of MLV.
- Lu, Y.,¹ Bachelor, L.T.,² Dept. of Biology, ²Fels Research Institute, Temple University School of Medicine, Philadelphia, Pennsylvania: Promoter activities of endogenous MLV LTRs.
- Rassart, E., Savard, P., Jolicoeur, P., Clinical Research Institute of Montreal, Canada: The wild mouse neurotropic MLV—Construction of "neurotropic"-specific probes and physical mapping of its leukemogenic potential.
- Celander, D., Haseltine, W., Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Identification and characterization of a glucocorticoid responsive element in the enhancer region of MLV control regions.
- Takeya, T., Onuki, Y., Ohshima, A., Takahashi, M., Institute for Chemical Research, Kyoto University, Japan: RNA sequence analysis of the 3' terminal regions of ALV and RSV—Comparison and possible genealogical relationship between ALV and RSV.
- Iwasaki, K.,¹ Niwa, O.,² Dept. of Tropical Agriculture, Kyoto University; ²Dept. of Pathology, Hiroshima University, Japan: Undifferentiated murine teratocarcinoma cells lack condensed chromatin structure associated with transcriptionally inactive and methylated DNA sequences.
- Catala, F., Institut Curie, Orsay, France: DNA methylation and transcriptional controls of proviral DNA in ASV-transformed mammalian cells.
- Pedersen, F.S., Etzerodt, M., Jensen, N.A., Jørgensen, P., Kjeldgaard, N.O., Dept. of Molecular Biology, Aarhus University, Denmark: Structural and functional analysis of cis-acting elements in MLV genetic variants.
- Hess, N.,¹ Stocking, C.E.,¹ Kollek, R.,¹ Mushinski, J.F.,² Ostertag, W.,¹ Heinrich-Pette-Institut, Hamburg, Federal Republic of Germany; ²NCI, National Institutes of Health, Bethesda, Maryland: Retroviral vectors with LTR regulated coexpression of the *neo* resistance gene and oncogenes.
- Günzburg, W.H., Groner, B., Salmons, B., Ludwig Institute for Cancer Research, Bern, Switzerland: A retroviral vector based on MMTV.
- Salmons, B., Jaggi, R., Muellerner, D., Kozma, S., Groner, B., Ludwig Institute for Cancer Research, Bern, Switzerland: MMTV LTR-regulated conditional transformation of cultured cells.
- Lipsick, J.S., Ibañez, C.E., Baluda, M.A., Jonsson Comprehensive Cancer Center and Dept. of Pathology, University of California School of Medicine, Los Angeles: A selectable ALV vector system.
- Bosseiman, R., Jones, T., Hsu, R., Amgen, Thousand Oaks, California: Deletion of U₃ sequences from the 3' LTR of a

provirus carrying the neomycin resistance gene—Effect on titer following rescue by infection of primary transfectants and passage by infection.

Emerman, M., Temin, H.M., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Suppression of linked transcriptional units in retroviruses vectors.

Wong, T.C.,¹ Goodenow, R.S.,² Sher, B.T.,³ Davidson, N.,⁴ ¹Dept. of Microbiology and Immunology, University of Washington School of Medicine, Seattle; ²Dept. of Genetics, University of California, Berkeley; ³Dept. of Pathology, Stanford University School of Medicine, California; ⁴Dept. of Chemistry, California Institute of Technology, Pasadena: The promoter of the LTR of FeLV is effective for expression of a mouse *H-2* histocompatibility gene in mouse and human cells.

Chang, S.M., Tsao, T.Y., Vaishnav, S., Caskey, C.T., Howard Hughes Medical Institute, Baylor College of Medicine, Houston, Texas: Defective HPRT retroviruses containing genomic control sequences.

Flavell, A., Reid, C., Dept. of Biochemistry, University, Dundee, Scotland: Structure and origin of *cop*ia extrachromosomal elements.

Lambert, M.E.,¹ McDonald, J.,² Strand, D.J.,² Fisher, P.B.,¹ Weinstein, I.B.,¹ ¹Columbia University, New York, New York; ²Dept. of Genetics, University of Georgia, Athens: Regulation of *cop*ia expression of E1A or heat shock after introduction into rat fibroblasts.

Strand, D.,¹ Lambert, M.,¹ Arthur, W.L.,¹ McDonald, J.,¹ ¹Dept. of Genetics, University of Georgia, Athens; ²Institute of Cancer Research, Columbia University, New York, New York: The *Drosophila* retroviral-like element *cop*ia is transcriptionally and topographically responsive to heat-shock stress.

Conrad, D.,¹ Friedman-Kien, A.,² Blair, D.,³ Tomar, R.,³ Ehrlich, G.,² Ruscetti, F.,⁵ Han, T.,⁶ Moore, J.,³ Montagna, R.,¹ Halbert, S.,⁷ Poiesz, B.,^{3,4} ¹Cellular Products, Inc., Buffalo, New York; ²New York University, New York; ³State University of New York, Upstate Medical Center, Syracuse; ⁴Veterans Administration Medical Center, Syracuse, New York; ⁵NCI-Frederick Cancer Research Center, Frederick, Maryland; ⁶Roswell Park Memorial Institute, Buffalo, New York; ⁷Cordis Laboratories, Miami, Florida: Seroreactivity to AIDS-associated virus (AAV).

Krone, W.J.A.,¹ Hellings, J.A.,¹ Smit, L.,² Bakker, M.,² van de Noordaa, J.,² Goudsmit, J.,² ¹Organon Diagnostics Research Laboratories, Oss; ²Dept. of Virology, University of Amsterdam, The Netherlands: Specificity and sensitivity of an "ELISA" specific for HTLV-III antibodies using crude antigens.

Maeda, S., Yonezawa, K., Akahonai, Y., Yachi, A., Dept. of Internal Medicine, Sapporo Medical College, Japan: Antibody reactive to human retrovirus-related antigen detected in sera of patients with leukemia/lymphoma.

Allan, J.S.,¹ Coligan, J.E.,² Barin, F.,¹ McLane, M.F.,¹ Lee, T.H.,¹ Essex, M.,¹ ¹Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts; ²NIAID, National Institutes of Health, Bethesda, Maryland: Serological and biochemical analysis of the envelope proteins of HTLV-III.

Lee, T.H.,¹ Essex, M.,² Klein, E.,¹ Klein, G.,¹ ¹Dept. of Tumor Biology, Karolinska Institute, Stockholm, Sweden; ²Dept. of Cancer Biology, Harvard School of Public Health,



A. Skalka

Boston, Massachusetts: Identification of a HTLV-associated antigen (TILNA).

Fultz, P.,^{1,2} McGrath, C.,¹ McClure, H.,² Broderson, R.,¹ Jensen, F.,³ Francis, D.,¹ ¹Centers for Disease Control, Atlanta, Georgia; ²Yerkes Regional Primate Research Center, Emory University, Atlanta, Georgia; ³Cytotech, San Diego, California: HTLV-III/LAV persistence in chimpanzees.

Anand, R., Srinivasan, A., Moore, J.L., Francis, D.P., Feorino, P.M., Curran, J.W., AIDS Branch Center for Disease Control, Atlanta, Georgia: Inhibition of the production of HTLV-III/LAV in primary lymphocyte cultures and a virus producer cell line by ansamycin LM 427.

Miedema, F.,¹ Tersmette, M.,¹ Huisman, H.G.,¹ Goudsmit, J.,² Melief, C.J.M.,¹ ¹Central Laboratory Netherlands Red Cross Blood Transfusion Service and Laboratory for Experimental and Clinical Immunology; ²Dept. of Virology, University of Amsterdam, The Netherlands: Productive infection of human EBV-transformed lymphoblastoid cell lines with HTLV-III/LAV.

Shearer, G.M.,¹ Salahuddin, S.Z.,¹ Bernstein, D.C.,¹ Markham, P.D.,² Tung, K.S.,³ Gallo, R.C.,¹ ¹NCI, National Institutes of Health, Bethesda; ²Dept. of Cell Biology, Litton Bionetics, Kensington, Maryland; ³Dept. of Pathology, University of New Mexico, Albuquerque: Enhanced infection of human peripheral blood leukocytes with HTLV-III after stimulation with HLA alloantigens.

Markham, P., Salahuddin, S., Popovic, M., Gallo, R., NCI, National Institutes of Health, Bethesda, Maryland: Tissue and patient sources of more than 100 isolates of HTLV-III.

Shaw, G.M., Hahn, B.H., Salahuddin, S.Z., Markham, P., Popovic, M., Gallo, R.C., Wong-Staal, F., NCI, National Institutes of Health, Bethesda, Maryland: Genomic diversity of the AIDS virus, HTLV-III.

Okamoto, T.,¹ Reitz, M., Jr.,¹ Clarke, M.,¹ Jagodzinski, L.,² Gallo, R.,¹ Wong-Staal, F.,¹ ¹NCI, National Institutes of Health, Bethesda; ²Biotech Research Laboratories, Inc., Rockville, Maryland: Evidence of cis transduction of *KpnI* transcripts in an HTLV-I immortalized cell line.

Arya, S.K., Wong-Staal, F., Gallo, R.C., NCI, National Institutes of Health, Bethesda, Maryland: Human T-cell growth factor and gamma interferon genes—Expression in HTLV-I (leukemia virus) and type-III (AIDS virus) infected cells.

Jendis, J.,¹ Salahuddin, S.,¹ Markham, P.,¹ Franchini, G.,¹ Gurgo, C.,² Gallo, R.C.,¹ ¹NCI, National Institutes of Health, Bethesda, Maryland; ²CEOS, Naples, Italy: Development and characterization of human T-cell line non-productively transformed by HTLV-II.

Gartner, S., Popovic, M., Stockbauer, P., Read-Connole, E., Gallo, R.C., NCI, National Institutes of Health, Bethesda, Maryland: HTLV-III infection of cultured human normal and neoplastic brain-derived cells.

SESSION 7 ONCOGENES III: SRC, FOS/FES, ROS, FGR

Chairpersons: **T. Parsons**, University of Virginia Medical School, Charlottesville, Virginia
D. Stehelin, Institut Pasteur, Paris, France

Wang, J.Y.J.,¹ Maher, P.,¹ Pasqual, E.,¹ Morla, A.O.,¹ Singer, J.S.,¹ Williams, L.T.,² ¹Dept. of Biology, University of California, San Diego, La Jolla; ²Howard Hughes Medical Institute of California, San Francisco: Detection of tyrosine-phosphorylated proteins in normal and transformed cells using antibodies for phosphotyrosine isolated by immunization with a *v-abf*-encoded protein.

Garber, E.A., Cross, F.R., Hanafusa, H., Rockefeller University, New York, New York: Mapping aminoterminal functional domains of p60^{src} - Comparison of wild-type RSV and in vitro constructed deletion mutants.

Kamps, M., Buss, J., Sefton, B., Molecular Biology and Virology Laboratory, Salk Institute, San Diego, California: Nontransforming p60^{src} mutants lacking myristic acid induce high levels of tyrosine protein phosphorylation but demonstrate an altered spectrum of substrate phosphorylation.

Takeya, T.,¹ Kato, J.-Y.,¹ Grandori, C.,² Iba, H.,² Hanafusa, H.,² ¹Institute for Chemical Research, Kyoto University, Japan; ²Rockefeller University, New York, New York: Amino acid substitutions sufficient to convert the non-transforming p60^{src} to a transforming protein.

Snyder, M.A.,¹ Bishop, J.M.,¹ McGrath, J.P.,² Levinson, A.R.,² ¹G.W. Hooper Foundation and Dept. of Microbiology and Immunology, University of California; ²Genentech Inc., South San Francisco, California: A mutation at the ATP binding site of pp60^{src} abolishes kinase activity, transformation, and tumorigenicity.

Tanaka, A., Fujita, D.J., Cancer Research Laboratory, University of Western Ontario, Canada: Expression and oncogenic activation of a molecularly cloned human *c-src* gene.

Sugimoto, Y., Erikson, R.L., Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: Phosphatidylinositol kinase activities in normal and RSV-transformed cells.

Gentry, L., Shoyab, M., Purchio, T., Oncogen, Seattle, Washington: Increased phosphorylation of pp60^{src} in RSV-transformed cells by treatment with TPA.

Skalka, A.M.,¹ Lehrman, S.R.,¹ Langer-Safer, P.R.,¹ ¹Dept. of Molecular Genetics, Hoffmann-La Roche, Nutley, New Jersey; ²Genetics Institute, Cambridge, Massachusetts: *v-src* suppresses differentiation via extracellular intermediate(s).

Shalloway, D., Johnson, P.J., Freed, E.O., Coulter, D., Dept. of Molecular and Cell Biology, Pennsylvania State University, University Park: Cooperation between *c-src* and polyoma large T, *c-myc*, *adeno E1A*, and *adeno E1B* genes to transform NIH-3T3 cells.

Golden, A.,¹ Nemeth, S.,¹ Keane, R.W.,² Lustig, A.,¹ Cotton, P.C.,¹ Brugge, J.,¹ ¹State University of New York, Stony Brook; ²Dept. of Physiology and Biophysics, University of Miami, Florida: Regulation of the expression of the cellular *src* protein in neural cells and peripheral blood cells.

Foster, D.A., Shibuya, M., Hanafusa, H., Rockefeller University, New York, New York: Viral gag sequences activate the transformation potential of cellular *fps* gene sequences.

Neckameyer, W.,¹ Shibuya, M.,² Wang, L.-H.,¹ ¹Rockefeller University, New York, New York; ²University of Tokyo, Japan: Nucleotide sequence and expression of cellular *ros*.
Nishizawa, M.,¹ Semba, K.,¹ Yamamoto, T.,¹ Yoshida, M.,² Toyoshima, K.,¹ ¹Dept. of Oncology, Institute of Medical Science, University of Tokyo; ²Chromosome Research Unit, Hokkaido University, Sapporo, Japan: Structural analysis of human *c-igr* gene.

Soong, M.-M.,¹ Iijima, S.,² Wang, L.-H.,¹ ¹Rockefeller University, New York, New York; ²Nagoya University, Japan: Mechanism of transduction of the *c-src* sequences by a *src* deletion mutant of RSV.

SESSION 8 CIS AND TRANS ACTING TRANSCRIPTIONAL CONTROL

Chairpersons: **H. Fan**, University of California, Irvine, California
A. Skalka, Hoffmann-La Roche, Inc., Nutley, New Jersey

Derse, D., Casey, J., NCI-Frederick Cancer Research Facility, Frederick, Maryland: The BLV LTR possesses two independent elements controlling gene expression.

Srinivasan, A.,¹ Des Rosiers, G.,¹ Kalyanaraman, V.S.,¹ Anand, R.,¹ Narayanan, R.,¹ Kashmiri, S.V.S.,² Ferrer, J.F.,² Venkat Gopal, T.,³ ¹Centers for Disease Control, Atlanta, Georgia; ²New Bolton Center, Kennett Square, Pennsyl-

vania; ³NCI, National Institutes of Health, Bethesda, Maryland: *Trans*-acting transcriptional activation of BLV LTR - Evidence for positive and negative regulation in BLV-infected cells.

Wachsman, W., Slamon, D., Chen, I.S.Y., Dept. of Medicine, University of California School of Medicine, Los Angeles: Structure and function of the HTLV X gene.

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Rosen, C.A., Sodroski, J.G., Haseltine, W.A., Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Localization of enhancer sequences responsive to viral associated *trans*-acting factors in the HTLV-III LTR.

SESSION 9 POSTER SESSION III

Strebhardt, K.,¹ Rübbsamen-Waigmann, H.,² Bruck, C.,^{1,3} Mullins, J.L.,¹ ¹Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts; ²Paul Ehrlich Institute, Frankfurt, Federal Republic of Germany; ³Tufts University, Boston, Massachusetts: Molecular cloning of chicken *c-src* cDNA.

Yonemoto, W., Filson, A., Lustig, A., Brugge, J.S., Dept. of Microbiology, State University of New York, Stony Brook: Characterization of the pp60^{src}-middle T antigen complex in polyoma virus-transformed cells.

Rabotti, G.C.,¹ Mariller, M.,¹ Teutsch, B.,¹ Semmel, M.,² Pavloff, N.,² ¹Laboratoire de Médecine Expérimentale, Collège de France, Paris; ²Institut Gustave Roussy, Villejuif, France: Provirus integration and expression in ASV-transformed human diploid fibroblasts.

Anderson, S.M.,¹ Rands, E.,¹ Fincham, V.J.,² Wyke, J.,² ¹Virus and Cell Biology, Merck Sharp and Dohme Research Laboratories, Swedeland, Pennsylvania; ²Imperial Cancer Research Fund, London, England: Construction of a *src*-containing murine retrovirus with a temperature-sensitive lesion in *src*.

Piwica-Worms, H.,¹ Kaplan, D.R.,¹ Whitman, M.,² Roberts, T.M.,¹ ¹Dana-Farber Cancer Institute, Boston; ²Harvard University, Cambridge, Massachusetts: Efficient transmission, expression and rescue of *c-src* carried in a recombinant murine retrovirus.

Jong, S.-M., Wang, L.-H., Rockefeller University, New York, New York: Study on the transforming ability of ASV UR2 *src* deletion mutants and *src-ros* recombinants.

Manger, R., Ewing, L., Rohrschneider, L., Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Se-

Norton, P.A., Coffin, J.M., Tufts University School of Medicine, Boston, Massachusetts: Multiple roles of the RSV LTR.

Davis, B.,¹ Hanecak, R.,¹ Rockford, R.,² Villarreal, L.,² van der Putten, H.,³ Fan, H.,¹ ¹Dept. of Molecular Biology and Biochemistry, University of California, Irvine; ²Dept. of Microbiology and Immunology, University of Colorado School of Medicine, Denver; ³Molecular Biology and Virology Laboratory, Salk Institute, San Diego, California: Effect of LTR alterations upon the biological activity of Mo-MLV.

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Cizdziel, P.E., Nash, M.A., Murphy, E.C., Jr., Dept. of Tumor Biology, University of Texas, M.D. Anderson Hospital, Houston: The molecular basis for phenotypic reversal in cells infected with MSVts110.

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Lee, W.-H., Chen, L.-H., Wheatley, W., Dept. of Pathology, University of California, San Diego, La Jolla: Mapping *ts* mutations of FSV responsible for temperature-sensitive transformation by *in vitro* recombination.

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S. Hughes

Antler, A.M., Kidd, S., Young, M., Hanafusa, H., Rockefeller University, New York, New York: Cloning and characterization of a *Drosophila melanogaster* gene homologous to the *erb-B* oncogene.

Gentry, L., Lawton, A., Oncogen, Seattle, Washington: Inhibition of EGF receptor tyrosine kinase activity with site-specific antibodies.

Richards, C.S.,¹ Hender, F.,² Burns, D.,³ Gusterson, B.,⁴ Ozanne, B.,¹ Depts. of ¹Microbiology, ²Internal Medicine, ³Pathology, University of Texas Health Science Center, Dallas; ⁴Ludwig Institute for Cancer Research, Sutton, England: EGF receptor functions as an oncogene in epidermoid malignancies.

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Rothwell, V.M., Lyman, S.D., Rohrschneider, L.R., Fred Hutchinson Cancer Research Center, Seattle, Washington: Production and characterization of antibodies to two domains of the *v-fms* protein.

Mahadevan, L.,¹ Heath, J.K.,² Foulkes, J.G.,² ¹Dept. of Zoology, University of Oxford, ²National Institute for Medical Research, London, England: ECDGF—A growth factor which reduces phosphotyrosine levels?

Lin, M.-F., Clinton, G.M., Dept. of Biochemistry, Louisiana State University Medical Center, New Orleans: Decreased prostatic acid phosphatase corresponds to amplified tyrosine phosphorylation and tyrosyl kinase activity in human prostate carcinoma cells.

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Maness, P., Shores, C., Dept. of Biochemistry, University of North Carolina School of Medicine, Chapel Hill: A novel *src*-related protein kinase in the electric eel.

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Evinger-Hodges, M.J.,¹ Dicke, K.A.,¹ Gutterman, J.U.,² Blick, M.,² Depts. of ¹Hematology, ²Clinical Immunology, University of Texas, M.D. Anderson Hospital, Houston: Oncogene expression in human normal bone marrow.

Niman, H.L., Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California: Detection of oncogene-related proteins in urine.

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- Anderson, G.R., Farkas, B.K., Polonis, V.R., Cell and Tumor Biology, Roswell Park Memorial Institute, Buffalo, New York: The VL30 sequences of K1SV as anoxic stress response genes.
- Nishikura, K., Goldflam, S., Vuocolo, G.A., Wistar Institute, Philadelphia, Pennsylvania: Accurate and efficient transcription of human *c-myc* genes injected into *Xenopus laevis* oocytes.
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- T-Yokota, Y.,¹ Yokota, J.,¹ Battifora, H.,² Slamon, D.,¹ Cline, M.J.,² ¹Dept. of Medicine, University of California School of Medicine, Los Angeles; ²Dept. of Anatomic Pathology, City of Hope National Medical Center, Duarte, California: Amplification of the *c-myc* oncogene occurs in vivo in variety of human cancers.
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- Henry, C.,¹ Coquillaud, M.,¹ Martin, P.,² Ferre, F.,² Saule, S.,² Stehelin, D.,² Béchade, C.,³ Calothy, G.,³ Debuire, B.,¹ ¹INSERM, Lille; ²INSERM, Institute Pasteur, Lille; ³Institut Curie, Orsay, France: Characterization of a *myc*-containing retrovirus generated by propagation of the MH2 subgenomic RNA.
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- Braun, M.J.,¹ Deininger, P.L.,² Casey, J.,¹ ¹NCI-Frederick Research Facility, Maryland; ²Dept. of Biochemistry, Louisiana State University Medical Center, New Orleans: Nucleotide sequence of a transduced *myc* gene from a defective feline leukemia provirus.
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- Klempnauer, K.-H., Biedenknapp, H., Sippel, A.E., University of Heidelberg, Federal Republic of Germany: DNA-binding activity of bacterially expressed viral *myb* protein.

SESSION 10 RETROVIRUS BIOLOGY AND GENETICS

Chairpersons: S. Ruscetti, NCI, National Institutes of Health, Bethesda, Maryland
N. Jenkins, NCI, National Institutes of Health, Bethesda, Maryland

DesGroseillers, L., Jolicoeur, P., Clinical Research Institute of Montreal, Canada: LTR sequences greatly influence the

leukemogenic and paralysis-inducing potential of the wild mouse neurotropic MLV.

- Offit, A., Agranovsky, O., McKinney, M., Memorial Sloan-Kettering Cancer Center, New York, New York: Fr-MLV immortalized myeloid cells are converted into tumorigenic cells by AblV.
- Heard, J.M., Sola, B., Martial, M.A., Fichelson, S., Gisselbrecht, S., INSERM, Paris, France: Isolation of clonal pre-leukemic myelomonocytic cells at early time of the Fr-MLV infection.
- Wolff, L., Tambourin, P., Ruscetti, S., NCI, National Institutes of Health, Bethesda, Maryland: Helper-virus is not required for multiple stages of SFFV-induced erythroid cell transformation.
- Wolff, L., Ruscetti, S., NCI, National Institutes of Health, Bethesda, Maryland: Biological effects of the envelope gene of SFFV under control of LTRs from various MLVs.
- Bestwick, R.,¹ Hankins, D.,² Kabat, D.,¹ ¹Dept. of Biochemistry, Oregon Health Sciences University, Portland; ²NCI, National Institutes of Health, Bethesda, Maryland: Rauscher SFFV leukemogenesis may require RNA co-packaging with helper MLV.
- Laigret, F., Ikeda, H., Martin, M., Repaske, R., NCI, National Institutes of Health, Bethesda, Maryland: Expression of the *Fv-4* gene, a truncated provirus lacking a 5' LTR.
- Buller, R., Portis, J., National Institutes of Health, Rocky Mountain Laboratories, Hamilton, Montana: Evidence that expression of an endogenous MCF gp70 in murine embryo cell cultures may correlate with the Rmcl resistance phenotype.
- Cloyd, M.W.,¹ Chattopadhyay, S.K.,² ¹Depts. of Surgery and
- Microbiology, Duke University Medical Center, Durham, North Carolina; ²NIAID, National Institutes of Health, Bethesda, Maryland: A new class of recombinant MLV present in many murine leukemia systems.
- Thomas, C.T., Dept. of Medicine and Microbiology, University of Virginia, Charlottesville: The leukemogenic AKR ecotropic virus SL3-3 forms envelope gene recombinants in vivo.
- Bacheler, L., Fels Research Institute, Temple University School of Medicine, Philadelphia, Pennsylvania: Transcriptional activity of integration sites for Mo-MLV.
- Vijaya, S., Steffen, D., Robinson, H.L., Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Five sequences with acceptor sites for proviral integrations map near DNase-hypersensitive site in chromatin.
- Connors, R.W., Vaidya, A.B., Hahnemann University, Philadelphia, Pennsylvania: Structural analyses and biological activity of cloned MMTV proviruses that are differentially regulated in vivo.
- Horowitz, J.M., Risser, R., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Novel integration site and biological activity of the BALB/c endogenous ecotropic provirus.
- Horn, T.,¹ Fetherston, J.,¹ Mariani-Costantini, R.,¹ Huebner, K.,² Croce, C.,² Callahan, R.,² ¹NCI, National Institutes of Health, Bethesda, Maryland; ²Wistar Institute, Philadelphia, Pennsylvania: Human endogenous retroviral sequences related to MMTV.

SESSION 11 ONCOGENES IV: MYC, MYB, FOS, SKI

Chairpersons: **R. Eisenman**, Fred Hutchinson Cancer Research Center, Seattle, Washington
B. Vennström, European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany

- Hann, S.R., Thompson, C.B., Challoner, P.B., Groudine, M., Eisenman, R.N., Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington: Expression of the *myc* oncogene is independent of the cell cycle in avian and human cells.
- Lee, W., Jakobovits, E., Varmus, H.E., Dept. of Microbiology, University of California, San Francisco: Cotransformation of primary rat embryo cells with *c-myc* genes regulated by inducible promoters.
- Stone, J.,¹ Lee, W.,² Jakobovits, E.,² Ramsay, G.,² Bishop, J.M.,² Varmus, H.E.,² ¹Hospital for Sick Children, Toronto, Canada; ²University of California, San Francisco: Cotransforming activity of linker-insertion and deletion mutants of human *c-myc*.
- Heaney, M.L.,¹ Morgan, J.H.,¹ Pierce, J.H.,² Parsons, J.T.,¹ ¹Dept. of Microbiology, University of Virginia School of Medicine, Charlottesville; ²NCI, National Institutes of Health, Bethesda, Maryland: Site-directed mutagenesis of the *v-myc* gene—Correlation of structure and function.
- Kelekar, A., Keath, E.J., Caimi, P.G., Cole, M.C., Dept. of Molecular Biology, Princeton University, New Jersey: Transformation and immortalization by cellular oncogenes.
- Vennström, B., Bravo, R., EMBL, Heidelberg, Federal Republic of Germany: *v-myc*-transformed BALB-c/3T3 cells require FGF or PDGF for expression on the transformed phenotype.
- Brightman, B.K., Fan, H., University of California, Irvine: In vitro and in vivo effects of a MLV recombinant containing *v-myc*.
- Thompson, C.B., Challoner, P.B., Groudine, M., Neiman, P.E., Fred Hutchinson Cancer Research Center, Seattle, Washington: *c-myc* mRNA levels during in vivo activation and proliferation of lymphocytes—Evidence that unregulated *myc* expression maintains lymphocytes within the cell cycle.
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- Gerondakis, S., Chen, L., Bishop, J.M., Dept. of Microbiology, University of California School of Medicine, San Francisco: The structure and function of avian *c-myc*.
- Boyle, W.J.,¹ Lampert, M.A.,¹ Li, A.C.,² Lipsick, J.S.,¹ Ong, J.M.,¹ Baluda, M.A.,¹ ¹Dept. of Pathology, University of California School of Medicine and Johnsons Comprehensive Cancer Center; ²Dept. of Biology, University of California, Los Angeles: Analysis of p48^{v-myc} interaction with nuclear components by subnuclear fractionation and chemical cross-linking.
- Weinstein, Y., Ihle, J.N., NCI-Frederick Cancer Research Facility, Frederick, Maryland: *c-myc* rearrangements in interleukin-3-dependent myeloid leukemia cell lines.

Krujer, W.,¹ Schubert, D.,¹ van der Putten, H.,¹ Leffert, H.L.,² Verma, I.M.,¹ ¹Molecular Biology and Virology Laboratory, Salk Institute, San Diego; ²Dept. of Medicine, University of California, San Diego: Induction of *c-fos* expression after mitogenic stimulation.

Jenuwein, T.,¹ Müller, D.,¹ Curran, T.,² Müller, R.,¹ ¹EMBL, Heidelberg, Federal Republic of Germany; ²Dept. of Molecular Genetics, Roche Research Center, Hoffmann-La

Roche Inc., Nutley, New Jersey: The *fos* oncogene of FBR-MSV can induce transformation and extended self-renewal of nonestablished mouse connective tissue cells.

Teumer, J., Broder, D., Stavnezer, E., Sloan-Kettering Cancer Center, New York, New York: Constructed nondefective SKVs and natural variants indicate no requirement for helper sequences in *v-ski* transforming proteins.

SESSION 12 VECTORS

Chairpersons: E. Gilboa, Princeton University, Princeton, New Jersey
S. Hughes, NCI-Frederick Cancer Research Facility, Frederick, Maryland

Hwang, L.-H.,¹ Chern, Y.-D.,² Gilboa, E.,² ¹Wistar Institute, Philadelphia, Pennsylvania; ²Dept. of Molecular Biology, Princeton University, New Jersey: Specific regions in the genome of Mo-MLV control the accumulation of the unspliced and spliced RNA form in the cytoplasm.

Armentano, D., Shimamura, A., Gilboa, E., Dept. of Molecular Biology, Princeton University, New Jersey: Three distinct regions in the genome of Mo-MLV are required for the packaging of the viral RNA into virions.

Scott, M., Varmus, H., Dept. of Microbiology and Immunology, University of California, San Francisco: Expression and transmission of foreign genes installed in retrovirus vectors.

Hughes, S.H.,¹ Ordahl, C.P.,² Crittenden, L.B.,³ ¹LBI-Basic Research Program, NCI-Frederick Cancer Research Facility, Frederick, Maryland; ²Dept. of Anatomy, University of California, San Francisco; ³USDA Regional Poultry Research Laboratory, East Lansing, Michigan: Introducing foreign promoters and enhancers into retrovirus vectors.

Embretson, J.E., Temin, H.M., McArdle Laboratory for Can-

cer Research, University of Wisconsin, Madison: Efficiency of maturation of pseudotypes of avian or murine recombinant retroviral genomes with REV-A or murine amphitropic virus proteins.

Miller, C.K., Temin, H.M., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Mechanism of suppression of REV-T transformation by helper virus sequences.

Gilboa, E.,¹ Kantoff, P.,² Yu, S.-F.,¹ Eglidis, M.,² von Ruden, T.,¹ Anderson, F.,² ¹Dept. of Molecular Biology, Princeton University, New Jersey; ²National Institutes of Health, Bethesda, Maryland: Use of retrovirus derived vectors in somatic gene therapy—The human adenine deaminase (ADA) deficiency system.

Keller, G.,¹ Gilboa, E.,² Wagner, E.,³ ¹Basel Institute for Immunology, Switzerland; ²Dept. of Molecular Biology, Princeton University, New Jersey; ³EMBL, Heidelberg, Federal Republic of Germany: Gene transfer to hematopoietic recursors of the mouse.

SESSION 13 ONCOGENESIS BY VIRUSES LACKING ONCOGENES

Chairpersons: P. Jolicoeur, Montreal Institute of Research Clinic, Montreal, Canada
P. Tschisli, Fox Chase Cancer Center, Philadelphia, Pennsylvania

Nusse, R., van Ooyen, A., Schuurig, E., Rijsewijk, F., Netherlands Cancer Institute, Amsterdam: Structural and biological properties of the *int-1* oncogene.

Brown, A.M.C., Fung, Y.-K.T., Wildin, B.S., Varmus, H.E., Dept. of Microbiology and Immunology, University of California, San Francisco: Synthesis in vitro of the protein product of the *int-1* gene.

Dickson, C., Casey, G., Brookes, S., Smith, R., Peters, G., Imperial Cancer Research Fund Laboratories, London, England: Isolation and sequence analysis of the human *int-2* locus.

Moore, R., Dixon, M., Peters, G., Dickson, C., Imperial Cancer Research Fund Laboratories, London, England: The sequence and structure of the MMTV *int-2* locus—Isolation of cDNA clones.

Escot-Thellett, C., Hogg, E., Callahan, R., NCI, National Institutes of Health, Bethesda, Maryland: Mammary tumorigenesis in leral *Mus cervicolor popaeus*—MMTV proviral insertion in common *int* loci.

Gallahan, D., Hogg, E., Kozak, C., Callahan, R., NCI, National Institutes of Health, Bethesda, Maryland: Analysis of

a new common insertion region (*int-3*) in MMTV (Czech II)-induced mammary tumors of *M. musculus musculus*.

Tschisli, P.N., Strauss, P.G., Lohse, M.A., Fox Chase Cancer Center, Philadelphia, Pennsylvania: Concerted DNA rearrangements in Mo-MLV-induced thymomas.

Villeneuve, L., Leganière, B., Rassart, E., Jolicoeur, P., Clinical Research Institute of Montreal, Canada: The frequency of *Mis-1* rearrangement in Mo-MLV-induced rat thymomas is host dependent.

Selten, G., Cuyper, H.T., Berns, A., Dept. of Biochemistry, University of Nijmegen, The Netherlands: Expression of the putative oncogene *Pim-1* is restricted to distinct hematopoietic cell lineages.

O'Donnell, P., Fleissner, E., Lonial, H., Koehne, C., Reicin, A., Woller, R., Memorial Sloan-Kettering Cancer Center, New York, New York: Multistage events in MCF virus-accelerated leukemia in AKR mice.

Mucenski, M.,¹ Taylor, B.,² Jenkins, N.,¹ Copeland, N.,¹ ¹Laboratory of Mammalian Genetics, NCI-Frederick Cancer Research Facility, Frederick, Maryland; ²Jackson Laboratory, Bar Harbor, Maine: AKXD recombinant inbred

mice—Molecular genetic approach for studying mouse lymphomas.
Simon, M.C.,^{1,3} Smith, R.E.,² Hayward, W.S.,¹ ¹Memorial Sloan-Kettering Cancer Center, New York, New York; ²Colorado State University, Fort Collins; ³Rocketfeller University, New York, New York: Pathogenicity of subgroup ALVs is associated with the 3' portion of the viral genome.

Shipp, M.,¹ Diamond, A.,² Billings, P.,³ Ritz, J.,¹ Cooper, G.,² ¹Division of Tumor Immunology, ²Molecular Carcinogenesis, Dana-Farber Cancer Institute and Harvard Medical School, Boston; ³Division of Radiobiology, Harvard School of Public Health, Boston, Massachusetts: Identification of the putative protein product of the human *Blym* transforming gene.

Molecular Biology of Yeast

August 13 – August 18

ARRANGED BY

James Hicks, Amar Klar, David Beach,
Mark Zoller, Michael Wigler, Cold Spring Harbor Laboratory

437 participants

Continuing the trend of the last three biennial yeast meetings at Cold Spring Harbor, the 1985 meeting brought more than 450 scientists to the laboratory for ten intensive sessions of oral presentations and three large poster sessions. As usual, much of the excitement centered on developments in the molecular genetics of gene regulation and expression, where the ease of nucleic acid manipulation has clearly made *Saccharomyces cerevisiae* the organism of choice. The detailed structure of several yeast promoters and their associated regulatory sites is rapidly being revealed. In some systems, both *cis*- and *trans*-acting elements for gene control have been identified.

This year, however, more attention than ever was paid to genetic and biochemical studies of cell structure and whole-cell processes, including protein localization, chromosome structure, and RNA processing and control of the cell cycle. Now that homologs for many so-called higher eukaryote functions have been found, yeast is rapidly becoming a fashionable subject for cell biology as well as genetics.

This meeting was funded in part by the Anheuser-Busch Companies, Gist-Brocades, Miller Brewing Company, Smith Kline & French Laboratories, and Zymo-Genetics, Inc.

SESSION 1 GENE REGULATION I-TY ELEMENTS AND CELL TYPE

Chairperson: A. Hinnebusch, National Institutes of Health

Boeke, J.D., Garfinkel, D.J., Styles, C.A., Fink, G.R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Reverse transcription and Ty transposition.

Gafner, J., Müller, F.T., Ciriacy, M., Institut für Mikrobiologie, Universität Dusseldorf, Federal Republic of Germany: A Ty element encodes both a δ -specific endonuclease and a reverse transcriptase.

Garfinkel, D.J., Boeke, J.D., Fink, G.R., Whitehead Institute for Biomedical Research, Massachusetts Institute of Tech-

nology, Cambridge: Ty element transposition and virus-like particles.

Tavianini, M.A., Worthington, M., Young, E.T., Dept. of Biochemistry, University of Washington, Seattle: Characterization of *cis*-acting control sequences in Ty1 elements.

Company, M., Yarnell, W., Connell, L., Errede, B., Dept. of Chemistry, University of North Carolina, Chapel Hill: A multicomponent structure for the Ty1 activator.

Winston, F., Minehart, P., Dept. of Genetics, Harvard Medi-

- cal School, Boston, Massachusetts: Analysis of *SPT3* and newly identified genes that affect Ty transcription.
- Margolskøe, J., Kassir, Y., Simchen, G., Dept. of Genetics, Hebrew University, Jerusalem, Israel: Positive regulation is involved in the genetic control of meiosis.
- Mitchell, A.P., Herskowitz, I., Dept. of Biochemistry and Biophysics, University of California, San Francisco: Isolation of the *RME1* gene, a gene that mediates mating-type control of sporulation.
- Marshall, M.,¹ Hicks, J.,² Broach, J.,¹ ¹Dept. of Molecular Biology, Princeton University, New Jersey; ²Cold Spring Harbor Laboratory, New York: Structural and genetic analysis of *SIR4*.
- Schnell, R., Kimmerly, W., Rine, J., Dept. of Biochemistry, University of California, Berkeley: Genetic and biochemical characterization of the regulation of the silent mating-type genes of *S. cerevisiae*.



M. Rolfe, D. Beach

SESSION 2 RNA PROCESSING

Chairperson: C. Guthrie, University of California, San Francisco

- Rymond, B.C., Jacquier, A., Rosbash, M., Dept. of Biology, Brandeis University, Waltham, Massachusetts: A scanning model for lariat formation of the RP51 intron.
- Fouser, L., Friesen, J.D., Dept. of Medical Genetics, University of Toronto, Canada: Mutations in the *S. cerevisiae* actin intron define nucleotides required for the two stages in mRNA splicing.
- Patterson, B., Guthrie, C., Dept. of Biochemistry and Biophysics, University of California, San Francisco: Analysis of an essential snRNA in yeast.
- Zagorski, J.,¹ Thompson, J.R.,¹ Woolford, J.L.,² Fournier, M.J.,¹ ¹Dept. of Biochemistry, University of Massachusetts, Amherst; ²Dept. of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania: Characterization of the small nuclear RNA genes of yeast—Structure and demonstration of nonessentiality of a gene for a 189-base snRNA.
- Atkinson, N.S., Dunst, R.W., Traglia, H.M., Hopper, A.K., Dept. of Biological Chemistry, M.S. Hershey Medical Center, Pennsylvania: *RNA1*—Characterization of the gene, its product, and a new allele with a novel phenotype.
- Phizicky, E., Clark, M., Westaway, S., Abelson, J., Division of Biology, California Institute of Technology, Pasadena: *S. cerevisiae* tRNA ligase—Isolation of the structural gene and intracellular localization of the protein.
- Greer, C.L., Dept. of Biological Chemistry, University of California College of Medicine, Irvine: Assembly of a tRNA splicing complex in vitro.
- Nichols, M., Willis, I., Söll, D., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: In vitro generated mutations in *Schizosaccharomyces pombe* UGA suppressor serine tRNA genes—Studies of transcription, processing, and suppression.
- Cumsky, M.G., Trueblood, C.E., Poyton, R.O., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: RNA processing regulates expression of the *COX5b* gene product in yeast.
- Peebles, C.L.,¹ Mecklenburg, K.L.,² Perlman, P.S.,² Petrillo, M.L.,¹ Dietrich, R.C.,² Romiti, L.,¹ ¹Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania; ²Dept. of Genetics, Ohio State University, Columbus: *oxi3* intron 7 represents a new type of self-splicing intron.

SESSION 3 POSTER SESSION I

Ty Elements

- Stolz, A., Pridmore, D., Fleig, U., Jäger, D., Philippsen, P., Dept. of Microbiology, Biozentrum, University of Basel, Switzerland: Ty transposition mechanism.
- Schirmaier, F., Philippsen, P., Dept. of Microbiology, Biozentrum, University of Basel, Switzerland: Site-directed mutagenesis of the yeast translation elongation factor EF1 α .
- Jäger, D., Philippsen, P., Dept. of Microbiology, Biozentrum, University of Basel, Switzerland: Length polymorphism in chromosomal DNA from *Saccharomyces* strains.
- Yu, K., Elder, R.T., Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois: The *Ty-D15* element contains an internal transcriptional activating sequence that has properties significantly different from those of an enhancer sequence.
- Heidler, S., Liebman, S.W., Dept. of Biological Sciences, University of Illinois, Chicago: Ty transposition into the *CAN1* gene.
- Fulton, A., Malim, M., Mellor, J., Wilson, W., Kingsman, A., Kingsman, S., Dept. of Biochemistry, University of Oxford, England: Variants of the yeast transposable element, Ty.
- Liao, X., Clare, J., Farabaugh, P., Dept. of Microbiology, University of Connecticut Health Center, Farmington: Deletion analysis of a Ty promoter.
- Kapakos, J., Farabaugh, P., Dept. of Microbiology, Univer-

- sity of Connecticut Health Center, Farmington: Comparison of the mating-type regulation of Ty917 transcription to adjacent transcription.
- Errede, B.,¹ Hutchison, C.A. III,² Depts. of ¹Chemistry, ²Microbiology and Immunology, University of North Carolina, Chapel Hill: Replacement of a Ty1 activator region with a synthetic regulatory element.
- RNA Processing and Translation**
- Strobel, M.C.,^{1,2} Abelson, J.,² ¹Dept. of Biology, University of California, San Diego; ²Division of Biology, California Institute of Technology, Pasadena: Effect of intron mutations on splicing of *S. cerevisiae* SUP53 pre-tRNA.
- Käuler, N.F.,¹ Simonis, V.,² Nurse, P.,² ¹Institut für Biochemie und Molekularbiologie, Freie Universität Berlin, Federal Republic of Germany; ²Imperial Cancer Research Fund Laboratories, London, England: Splicing of foreign introns in fission yeast.
- Last, R.L., Woodford, J.L., Carnegie-Mellon University, Pittsburgh, Pennsylvania: Evidence for related function of the *RNA2*, *RNA3*, and *RNA4* genes of *S. cerevisiae*.
- Nagasu, T.,¹ McConaughy, B.L.,² Hall, B.D.,² ¹Eisai Co., Ltd., Tsukuba Research Laboratories, Japan; ²Dept. of Genetics, University of Washington, Seattle: Splicing of a neurospora intron by *Schizosaccharomyces pombe*.
- Jacquier, A., Rosbash, M., Dept. of Biology, Brandeis University, Waltham, Massachusetts: Genetic analysis of cis-acting elements involved in splicing of the RP51 intron.
- Knapp, G., Lee, M.-C., Solvason, H.B., Dept. of Microbiology, University of Alabama, Birmingham: Yeast tRNA splicing requires that the 3' splice junction be single-stranded.
- Jackson, S.P., Beggs, J.D., Dept. of Molecular Biology, University of Edinburgh, Scotland: Molecular cloning of *S. cerevisiae* genes implicated in RNA splicing.
- Dihanich, M.,¹ Najarian, D.,² Gillman, E.,¹ Martin, N.C.,² Hopper, A.K.,¹ ¹Dept. of Biological Chemistry, M.S. Hershey Medical Center, Hershey, Pennsylvania; ²Dept. of Biochemistry, University of Texas Health Science Center, Dallas: *mod5*, a gene involved in modification of both cytoplasmic and mitochondrial tRNAs.
- Fabian, G.R., Hopper, A.K., M.S. Hershey Medical Center, Hershey, Pennsylvania: Molecular and genetic analysis of *rpt1* – A mutation affecting ribosomal RNA processing.
- Couto, J.R., Tamm, J.A., Parker, R., Guthrie, C., Dept. of Biochemistry and Biophysics, University of California, San Francisco: Suppressors that restore RNA splicing.
- Cigan, M., Donahue, T.F., Dept. of Molecular Biology, Northwestern University Medical School, Chicago, Illinois: Translation initiation – Mutational analysis of tRNA^{Met}.
- Byström, A.S., Fink, G.R., Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: Cloning of the methionine initiator tRNA genes in yeast.
- Wilson, P.G., Culbertson, M.R., University of Wisconsin, Madison: Analysis of the *sufl2* gene and gene product.
- Winey, M., Mendenhall, M., Culbertson, M., University of Wisconsin, Madison: Intron splicing of frameshift suppressor pre-tRNA.
- Ranu, R.S., Dept. of Microbiology, Colorado State University, Fort Collins: Protein kinases and the regulation of protein synthesis in *S. cerevisiae*.
- Gill, G.,¹ Zaworski, P.,¹ Sharma, S.,¹ Adams, L.,¹ Murakami, K.,² ¹Research Laboratories, Upjohn Company, Kalamazoo, Michigan; ²Tsukuba University, Japan: Recognition of an *S. cerevisiae* protein by an antibody to human renin.
- Maicas, E., Pluthero, F.G., Friesen, J.D., Dept. of Medical Genetics, University of Toronto, Canada: Posttranscriptional control of the synthesis of ribosomal protein L3 in *S. cerevisiae*.
- Chen, J.-Y., Bodley, J.W., Livingston, D.M., Dept. of Biochemistry, University of Minnesota, Minneapolis: Yeast mutants resistant to the in vitro action of diphtheria toxin.
- Cottrelle, P., Buhler, J.-M., Cool, M., Thiele, D., Huet, J., Sentenac, A., Service de Biochimie du Département de Biologie, Cen Saclay, Gil-sur-Yvette, France: Expression of the TEF1 and TEF2 genes encoding yeast elongation factor EF-1 α .
- Warner, J.R., Elion, E.A., Post-Beitlenmiller, M., Dept. of Cell Biology, Albert Einstein College of Medicine, Bronx, New York: A run-on transcription system for yeast.
- Kruse, C., Johnson, S.P., Warner, J.R., Dept. of Cell Biology, Albert Einstein College of Medicine, Bronx, New York: Phosphorylation of the yeast equivalent of ribosomal protein S6 is not essential for growth.
- Hussain, I., Leibowitz, M.J., Dept. of Microbiology, UMDNJ-Rutgers Medical School, Piscataway, New Jersey: Translation of homologous and heterologous mRNAs in a yeast cell-free system.
- McCusker, J.H., Haber, J.E., Rosenstiel Center, Brandeis University, Waltham, Massachusetts: Characterization of cycloheximide-resistant, temperature-sensitive lethal (*crt1*) mutations.
- Friden, P., Schimmel, P., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Yeast leucyl-tRNA synthetase – Evidence for isolation of mutants and cloning of the gene.
- Liebman, S.W., Robyn, J.A., Griffin, E.W., Song, J.M., Dept. of Biological Sciences, University of Illinois, Chicago: New omnipotent suppressors and allosuppressors.
- Tricks of the Trade**
- Tschopp, J.F., Brust, P.F., Cregg, J.M., Stillman, C.A., Gingers, T.R., Salk Institute Biotechnology/Industrial Associates, Inc., La Jolla, California: Two methanol-regulated promoters used in the construction of yeast expression vectors – Studies on the mechanism of regulation and comparative promoter strengths in *Pichia pastoris* and *S. cerevisiae*.
- Klein, H., Dept. of Biochemistry, New York University Medical Center, New York, New York: Separation of whole yeast chromosomes – Altered mobility in transformed strains.
- Hadfield, C., Cashmore, A., Meacock, P., Leicester Biocentre, University of Leicester, England: A shuttle chloramphenicol-resistance marker for the efficient transformation of haploid and polyploid strains of *S. cerevisiae* and *E. coli*.
- Hadfield, C., Cashmore, A., Meacock, P., Leicester Biocentre, University of Leicester, England: The construction of APT gene cartridges and their use in *E. coli* – yeast shuttle resistance determinants against aminoglycoside antibiotics.

Melnick, L., Sherman, F., University of Rochester, New York: Gene cluster associated with the *CYC1* and *CYC7* genes.
Wakem, P., Sharma, G., Sherman, F., University of Rochester, New York: A new method for chromosomal assignment of mutations in yeast.
Chisholm, V.T., Kline, C.J., Willis, I., Burkard, U., Nichols, M., Söll, D., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Classical genetics with a molecular twist: A novel approach to the isolation of genes associated with tRNA metabolism.

Expression of Foreign Genes

Hitzeman, R., Chen, C., Dept. of Cell Genetics, Genentech, Inc., South San Francisco, California: Human, yeast, and hybrid 3-phosphoglycerate kinase gene expression in yeast.
Davidow, L.S., Apostolakis, D., Stasko, I., DeZeeuw, J.R., Dept. of Molecular Genetics, Pfizer Central Research, Groton, Connecticut: Cloning genes by complementation form an integrating vector library in the yeast *Y. lipolytica*.
Wright, C., Thiele, D., Walling, M.J., Hamer, D., NCI, National Institutes of Health, Bethesda, Maryland: Structure and function of yeast copperthionein.
Schenberg-Frascino, A.C.,¹ Filho, S.A.,² Galembeck, E.V.,¹ Faria, J.B.,¹ ¹Chemistry Institute, Universidade de São Paulo, Spain; ²Biological Sciences Institute, Universidade de Brasília, Brazil: Stable yeast transformants secreting mouse pancreatic α -amylase.
Meyhack, B., Märki, W., Heim, J., Hinnen, A., Ciba-Geigy AG, Biotechnology, Basel, Switzerland: High-level expression of a proteinase inhibitor in yeast.
Kurtz, M.B., Cortelyou, M.W., Kirsch, D.R., Dept. of Cellular Biology, Squibb Institute for Medical Research, Princeton, New Jersey: Integrative transformation of *Candida albicans* using a cloned *Candida ade2* gene.
Ying, T., Kuang, D., Shanghai Institute of Cell Biology, China: Expression of β -hCG (β -subunit of human chorionic gonadotropin) gene in yeast and secretion of the gene product.
Egan, K.M., Koski, R.A., Jones, M., Fieschko, J.C., Bitter, G.A., Amgen, Thousand Oaks, California: Regulated expression of human immune interferon in *S. cerevisiae*.
Kim, S., Mellor, J., Dobson, M., James, P., Kingsman, A., Kingsman, S., Dept. of Biochemistry, University of Oxford, England: Structural analysis of the *TRP1* promoter directing human interferon α -2 gene expression on a high-copy-number plasmid.
Itaya, M., Hinnebusch, A.G., Crouch, R.J., NICHD, National Institutes of Health, Bethesda, Maryland: Cloning of a yeast ribonuclease H gene by complementing an *E. coli rnh* deficiency.
Hsiao, C.-L., Mason, B.B., Dheer, S.K., Hung, P.P., Davis, A.R., Microbiology Division, Wyeth Laboratories, Inc., Philadelphia, Pennsylvania: Expression of rotavirus glycoprotein VP7 in yeast.

Growth Control and Cell Cycle, Part I

Thill, G.,¹ Barber J.,² Deschamps, J.,² Lair, S.,¹ Verma, I.,² ¹Salk Institute Biotechnology/Industrial Associates, Inc., San Diego; ²Molecular Biology and Virology Laboratory,

Salk Institute for Biological Studies, La Jolla, California: Expression of fos proteins in *S. cerevisiae*.
Schmitt, H.D., Molenaar, C., Rauh, A., Wagner, P., Gallwitz, D., Physiologisch-Chemisches Institut, Universität Marburg, Federal Republic of Germany: The *ras*-related *YP2* gene of *S. cerevisiae*—Characterization of insertion and point mutations.
Hanes, S.D., Shank, P.R., Bostian, K.A., Division of Biology and Medicine, Brown University, Providence, Rhode Island: Characterization of a potential yeast homolog of the simian sarcoma virus oncogene *v-sis*.

DNA Replication and Chromosome Structure, Part I

Fournier, P.,¹ Gaillardin, C.,¹ Persuy, M.-A.,¹ Klootwijk, J.,² van Heerikhuizen, H.,² ¹Laboratoire Génétique, Institut National Agronomique, Paris, France; ²Biochemisch Laboratory, Vrije Universiteit, Amsterdam, The Netherlands: Heterogeneity in the ribosomal DNA family of the yeast *Yarrowia lipolytica*—Genomic organization and segregation studies.
Klootwijk, J.,¹ van Heerikhuizen, H.,¹ Ykema, A.,¹ Gaillardin, C.,² Ballas, C.,² Fournier, P.,² ¹Biochemisch Laboratory, Vrije Universiteit, Amsterdam, The Netherlands; ²Laboratoire Génétique, Institut National Agronomique, Paris, France: Heterogeneity in the ribosomal DNA family of the yeast *Yarrowia lipolytica*—Molecular cloning and analysis of two different repeated units.
Wu, L.-C.C.,¹ Broach, J.R.,² Fisher, P.A.,¹ ¹Dept. of Pharmacological Sciences, State University of New York, Stony Brook; ²Dept. of Molecular Biology, Princeton University, New Jersey: Identification and characterization of the yeast nuclear matrix.
Conrad, M.N., Zakian, V.A., Fred Hutchinson Cancer Research Center, Seattle, Washington: Plasmid interactions with the yeast nuclear matrix.
Sweeney, R., Zakian, V., Fred Hutchinson Cancer Research Center, Seattle, Washington: The nibbled phenotype and 2-micron DNA.
Conrad, M.N., Hager, L.J., Zakian, V.A., Fred Hutchinson Cancer Research Center, Seattle, Washington: ARS behavior on plasmids and chromosomes.
Peterson, B.C., Pausch, M.H., Dumas, L.B., Dept. of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, Illinois: Purification and analysis of the yeast DNA polymerase-DNA primase complex using monoclonal antibodies.
Pocklington, M.J., Orr, E., Dept. of Genetics, University of Leicester, England: Genes that determine resistance to novobiocin in *S. cerevisiae*.
Goetsch, L., Byers, B., Dept. of Genetics, University of Washington, Seattle: Mutational analysis of a major chromosomal protein abundant in meiotic yeast.
Gibson, S., Surosky, R., Tye, B.-K., Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: A minichromosome maintenance mutant with a possible defect in initiation of DNA replication.
Giroux, C.N.,¹ Tiano, H.F.,¹ Dresser, M.,² Moses, M.,² ¹National Institute of Environmental Health Sciences, Research Triangle Park; ²Dept. of Anatomy, Duke University Medical School, Durham, North Carolina: Functional and structural analysis of meiotic chromosome metabolism



C. McGill, J. Strathern

during meiosis—*SPO11*.

Zealey, G.,¹ Wheals, A.,¹ Goodey, A.,² Carter, B.,²
¹Microbiology Group, School of Biological Sciences, University of Bath; ²G.D. Searle Ltd., High Wycombe, England: Studies on plasmid copy number in *S. cerevisiae*.

Norris, D.N., Herelford, L.M., Dana-Farber Cancer Institute, Boston, Massachusetts: Genetic analysis of histone function.

Thompson, A., Oliver, S.G., Dept. of Biochemistry and Applied Molecular Biology, UMIST, Manchester, England: DNA fragments with *ARS* activity in both *S. cerevisiae* and *Kluyveromyces fragilis*.

Palzkill, T.,^{1,2} Matz, K.,¹ Hardeman, K.,^{1,2} Kim, K.,¹ Newlon, C.S.,² ¹Dept. of Biology, University of Iowa, Iowa City; ²Dept. of Microbiology, UMDNJ-New Jersey Medical School, Newark: Autonomously replicating sequences from chromosome III.

Warrington, J.R.,¹ Anwar, R.,¹ Pizzigallo, P.,¹ Patel, N.,¹ Waring, R.B.,¹ Indge, K.J.,¹ Newlon, C.S.,² Oliver, S.G.,¹
¹Dept. of Biochemistry and Applied Molecular Biology, UMIST, Manchester, England; ²Dept. of Microbiology, UMDNJ-New Jersey Medical School, Newark: Nucleotide sequence of chromosome III from yeast.

Amin, A.A., Pearlman, R.E., Dept. of Biology, York University, Toronto, Canada: Nucleotide sequence requirements for *ARS* function of a class of non-yeast replication origins.

O'Rear, J., Rine, J., Dept. of Biochemistry, University of California, Berkeley: Precocious centromere division of a novel yeast chromosome.

Murray, J.A.H., Cesareni, G., European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: Effects of transcription in the ORI region of the stability of 2-micron plasmid derivatives.

Mittman, B.A., Smith, M.M., Dept. of Microbiology, University of Virginia, Charlottesville: Generation and analysis of histone H4 mutations in yeast.

Moore, C., Jones, C., Wall, L., University of Rochester School of Medicine, New York: Treated intact (whole) cells exhibit internucleosomal chromatin cleavage.

Mead, D.J., Gardner, D.C.J., Oliver, S.G., Dept. of Biochemistry and Applied Molecular Biology, UMIST Manchester, England: Profound differences in the stability of the yeast 2-micron plasmid between haploid and diploid cells.

Fragrelis, T.J., Strand, A.D., Livingston, D.M., Dept. of Biochemistry, University of Minnesota, Minneapolis: An analysis of the effect of 2-micron DNA gene products on DNAase-I-sensitive sites in plasmid chromatin.

Haggren, W., Burgum, A., Kolodrubetz, D., Dept. of Microbiology, University of Texas Health Science Center, San Antonio: Analysis of genes encoding the chromatin-associated HMG proteins of yeast.

Brierley, R.A.,^{1,2} Groppi, V.E.,³ Klein, R.D.,² Worcester Polytechnic Institute, ²Molecular Biology Research, ³Cell Biology Research, Upjohn Company, Kalamazoo, Michigan: Plasmid stability and segregation under various fermentation conditions.

Heyer, W.-D.,¹ Sipiczki, M.,² Kohli, J.,¹ ¹Institute of General Microbiology, University of Bern, Switzerland; ²Dept. of Genetics, University of Debrecen, Hungary: Replicating plasmids in *Schizosaccharomyces pombe*—Improvement of symmetric segregation by a new genetic element.

Harford, N., Gathoye, A.-M., Fiévet, F., De Wilde, M., Dept. of Molecular Genetics, Smith Kline-RIT, Rixensart, Belgium: Displacement of endogenous 2-micron circles by *LEU2*-based, moderate-copy-number vectors.

Horowitz, H., Haber, J.E., Rosenstiel Center, Brandeis University, Waltham, Massachusetts: The behavior of marked telomeres in *S. cerevisiae*.

Fournier, P.,¹ Heslot, H.,¹ Goursot, R.,² Dedieu, A.,² Ehrlich, S.D.,² ¹Laboratoire Génétique, Institut National Agronomique; ²CNRS, Institut J. Monod, Université Paris, France: Behavior of a *Staphylococcus aureus* plasmid in the yeast *Schizosaccharomyces pombe*.

Gaudet, A., McGrew, J., Fitzgerald-Hayes, M., Dept. of Biochemistry, University of Massachusetts, Amherst: Single-base-pair mutations in centromere element III can cause aberrant chromosome segregation.

Cramer, J.H.,¹ Bowen, B.,² Lea, K.,¹ Idler, K.,¹ Cullis, C.,²
¹Arigenetics Corp., Advanced Research Division, Madison, Wisconsin; ²John Innes Institute, Norwich, England: Characterization of a monocot chloroplast DNA restriction fragment displaying *ARS* activity in *S. cerevisiae*.

Crowley, J.C.,¹ Steensma, H.Y.,² Lamb, J.,¹ Koepf, L.,³ Kaback, D.B.,¹ ¹UMDNJ, New Jersey Medical School, Newark; ²Delft University of Technology, The Netherlands; ³Bloomfield College, New Jersey: The molecular organization of chromosome I.

Cregg, J.M., Barringer, K.J., Madden, K.R., Salk Institute Biotechnology/Industrial Associates, Inc., La Jolla, California: *Pichia pastoris* and *S. cerevisiae ARSs*—Heterologous host function analysis and DNA sequence comparison.

Genbautte, F., Cooper, T., University of Tennessee Center for the Health Sciences, Memphis: Sequences required for induction and repression of the urea amidolyase (*DUR12*) gene.

Gorman, J.A.,¹ Kollin, Y.,² Clark, P.,¹ ¹Dept. of Molecular Genetics, Smith Kline and French Laboratories, Philadelphia, Pennsylvania; ²Dept. of Microbiology, Tel-Aviv University, Israel: Isolation and characterization of genes involved in sorbitol utilization from *Candida albicans*.

Heinisch, J., Zimmermann, F.K., Institut für Mikrobiologie, Technische Hochschule Darmstadt, Federal Republic of Germany: Isolation of the genes coding for yeast phosphofructokinase and increasing the enzyme activity.

Janowicz, Z.A., Eckart, M., Roggenkamp, R., Hollenberg,

- C.P. Institut für Mikrobiologie, Universität Dusseldorf, Federal Republic of Germany: Characterization of genes involved in methanol utilization from *Hansenula polymorpha*.
- Trumbly, R.J., Wadsworth Center for Laboratories and Research, New York State Dept. of Health, Albany: Characterization of mutants resistant to catabolite repression of invertase.
- Williamson, V.M., Paquin, C., Walton, J., ARCO Plant Cell Research Institute, Dublin, California: A new yeast alcohol dehydrogenase—Increased expression due to Ty insertion or gene amplification.
- Wing, R.A., Ogrzydzki, D.M., Institute of Marine Resources, University of California, Davis: Cloning of the alkaline extracellular protease gene from *Yarrowia lipolytica*.
- Yoo, H.-S., Cooper, R.G., Dept. of Microbiology and Immunology, University of Tennessee Center for the Health Sciences, Memphis: A new allantoin degradative gene in the DAL gene cluster of chromosome IX.
- Chen, W., Struhl, K., Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Yeast transcriptional initiation sites are determined primarily by specific sequences, not by the distance from the TATA element.

SESSION 4 RECOMBINATION/DNA REPAIR

Chairperson: J. Strathern, NCI, Frederick Cancer Research Facility

- Strathern, J.,¹ McGill, C.,¹ Shafer, B.,¹ Klar, A.,² Hicks, J.,² Model, P.,³ Fulton, W. III,³ ¹NCI-Frederick Cancer Research Facility, Frederick, Maryland; ²Cold Spring Harbor Laboratory, New York; ³Rockefeller University, New York, New York: Site-specific gene conversion.
- Zoller, M.J., Kostriken, R.G., Johnson, K.E., Cold Spring Harbor Laboratory, New York: Point mutations in the yeast *MATa* gene that affect cleavage by HO endonuclease.
- Kolodkin, A.,¹ Klar, A.,² Stahl, F.,¹ ¹Institute of Molecular Biology, Eugene, Oregon; ²Cold Spring Harbor Laboratory, New York: Double-strand breaks and meiotic recombination.
- Klar, A., Cold Spring Harbor Laboratory, New York: Double-stranded DNA break, meiotic gene conversion, and chromosomally inherited developmental potential for fission yeast *MAT* switching.
- Rudin, N., Connolly, B., Klucznik, M., Haber, J., Rosenstiel Center, Brandeis University, Waltham, Massachusetts: Effect of interchromosomal mating-type switching in *S. cerevisiae*.
- Colleaux, L.,¹ d'Auriol, L.,² Demariaux, M.,¹ Dujon, B.,¹ Galibert, F.,² Jacquier, A.,¹ ¹Centre de Genetique moleculaire du CNRS, ²Laboratoire d'Hematologie experimen-
- tale, Centre Hayem, Hopital Saint Louis, Paris, France: Expression in *E. coli* of a universal code equivalent of a yeast mitochondrial intron reading frame involved in the integration of an intron within a gene.
- Zinn, A., Zhu, H., Butow, R., Macreadie, I., Dept. of Biochemistry, University of Texas Health Science Center, Dallas: A yeast mitochondrial intron encodes a protein required for transposition of that intron.
- Ronne, H., Røthstein, R., Dept. of Human Genetics and Development, Columbia University, New York, New York: Genetic evidence for heteroduplex DNA formation during mitotic recombination.
- Resnick, M.A.,^{1,2} Westmoreland, J.,¹ Bloom, K.,² ¹National Institute of Environmental Health Sciences, Research Triangle Park; ²University of North Carolina, Chapel Hill: DNA repair, damage, and chromosome stability in yeast.
- Elledge, S.J., Davis, R.W., Dept. of Biochemistry, Stanford University School of Medicine, California: The isolation of genes from yeast encoding proteins immunologically related to the RecA protein.
- Wagstaff, J., Gottlieb, S., Easton Eposito, R., University of Chicago, Illinois: *RAD50*-dependent and -independent pathways of meiotic intrachromosomal recombination.

SESSION 5 DNA/REPLICATION AND CHROMOSOME STRUCTURE

Chairperson: J. Broach, Princeton University

- Brill, S.J., Thrash, C., DiNardo, S., Voelkel-Meiman, K., Sternglanz, R., State University of New York, Stony Brook: Roles of yeast DNA topoisomerases I and II in DNA replication and transcription.
- Volkert, F.,¹ Armstrong, K.,¹ Som, T.,¹ Seale, R.,¹ Wu, L.,² Fisher, P.,² Broach, J.,¹ ¹Dept. of Molecular Biology, Princeton University, New Jersey; ²Dept. of Pharmacology, State University of New York, Stony Brook: Mechanism of stable propagation of yeast plasmid 2-micron circle.
- McCarroll, R.M., Fangman, W.L., Dept. of Genetics, University of Washington, Seattle: Replication timing during the S phase.
- Passmore, S., Chan, C., Tye, B.-K., Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Hierarchy of DNA replication origins in yeast.
- Zakian, V.A., Blanton, H.M., Wetzel, L., Dani, G.M., Fred Hutchinson Cancer Research Center, Seattle, Washington: Creation and maintenance of telocentric chromosomes.
- Walmsley, R.M., Dept. of Biochemistry and Applied Molecular Biology, UMIST, Manchester, England: Telomere length is affected by the number of telomere-associated sequences present in a strain.
- Hill, A., Bloom, K., Dept. of Biology, University of North Carolina, Chapel Hill: Genetic manipulation of yeast centromeres.
- Hegemann, J.H.,¹ Pridmore, D.,¹ Panzeri, L.,² Landonio,

L.,² Stotz, A.,¹ Huberman, J.,¹ Jäger, D.,¹ Zonneveld, B.,¹ Philippsen, P.,¹ ¹Dept. of Microbiology, Biozentrum, Basel, Switzerland; ²Instituto de Genetica, University di Milano, Italy: In-vitro-mutated yeast *CEN* DNA.

Kaback, D.B., Fajardo, J.E., UMDNJ Graduate School of Biomedical Sciences, New Jersey Medical School, Newark: Segregation of *CEN*-containing plasmids away from intact chromosomes during meiosis I in *S. cerevisiae*.

SESSION 6 POSTER SESSION II

DNA Replication and Chromosome Structure, Part II

Cashmore, A., Hadfield, C., Albury, M., Meacock, P., Leicester Biocentre, University of Leicester, England: The role of 2-micron-encoded functions in plasmid maintenance.

Brown, J.A., Smith, M.M., Dept. of Microbiology, University of Virginia, Charlottesville: Analysis of yeast H3 and H4 histone gene chromatin structure.

Lucchini, G., Brandazza, A., Foiati, M., Plevani, P., Badaracco, G., Dept. di Biologia, Università de Milano, Italy: Yeast DNA polymerase I gene—Cloning by immunoscreening.

Blowers, A., DiGate, R.J., Hinkle, D.C., Dept. of Biology, University of Rochester, New York: Purification, characterization, and cloning of yeast DNA polymerase I.

Araki, H., Jearnpipatkul, A., Kaisho, Y., Matsuzaki, H., Oshima, Y., Dept. of Fermentation Technology, Osaka University, Japan: Functional organization of yeast plasmid pSR1.

Jong, A.Y.-S., Campbell, J.L., Divisions of Biology and Chemistry, California Institute of Technology, Pasadena: Single-stranded DNA-binding proteins of yeast.

Hamatake, R.K., Sakai, A., Alexander, P.S., Desai, R., Sugino, A., NIEHS, National Institutes of Health, Research Triangle Park, North Carolina: Purification, characterization, and gene cloning of three different yeast single-stranded DNA-binding proteins.

Berman, J., Tye, B.-K., Dept. of Biochemistry, Molecular, and Cellular Biology, Cornell University, Ithaca, New York:

Campbell, J.L.,¹ Johnson, L.M.,¹ Jong, A.Y.S.,¹ Srienc, F.,¹ Sweder, K.,¹ Snyder, M.P.,² ¹Divisions of Biology and Chemistry, California Institute of Technology, Pasadena; ²Stanford University School of Medicine, California: Dissection of the yeast DNA replication apparatus.

Identification and characterization of a telomere-binding protein from *S. cerevisiae*.

Alonso, W., Nelson, D., Dept. of Biochemical and Biophysical Sciences, University of Houston, Texas: A novel histone deacetylase activity colocalized with yeast nuclei.

Snyder, M., Davis R.W., Dept. of Biochemistry, Stanford University School of Medicine, California: Molecular analysis of chromosome segregation in yeast.

Recombination/DNA Repair

Kunes, S., Bolstein, D., Fox, M., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: On a mechanism of double-strand-break repair that yields inverted repeats.

Muster-Nassal, C., Kolodner, R., Dana-Farber Cancer Institute, and Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Repair of mismatched nucleotides by cell-free extracts of *S. cerevisiae*.

Naumovski, L., Friedberg, E.C., Dept. of Pathology, Stanford University School of Medicine, California: Functional organization of the *RAD3* gene of *S. cerevisiae*.

Willis, K., Klein, H.L., Dept. of Biochemistry, New York University Medical Center, New York, New York: Intrachromosomal recombination events leading to increased expression of a selectable marker.

Vincent, A., Petes, T.D., Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois: The effect of mitotic gene conversion on the number of Ty elements in yeast.

Jinks-Robertson, S., Petes, T.D., Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois: Recombination between repeated genes in *S. cerevisiae*.

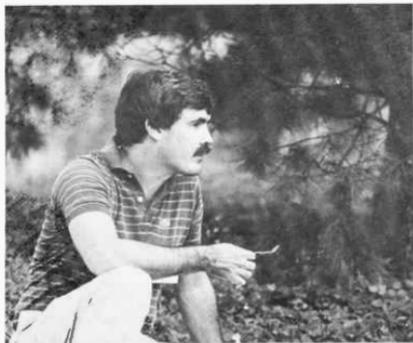
Wallis, J.W., Chretien, G., Benjaminovitz, A., Rothstein, R., Dept. of Human Genetics and Development, Columbia University College of Physicians & Surgeons, New York, New York: Control of *delta* recombination.

Keil, R.L., Voelkel-Meiman, K., Roeder, G.S., Dept. of Biology, Yale University, New Haven, Connecticut: A mitotic recombination-stimulating activity in the ribosomal DNA of yeast.

Sutton, P., Piloglou, S., Downs, K., Diczg, M., Kovarik, P., Leibman, S., Dept. of Biological Sciences, University of Illinois, Chicago: Ty-mediated rearrangements and the effect of *rad52*.

White, J.H., DiMartino, J.F., Fogel, S., Dept. of Genetics, University of California, Berkeley: Gene conversion and postmeiotic segregation patterns in graded deletion series at *ADE8*.

Blechl, A.E., Whittaker, S.G., Fogel, S., Dept. of Genetics, University of California, Berkeley: Characterization of the *arg4-8* allele.



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- Maloney, D.H., Fogel, S., Dept. of Genetics, University of California, Berkeley: Recombination and unequal crossing over at a non-tandem duplication of the *ade8* locus in yeast.
- Fasullo, M.T., Davis, R.W., Dept. of Biochemistry, Stanford University School of Medicine, California: Characterization of the *RAD52* dependence and the environmental induction of specific chromosome rearrangements in *S. cerevisiae*.
- Vollrath, D.,¹ Hieter, P.,² Davis, R.W.,¹ ¹Dept. of Biochemistry, Stanford University School of Medicine, California; ²Dept. of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, Maryland: Physical mapping of chromosomal sites using orthogonal field alternation gel electrophoresis and integrative recombination.
- Schmitt, E.S., Olson, M.V., Dept. of Genetics, Washington University School of Medicine, St. Louis, Missouri: The development of UGA suppressor genes for the study of intergenic conversion in *S. cerevisiae*.
- Rolle, M., National Institute for Medical Research, London, England: UV-inducible transcripts from *S. cerevisiae*.
- Chaudhury, A., Resnick, M.A., National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Mutants of *S. cerevisiae* elevated in sister chromatid exchange.
- Nitiss, J.L.,^{1,2} Resnick, M.A.,^{1,2} Edwards, C.,³ Malone, R.,³ ¹Illinois Institute of Technology, Chicago; ²National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina; ³Loyola Medical Center, Maywood, Illinois: Meiosis induced recombination in *rad52* and *rad57* mutants.
- Nitiss, J.L.,^{1,2} Resnick, M.A.,² ¹Illinois Institute of Technology, Chicago; ²National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: DNA strand breaks in *rad52* and *rad57* mutants during meiosis.
- Chow, T.Y.-K., Resnick, M.A., National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Identification and characterization of a *rad52*-controlled deoxyribonuclease in *S. cerevisiae*.
- Malone, R.E., Cramton, S., Dept. of Microbiology, Stritch School of Medicine, Loyola University, Chicago, Illinois: Gene conversion in the *HIS2* region of *S. cerevisiae*.
- Hoekstra, M.F., Malone, R.E., Dept. of Microbiology, Loyola University Medical School, Maywood, Illinois: Interactions between repair functions involved in mitotic recombination and the cloning of *REM1*.
- Lovett, S.T., Mortimer, R.K., Dept. of Biophysics, University of California, Berkeley: Characterization of the *RAD55* gene.
- Lichten, M., Borts, R.H., Haber, J.E., Rosenstiel Center, Brandeis University, Waltham, Massachusetts: Physical and genetic analysis of meiotic exchange between dispersed, homologous sequences.
- Larimer, F.W., Hardigree, A.A., Biology Division, Oak Ridge National Laboratory, Tennessee: Isolation and partial characterization of the *REV1* gene of *S. cerevisiae*.
- Jayaram, M., Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California: 2-micron circle – A model substrate for gene conversion.
- Bruschi, C.V.,^{1,2} Howe, G.A.,² ¹Dept. of Microbiology, School of Medicine, ²Dept. of Biology, East Carolina University, Greenville, North Carolina: Visual screen of in vivo phenotypic expression of 2-micron site-specific recombination in the yeast *S. cerevisiae*.
- Bishop, D.,^{1,2} Kolodner, R.,¹ ¹Dana Farber Cancer Institute, Boston; ²Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: Mitotic mismatch repair in vivo.
- Proteau, G., Gronostajski, R., Sadowski, P., Dept. of Medical Genetics, University of Toronto, Canada: Determination of the minimal DNA sequences required for site-specific recombination catalyzed by the FLP protein of the yeast 2-micron plasmid in vitro.
- Friesen, H., Sadowski, P.D., Dept. of Medical Genetics, University of Toronto, Canada: Mating-type switching in an *a/a* diploid.

Gene Regulation and Transcription, Part I

- Helser, T.L.,² Healy, A.M.,¹ Zitomer, R.S.,¹ ¹Dept. of Biological Sciences, State University of New York, Albany; ²Dept. of Chemistry, State University of New York College, Oneonta: Transcriptional start signals in the *CYC7* gene.
- Hahn, S., Hoar, E., Guarente, L., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Multiple TATA elements promote transcription from *CYC1* – Identification of a new element that determines the site of transcription initiation.
- Pinkham, J.L., Hahn, S., Guarente, L., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Cloning and molecular analysis of the *HAP2* and *HAP3* loci, global regulators of respiratory genes in *S. cerevisiae*.
- Spevak, W., Hartig, A., Bissinger, P., Schanz, M., Ruis, H., Institut für Allgemeine Biochemie der Universität Wien and Ludwig Boltzmann-Forschungsstelle für Biochemie, Vienna, Austria: Heme control regions of the catalase T (*CTT1*) gene of *S. cerevisiae*.
- Van Arsdell, S.W., Stetler, G.L., Thorne, J., Dept. of Microbiology and Immunology, University of California, Berkeley: Yeast transposable element, *sigma*, functions as a hormone-inducible promoter.
- Sandmeyer, S.B.,¹ Clark, D.J.,¹ Brodeur, G.M.,² ¹Dept. of Microbiology and Molecular Genetics, University of California, Irvine; ²Dept. of Pediatrics and Genetics, Washington University School of Medicine, St. Louis, Missouri: Characterization of the *sigma* family of transposable elements.
- Kurjan, J., Dept. of Biological Sciences, Columbia University, New York, New York: The *mta1::URA3A* mutation produces a hybrid gene product that is inhibitory to *a* mating.
- Klar, A.J.S., Kakar, S.N., Ivy, J.M., Hicks, J.R., Livi, G.P., Miglio, L.M., Cold Spring Harbor Laboratory, New York: *SUM1* – An apparent positive regulator of the cryptic mating-type loci in *S. cerevisiae*.
- Lin, C.-I.P., Ivy, J.M., Klar, A.J.S., Cold Spring Harbor Laboratory, New York: Isolation and analysis of suppressors of *mar2* mutations in *S. cerevisiae*.
- Jarvis, E.,¹ Hagen, D.,¹ Clark, K.,¹ Sprague, G.,¹ Chalfell, D.,² ¹Institute of Molecular Biology, University of Oregon, Eugene; ²E. I. du Pont Co., Wilmington, Delaware: Identification of an element upstream from the *a*-specific *STE3* gene of yeast that is sufficient for regulation by mating type.

- Fields, S., Herskowitz, I., Dept. of Biochemistry and Biophysics, University of California, San Francisco: Activators of cell-type-specific genes.
- Michaelis, S., Herskowitz, I., Dept. of Biochemistry and Biophysics, University of California, San Francisco: Genetic and molecular characterization of *t*-factor biosynthesis.
- Sternberg, P., Stern, M., Herskowitz, I., Dept. of Biochemistry and Biophysics, University of California, San Francisco: Mutations that allow *H*O expression in the absence of *swi* gene products.
- Holmberg, S.,¹ Petersen, J.G.L.,¹ Nilsson-Tillgren, T.,² Kieland-Brandt, M.C.,¹ ¹Dept. of Physiology, Carlsberg Laboratory; ²Institute of Genetics, University of Copenhagen, Denmark: Multivalent repression of the *ILV1* and *ILV5* genes of *S. cerevisiae* is caused by general control.
- Potier, S., Souciet, J.L., Hubert, J.C., Lacroute, F., Laboratoire de Génétique Physiologique, IMBC du CNRS, Strasbourg, France: Aspects of the *URA2* gene of *S. cerevisiae*.
- Craebel, M., Devos, K., Research Institute CERIA-VUB, Brussels, Belgium: Sequence similarities between the regulatory regions of *ARG3* and newly isolated *ARG1* genes.
- Scherens, B.,¹ Messenguy, F.,² ¹Dept. of Microbiologie, Vrije Universiteit Brussels; ²Institut de Recherches du CERIA, Brussels, Belgium: Nucleotide pools and amino acid pool compartmentation in mutants affected in the "general control" of amino acid biosynthesis.
- Verschueren, K.,¹ Tylzanowski, P.,² Messenguy, F.,² Dubois, E.,² ¹Dept. of Microbiology, Vrije Universiteit Brussel; ²Institut de Recherches du CERIA, Brussels, Belgium: Measurements of *ARG3*, *ARG5-6*, *CPA1*, and *CAR1* mRNAs isolated from polyomes in *S. cerevisiae*.
- Ramos, F.,¹ Dubois, E.,² Piérard, A.,^{1,2} ¹Laboratoire de Microbiologie, Université Libre de Bruxelles; ²Institut de Recherches du CERIA, Brussels, Belgium: Regulation of genes *LYS9* and *LYS14* required for the synthesis of saccharopine dehydrogenase (glutamate-forming) in *S. cerevisiae*.
- Verschueren, K.,¹ Tylzanowski, P.,² Messenguy, F.,² Glansdorff, N.,^{1,2} ¹Dept. of Microbiology, Vrije Universiteit Brussel; ²Institut de Recherches du CERIA, Brussels, Belgium: Nuclease hypersensitive sites in *ARG3* chromatin.
- Scherens, B.,¹ Messenguy, F.,² ¹Microbiologie, Vrije Universiteit Brussel; ²Institut de Recherches du CERIA, Brussels, Belgium: Nucleotide pools and amino acid pool compartmentation in mutants affected in the "general control" of amino acid biosynthesis.
- Dubois, E.,¹ Messenguy, F.,¹ Descamps, F.,² ¹Institut de Recherches du CERIA, ²Microbiologie, Université Libre de Bruxelles, Belgium: Sense and anti-sense RNAs could be necessary to produce the *argR1* regulatory molecule.
- Percival-Smith, A., Segall, J., Dept. of Biochemistry, University of Toronto, Canada: Mutation of *SPS2*, a gene preferentially expressed during sporulation in *S. cerevisiae*, results in an asporogenous phenotype.
- Greenberg, M.L.,¹ Skvirsky, R.C.,^{1,2} Myers, P.L.,¹ Landry, D.,¹ Greer, H.,¹ ¹Dept. of Cellular and Developmental Biology, Harvard University, Cambridge; ²Dept. of Biology, Simmons College, Boston, Massachusetts: A new negative regulatory gene (*GCD5*) and new positive regulatory genes (*GCN6*, 7, 8, and 9) involved in the general control of amino acid biosynthesis.
- Drain, P., Schimmel, P., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: A yeast locus that functions in leucine biosynthesis is required for growth on acetate, glycerol, or lactate as the sole carbon source.
- Beltzer, J.P., Chang, L.-F.L., Hinkkanen, A., Kohlhaas, G.B., Dept. of Biochemistry, Purdue University, West Lafayette, Indiana: The yeast *LEU4* gene—Structure, function, and regulation.
- Benjamin, P., Wu, J.L., Mitchell, A.P., Magasanik, B., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Regulation of glutamine synthetase in *S. cerevisiae* occurs at the transcriptional level.
- Braus, G., Furter, R., Prantl, F., Niederberger, P., Huetter, R., Mikrobiologisches Institut, Zürich, Switzerland: Expression of the *TAP1* gene of *S. cerevisiae*.
- Folch Mallol, J.L., González, A., Centro de Investigación sobre Fijación de Nitrógeno, Cuernavaca, Mexico: Genetic and phenotypic characterization of a mutant of *S. cerevisiae* that lacks glutamate synthase.
- Soberón, M., González, A., Control de Investigación sobre Fijación de Nitrógeno, Cuernavaca, Mexico: Glutamine assimilation in *S. cerevisiae*—Regulation of the enzymes of the ω -amidase pathway and of a glutaminase.
- Rodríguez, L., Lara, F., Soberón, M., González, A., Centro de Investigación sobre Fijación de Nitrógeno, Cuernavaca, Mexico: Nitrogen distribution in a glutamate auxotroph of *S. cerevisiae*.
- Jund, R., Weber, E., Chevallier, M.R., Laboratoire de Génétique Physiologique, CNRS, Strasbourg, France: DNA sequence of the uracil permease gene of *S. cerevisiae*.
- Falco, S.C., Dumas, K.S., Ward, R.T., Dept. of Central Research and Development, E.I. du Pont de Nemours and Co., Wilmington, Delaware: Regulation of the *S. cerevisiae* *ILV2* gene by the general amino acid control system.
- Furter, R., Prantl, F., Braus, G., Niederberger, P., Hütter, R., Mikrobiologisches Institut, ETH-Zentrum, Zürich, Switzerland: Structure, expression, and regulation of the *TAP4* gene of *S. cerevisiae*.
- Harashima, S., Hinnebusch, A.G., NICHD, National Institutes of Health, Bethesda, Maryland: Isolation and characterization of new *gcd⁻* mutations in the general amino acid control of *S. cerevisiae*.
- Keng, T., Alani, E., Guarente, L., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Targeting of *HEMI-lacZ* fusion proteins to the mitochondria.
- Kim, K.S.,¹ Rosenkrantz, M.S.,² Guarente, L.,² ¹Eugene Tech International, Allendale, New Jersey; ²Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Two genes encode citrate synthase in the yeast *S. cerevisiae*.
- Lee, G.S.-F., Ritzel, R.G., von Borstell, R.C. II, Savage, E.A., Bendiak, D.S., Tyler, J.M., Quah, S.-K., von Borstell, R.C., Dept. of Genetics, University of Alberta, Canada: Base-sequence alterations of mutants of *URA3⁺* in *S. cerevisiae*.
- Lowry, C.V., Lieber, R.H., Verbeck, J., Dept. of Biological Sciences, State University of New York, Albany: Regulation of anaerobic genes in *S. cerevisiae*.
- Teshiba, S., Braus, R., Furter, R., Niederberger, P., Hütter, R., Mikrobiologisches Institut, ETH-Zentrum, Zürich, Switzerland: Cloning and characterization of the *ARO3* gene of *S. cerevisiae*.
- Wang, S.-S., Brandriss, M.C., Dept. of Microbiology, UMDNJ-

New Jersey Medical School, Newark: Cloning and characterization of the *POT1* gene of *S. cerevisiae*.

Porter, S., Smith, M., Dept. of Biochemistry, University of British Columbia, Canada: Location of functional domains of the *MATa2* gene product.

Siliciano, P., Tatchell, K., Dept. of Biology, University of Pennsylvania, Philadelphia: The *MATa* regulatory region can put heterologous genes under mating type control.

Rosenberg, F., Kurtz, S., Lindquist, S., Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois: Gene expression during sporulation.

Wang, H.-T., Buckingham, L., Frackman, S., Jensen, L., Easton Esposito, R., Elder, R., University of Chicago, Illinois: *SPO13* encodes a developmentally regulated transcript.

Easton Esposito, R., Klapholz, S., Wagstaff, J.E., Di-Domenico, B., Frackman, S., Waddell, C.S., Jensen, L., Atecheson, C., Elder, R.T., University of Chicago, Illinois: The *SPO11* gene.

Elder, R., Malavasic, M., Frackman, S., Jensen, L., Esposito, R.E., Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois: The *spo12* region contains two sporulation genes and the transcripts from these two genes are complementary at their 3' ends.

Fassler, J.S., Winston, F., Dept. of Genetics, Harvard Medical School, Boston, Massachusetts: Phenotypes of *sp14* mutants include suppression of insertion mutations, γ -ray sensitivity, and cell lysis.

Growth Control and Cell Cycle, Part II

Nadin-Davis, S.A., Yang, R.C.A., Narang, S.A., Nasim, A., Molecular Genetics Section, Division of Biology, National Research Council, Ontario, Canada: Characterization of a *RAS* gene from the fission yeast *Schizosaccharomyces pombe*.

Levin, D.E., Hammond, C.I., Ralston, R., Bishop, J.M., G.W. Hooper Foundation, Dept. of Microbiology and Immunology, University of California, San Francisco: A yeast gene homologous to protein kinase oncogenes.

SESSION 7 GENE REGULATION II

Chairperson: F. Sherman, University of Rochester

Hampsey, D.M., Sherman, F., Dept. of Radiation Biology, and Biophysics and Biochemistry, University of Rochester, New York: A comparison of mutationally altered forms of yeast ISO-1-cytochrome c to the phylogenetic series of eukaryotic cytochromes c.

Wright, C.F., Zitomer, R.S., Dept. of Biological Sciences, State University of New York, Albany: Point mutations in the regulatory sequences of the *CYC7* gene.

Arcangioli, B., Lescure, B., Dept. of Molecular Biology, Institut Pasteur, Paris, France: Identification of proteins involved in the regulation of iso-1-cytochrome c expression by oxygen.

Hill, D.E.,^{1,2} Struhl, K.,¹ Dept. of Biological Chemistry, Harvard Medical School, Boston; ²Genetics Institute, Inc., Cambridge, Massachusetts: Regulation of the *HIS3* gene—Single-base-pair changes within a conserved regulatory element alter *HIS3* expression.



R. Yocum, J. Marmur

Fujiyama, A., Tamanoi, F., Dept. of Biochemistry and Molecular Biology, University of Chicago, Illinois: Biosynthesis of yeast *RAS* proteins.

Deschenes, R.,¹ Silberberg, S.,¹ Camerson, S.,² Wigler, M.,² Broach, J.,² ¹Dept. of Molecular Biology, Princeton University, New Jersey; ²Cold Spring Harbor Laboratory, New York: Genetic analysis of *RAS* function in yeast.

Cannon, J.,¹ Tatchell, K.,¹ Gibbs, J.,² Sigal, I.,² Scolnick, E.M.,² ¹Dept. of Biology, University of Pennsylvania, Philadelphia; ²Dept. of Virus and Cell Biology, Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania: Suppressors of the *RAS2* mutation.

Mattoon, J.,¹ Tatchell, K.,² ¹Dept. of Biology, University of Colorado, Colorado Springs; ²Dept. of Biology, University of Pennsylvania, Philadelphia: Alterations in biosynthesis of glycogen, porphyrins, and cytochromes associated with *ras* mutations and suppressors.

Castilho, B.A., Donahue, T.F., Dept. of Molecular Biology, Northwestern University Medical School, Chicago, Illinois: Translation initiation at the *HIS4* gene.

Roeder, G.S.,¹ Pearlman, R.E.,² Rose, A.B.,² ¹Dept. of Biology, Yale University, New Haven, Connecticut; ²Dept. of Biology, York University, Toronto, Canada: Control of *HIS4* expression by Ty elements.

Arndt, K.T., Fink, G.R., Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: *cis*- and *trans*-acting elements in *HIS4* regulation in yeast.

Hinnebusch, A.G., Mueller, P., NICHD, National Institutes of Health, Bethesda, Maryland: A hierarchy of factors modulate translation of a positive effector of amino acid biosynthetic genes in yeast.

Skvinsky, R.C.,^{1,2} Myers, P.L.,¹ Greenberg, M.L.,¹ Greer, H.,¹ ¹Dept. of Cellular and Developmental Biology, Harvard University, Cambridge; ²Dept. of Biology, Simons

College, Boston, Massachusetts: A negative regulatory gene for general control of amino acid biosynthesis. Messenguy, F., Dubois, E., Bercy, J., Institut de Recherches

du CERIA, Université Libre de Bruxelles, Belgium: Control of the *ARGRI* regulatory gene by the *ARGRIII* regulatory molecule.

SESSION 8 GROWTH CONTROL/CELL CYCLE

Chairperson: J. Hicks, Cold Spring Harbor Laboratory

Kataoka, T., Broek, D., Powers, S., Toda, T., Wigler, M., Cold Spring Harbor Laboratory, New York: Interaction of RAS proteins with yeast adenylate cyclase.

Robinson, L.C., Tatchell, K., Dept. of Biology, University of Pennsylvania, Philadelphia: The interaction of the cell-division cycle mutations *cdc25* and *cdc35* with *RAS2^{val19}* and the *RAS* suppressor *sra1*.

Toda, T., Kataoka, T., Sass, P., Cameron, S., Powers, S., Levin, L., Zoller, M., Wigler, M., Cold Spring Harbor Laboratory, New York: Structural and genetic analysis of protein kinase genes in yeast.

Patterson, M., Moore, D., Bahman, M., Rosamond, J., Dept. of Biochemistry, University of Manchester, England: *CDC7* is homologous to *CDC28* and oncogene protein kinases.

Mendenhall, M.D., Hadwiger, J.A., Horwitz, R., Reed, S.I., Dept. of Biological Sciences, University of California, Santa Barbara: Genetic analysis of the *CDC28* gene.

Russell, P., Nurse, P., Imperial Cancer Research Fund, Lon-

don, England: Mitotic control and mitotic catastrophe in fission yeast — *cdc25* and *nim1* encode mitotic inducers. Booher, R., Beach, D., Cold Spring Harbor Laboratory, New York: Site-specific mutagenesis of the *cdc2+* gene of the fission yeast.

Smith, M.M., Karns, L., Cross, S., Freeman, K., Dept. of Microbiology, University of Virginia Medical School, Charlottesville: Identification of DNA sequences regulating the cell-division cycle expression of histone H3- β -galactosidase gene fusions.

Bender, A., Hagen, D., McCaffrey, G., Sprague, G., Institute of Molecular Biology, University of Oregon, Eugene: Peptide pheromones α -factor and α -factor create a common signal in target cells.

Haarer, B., Lillie, S.H., Bloom, L., Coleman, K., Ketcham, S., Johnson, D., Pringle, J.R., Division of Biological Sciences, University of Michigan, Ann Arbor: Molecular studies of genes involved in cellular morphogenesis in *S. cerevisiae*.

SESSION 9 POSTER SESSION III

Growth Control and Cell Cycle, Part III

Olempska-Beer, Z., Freese, E., National Institutes of Health, Bethesda, Maryland: Intracellular concentrations of cAMP and cGMP during sporulation of various strains of *S. cerevisiae*.

Yeh, E., Schwartz, L., Bloom, K., Dept. of Biology, University of North Carolina, Chapel Hill: Functional analysis of a gene required for meiotic division in *S. cerevisiae*.

Pettitjean A.,¹ Vidal, M.,² Hilger, F.,² ¹Laboratoire de Chimie Biologique, Université libre de Bruxelles; ²Laboratoire de Microbiologie, Faculté des Sciences Agronomiques de Gembloux, Brussels, Belgium: The *S. cerevisiae cdc25* mutation alters an element of the cAMP pathway.

Hanic-Joyce, P.,¹ Johnston, G.C.,¹ Singer, R.A.,² Depts. of ¹Microbiology, ²Biochemistry, Dalhousie University, Halifax, Canada: Cloning a cell-cycle regulatory gene from the yeast *S. cerevisiae*.

Moore, S.A., Dept. of Chemistry and Biochemistry, University of Guelph, Canada, and Dept. of Chemistry, California State University, Fullerton: Evidence for a critical rate of protein synthesis prior to "start" in the *S. cerevisiae* cell cycle.

Wittenberg, C., Hadwiger, J.A., Jones, C., Lürincz, A., Mendenhall, M.D., Richardson, S., Reed, S.I., Dept. of Biological Sciences, University of California, Santa Barbara: Molecular analysis of the *CDC28* gene product — A gene controlling cell division in yeast.

Camonis, J., Boy-Marcotte, E., Gondre, B., Garreau, H., Ka-

lekine, M., Jacquet, M., Laboratoire d'Information Génétique et Développement, Université de Paris, Orsay, France: Cloning and sequence analysis of the *CDC25* gene which controls the cyclic AMP level of *S. cerevisiae*.

Martegani, E., Baroni, M., Frascotti, G., Vanoni, M., Alberghina, L., Dept. de Fisiologia e Biochimica Generali, Università di Milano, Italy: Characterization of the start mutant *CDC25* of budding yeast and identification of a cloned genomic sequence that complements the mutation.

Vienot-Drebot, L.,¹ Johnston, G.C.,¹ Singer, R.A.,² Depts. of ¹Microbiology, ²Biochemistry, Dalhousie University, Halifax, Canada: Macromolecular synthesis and cell-cycle regulation in yeast.

Singer, R.A.,¹ Johnston, G.C.,² Depts. of ¹Medicine and Biochemistry, ²Microbiology, Dalhousie University, Halifax, Canada: Regulation of mass accumulation in *Schizosaccharomyces pombe*.

Iida, H., Yahara, I., Tokyo Metropolitan Institute of Medical Science, Japan: Involvement of heat-shock proteins in regulation of cell proliferation in *S. cerevisiae*.

Hughes, D.A., Fantes, P.A., Dept. of Zoology, University of Edinburgh, Scotland: Cloning of the *cdc27* gene of *Schizosaccharomyces pombe*; a gene required for the initiation of mitosis.

Iino, Y.,¹ Watanabe, Y.,¹ Inoue, J.,¹ Shimoda, C.,² Yamamoto, M.,¹ ¹Institute of Medical Science, University of Tokyo; ²Dept. of Biology, Osaka City University, Japan: Analysis of genes involved in the initiation mechanism of meiosis in *Schizosaccharomyces pombe*.

- McLeod, M., Beach, D., Cold Spring Harbor Laboratory, New York: The *ran1+* gene of fission yeast encodes a product that is homologous to protein kinases.
- Transcription**
- West, R.W., Jr.,¹ Banerji, M.,¹ Yocum, R.R.,² Guarente, L.,³
¹Dept. of Biochemistry, State University of New York Upstate Medical Center, Syracuse; ²Biotechnica International, Cambridge; ³Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Synergy and distance effects control UAS function in yeast.
- Riva, M., Buhler, J.-M., Dassa, J., Huet, J., Mann, C., Mariotte, S., Memet, S., Micouin, J.-Y., Thuriaux, P., Treich, I., Sentenac, A., Service de Biochimie du Dept. de Biologie, Gif-sur-Yvette, France: Cloning of genes coding for the three yeast nuclear RNA polymerases.
- McNeil, J.B., Smith, M., Dept. of Biochemistry, University of British Columbia, Vancouver, Canada: Site selection for mRNA 5'-ends of the *S. cerevisiae* *CYC1*.
- Yarger, J.G., Armilei, G., Gorman, M.C., Biosynthesis Research, Miles Laboratories, Inc., Elkhart, Indiana: A yeast transcription terminator works efficiently in both orientations.
- Veit, B., Fangsman, W.L., Dept. of Genetics, University of Washington, Seattle: Regulation of 2-micron plasmid transcription by plasmid-encoded products.
- Slater, M.R., Stone, D.E., Boorstein, W.R., Craig, E.A., Dept. of Physiological Chemistry, University of Wisconsin, Madison: Regulation of yeast heat-shock promoters.
- Rotenberg, M., Eastman, C., Larkin, J., Anthony, J., Woolford, J., Dept. of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania: Regulation of expression of yeast ribosomal protein genes.
- Ruohola, H.,¹ Parker, R.,² Osborne, B.,³ Platt, T.,⁴
¹Dept. of Cell Biology, Yale University, New Haven, Connecticut; ²Dept. of Biochemistry and Biophysics, University of California, San Francisco; ³Dept. of Biology, Massachusetts Institute of Technology, Cambridge; ⁴Dept. of Biochemistry, University of Rochester Medical Center, New York: Orientation-dependent function of the 80-bp transcription terminator of *CYC1* inserted in the actin intron of yeast.
- Mellor, J., Kim, S., Dobson, M., Odgen, J., Roberts, N., Kingsman, A., Kingsman, S., Dept. of Biochemistry, University of Oxford, England: Identification of upstream and downstream activator sequences in the 3-phosphoglycerate kinase (*PGK*) gene in *S. cerevisiae*.
- Klekamp, M., Weil, T., Dept. of Biochemistry, University of Iowa Medical School, Iowa City: Purification and functional characteristics of the yeast class III gene transcription factor TFIIIB.
- Johnston, S.A., Dincer, S., Duke University, Durham, North Carolina: GAL4 protein has two separable domains for the activation of transcription.
- Himmelfarb, H.J., Friesen, J.D., Dept. of Medical Genetics, University of Toronto, Canada: Genetic and phenotypic analysis of the RNA polymerase II large subunit gene (*RPO21*).
- Elion, E.A., Warner, J.R., Dept. of Cell Biology, Albert Einstein College of Medicine, Bronx, New York: An enhancer element in rDNA.
- Di Mauro, E., Camilloni, G., Della Seta, F., Ficca, A.G., Negri, R., C.S. Dept. of Genetics and Molecular Biology, University of Rome, Italy: Upstream activator sequences as preferential interaction site with purified yeast RNA polymerase II.
- Allison, L.A., Moyle, M., Shales, M., Ingles, C.J., Banting and Best Dept. of Medical Research, University of Toronto, Canada: The largest subunits of yeast RNA polymerase II and III are homologous to the *E. coli* RNA polymerase subunit β' .
- Killer**
- Panchal, C.J., Stewart, G.G., Production Research Dept., Labatt Brewing Company Limited, London, Canada: Sensitivity of *S. cerevisiae* *sst1* and *sst2* mutants to *Kluyveromyces lactis* killer toxin.
- Fujimura, T., Wickner, R.B., NCI, National Institutes of Health, Bethesda, Maryland: Thermolabile RNA polymerase activity of L-A virus-like particles from *pet18* mutants of *S. cerevisiae*.
- Icho, T., Sommer, S.S., Lee, H.-S., Wickner, R.B., NIADDK, National Institutes of Health, Bethesda, Maryland: Cloning of chromosomal genes affecting killer double-stranded RNA replication.
- Hannig, E.M.,¹ Leibowitz, M.J.,²
¹NCI, National Institutes of Health, Bethesda, Maryland; ²Dept. of Microbiology, UMDNJ-Rutgers Medical School, Piscataway, New Jersey: Characterization of M_2 double-stranded RNA from the type-2 killer virus of *S. cerevisiae*.
- Williams, T.L., Hannig, E.M., Leibowitz, M.J., Dept. of Microbiology, UMDNJ-Rutgers Medical School, Piscataway, New Jersey: Variability in length of the internal polyadenylation tract of killer virus M, double-stranded RNA.
- Romanos, M., Boyd, A., Mileham, A., Stark, M., Leicester Biocentre, University of Leicester, England: The plasmid-coded killer phenotype of *Kluyveromyces lactis*—Analysis of gene function.
- Vernet, T.,¹ Boone, C.,² Greene, D.,² Bussey, H.,² Thomas, D.Y.,¹
¹National Research Council of Canada, Biotechnology Research Institute; ²Dept. of Biology, McGill University, Montreal, Canada: Location of the immunity domain of the yeast K1 preprotoxin.
- Mitochondria**
- Myers, A.M., Pape, L.K., Hill, J.E., Koerner, T.J., Crivellone, M., Gampel, A., Tzagoloff, A., Dept. of Biological Sciences, Columbia University, New York, New York: Isolation of nuclear genes required for mitochondrial protein synthesis in yeast.
- Nagley, P., Ooi, B.G., Novitski, C.E., Lukins, H.B., Linnane, A.W., Dept. of Biochemistry, Monash University, Clayton, Australia: Sequence changes in the yeast mitochondrial *OL11* gene conferring oligomycin resistance or functional deficiencies on subunit 9 of mitochondrial ATPase.
- Jarrell, K., Conrad, H., Perlman, P., Dept. of Genetics, Ohio State University, Columbus: Analysis of mutations mapping to intergenic regions of the mitochondrial genome.
- Devenish, R.J., Gearing, D.P., McMullen, G.L., Tymms, M.J.,

Nagley, P., Centre for Molecular Biology and Medicine, Monash University, Clayton, Australia: Chemical synthesis and properties of genes coding for yeast mitochondrial ATPase subunit 8 redesigned for expression outside mitochondria.

Fearon, K., Partaleidis, J.A., Sirum, K.L., Mason, T.L., Dept. of Biochemistry, University of Massachusetts, Amherst: Identification of nuclear genes for proteins of the mitochondrial ribosome.

Wright, R.M., Poyton, R.O., Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: Differential regulation of two nuclear genes for subunits of cytochrome c oxidase.

Poutre, C.G., Strick, C.A., Fox, T.D., Sections of Genetics and Development; Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: *PET111*, a nuclear gene required for the accumulation of subunit II of cytochrome c oxidase.

Séraphin, B., Simon, M., Faye, G., Institut Curie, Orsay, France: Processing of the *oxi3* transcript in yeast mitochondria.

Protein Traffic/Cell Surface

Stevens, T.H., Valls, L.A., Hunter, C.P., Rothman, J.H., Institute of Molecular Biology, University of Oregon, Eugene: Identification of the localization determinants on vacuolar proteins.

Keng, T., Guarente, L., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Regulation of *HEM1* gene expression.

Willsky, G.R., White, D.A., Dosch, S.F., Sigmund, R.D., Dept.

of Biochemistry, State University of New York, Buffalo: Vanadate metabolism and the plasma membrane Mg^{2+} -ATPase in *S. cerevisiae*.

Ohsumi, Y., Anraku, Y., Dept. of Biology, University of Tokyo, Japan: Specific induction of Ca^{2+} transport activity in *MATa* cells of *S. cerevisiae* by a mating pheromone, α -factor.

Gaber, R.F.,¹ Kielland-Brandt, M.C.,² Fink, G.R.,¹ ¹Whitehead Institute, Cambridge, Massachusetts; ²Carlsberg Laboratory, Copenhagen, Denmark: Yeast genes involved in the transport of cations across the cytoplasmic membrane.

Fernandez, S., Henry, S., Depts. of Genetics and Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Effects of inositol on phospholipid metabolism in *Schizosaccharomyces pombe*.

Henry, S., Hirsch, J., Hoshizaki, D., Johnson, M., Loewy, B., Depts. of Genetics and Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Regulation of phospholipid synthesis in yeast.

Kukuruzinska, M.A., Robbins, P.W., Massachusetts Institute of Technology, Cambridge: Studies of the lipid-linked oligosaccharide assembly in *S. cerevisiae*.

Ngsee, J.K., Smith, M., Dept. of Biochemistry, University of British Columbia, Canada: Cassette mutagenesis on the signal peptide of yeast invertase and bovine prolactin.

Ruohola, H.,¹ Yocum, R.,² Platt, T.,³ ¹Dept. of Cell Biology, Yale University, New Haven, Connecticut; ²BioTechnica International, Cambridge, Massachusetts; ³Dept. of Biochemistry, University of Rochester Medical Center, New York: Preliminary results of selections to obtain extragenic mutations that affect nuclear import of *GAL4*.

Sturley, S.L.,¹ Hanes, S.D.,¹ Burn, V.,¹ Tipper, D.J.,² Boston, K.A.,¹ ¹Section of Biochemistry, Division of Biology and Medicine, Brown University, Providence, Rhode Island; ²Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester: Factors influencing the maturation and secretion of yeast killer toxin.

Teem, J., Fink, G., Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: Temperature-sensitive mutations affecting the entry of ribosomal proteins to the nucleus.

Hishinuma, F., Nishizawa, R., Kitada, K., Mitsubishi-Kasei Institute of Life Sciences, Tokyo, Japan: Effects of glycosylation on secretion of proteins.

Etcheverry, T., Forrester, W., Hitzeman, R., Dept. of Cell Genetics, Genentech, Inc., South San Francisco, California: A human serum albumin-yeast chelatin fusion protein mediates copper resistance in yeast.

Bergh, M.L.E., Robbins, P.W., Massachusetts Institute of Technology, Cambridge: Cloning of a yeast gene conferring resistance to the glycosylation inhibitor 4-deoxy-4-fluoro-D-mannose.

Deeley, M.C., Price, V.L., Immunex Corporation, Seattle, Washington: Secretion of human and murine lymphokines from yeast.

Runge, K.,¹ Robbins, P.,² ¹Fred Hutchinson Cancer Research Center, Seattle, Washington; ²Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Early steps in protein glycosylation.

Meade, J.H., Wittman, V.P., Inlow, D., Flatgaard, J.E., Watt,



S. Johnson

- K.W.K., Chang, S., Cetus Corporation, Emeryville, California: Effects of leader sequence mutations on the posttranslational processing and secretion of an *Aspergillus* glucoamylase by yeast.
- Cheng, S.C., Matoba, S., Fukayama, J., Ogrzydzak, D.M., Institute of Marine Resources, University of California, Davis: Intracellular precursors of the ribonuclease and the alkaline extracellular protease of *Yarrowia lipolytica*.
- Kronstad, J., Holly, J., MacKay, V.L., ZymoGenetics, Inc., Seattle, Washington: Expression of barrier activity.
- Reipen, G., Roggenkamp, R., Bielefeld, M., Hollenberg, C.P., Institut für Mikrobiologie, Universität Düsseldorf, Federal Republic of Germany: Analysis of yeast transport and secretion signals by fusion to bacterial β -lactamase.
- Petko, L., Rossi, J., Susek, R., Lindquist, S., Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois: A mutational analysis of heat-shock genes in yeast.
- Ohya, Y.,¹ Uno, I.,² Ishikawa, T.,² Anraku, Y.,¹ ¹Dept. of Biology, ²Institute of Applied Microbiology, University of Tokyo, Japan: Purification and molecular properties of calmodulin in *S. cerevisiae*.
- Hodgson, J.A., De Zoysa, P., Johnston, J.R., Dept. of Bioscience and Biotechnology, University of Strathclyde, Glasgow, Scotland: Genetics and Biochemical phenotypes of flocculent strains of *S. cerevisiae*.
- Hanson, B.A., University of Kentucky School of Biological Sciences, Lexington: Characterization of the intracellular membranes in which the zymogen form of chitin synthetase is localized in yeast.
- Gene Regulation, Part II*
- Padmanabha, R., Glover, C., Dept. of Biochemistry, University of Georgia, Athens: Casein kinase II of *S. cerevisiae*—Subunit composition and immunological cross-reactivity.
- Golin, J.E., McDevitt, R.E., Van Dyk, T.K., Falco, S.C., Dept. of Central Research and Development, E.I. du Pont de Nemours and Co., Experimental Station, Wilmington, Delaware: Molecular cloning of a pleiotropic drug resistance locus of *S. cerevisiae*.
- Bulawa, C.E., Massachusetts Institute of Technology, Cambridge: Characterization of yeast mutants defective in chitin synthesis in vitro.
- Briza, P.,¹ Winkler, G.,³ Kalchauer, H.,² Breitenbach, M.,¹ ¹Institut für Biochemie und Ludwig Boltzmann-Forschungsstelle für Biochemie; ²Institut für Organische Chemie; ³Hygieneinstitut, Vienna, Austria: Dityrosine is a major and sporulation-specific component of the yeast ascospore wall.
- Dunn, T., Shortle, D., Johns Hopkins University School of Medicine, Baltimore, Maryland: Genetic analysis of actin expression and function.
- Griz, L., Abovich, N., Teem, J.L., Rosbash, M., Dept. of Biology, Brandeis University, Waltham, Massachusetts: Regulation of a yeast ribosomal protein- β -galactosidase fusion is coupled to ribosome assembly.
- Cohen, R., Yokoi, T., Holland, J., Brindle, P., Kim, D., Holland, M., Dept. of Biological Chemistry, University of California School of Medicine, Davis: Regulation of expression of the two yeast enolase genes.
- Tait-Kramrdt, A., Lemire, J., Koren, R., Bostian, K., Division of Biology and Medicine, Brown University, Providence, Rhode Island: Regulation of the *PHO5/PHO3* gene cluster.
- Koren, R., LeVitre, J., Litanova, O., Bostian, K., Division of Biology and Medicine, Brown University, Providence, Rhode Island: Characterization of the *PHO4* gene in yeast.
- Legrain, M.,¹ Martin, R.,¹ De Wilde, M.,² Hilger, F.,¹ ¹Faculté des Sciences Agronomiques, Gembloux; ²Dept. of Molecular Genetics, Smith-Kline RIT, Rixensart, Belgium: Characterization and DNA sequence analysis of the regulatory gene *PHO4*.
- Denis, C.L., Bemis, L.T., Chert, J.R., Stewart, G., Dollard, K., MacLachlan, A.L., Dept. of Biochemistry, University of New Hampshire, Durham: Characterization and function of the *ADP1* regulatory gene.
- Loon, A.V.,¹ Young, E.T.,² ¹Biozentrum, Basel, Switzerland; ²Dept. of Biochemistry, University of Washington, Seattle: Intracellular sorting of alcohol dehydrogenase isoenzymes in yeast.
- Taguchi, A., Young, E., University of Washington, Seattle: *ADR6*, a new positive regulatory element for yeast alcohol dehydrogenase II.
- Driscoll, R.M., Gesteland, R.F., Dept. of Biology, University of Utah, Salt Lake City: Assessment of roles of the two *gal4* proteins with respect to the known *gal4* functions.
- Liljeström, P., Ruohola, H., Torkkeli, T., Koppu, H., Lehtinen, P., Korhola, M., Finnish State Alcohol Company, Aldo Ltd., Helsinki, Finland: Structure and expression of yeast *MEL1* gene.
- Celenza, J.L., Carlson, M., Dept. of Human Genetics and Development, Columbia University College of Physicians & Surgeons, New York, New York: Characterization of the *SNF1* gene product—A protein required for glucose depression.
- Sarokin, L., Carlson, M., Dept. of Human Genetics and Development, Columbia University College of Physicians & Surgeons, New York, New York: Structure of the *SUC2* upstream regulatory region.
- Ma, H., Bostein, D., Dept. of Biology, Massachusetts Institutes of Technology, Cambridge: Genetic and physiological analysis of yeast hexokinases.
- Sidhu, R.S., Bollon, A.P., Dept. of Molecular Genetics, Wadley Institutes of Molecular Medicine, Dallas, Texas: Analysis of genes by fusion with acid phosphatase in yeast.
- Khan, N., Millington, D., Lee, E., D'Orazio, I., Stepyr, R., Dept. of Biology, Brooklyn College, New York: A novel mechanism for maltose utilization in yeast.
- Charron, M.J.,¹ Needleman, R.B.,² Michels, C.A.,¹ ¹Dept. of Biology, Queens College of the City University of New York, Flushing; ²Dept. of Biochemistry, Wayne State University College of Medicine, Detroit, Michigan: Genetic organization of the *MAL* family of loci.
- Hong, S.H.,¹ Chow, T.,¹ Jedge, M.V.,² Marmur, J.,¹ ¹Albert Einstein College of Medicine, Bronx, New York; ²University of Poona, India: Nucleotide sequence of maltase gene from the *MAL6* locus of yeast *S. carlsbergensis*.
- Modena, D.,¹ Vanoni, M.,^{1,2} England, S.,¹ Marmur, J.,¹ ¹Dept. of Biochemistry, Albert Einstein College of Medicine, Bronx, New York; ²Dept. of Biochemistry, Università di Milano, Italy: Biochemical and immunological properties of an external glucoamylase from *S. diastaticus*.
- Cohen, J.,¹ Gorman, J.,¹ Kollin, Y.,² De Wilde, M.,³ ¹Dept.

- of Molecular Genetics, Smith Kline and French Laboratories, Philadelphia, Pennsylvania; ²Dept. of Microbiology, Tel Aviv University, Israel; ³Dept. of Molecular Genetics, Smith Kline-RIT, Rixensart, Belgium: Molecular cloning of a glucosylase gene *Candida albicans*—Expression and secretion in *S. cerevisiae*.
- Erratt, J.A., Nasim, A., Division of Biological Sciences, National Research Council, Ottawa, Canada: Molecular cloning of glycoamylase from *S. diastaticus*.
- Taguchi, A., Young, E., University of Washington, Seattle: ADG6, a new positive regulatory element for yeast alcohol dehydrogenase II.
- Barker, D.G., Johnson, A.L., White, J.H.M., Johnston, L.H., MRC, National Institute for Medical Research, London,

- England: Structure and cell-cycle regulation of the DNA ligase genes of yeast.
- Seifert, H., Heffron, F., Dept. of Molecular Biology, Scripps Clinic and Research Foundation, La Jolla, California: Shuttle mutagenesis of yeast.
- Ruzzi, M., Breunig, K., Walker-Jonah, A., Das, S., Hollenberg, C.P., Institute für Mikrobiologie, Universität Düsseldorf, Federal Republic of Germany: Regulation of the β -galactosidase gene from *Kluyveromyces fragilis*.
- Overbeeke, N., Verriss, C.T., Unilever Research Laboratory, Vlaardingen, The Netherlands: Expression and stability of the gene encoding the sweet-tasting protein thaumatin in *S. cerevisiae* under various growth conditions.

SESSION 10 TRANSCRIPTION

Chairperson: L. Guarente, Massachusetts Institute of Technology

- Baker, R., Hall, B., Dept. of Genetics, University of Washington, Seattle: Effects of tRNA^{Trp} gene promoter mutations in transcription factor binding.
- Hope, I.A., Struhl, K., Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: General amino acid control—GCN4 protein binds specifically to the upstream region of *HIS3*.
- Lalonde, B., Prezant, T., Guarente, L., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Coordination in transcription of the *CYC1* and *CYC7* genes of *S. cerevisiae*.
- Oeller, P., Johnston, M., Dept. of Genetics, Washington University School of Medicine, St. Louis, Missouri: Unobstructed transcription from opposing yeast promoters.
- Keegan, L., Gill, G., Brent, R., Silver, P., Ptashne, M., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: The yeast GAL4 protein binds to upstream activation sequences by a small amino-terminal domain.
- McNeil, J.B., Smith, M., Dept. of Biochemistry, University of

- British Columbia, Canada: Functional and spatial relationship between TATA sequences and transcription start sites of the *S. cerevisiae* *CYC1* gene.
- Nam, H.G.,¹ Fried, H.M.,² Depts. of ¹Chemistry, ²Biochemistry, University of North Carolina, Chapel Hill: Sequences specifying the constitutive and temperature-sensitive transcription of ribosomal protein gene *TCM1*.
- Giniger, E., Lamphier, M., Ptashne, M., Dept. of Biochemistry and Molecular Biology, Harvard University, Boston, Massachusetts: Specific protein binding to the *GAL1* promoter.
- Brow, D.A., Geiduschek, E.P., Dept. of Biology, University of California, San Diego, La Jolla: Interaction of 5S ribosomal RNA with components of a yeast cell-free polymerase III transcription system.
- Struhl, K., Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Constitutive and regulatory promoters—Evidence for two distinct molecular mechanisms.

SESSION 11 EXTRACHROMOSOMAL ELEMENTS

Chairperson: R. Wickner, National Institutes of Health

- Leibowitz, M.J., Georgopoulos, D.E., Dept. of Microbiology, UMDNJ-Rutgers Medical School, Piscataway, New Jersey: Nucleotide metabolic activities associated with the killer virus of yeast.
- Esteban, R., Wickner, R.B., NIADDK, National Institutes of Health, Bethesda, Maryland: Two different virus-like particle types containing M, double-stranded RNA in *S. cerevisiae*—One may be involved in transcription, the other in replication.
- El-Sherbeini, M., Bostian, K.A., Division of Biology and Medicine, Brown University, Providence, Rhode Island: Infection of yeast protoplasts with the K1 and K2 dsRNA killer viruses.
- van Loon, A.P.G.M., Hurt, E., Biocenter, University of Basel,

- Switzerland: Cleavable presequences of imported mitochondrial precursor proteins contain information for intracellular targeting and for intramitochondrial sorting.
- Brandriss, M.C., Krzywicki, K.A., Dept. of Microbiology, UMDNJ-New Jersey Medical School of New Jersey, Newark: Targeting of two pyrroline carboxylate dehydrogenase- β -galactosidase hybrid proteins to different submitochondrial compartments in *S. cerevisiae*.
- Koerner, T.J., Tzagoloff, A., Dept. of Biological Sciences, Columbia University, New York, New York: Mitochondrial biogenesis—Nuclear mutants that affect cytochrome c oxidase assembly.
- Patterson, T.E., Wright, R.M., Power, S.D., Poyton, R.O., Dept. of Molecular, Cellular and Developmental Biology,

University of Colorado, Boulder: Comparison of the polyoxidase and nucleotide sequences of yeast cytochrome c oxidase subunits VIII and VIIa suggests carboxyterminal processing.

Anziano, P.O., Lanner-Herrera, C., Gerber, D., Perlman, P.S., Dept. of Genetics, Ohio State University, Columbus: Unstable revertants of mitochondrial maturaseless mutants—

Cells requiring two mutant mitochondrial genomes for respiratory growth.

Costanzo, M.C., Fox, T.D., Section of Genetics and Development, Cornell University, Ithaca, New York: The product of the nuclear gene *PET494* promotes translation of a specific mitochondrial mRNA.

SESSION 12 CELL STRUCTURE AND PROTEIN LOCALIZATION

Chairperson: I. Herskowitz, University of California, San Francisco

Rose, M., Fink, G.R., Whitehead Institute for Biomedical Research, Massachusetts Institute of Technology, Cambridge: Role of the *KAR1* gene in the cell-division cycle.

Baum, P., Diem, K., Yochem, J., Byers, B., Dept. of Genetics, University of Washington, Seattle: Genetic evidence for calcium regulation of spindle pole body duplication.

Davis, T.N.,¹ Urdea, M.S.,² Masiarz, F.R.,² Thorer, J.,¹ ¹Dept. of Microbiology and Immunology, University of California, Berkeley; ²Chiron Corporation, Emeryville, California: Isolation of yeast calmodulin and its structural gene.

Garlow, S.J., Hospodar, M.E., Jones, E.W., Dept. of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania: *pep7*—A gene essential for synthesis and processing of vacuolar hydrolases.

Bohni, P.C.,¹ Tekamp-Olson, P.,² Schauer, I.,¹ Schekman, R.,¹ ¹Dept. of Biochemistry, University of California, Berkeley; ²Chiron Corporation, Emeryville, California: Impaired proteolytic processing and secretion of invertase mutants carrying altered signal peptide cleavage sites.

Kaiser, C., Botstein, D., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Mutations in the signal sequence of invertase.

Bankaitis, V.A., Johnson, L.M., Emr, S.D., Division of Biology, California Institute of Technology, Pasadena: Genetic studies of the mechanism of vacuole assembly in yeast.

Stevens, T.H., Valls, L.A., Hunter, C.P., Rothman, J.H., Institute of Molecular Biology, University of Oregon, Eugene: Identification of the localization determinants on vacuolar proteins.

Elliott, S., Zsebo, K.M., Giffin, J.C., Koski, R., Jones, M., Bitter, G.A., Amgen, Thousand Oaks, California: Heterologous protein secretion from yeast directed by the prepro- α -factor leader region.

Fuller, R.S.,¹ Brake, A.J.,² Thorer, J.,¹ ¹Dept. of Microbiology and Immunology, University of California, Berkeley; ²Chiron Corporation, Emeryville, California: Yeast prepro- α -factor convertase (*KEX2* gene product)—A calcium-dependent, Lys-Arg-specific endopeptidase.

SESSION 13 GENE REGULATION III

Chairperson: A. Klar, Cold Spring Harbor Laboratory

Osley, M.A., Gould, J., Kim, S., Kane, M., Herelof, L., Dana-Farber Cancer Institute, Boston, Massachusetts: Negative and positive control sites in histone gene expression.

Michels, C.A.,¹ Dubin, R.A.,¹ Perkins, E.L.,² Needleman, R.B.,² ¹Dept. of Biology, Queens College of the City of New York, Flushing, New York; ²Dept. of Biochemistry, Wayne State University College of Medicine, Detroit, Michigan: Characterization of a second *trans*-acting regulatory element controlling maltose fermentation.

Chang, Y.S.,¹ Dubin, R.,² Perkins, E.,¹ Michels, C.,² Needleman, R.,¹ ¹Dept. of Biochemistry, Wayne State University School of Medicine, Detroit, Michigan; ²Dept. of Biology, Queens College, Flushing, New York: *MAL63* is a positive regulatory gene at the *MAL6* locus of *S. carlsbergensis*.

Pretorius, I.S.,¹ Chow, T.,² Modena, D.,² Marmur, J.,² ¹University of the Orange Free State, Bloemfontein, South Africa; ²Albert Einstein College of Medicine, Bronx, New York: Characterization of the glucoamylase genes of *S. diastolicus*.

Riley, M.,¹ Hopper, J.,² Johnston, S.,³ Dickson, R.,¹ ¹Dept. of Biochemistry, University of Kentucky, Lexington; ²Dept. of Biological Chemistry, Pennsylvania State University,

Hershey; ³Dept. of Botany, Duke University, Durham, North Carolina: Involvement of *GAL4* in glucose repression.

Carlson, M., Neigeborn, L., Sarkin, L., Dept. of Human Genetics and Development, Columbia University College of Physicians & Surgeons, New York, New York: Regulation of *SUC2* expression.

Taylor, W.,¹ Blumberg, H.,¹ Hartshorne, T.,¹ Yu, J.,¹ Irani, M.,¹ Chan, R.,² Smith, M.,² Young, E.,¹ ¹Dept. of Biochemistry, University of Washington, Seattle; ²Dept. of Biochemistry, University of British Columbia, Vancouver, Canada: Positive regulation of *ADH2* expression by *ADRI*.

Sumrada, R., Cooper, T., Dept. of Microbiology and Immunology, University of Tennessee Center for the Health Sciences, Memphis: Dual regulation of the arginase gene.

Holland, M., Yip, M., Holland, J., Dept. of Biological Chemistry, University of California School of Medicine, Davis: Regulation of ribosomal RNA synthesis in yeast.

Baker, S.M., Jerome, J.F., Karl, M.R., Jaehning, J.A., Dept. of Biochemistry, University of Illinois, Urbana: The effect of copy number and regulatory gene mutants on the transcription of the *GAL7* gene in vivo and in isolated nuclei.



Molecular Genetics of Bacteria and Phages

August 20 – August 25

ARRANGED BY

Sankar Adhya, National Cancer Institute

Pablo Scolnik, Cold Spring Harbor Laboratory

324 participants

The study of prokaryotes continues to be essential for our understanding of basic processes in molecular biology. The simplicity of the systems used and the knowledge accumulated during the years provide a solid base for new approaches such as the study of structure-function relationships by X-ray crystallography. To reflect the growth of the "non-phage" area of the meeting, the title has been changed from Bacteriophage to the present title. However, this meeting continues to be an almost unique opportunity for the interaction of scientists from different areas of prokaryotic molecular genetics. The meeting was divided into ten sessions covering different molecular processes and one general poster session.

SESSION 1 TRANSPOSONS

Bourret, R.B., Fox, M.S., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Lysogenization by bacteriophage Mu.

Groenen, M.A.M., van de Putte, P., Laboratory of Molecular Genetics, State University of Leiden. The Netherlands: Analysis of the attachment sites of bacteriophage Mu using site-directed mutagenesis.

Szatmari, G.B., Kahn, J.S., DuBow, M.S., Dept. of Microbiology and Immunology, McGill University, Montreal, Canada: Characterization of bacteriophage Mu and D108 insertion in pSC101.

Craigie, R., Mizuuchi, K., NIADDK, National Institutes of Health, Bethesda, Maryland: Protein and DNA substrate requirements for the DNA strand transfer step in transposition of bacteriophage Mu.

Miller, J.L., Chaconas, G., Depts. of Biochemistry and Microbiology, University of Western Ontario, London, Canada: Identification and characterization of *in vitro* transposition intermediates of Mu DNA by electron microscopy.

Chaconas, G., Giddens, E.B., Miller, J.L., Gloor, G., Depts. of Biochemistry and Microbiology, University of Western Ontario, Canada: A truncated form of the bacteriophage

Mu B protein promotes conservative integration but not replicative transposition of Mu DNA.

LaRossa, R.A., Central Research and Development Dept., E.I. du Pont de Nemours and Co., Wilmington, Delaware: Inhibition of transposase action by the repressor of bacteriophage Mu.

Craig, N.L., Arciszewska, L., McKown, R., Waddell, C., Dept. of Microbiology and Immunology and The Hooper Research Foundation, University of California, San Francisco: Transposition of Tn7.

De Long, A., Syvanen, M., Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massa-

chusetts: Cellular localization of Tn5 transposase and inhibitor proteins is differential.

Yin, J., Krebs, M., Reznikoff, W., Dept. of Biochemistry, University of Wisconsin, Madison: The role of the *dnaA* protein in Tn5 transposition.

Salvo, J.J., Grindley, N.D.F., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Resolvase protein bends *res*-site DNA.

Bhagwat, A.S., Cold Spring Harbor Laboratory, New York: Relative frequencies of intermolecular and intramolecular transposition for Tn3.

SESSION 2 PROMOTERS, ACTIVATORS, AND RNA POLYMERASES

Reznikoff, W.S., Peterson, M., Yu, X.-M., Dept. of Biochemistry, University of Wisconsin, Madison: RNA polymerase binding sites in the *lac* controlling elements.

Shanblatt, S.H., Revzin, A., Dept. of Biochemistry, Michigan State University, East Lansing: CAP and RNA polymerase contacts at the *E. coli* galactose promoter.

Hoopes, B.C., McClure, W.R., Dept. of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania: Comparison of the promoter strengths of the three *cil*-dependent promoters of bacteriophage λ .

Basu, S., Maitra, U., 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).

Garges, S., Adhya, S., NCI, National Institutes of Health, Bethesda, Maryland: Sites of allosteric shift in the structure of the cAMP receptor protein of *E. coli*.

McKenny, K., NCI, National Institutes of Health, Bethesda, Maryland: Relating structure to function for the cAMP receptor protein in *E. coli* by analysis of *crp* mutants.

Daniels, D.W., Bertrand, K.P., University of California, Irvine: Overlapping Tn10 *tet* promoters compete for RNA polymerase.

Hunt, T., Magasanik, B., Dept. of Biology, Massachusetts In-

stitute of Technology, Cambridge: *E. coli* nitrogen regulation—In vitro transcription of *glnA*.

Jin, D.J., Gross, C.A., Dept. of Bacteriology, University of Wisconsin, Madison: Search for structural and functional domains in the β subunit of *E. coli* RNA polymerase by studying rifampicin-resistant mutations. II. Sequencing of *rif^r* mutations.

Siegele, D.A., Hu, J.C., Gross, C.A., Dept. of Bacteriology, University of Wisconsin, Madison: Mutations of *rpoD* that change the promoter-recognition properties of *E. coli* RNA polymerase.

Tobe, T., Yano, R., Yura, T., Institute for Virus Research, Kyoto University, Japan: Extragenic suppressors of missense and nonsense *rpoH* (= *htpR*, *hin*) mutations in *E. coli*.

Glucksmann, S., Markiewicz, P., Rothman-Denes, L.B., Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois: DNA sequences and host factors required for N4 virion RNA polymerase transcription.

Abravaya, K., Zehring, W., Rothman-Denes, L.B., Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois: N4 RNA polymerase II activity—Structure and template requirements.

SESSION 3 REPRESSORS: PHAGE MORPHOGENESIS

Krause, H.M., Higgins, N.P., Dept. of Biochemistry, University of Alabama, Birmingham: Positive and negative regulation of the Mu operator by Mu repressor and *E. coli* integration host factor.

Polymeropoulos, M., Majumdar, A., Adhya, S., NCI, National Institutes of Health, Bethesda, Maryland: Mechanism of repression of *gal* operon.

Bushman, F.D., Anderson, J.E., Harrison, S.C., Ptashne, M., Harvard University, Cambridge, Massachusetts: Ethylation interference and x-ray crystallography suggest identical interactions between 434 repressor and the operator phosphate backbone.

Maloy, S., Szymski, G., Dept. of Microbiology, University of Illinois, Urbana: Autogenous regulation of the *put* operon—The *putA* protein is a DNA-binding protein in vitro.

Huo, L., Schleif, R., Dept. of Biochemistry, Brandeis University, Waltham, Massachusetts: In vivo experiments dem-

onstrating that AraC protein binds to the *araI* site during both inducing and repressing conditions.

Isackson, P.J., Bertrand, K.P., University of California, Irvine: DNA-binding mutations in the Tn10 *tet* repressor.

Sternberg, N., Couly, J.N., E.I. du Pont de Nemours and Company, Inc., Wilmington, Delaware: Cleavage of the bacteriophage P1 *pac* (packaging) site is regulated by DNA methylation.

King, J., Yu, M.-h., Haase, C., Siddiqui, J., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Temperature-sensitive folding mutations in gene 9 of P22.

Six, E.W.,¹ Sunshine, M.G.,¹ Lindqvist, B.H.,² ¹Dept. of Microbiology, University of Iowa, Iowa City; ²Institute of Medical Biology, University of Tromsø, Norway: Head-size control mutants of phage P2.

Drexler, H., Dept. of Microbiology and Immunology, Wake Forest University Medical Center, Winston-Salem, North

Carolina: T1*pip*—A mutant that affects both packaging initiation and processive packaging of T1 DNA.

Chandrasekhar, G.N.,¹ Georgopoulos, C.,¹ Woolford, C.,² Hendrix, R.,² Tilly, K.,³ ¹Dept. of Cellular, Viral, and Molecular Biology, University of Utah, Salt Lake City; ²Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania; ³Dept. of Physiological Chemistry, University of Wisconsin, Madison: Purification of the *E. coli* groES morphogenetic protein and evidence that it interacts with the groEL protein.

Bazinet, C., King, J., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Initiation complex for P22 procapsid assembly.

White, J.H., Richardson, C.C., Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Two phage proteins involved in processing of concatamers of bacteriophage T7—Gene 18 and 19 proteins.

Rennell, D., Potete, A.R., Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester: Genetic analysis of phage lysozyme structure.

SESSION 4 TRANSCRIPTION TERMINATION CONTROL

Friedman, D.I., Eades, L.J., Schauer, A.T., Carver, D.L., Granston, A.E., Dept. of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor: Studies on NusA protein and boxA.

Ghosh, B., Barik, S., Whalen, W., Warren, F., Wolska, K., Sparkowski, J., Das, A., Program in Molecular Biology and Biochemistry, University of Connecticut, Farmington: Elements of N-transcription antitermination system—Studies with cell-free extracts.

Warren, F.,¹ Lazinski, S.,² Das, A.,² ¹Dept. of Molecular Biology, Princeton University, New Jersey; ²Program in Molecular Biology and Biochemistry, University of Connecticut, Farmington: Analysis of a silent Rho-dependent transcriptional terminator located beyond the N cistron of phage lambda.

Yang, X., Goliger, J.G., Hart, C.M., Grayhack, E.J., Roberts, J.W., Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Function of an early pause site in antitermination by Q gene proteins of phages lambda and 82.

Goliger, J.G., Martin, C.S., Yang, X., Roberts, J.W., Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Mapping a recognition site of the late gene regulator of phage 82.

Eller, R.,^{1,3} Edlind, T.,¹ Ihler, G.,¹ Benedik, M.,² Depts. of ¹Medical Biochemistry, ²Biology, ³Biochemistry and Biophysics, Texas A&M University, College Station: Termination of transcripts mediated by gpQ product of bacteriophage lambda.

Hasan, N., Szybalski, W., McArdle Laboratory for Cancer

Research, University of Wisconsin, Madison: Analysis of the coliphage lambda *nutL* antitermination module.

Somasekhar, G., Szybalski, W., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: The left boundary of the O-utilization (*gut*) site required for transcription antitermination in coliphage lambda.

Zuber, M., Honigman, A., Court, D., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Analysis of a p-dependent terminator—*t_{a1}* of lambda.

Zuber, M., Patterson, T., Court, D., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Deletion analysis of the lambda *boxA*—*nutR* region.

Hyman, H.C., Honigman, A., Dept. of Molecular Genetics, Hadassah Medical School, Jerusalem, Israel: Transcription termination and processing sites in the lambda *P_l* operon.

Robert, J., Gottesman, M.E., Weisberg, R.A., NCI, National Institutes of Health, Bethesda, Maryland: *nun*—A phage-encoded transcription-termination factor.

Greenblatt, J., Li, J., Goda, Y., Peritz, L., Banting and Best Dept. of Medical Research, University of Toronto, Canada: Structures of transcription complexes made termination resistant at the antitermination utilization sites (*aut* sites) of the leftward early operon of bacteriophage lambda and the *rrmB* operon of *E. coli*.

Sigmund, C.D., Morgan, E.A., Roswell Park Memorial Institute, Buffalo, New York: NusA-dependent antitermination of ribosomal RNA transcription *in vitro*.

Stewart, V., Yanofsky, C., Dept. of Biological Sciences, Stanford University, California: Transcription antitermination control of tryptophanase gene expression in *E. coli*.

SESSION 5 POSTER SESSION

Polymeropoulos, M., Majumdar, A., Adhya, S., NCI, National Institutes of Health, Bethesda, Maryland: A promoter vector for divergent transcription.

Bergsland, K., Kao, C., Green, R., Gumbs, E., Snyder, L., Dept. of Microbiology, Michigan State University, East Lansing: Studies on the *E. coli* *lit* gene and its effect on T4 gene expression.

Siliaty, S.N., Rupley, J.A., Cegielska, A., Little, J.W., Dept. of Biochemistry, University of Arizona, Tucson: Mechanism of LexA repressor autoinduction.

Patterson, T., Sisk, W., Court, D., Laboratory of Molecular Oncology, NCI-Frederick Cancer Research Facility, Fred-

erick, Maryland: Role of IHF in the expression of the bacteriophage Mu early operon.

Pedersen, D.,¹ Pedersen-Lane, J.,¹ Herrington, M.,² Belfort, M.,¹ ¹Wadsworth Center for Laboratories and Research, New York State Dept. of Health, Albany; ²Dept. of Biology, Concordia University, Montreal, Canada: Is the *thyA* gene of *E. coli* regulated by an overlapping 5' gene?

Gruss, A., Novick, R., Public Health Research Institute, New York, New York: Palindromic sequences are required for plasmid maintenance in *S. aureus* but not in *B. subtilis*.

Majumder, S., Novick, R.P., Dept. of Plasmid Biology, Public Health Research Institute, New York, New York: A unique

hypersupercoiled molecule as one of the replication products of plasmid pT181.

Gennaro, M.L., Kornblum, J., Novick, R.P., Dept. of Plasmid Biology, Public Health Research Institute, New York, New York: *S. aureus* plasmid pT181 encodes a site-specific recombination protein.

Brown, S., Fournier, M.J., Dept. of Biochemistry, University of Massachusetts, Amherst: Mutational analysis of the 4.5S RNA of *E. coli*.

Adams, T.H., Guerinot, M.L., Chelm, B.K., Michigan State University, DOE Plant Research Laboratory, East Lansing: Transcriptional control of *nif* genes in the *B. japonicum* soybean symbiosis.

Scott-Craig, J., Chelm, B.K., MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing: Isolation of bacterial genes expressed during *B. japonicum*/soybean nodule development.

Garry, R.R., Rutherford, T.J., McCorquodale, D.J., Dept. of Biochemistry, Medical College of Ohio, Toledo: Apparent identity of *cmrA* with *trkA* of *E. coli*, and a mechanism for abortive infection of *E. coli* (ColIb) with bacteriophage T5.

Hughes, K.T., Olivera, B.M., Roth, J.R., Dept. of Biology, University of Utah, Salt Lake City: Transposition of defective Mu by P22 transduction is rec-dependent.

Brunelle, A., Schleif, R., Dept. of Biochemistry, Brandeis University, Waltham, Massachusetts: Determination of guanine, cytosine, thymine, and adenine contacts between the *E. coli* AraC protein and DNA of the *araI* site.

Wilkinson, R.,¹ Manning, F.,² Craven, D.,² Hansen, P.,³ Rubin, R.,¹ Goldstein, R.,² Infectious Disease Unit, Massachusetts General Hospital, Boston; ²Dept. of Molecular Genetics and Epidemiology, Boston University School of Medicine; ³Cambridge Research Laboratory, Massachusetts: Uropathogenic *E. coli*—Expression and molecular epidemiology of "virulence" factor operons.

Manning, F.,¹ Wilkinson, R.,² Christiansen, G.,³ Rosas, D.,¹ Hansen, P.,⁴ Rubin, R.,² Goldstein, R.,¹ ¹Dept. of Molecular Genetics and Epidemiology, Boston University School of Medicine, Massachusetts; ²Infectious Disease Unit, Massachusetts General Hospital, Boston; ³Dept. of Medical Microbiology, Aarhus University, Denmark; ⁴Cambridge Research Laboratory, Massachusetts: "Virulence" pil—Cloning, expression, assembly, and function.

Card, C., Croft, R., Jager, T., Moran, L., Lunnen, K., Nwankwo, D., Siatko, B., Wilson, G., New England Biolabs, Inc., Beverly, Massachusetts: Cloning, properties and expression of restriction and modification genes.

Selick, H.E., Alberts, B.M., Dept. of Biochemistry and Biophysics, University of California, San Francisco: Probing potential replication genes with a T4 insertion/substitution vector system.

Takiff, H.E., Court, D., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Characterization of *E. coli* RNase III gene.

Bedwell, D.M., Nomura, M., Dept. of Biological Chemistry, University of California, Irvine: Physiological and regulatory effects of increased RNA polymerase concentration in *E. coli*.

Greenstein, D., Horiuchi, K., Rockefeller University, New York, New York: Specific binding of gene II protein to the replication origin of bacteriophage ϕ 1.

Nakamura, Y., Tsugawa, A., Institute of Medical Science,



F. Stahl, P. Scolnick

University of Tokyo, Japan: Isolation and characterization of the conditionally lethal *nusA*s mutant of *E. coli*.

Engelberg-Kulka, H., Dekel-Gorodetsky, L., Schoulaker-Schwarz, R., Dept. of Molecular Biology, Hebrew University-Hadassah Medical School, Jerusalem, Israel: In vivo translation products of the *E. coli* tryptophan operon leader region.

Lee, F.,¹ Kurnit, D.,¹ Lagos, R.,² Lin, C.-T.,² Kim, S.,² Goldstein, R.,² ¹Genetics Division, Children's Hospital; ²Dept. of Molecular Genetics and Epidemiology, Boston University School of Medicine, Massachusetts: Antagonism of host factor ρ activity by plasmid P4—Construction and characterization of a *psu*⁺ *sid*⁻ mutant.

Shurvinton, C.E., Stahl, M.M., Stahl, F.W., Institute of Molecular Biology, University of Oregon, Eugene: A large palindromic in λ .

Reddy, P., Peterkofsky, A., McKenney, K., National Institutes of Health, Bethesda, Maryland: Translational efficiency of the *E. coli* adenylate cyclase gene—Mutating the UUG initiation codon to GUG or AUG results in increased gene expression.

Horabin, J.I., Webster, R.E., Dept. of Biochemistry, Duke University, Durham, North Carolina: Interaction of the gene I protein of the filamentous bacteriophage ϕ 1 with the bacterial membrane.

LaRossa, R.A., Van Dyk, T.K., Central Research and Development Dept., E.I. du Pont de Nemours and Co., Wilmington, Delaware: Interactions between the branched chain amino acid and energy-generating systems of *S. typhimurium*.

Myers, J.A., Beauchamp, B.B., Richardson, C.C., Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Purification and characterization of gene product 1.2 from phage T7.

Downs, D., Roth, J., Dept. of Biology, University of Utah, Salt Lake City: Cryptic phage release after purine starvation in *S. typhimurium*.

Condreay, J.P., Molineux, I.J., Dept. of Microbiology, University of Texas, Austin, Pseudorevertants of T3 mutants that overcome F-mediated restriction.

Debouck, C., Rosenberg, M., Gross, M., Dept. of Molecular



S. Adhya

Genetics, Smith, Kline and French Laboratories, Swedeland, Pennsylvania: Inhibition of gene expression from P_{λ} -containing plasmid vectors by functions of bacteriophage λ .

Paruchuri, D.K., Harshey, R.M., Scripps Clinic and Research Foundation, La Jolla, California: Color variation in *S. marcescens*—Correlation with variation of a surface protein.

Kemper, B., Jensch, F., Institute of Genetics, Cologne, Federal Republic of Germany: Endonuclease VII (gp49) of phage T4 resolves three-way junctions of recombinant DNA intermediates *in vitro*.

Lopes, J.M., Lawther, R.P., Dept. of Biology, University of South Carolina, Columbia: Genetic and molecular analysis of an intercistronic promoter ρ E in the *ilvGEDA* operons of *E. coli* and *S. typhimurium*.

Hejna, J.A., Warner, R.C., Dept. of Molecular Biology and Biochemistry, University of California, Irvine: Resolution of G4 figure-8 dimers in extracts of *E. coli*.

Kameyama, L., Guarneros, G., Dept. of Genetics and Molecular Biology, CINVESTAV-IPN, Mexico City, Mexico: *sib* inhibition of a λ *int-lacZ* gene fusion.

Beck, P.J., Molineux, I.J., Dept. of Microbiology, University of Texas, Austin: Abortive infection of T7 in *Shigella sonnei*.

Nashimoto, H., Uchida, H., Institute of Medical Science, University of Tokyo, Japan: DNA sequencing of the *E. coli* ribonuclease III and its mutations.

Zagotta, M., Wilson, D.B., Section of Biochemistry, Molecular, and Cell Biology, Cornell University, Ithaca, New York: Construction of λ S-*lacZ* gene fusions.

Bastos, M., Huwyler, L., Murphy, E., Dept. of Plasmid Biology, Public Health Research Institute, New York, New York: Complementation analysis of three functions required for transposition of *S. aureus* transposon Tn554.

Daub, E., Walsh, C.T., Bolstein, D., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: The isolation and characterization of two alanine racemase genes in *S. typhimurium*.

Falvey, E., Grindley, N.D.F., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Contact points between resolvase and the *res* site.

Hayes, S., Hayes, C., Tuer, R., Gordon, A., Dept. of Microbiology, University of Saskatchewan, Saskatoon, Canada: Measurement of spontaneous and induced mutagenesis by RK mutates—Influence of λ , *E. coli*, and plasmid genes.

Hoffman, C.S., Fishman, Y., Wright, A., Dept. of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts: Two regions of the λ receptor protein in *E. coli* K-12 independently direct outer membrane localization.

Kleina, L.G.,¹ Masson, J.-M.,¹ Miller, J.H.,¹ Normanly, J.,² Abelson, J.N.,² ¹University of California, Los Angeles; ²California Institute of Technology, Pasadena: Construction and use of an *E. coli* amber suppressor tRNA bank.

Liao, S.-M., McClure, W.R., Dept. of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania: Bacteriophage p22 *sar* RNA binding to antirepressor mRNA.

Mossing, M., Record, M.T., Jr., Dept. of Chemistry and Biochemistry, University of Wisconsin, Madison: Thermodynamic and molecular basis of repression in the *lac* operon.

Peterson, K.R.,¹ Wertman, K.F.,¹ Mount, D.W.,¹ Marinus, M.G.,² ¹Dept. of Molecular and Cellular Biology, University of Arizona, Tucson; ²Dept. of Pharmacology, University of Massachusetts Medical School, Worcester: DNA methylation affects expression of certain genes in *E. coli*.

Plumbidge, J.A., Sacerdot, C., Grunberg-Manago, M., Institut de Biologie Physico-chimique, Paris, France: Effect of *nsuA* protein on the expression of the *nusA, intB* operon in *E. coli*.

Revel, H.R., Brusian, J., Ewen, M., Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois: Characterization and expression of cDNA clones of bacteriophage ϕ 6 dsRNA.

Sarubbi, E., Rudd, K., Cashel, M., NICHD, National Institutes of Health, Bethesda, Maryland: Studies on the *E. coli* *spot* gene.

Schlagman, S.L.,¹ Hattman, S.,¹ Marinus, M.G.,² ¹Dept. of Biology, University of Rochester, New York; ²Dept. of Pharmacology, University of Massachusetts Medical School, Worcester: Evidence for a direct role of the *E. coli* *dam* + DNA methyltransferase in methylation-directed mismatch repair.

Schwartz, M.L., McClure, W.R., Dept. of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania: *In vitro* studies of CAP-positive control mutants.

Schwartz, I., Pramanik, A., Schwartz, J., Dept. of Biochemistry, New York Medical College, Valhalla: Identification of a functional promoter for *intC*, the gene for *E. coli* translation initiation factor 3.

Schwarz, J., Bergert, P.B., Dept. of Biochemistry and Molecular Biology, University of Texas Medical School, Houston: A plasmid genetic system for the tail protein of phage P22.

Kazic, T., Gots, J.S., Dept. of Microbiology, University of Pennsylvania, Philadelphia: Structure and regulation of the *purHD* operon of *E. coli*.

van de Putte, P., Vollerling, M., Laboratory of Molecular Genetics, State University of Leiden, The Netherlands: Regulation of *mom* expression in bacteriophage Mu.

Grzesiuk, E., Franklin, N.C., Dept. of Biology, University of Utah, Salt Lake City: A two-plasmid system used to select mutations in the *N* antitermination function of bacteriophage λ .

- Peterson, K.R., Mount, D.W., Dept. of Molecular and Cellular Biology, University of Arizona, Tucson: Degradation of the *E. coli* *lexA41* repressor by the Lon protease and explanation of the "split-phenotype" associated with this *lexA* (Ts) allele.
- Newlon, M.C., Dept. of Microbiology, UMDNJ-Rutgers Medical School, Piscataway, New Jersey: Amino-acid-starvation-induced death in *E. coli*.
- Brown, J., Klement, J., McAllister, W.T., UMDNJ-Rutgers Medical School, Dept. of Microbiology, Piscataway, New Jersey: Cloning and sequencing of promoters for the Sp6 RNA polymerase.
- Middle, G.A., Murray, N.E., Dept. of Molecular Biology, University of Edinburgh, Scotland: T4 polynucleotide kinase.
- Malone, C., Chase, J.W., Rothman-Denes, L.B., ¹Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois; ²Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Mutational analysis of the N4 virion RNA polymerase promoters.
- Fairfield, F.R., Linn, J., von Hippel, P.H., Dept. of Chemistry, University of Oregon, Eugene: Two conformations of the bacteriophage T4 DNA polymerase during DNA synthesis.
- Shapiro, J.A., Dept. of Biochemistry and Molecular Biology, University of Chicago, Illinois: Development of bacterial colonies.
- Scherzinger, E.,¹ Scholz, P.,¹ Haring, V.,¹ Bagdasarian, M.M.,² Bagdasarian, M.,² ¹Max-Planck Institute for Molecular Genetics, Berlin, Federal Republic of Germany; ²Institute of Applied Cell and Molecular Biology, Umeå University, Sweden: Replication genes of the promiscuous plasmid RSF1010.
- Duncan, B.K., Ciabarra, A., Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania: Identification of "hyperrecombinogenic" uracil-DNA glycosylase (*ung*) mutations.
- Hammer, K., Dandanell, G., Institute of Biological Chemistry, University of Copenhagen, Denmark: *deoR* repression of the *deo* operon requires two operator sites 600 base pairs apart.

SESSION 6 POSTTRANSCRIPTIONAL AND HEAT-SHOCK CONTROL; RESTRICTION AND MODIFICATION

- Plunkett, G., Echols, H., Dept. of Molecular Biology, University of California, Berkeley: Retroregulation of bacteriophage λ *int* gene—Fate of the RNase-III-processed RNA.
- Mackie, G.A., Parsons, G.D., Donly, B.C., Dept. of Biochemistry, University of Western Ontario, London, Canada: Posttranscriptional control of the synthesis of ribosomal protein S20.
- Dul, E., Mahoney, M.E., Wulff, D.L., Dept. of Biological Sciences, State University of New York, Albany: Mutations in the aminoterminal region of the *cII* gene of bacteriophage λ that affect translation efficiency.
- Springer, M., Graffe, M., Butler, J.S., Dondon, J., Buckingham, R.H., Grunberg-Manago, M., Institut de Biologie Physico-Chimique, Paris, France: Characterization of the *cis*- and *trans*-acting elements involved in transcriptional control of expression of the gene for threonyl-tRNA synthetase in *E. coli*.
- McCormick, J.R., Zengel, J.M., Lindahl, L., Dept. of Biology, University of Rochester, New York: The effects of translational efficiency on mRNA stability.
- Matthews, L.C., Cerretti, D.P., Nomura, M., Dept. of Biological Chemistry, University of California, Irvine: Translational coupling of the *spc* operon in *E. coli*.
- Gardel, C., Michaelis, S., Hunt, J., Beckwith, J., Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: *secD*—A new mutation affecting secretion in *E. coli*.
- Boyd, D.,^{1,2} Manoil, C.,¹ Beckwith, J.,¹ ¹Dept. of Microbiology and Molecular Genetics, Harvard Medical School; ²Dept. of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts: Identifying protein export signals in an inner membrane protein using an alkaline phosphatase fusion transposon.
- Mandel, M., Wilson, D.B., Section of Biochemistry, Molecular, and Cell Biology, Cornell University, Ithaca, New York: The 6K bacteriophage λ protein is a heat-shock protein.
- Erickson, J., Cowing, D., Straus, D., Zhou, Y.-N., Gross, C., Dept. of Bacteriology, University of Wisconsin, Madison: Regulation of the heat-shock response in *E. coli*.
- Bahl, H.,¹ Echols, H.,¹ Georgopoulos, C.,² Court, D.,³ ¹Dept. of Molecular Biology, University of California, Berkeley; ²Dept. of Cellular, Viral, and Molecular Biology, University of Utah, Salt Lake City; ³NCI-Frederick Cancer Research Facility, Frederick, Maryland: Overproduction of heat-shock proteins of *E. coli* mediated by the *cII* protein of phage λ .
- Brooks, J.E.,¹ Howard, K.A.,¹ Benner, J.,¹ Theriault, G.,² Roy, P.,² Gingeras, T.R.,³ ¹New England Bio-Labs, Beverly, Massachusetts; ²University of Laval, Quebec, Canada; ³Salk Institute for Biotechnology and Industrial Associates, La Jolla, California: Characterization of *PaeR7*, a restriction-modification system cloned from *P. aeruginosa*.
- Raleigh, E.A., Trimarchi, R., Latimer, E., New England Biolabs Inc., Beverly, Massachusetts: *MRC*, a locus determining rejection of cytosine-methylated DNA by *E. coli*.

SESSION 7 DNA REPLICATION AND REPAIR

- Dodson, M.,¹ Echols, H.,¹ Wickner, S.,² Roberts, J.D.,³ Gomes, B.,³ Alfano, C.,³ LeBowitz, J.,³ McMacken, R.,³ ¹Dept. of Molecular Biology, University of California, Berkeley; ²NCI, Bethesda, Maryland; ³Dept. of Biochemistry, Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland: Specialized nucleoprotein structures at the replication origin of bacteriophage λ .
- Flensburg, J., Krevolin, M., Calendar, R., Christian, R., Dept.



J. Shapiro showing movie of bacterial development

- of Molecular Biology, University of California, Berkeley: P4 phage DNA replication.
- Mosig, G., Macdonald, P., Dept. of Molecular Biology, Vanderbilt University, Nashville, Tennessee: On structure and function of a phage T4 DNA replication protein—gp69.
- Rabkin, S., Richardson, C., Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Initiation of bacteriophage T7 DNA replication.
- Fullford, W., Horiuchi, K., Model, P., Rockefeller University, New York, New York: Elements of the circuit regulating f1 DNA synthesis.
- Konings, R.N.H., Peeters, B.P.H., Schoenmakers, J.G.G., Laboratory of Molecular Biology, University of Nijmegen, The Netherlands: Structural and functional differences between the origins of viral-strand replication of the distantly related filamentous bacteriophages I_{ke} and F1.
- Pruss, G.J., Drica, K., Dept. of Biology, University of Rochester, New York: Replicon-specific changes in DNA supercoiling in topoisomerase I mutants.
- Projan, S.J., Kornblum, J., Moghazeh, S., Edelman, I., Nov-

- ick, R.P., Public Health Research Institute, Dept. of Plasmid Biology, New York, New York: Identification of domains of specificity for replication origins and replication proteins in *S. aureus* plasmids pT181, pC221, and pS194.
- Lu, C., Scheuermann, R.H., Echols, H., Dept. of Molecular Biology, University of California, Berkeley: Capacity of RecA protein to bind preferentially to UV lesions and inhibit the po1III editing subunit—A possible mechanism for SOS-induced targeted mutagenesis.
- Fishel, R., Kolodner, R., Laboratory of Molecular Genetics, Dana-Farber Cancer Institute, Boston, Massachusetts: A partially purified enzymatic system that catalyzes the repair of heteroduplex DNA from *E. coli*.
- Ennis, D.G., Peterson, K.R., Mount, D.W., Dept. of Molecular and Cellular Biology, University of Arizona, Tucson: Genetic requirements of SOS mutagenesis.
- Heitman, J., Model, P., Rockefeller University, New York, New York: Site-specific methylases induce the SOS response in *E. coli*.

SESSION 8 PLASMID CONTROL, RECOMBINATION, AND SPLICING

- Abeles, A., LBI-Basic Research Program, Frederick Cancer Research Facility, Frederick, Maryland: Purification and binding activity of the P1 RepA protein.
- Pal, S.K., Chattoraj, D.K., NCI-Frederick Cancer Research Facility, Frederick, Maryland: Quantitation of a P1 plasmid replication protein by Western blot analysis.
- Friedman, S., Abeles, A., Austin, S., LBI-Basic Research Program, Frederick Cancer Research Facility, Frederick, Maryland: P1 partition region—Cloning, sequence, and in vivo activity.
- Hansen, E.B., Yarmolinsky, M.B., NCI, National Institutes of Health, Bethesda, Maryland: Host participation in plasmid maintenance—*dnaA* function is an absolute requirement from *ori-2* and for P1 replication from a corresponding origin.
- Lupski, J.,¹ Projan, S.,² Ozaki, L.,¹ Godson, G.N.,¹
¹Dept. of Biochemistry, New York University Medical Cen-

- ter, New York; ²Public Health Research Institute, New York, New York: A temperature-dependent pBR322::Tn5 copy number mutant is due to a transcript originating from within Tn5.
- Abremski, K., Wierzbicki, A., Frommer, B., Hoess, R., Central Research and Development Dept., E.I. du Pont de Nemours and Company, Wilmington, Delaware: Phage P1 Cre-*loxP* site-specific recombination—A mutation in the *loxP* site that reveals the topoisomerase activity of the Cre recombinase.
- Thaler, D., Liu, S., Stahl, M.M., Stahl, F.W., Institute of Molecular Biology, University of Oregon, Eugene: Conversion associated with incorporation of λ dv into λ via the Rec pathway of homologous recombination.
- Stahl, F., Stahl, M., Institute of Molecular Biology, University of Oregon, Eugene: Nonreciprocal recombination in phage λ initiated at the cohesive end site (cos).

Murphy, K.C., Fenton, A.C., Poteete, A.R., Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester: *abc* gene of bacteriophage P22.

deMassy, B.,¹ Studier, F.W.,² Weisberg, R.A.,¹ ¹NICHD, National Institutes of Health, Bethesda, Maryland; ²Dept. of Biology, Brookhaven National Laboratories, Upton, New York: Resolution of Holliday structures by T7 endonuclease I.

Margolin, P., Public Health Research Institute of the City of New York, New York: Factors affecting the stability of tandem chromosomal duplications and the role of DNA topoisomerase I.

Belfort, M., Ehrenman, K., Pedersen-Lane, J., West, D., Herman, R., Maley, G., Chu, F., Maley, F., Wadsworth Center for Laboratories and Research, New York State Dept. of Health, Albany: RNA splicing and a circular intron in the expression of the interrupted *td* gene of phage T4.

Chu, F.K., Maley, G., Maley, F., Wadsworth Center for Laboratories and Research, New York State Dept. of Health, Albany: In vitro expression of the intron-containing *td* gene of T4 phage involving autocatalytic intron excision and exon splicing.



Hall, D.H.,¹ Povinelli, C.M.,¹ Ehrenman, K.,² Pedersen-Lane, J.,² Belfort, M.,² ¹School of Applied Biology, Georgia Tech, Atlanta; ²Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany: Intron mutations in the T4 gene coding for thymidylate synthase.

SESSION 9 TRICKS AND PHAGE REGULATION I

Hillyard, D.R., Roth, J., University of Utah, Salt Lake City: Generation of Mud(lac) fusions to essential genes in *S. typhimurium*.

Backman, K., Balakrishnan, R., BioTechnica International, Inc., Cambridge, Massachusetts: Engineered use of site-specific recombination in vivo for the control of cellular physiology.

Oliphant, A., Struhl, K., Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Selection of bacterial transcription signals from random-sequence DNA.

Sisk, W.P.,¹ Watson, D.,² Court, D.,² Tschlis, P.,³ ¹Georgetown University Medical Center, Washington, D.C.; ²NCI-Frederick Cancer Research Facility, Frederick, Maryland; ³Fox Chase Institute for Cancer Research, Philadelphia, Pennsylvania: The potential of *E. coli* to select exons utilizing an ORF vector.

Wang, P.-Z., Novick, R., Dept. of Plasmid Biology, Public Research Institute, New York, New York: Construction of an inducible expression vector with multiple functions to study the expression of pT181 *repC* gene.

Christie, G.E.,^{1,2} Dale, E.C.,¹ Feiwel, R.,¹ Halling, C.,¹ Calendar, R.,¹ Ljungquist, E.,³ ¹Dept. of Molecular Biology,

University of California, Berkeley; ²Dept. of Microbiology and Immunology, Virginia Commonwealth University, Richmond; ³Dept. of Microbiol Genetics, Karolinska Institute, Stockholm, Sweden: P2 and P4 phage late gene expression.

Birkeland, N.K., Lindqvist, B.H., Institute of Medical Biology, University of Tromsø, Norway: Control of P2 *ogr* gene expression.

Dehò, G., Zangrossi, S., Benatti, L., Sironi, G., Dept. de Biologia, Università di Milano, Italy: Transcription regulation of the phage-plasmid P4.

Kim, S., Lagos, R., Lozano, P., Lin, C.-T., Goldstein, R., Dept. of Molecular Genetics and Epidemiology, Boston University School of Medicine, Massachusetts: The "nonessential" region of satellite phage P4 is required during its alternate mode of propagation as a plasmid.

Lane, K.B., Lin, C.S., Six, E.W., Dept. of Microbiology, University of Iowa, Iowa City: P4 *ach* mutants and virulence suppression.

Bergsland, K., Kao, C., Green, R., Gumbs, E., Snyder, L., Dept. of Microbiology, Michigan State University, East Lansing: Studies on the *E. coli* *lit* gene and its effect on T4 gene expression.

SESSION 10 PHAGE REGULATION II

Lieb, M., Dept. of Microbiology, University of Southern California School of Medicine, Los Angeles: λ cl mutations that increase and decrease repressor synthesis.

MacHattie, L.A., Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois: New positive role for λ gene *rex*—Plasmid establishment.

Guzman, P., Guarneros, G., Dept. of Genetics and Molecular

Biology, CINVESTAV-IPN, Mexico City, Mexico: Replicon inhibition in *E. coli* *rap*.

Young, R., Raab, R., Neal, G., Dept. of Medical Biochemistry, Texas A&M University, College Station: Mutational analysis of the λ S gene.

Tolias, P.P., DuBow, M.S., Dept. of Microbiology and Immunology, McGill University, Montreal, Canada: Cloning and

- characterization of the bacteriophage Mu and D108 DNA-binding regulatory protein *ner*.
- Margolin, W., Howe, M.M., Dept. of Bacteriology, University of Wisconsin, Madison: Activation of late transcription of bacteriophage Mu—Sequence of the positive activator gene C.
- Stoddard, S.F., Howe, M.M., Dept. of Bacteriology, University of Wisconsin, Madison: Mapping of transcription initiation sites in bacteriophage Mu.
- Schumann, W., Apfel, C., Otto, U., Institut für Organische Chemie und Biochemie, Technische Hochschule Darmstadt, Federal Republic of Germany: Regulation of the transposition functions of *E. coli* phage Mu.
- Hattman, S.,¹ Ives, J.,¹ Margolin, W.,² Howe, M.M.,²
¹Dept. of Biology, University of Rochester, New York;
²Dept. of Bacteriology, University of Wisconsin, Madison: Regulation and expression of the bacteriophage Mu *mom* gene—Mapping of the transactivation (Dad) function to the C gene.
- Harshey, R.M., Scripps Clinic and Research Foundation, La Jolla, California: Regulation of transposase expression in phage Mu.
- Scolnik, P.A., Schumann, P., Cold Spring Harbor Laboratory, New York: The gene transfer agent of *Rhodospseudomonas capsulata* as a genetic tool.

SESSION 11 BACTERIAL REGULATION

- Cole, J.R., Nomura, M., Dept. of Biological Chemistry, University of California, Irvine: Translational regulation of ribosomal proteins in *E. coli* is responsible for their normal regulation at different growth rates and during stringent control.
- Roof, D., Roth, J., Dept. of Biology, University of Utah, Salt Lake City: Genetics and regulation of ethanolamine utilization in *S. typhimurium*.
- Arps, P., Marvel, C.,² Winkler, M.,¹ ¹Dept. of Molecular Biology, Northwestern University Medical School, Chicago, Illinois; ²Dept. of Biochemistry, University of California, Berkeley: Structural features on the *hisT* operon of *E. coli* K-12.
- Rudd, K.,¹ Menzel, R.,² ¹NICHD, ²NIADDK, National Institutes of Health, Bethesda, Maryland: Effects of mutations in *gyrA* and *gyrB* on *his* operon expression in *E. coli* and *S. typhimurium*.
- Mahadevan, S.,¹ Reynolds, A.E.,² Wright, A.,¹ ¹Dept. of Molecular Biology and Microbiology, Tufts University Medical School; ²Dept. of Molecular Biology, Massachusetts General Hospital, Boston: Positive and negative regulation of the *bgl* operon in *E. coli* K-12.
- Lobocka, M., Wild, J., Hennig, J., Klopotoski, T., Instytut Biochemii i Biofizyki, Warsaw, Poland: Mutations in *E. coli* K-12 with opposite effects on genes that are inducible only aerobically or anaerobically.
- Guiso, N.,¹ Ladant, D.,¹ Brézin, C.,² Alonso, J.-M.,² Bärzu, O.,¹ Ullman, A.,¹ ¹Unité de Biochimie des Régulations Cellulaires; ²Unité d'Ecologie Bactérienne, Institut Pasteur, Paris, France: Some regulatory aspects of *B. pertussis* adenylate cyclase.
- Hirschmann, J., Wong, P.-K., Sei, K., Keener, J., Kustu, S., Dept. of Bacteriology, University of California, Davis: Products of nitrogen regulatory genes *ntrA* and *ntrC* of enteric bacteria activate *glnA* transcription in vitro—Evidence that the *ntrA* product is a σ factor.
- Imamoto, F., Kano, Y., Yoshino, S., Wada, M., Yokoyama, K., Dept. of Molecular Genetics, Riken, Wako, Japan: Molecular cloning and nucleotide sequence of the HU-1 gene of *E. coli*.
- Daldal, F., Applebaum, J., Cold Spring Harbor Laboratory, New York: *C. pasteurianum* galactokinase—A model system for gene structure, expression and regulation in strict anaerobes.

Heat Shock

August 28 – September 1

ARRANGED BY

Elizabeth Craig, University of Wisconsin
 Costa Georgopoulos, University of Utah
 George Hahn, Stanford University
 Nancy Petersen, University of Wyoming

160 participants

The second Heat Shock meeting was held at Cold Spring Harbor 3 years after the first meeting on this subject. The 1982 meeting emphasized the generality of the heat-shock response and the organization of the heat-shock genes, in partic-

ular identification of regulatory signals responsible for the expression of these genes in a wide variety of organisms, including bacteria, plants, and animals, under chemical as well as environmental stress. At the 1985 meeting, the major problems addressed were the identity of the major heat-shock proteins and their roles in normal growth and development as well as following environmental stress. The regulation of heat-shock gene expression continued to be a topic of interest particularly in *Escherichia coli* where the gene regulating the heat-shock response has turned out to be a unique sigma factor that alters polymerase to recognize specifically heat-shock promoter sequences. The complexity of the cellular response to stress was indicated by the large number of different genes involved, including genes involved in DNA, RNA, and protein synthesis and in protein processing and degradation. Particular attention was focused on protein degradation as an important factor with the discovery that the *lon* gene protease of *E. coli* is a heat-shock protein and that ubiquitin, also associated with protein degradation, is expressed following heat shock in vertebrates.

Attention was particularly focused on the role of the major (70K) heat-shock protein and its normally expressed counterparts in growth and development. Yeast mutational analysis identified several essential functions for the eight yeast 70K heat-shock genes. The abundant presence of 70K heat-shock proteins or their homologs in early *Drosophila* embryos and mouse embryos also suggests a role for these proteins in normal development. Interesting properties reported for the eukaryotic 70K heat-shock protein include ATP binding, autoproteolytic activity, and clathrin coating activity. Antibodies to the major heat-shock proteins allowed localization of the proteins in the cytoplasm and nucleus. Particularly interesting was the observation that the conserved portion of the 70K protein specifically migrates to the nucleolus following heat shock and appears to speed recovery of normal nucleolar morphology following heat shock, suggesting a mechanism by which heat-shock proteins might play an essential role in recovery of protein synthesis following heat shock.

Progress in application of new information to cancer treatment was also reported. Impressive results showed that the combination of heat and radiation is three times as effective in treatment of some types of cancer as radiation treatment alone. Preliminary studies on the use of synthesis of the 70K heat-shock protein as a diagnostic tool to estimate heat sensitivity of cancer cells were also reported.

This meeting was supported in part by the National Science Foundation.

Introduction: A. Tissières, University of Geneva

SESSION 1 MECHANISMS OF INDUCTION

Chairperson: A. Tissières, University of Geneva

Christman, M.F., Morgan, R.W., Jacobson, F.S., Ames, B.N., Dept. of Biochemistry, University of California, Berkeley: Heat-shock and oxidative stress in *S. typhimurium*.

VanBogelen, R.A., Kelley, P.M., Neidhardt, F.C., University of Michigan, Ann Arbor: Differential expression of stress regulators in *E. coli*.

Bonner, J.J., Parker-Thornburg, J., Dept. of Biology, Indiana University, Bloomington: Tissue-specific expression of heat shock proteins.

Sherwood, A., Sanders, M.M., Dept. of Pharmacology,

UMDNJ-Rutgers Medical School, Piscataway, New Jersey: Modification of a membrane protein induces the heat shock response in *Drosophila*.

Mirault, M.-E., Dept. of Molecular Biology, University of Geneva, Switzerland: Oxidative stress and induction of heat shock genes.

Kitchener, K.,¹ Kao, H.-T.,¹ Capasso, O.,¹ Heintz, N.,¹ Nevins, J.,¹ Voellmy, R.,² ¹Rockefeller University, New York, New York; ²University of Miami, Florida: Regulation of a human hsp70 gene by the adenovirus E1A gene.

SESSION 2 TRANSCRIPTIONAL REGULATION

Chairperson: C. Gross, University of Wisconsin

Yura, T., Tobe, T., Yano, R., Institute for Virus Research, Kyoto University, Japan: Genetic control of heat-shock response in *E. coli*—Extragenic suppressors of *rpoH* (*htrP*, *hin*) mutations.

Gross, C., Cowing, D., Erickson, J., Straus, D., Zhou, Y.-N., Dept. of Bacteriology, University of Wisconsin, Madison: Regulation of the heat shock response in *E. coli*.

Key, J.L.,¹ Nagao, R.,² Gurley, W.,³ Czarnicka, E.,² Kimpel, J.,² Vierling, E.,² Lin, C.Y.,² Roberts, J.,² ¹Agrigenetics Corporation, Boulder, Colorado; ²Dept. of Botany, University of Georgia, Athens; ³University of Florida, Gainesville: Sequence analysis of some heat shock genes and their expression in plants.

SESSION 3 POSTER SESSION I

Amin, J., Mestrl, R., Schiller, P., Voellmy, R., Dept. of Biochemistry, University of Miami School of Medicine, Florida: The *Drosophila* hsp70 gene promoter.

Arrigo, A.-P., Dept. of Molecular Biology, University of Geneva, Switzerland: Acetylation and methylation patterns of core histones are modified following heat or arsenite stresses of *Drosophila* and human cells.

Aujame, L., Dept. of Biology, Queen's University, Kingston, Canada: Nonexpression of hsp68 gene in murine plasmacytomas.

Banerji, S.S., Theodorakis, N.G., Morimoto, R.I., Dept. of Biochemistry, Molecular and Cell Biology, Northwestern University, Evanston, Illinois: Evidence for elongation control of hsp70 synthesis in avian reticulocytes.

Bardwell, J.C.A., Craig, E.A., Dept. of Physiological Chemistry, University of Wisconsin, Madison: Isolation and genetic analysis of an *E. coli* heat-inducible gene homologous to the *Drosophila* 83,000-M, heat shock protein gene.



E. Craig

Elgin, S.C.R., Amero, S.A., Cartwright, I.L., Dietrich, V., Eisenberg, J.C., Flick, J.T., James, T.C., Mila, K., Siegfried, E., Thomas, H.G., Washington University, St. Louis, Missouri: DNA and chromatin structural patterns of the small heat shock genes of *Drosophila*.

Wu, C., NCI, National Institutes of Health, Bethesda, Maryland: Isolation of two DNA-binding proteins implicated in heat shock gene regulation.

Wiederrecht, G., Parker, C., Division of Chemistry, California Institute of Technology, Pasadena: Purification and properties of the *Drosophila* heat shock transcription factor.

Bienz, M., MRC, Cambridge, England: Developmental regulation of the *Xenopus* hsp70 promoter.

Bendena, W.G., Pardue, M.L., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Study of translational control during heat shock.

Berger, E.,¹ Morganelli, C.,¹ Torrey, D.,¹ Pelham, H.R.B.,² ¹Dept. of Biology, Dartmouth College, Hanover, New Hampshire; ²MRC, Cambridge, England: Functional assay of regulatory sequences flanking hsp genes in *Drosophila*.

Bournias-Vardiabasis, N., Flores, J., Buzin, C., Division of Cytochemistry, City of Hope Medical Center, Duarte, California: Differential expression of heat shock proteins in *Drosophila* cells following metal ion exposure.

Calderwood, S.K., Stevenson, M.A., Hahn, G.M., Dept. of Radiology, Stanford University, California: Temperature shock triggers multiple transmembrane signals—Inositol phosphates, Ca²⁺, cyclic nucleotides, and arachidonate. Catelli, M.G.,¹ Binart, N.,¹ Baulieu, E.E.,² Welch, W.,² Helfman, D.,² Feramisco, J.,² ¹Lab Hormones, Bicêtre France; ²Cold Spring Harbor Laboratory, New York: Identification and cloning of the 90-kD protein, a heat shock protein interacting with steroid hormone receptor.

Cheney, C.M., Lang, T.J., Dept. of Biology, Johns Hopkins University, Baltimore, Maryland: Evidence that *Drosophila* hsp23 is part of the Triton-insoluble cytoskeleton.

Currie, R.W., Dept. of Anatomy, Dalhousie University, Halifax, Nova Scotia: Synthesis of stress protein *M*, 71,000 in perfused rat hearts.

Drabent, B., Benecke, B.J., Dept. of Biochemistry, Ruhr University, Federal Republic of Germany: Isolation and characterization of cDNA and genomic clones of the major human heat shock genes.

Dubois, M.F.,¹ Lebon, P.,¹ Bensaude, O.,² Morange, M.,² ¹INSERM, Hôpital Saint Vincent-de-Paul; ²Institut Pasteur, Paris, France: Interferon potentiates the heat shock response in mouse cells.

Dubrovsky, E.B., Zhumulev, I.F., Institute of Cytology and Genetics, Academy of Sciences of the USSR, Siberian Division, Novosibirsk: Genetic regulation of synthesis of *Drosophila* salivary gland small hsps during normal development.

Dworniczak, B.,¹ Mirault, M.E.,² ¹Institute of Molecular Ge-

netics, University of Heidelberg, Federal Republic of Germany; ²Dept. of Molecular Biology, University of Geneva, Switzerland: Characterization of the human hsp70 gene and its closely related cognate family.

Dykstra, C.C., Walker, G.C., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: A positive selection for the isolation of mutations increasing protein stability.

Edington, B.V., White, C.N., Hightower, L.E., Dept. of Molecular and Cell Biology, University of Connecticut, Storrs: Stress gene expression in cultured chicken embryo cells.

Findly, R.C.,¹ Molt, J.E.,² Platt, T.,² ¹Dept. of Genetics, University of Georgia, Athens; ²Dept. of Biochemistry, University of Rochester Medical Center, New York: Mutations affecting regulation of the hsp70 gene in yeast.

Fornace, A.J., Jr., NCI, National Institutes of Health, Bethesda, Maryland: B2 RNA is the major transcript induced by heat shock in Chinese hamster cells.

Franza, B.R.,¹ Ruley, H.E.,² Chang, C.,¹ Garrels, J.I.,¹ ¹Cold Spring Harbor Laboratory, New York; ²Massachusetts Institute of Technology, Cambridge: Oncogene expression affects the mammalian heat shock response.

Hallberg, R., McMullin, T., Kraus, K., Dept. of Zoology, Iowa State University, Ames: The selective polysomal localization of a minor heat shock protein.

Heikkilä, J.J., Ovsenek, N., Krone, P., Dept. of Biology, University of Waterloo, Canada: Accumulation of *X. laevis* heat shock mRNAs during early development.

Hickey, E., Brandon, S., Weber, L.A., Dept. of Biology, University of South Florida, Tampa: Structure and organization of the human genes encoding hsp27.

Hovemann, B., Tyseck, R.-P., Richter, S., Molekular Genetik, Universität Heidelberg, Federal Republic of Germany: Chromosomal organization and transcriptional analysis of heat shock locus 93D of *D. melanogaster*.

Kapoor, M., Dept. of Biology, University of Calgary, Canada: Expression of heat shock proteins in the filamentous fungus, *N. crassa*.

Kay, R., Russnak, R., Candido, E.P.M., University of British Columbia, Vancouver, Canada: Transcription of wild type and in vitro-mutated *C. elegans* heat shock genes in mouse cells transfected with bovine papilloma virus-derived vectors.

SESSION 4 REGULATION OF EXPRESSION

Chairperson: M.L. Pardue, Massachusetts Institute of Technology

Riddihough, G.T.D.E., Young, H.A.F., Weeks, J.R., Dudler, R., Travers, A.A., MRC Laboratory of Molecular Biology, Cambridge, England: Transcriptional and posttranscriptional regulation of *Drosophila* heat shock genes.

Kraus, K., Hallberg, R., Dept. of Zoology, Iowa State University, Ames: Characterization of a heat shock mutant of *T. thermophila* with a translational defect.

Weber, L., Sadis, S., Hickey, E., Dept. of Biology, University of South Florida, Tampa: Coordinate transcription and differential mRNA stability determine the pattern of heat shock protein synthesis in HeLa cells.

Duncan, R., Hershey, J.W.B., Dept. of Biological Chemistry, University of California, Davis: Different inducers of the



A. Tissières, E. Bautz, H. Mitchell

Kelley, P.M., VanBogelen, R.A., Neidhardt, F.C., Dept. of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor: The accumulation of adenylated nucleotides in *E. coli* by agents that induce the heat shock proteins.

Ketis, N.V., Karnovsky, M.J., Hoover, R.L., Harvard Medical School, Boston, Massachusetts: Varying patterns of protein synthesis in endothelial cells from different origins exposed to hyperthermia.

Kimpel, J.A.,¹ Lin, C.-Y.,² Key, J.L.,³ ¹Botany and Plant Pathology, Oregon State University, Corvallis; ²Dept. of Botany, National Taiwan University, Taipei; ³Dept. of Botany, University of Georgia, Athens: Parameters of heat shock mRNA protein accumulation in soybean seedlings.

Kirchner, G., Qoronfleh, M.W., Streips, U.N. Dept. of Microbiology and Immunology, University of Louisville School of Medicine, Kentucky: Genetic analysis of heat shock response in *B. subtilis*.

Klippstech, K.,¹ Meyer, G.,¹ Schuster, G.,² Ohad, I.,² ¹Botanik Institute, Universität Hannover, Federal Republic of Germany; ²Dept. of Biological Chemistry, Hebrew University, Jerusalem, Israel: Localization of a nuclear coded 22-kD heat-shock protein in the chloroplast membranes of peas and *Chlamydomonas reinhardtii*.

stress response affect the protein synthesis initiation factors in different ways.

Garbe, J.,¹ Lakhotia, S.C.,² Pardue, M.L.,¹ ¹Dept. of Biology, Massachusetts Institute of Technology, Cambridge; ²Dept. of Zoology, Banaras Hindu University, Varanasi, India: Molecular studies of the 93D heat shock locus of *Drosophila*.

Kloetzel, P.M., Schuldt, C., Hovemann, B., Bautz, E.K.F., Institute of Molecular Genetics, University of Heidelberg, Federal Republic of Germany: Organization and distribution of nuclear RNP complexes after heat shock in *D. melanogaster*.

SESSION 5 GENETIC ANALYSIS OF FUNCTION

Chairperson: F. Neidhardt, University of Michigan Medical School

- Zylicz, M., Tilly, K., Chandrasekhar, G.N., Ang, D., Sell, S., Johnson, C., Spence, J., King, J., Sharma, S., Myers, R., Cegielska, A., Fayet, O., Georgopoulos, C., Dept. of Cellular, Viral and Molecular Biology, University of Utah School of Medicine, Salt Lake City: Properties of heat shock proteins of *E. coli* and the regulation of their synthesis.
- Walker, G.C., Paek, K.-H., Dykstra, C., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Genetic studies of the *E. coli* heat shock response.
- Craig, E., Jacobsen, K., Werner-Washburne, M., Boorstein, W., Tilly, K., Dept. of Physiological Chemistry, University of Wisconsin, Madison: Mutational analysis of the *S. cerevisiae* hsp70 multigene family.

- Petko, L., Lindquist, S., Dept. of Biology, University of Chicago, Illinois: A mutational analysis of Hsp26 and Hsp 35.
- Iida, H., Yahara, I., Tokyo Metropolitan Institute of Medical Science, Japan: Yeast hsp48 is an isoprotein of enolase and is involved in both growth control and thermal tolerance.
- Ayme, A., Dept. of Molecular Biology, University of Geneva, Switzerland: Chromosomal site 67B of *D. melanogaster* contains seven, not four, heat shock genes.
- Thomas, G.P., Pasucci, D.D., Cold Spring Harbor Laboratory, New York: Inverted relationships between induced and noninduced 70K gene products among different mammals.

SESSION 6 POSTER SESSION II

- Laszlo, A., Dept. of Radiology, Washington University School of Medicine, St. Louis, Missouri: Altered expression of the 70-kD hsp in heat-resistant Chinese hamster cells.
- Leicht, B.,¹ Biessmann, H.,² Bonner, J.J.,¹ Dept. of Biology, Indiana University, Bloomington; ²Dept. of Biochemistry and Biophysics, University of California, San Francisco: The small heat shock proteins of *Drosophila* associate with the cytoskeleton.
- Mestrl, R.,¹ Schiller, P.,¹ Ananthan, J.,¹ Amin, J.,¹ Klapper, H.,¹ Rungger, D.,² Voellmy, R.,¹ Dept. of Biochemistry, University of Miami School of Medicine, Florida; ²Dept. of Animal Biology, University of Geneva, Switzerland: Promoter sequences involved in the heat and developmental regulation of the *D. melanogaster* hsp23 gene.
- Mivechi, N.F., Li, G.C., Dept. of Radiation Oncology, University of California, San Francisco: Early progenitors of mu-

rine bone marrow cells are not capable of development of thermotolerance.

- Morange, M., Mezger, V., Bensaude, O., Institut Pasteur, Paris, France: Teratocarcinoma stem cells are a model system to study heat shock protein synthesis during early mouse embryogenesis.
- Munro, S., Pelham, MRC Laboratory of Molecular Biology, Cambridge, England: Cloning and intracellular localization of hsp70 cognate proteins.
- Nicholson, R.C., Wan, K., Townsend, C.A., Moran, L.A., Dept. of Biochemistry, University of Toronto, Canada: The yeast hsp70 gene family.
- Ohki, M., Tamura, F., Nishimura, S., Division of Biology, National Cancer Center Research Institute, Tokyo, Japan: Defects in protein biosynthesis of *dnaJ* mutants closely resembling those of a cell cycle-control mutant *divE42*.
- Paek, K.-H., Walker, G.C., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Are *dnaK* and *hspR* only essential at high temperatures in *E. coli*?
- Palter, K.B.,¹ Watanabe, M.,² Stinson, L.,¹ Mohawald, A.P.,² Craig, E.A.,¹ Dept. of Physiological Chemistry, University of Wisconsin, Madison; ²Dept. of Anatomy and Developmental Biology, Case Western Reserve University, Cleveland, Ohio: The expression and localization of *Drosophila* hsp70 cognate proteins.
- Pauli, D., Dept. of Molecular Biology, University of Geneva, Switzerland: The expression of *Drosophila* hsp23 and hsp26 genes studied by promoter deletion analysis in transformed flies.
- Perry, M.D., Lowe, D.C., Moran, L.A., Dept. of Biochemistry, University of Toronto, Canada: The mouse hsp70 gene family.
- Piper, P.W., Patel, N., Lockheart, A., Dept. of Biochemistry, University College London, England: The yeast gene for phosphoglycerate kinase is activated by heat shock.
- Rees, C.A.B.,¹ Hogan, N.C.,² Walden, D.B.,¹ Atkinson, B.G.,² Depts. of ¹Plant Sciences, ²Zoology, University of Western Ontario, London, Canada: Characterization and localization of 70- and 18-kD hsp mRNAs in an inbred line of maize OH43.
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dology, Washington University, St. Louis, Missouri: The nuclear matrix from heat-shocked cells is resistant to RNase-induced disruption.

Russnak, R.H., Jones, D., Candido, E.P.M., Dept. of Biochemistry, University of British Columbia, Vancouver, Canada: The 16-kD heat shock protein gene family of *C. elegans*.

Sacco, M., Caruso, M., Maresca, B., International Institute of Genetics and Biophysics, Naples, Italy: Cloning of *H. capsulatum* gene coding for major heat shock protein hsp70 and its expression during differentiation.

Sanders, M.M., Triemer, D.F., Olsen, A.S., Dept. of Pharmacology, UMDNJ-Rutgers Medical School, Piscataway, New Jersey: The regulation of translation in heat shock in *Drosophila*.

Schiller, P.,¹ Ahmed, A.,¹ Amin, J.,¹ Mestrlil, R.,¹ Bromley, P.,² Rungger, D.,³ Voellmy, R.,¹ ¹Dept. of Biochemistry, University of Miami School of Medicine, Florida; ²Battelle Memorial Institute, Geneva Research Center, Switzerland; ³Dept. of Animal Biology, University of Geneva, Switzerland: Expression studies with a human 70-kD heat shock protein gene.

Sciandra, J.J.,¹ Hughes, C.S.,² Subject, J.R.,² ¹Division of Radiation Biology, Roswell Park Memorial Institute, Buffalo, New York; ²University of Rochester, New York: Repression of glucose-regulated proteins induces heat shock proteins.

Sherwood, S.W., Daggett, A.S., Schimke, R.T., Dept. of Biological Sciences, Stanford University, California: The heat shock response does protect cells.

Siegfried, E., Bond, U., Elgin, S.C.R., Dept. of Biology, Washington University, St. Louis, Missouri: Supercoil-dependent DNA structure at hsp26.

Silver, J.C., Pekkala, D., Life Sciences Division, University of Toronto, Canada: Alterations in translational machinery with heat shock in *Achlya*.

Sirkin, E., Lindquist, S., Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois: Nucleic acid binding properties of hsp70.

Slater, M.R., Stone, D.E., Boorstein, W.R., Craig, E.A., Dept. of Physiological Chemistry, University of Wisconsin, Madison: Regulation of yeast heat shock proteins.

Strand, D.J.,¹ Lambert, M.E.,² Arthur, W.L.,³ Weinstein, B.,³ Fisher, P.B.,⁴ McDonald, J.F.,¹ ¹Dept. of Genetics, University of Georgia, Athens; ²Depts. of ²Human Genetics and Development, ³Microbiology, ⁴Cancer Center, Columbia University, New York, New York: Regulation of *copia* expression by E1A or heat shock.

Tanguay, R.M.,¹ Duband, J.L.,¹ Lettre, F.,¹ Carbalaj, E.,¹ Valet, J.P.,¹ Arrigo, A.P.,² ¹Oncogénèse et Génétique Moléculaires, University of Laval Hospital Center, Quebec, Canada; ²Dep. Biologie Moléculaire, Université de Genève, Switzerland: Intracellular shuttling of heat shock proteins in stressed cells—Dynamics and significance.

Tanguay, R.M., Desrosiers, R., Ontogénèse et Génétique Moléculaires, University of Laval Hospital Center, Quebec, Canada: Posttranslational modifications of histones in response to heat or chemical stress—Relation to recovery of protein synthesis.

Tao, T.W., Leu, S.L., Duncan, A., Division of Nuclear Medicine, Stanford University, California: Preferential induction of nonconstitutive hsp68 in heat-sensitive cells and of con-



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stitutive hsp70 in heat-resistant mutants of mouse melanoma cells.

Theodorakis, N.G., Banerji, S.S., Morimoto, R.I., Dept. of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, Illinois: Translation of hsp70 is coupled to continuous transcription in avian reticulocytes.

Tilly, K.,¹ Sharma, S.,² Georgopoulos, C.,² ¹Dept. of Physiological Chemistry, University of Wisconsin, Madison; ²Dept. of Cellular, Viral and Molecular Biology, University of Utah, Salt Lake City: Regulation of the *E. coli* *hspR* (*ipbH*) gene.

Vierling, E., Mishkind, M.L., Roberts, J.K., Schmidt, G.W., Key, J.L., Dept. of Botany, University of Georgia, Athens: Heat shock proteins in chloroplasts.

Vokes, E.E., Brownstein, B.H., Golomb, H.M., Dept. of Medicine, University of Chicago, Illinois: Heat shock proteins in human leukemic cells.

Walsh, D.A.,¹ Hightower, L.E.,² Klein, N.W.,³ Edwards, M.J.,¹ ¹Dept. of Veterinary Clinical Studies, University of Sydney, Australia; ²Microbiology Section, ³Dept. of Animal Genetics, University of Connecticut, Storrs: The induction of heat shock proteins during early mammalian development.

Welch, W.J., Suhan, J.P., Cold Spring Harbor Laboratory, New York: Morphological study of the mammalian stress response.

Wohlhilt, A., Bonner, J.J., Dept. of Biology, Indiana University, Bloomington: Genetic analysis of hsp82 in *D. melanogaster*.

Wu, B.,¹ Kingston, R.,² Morimoto, R.I.,¹ ¹Dept. of Biochemistry, Molecular and Cell Biology, Northwestern University, Evanston, Illinois; ²Dept. of Molecular Genetics, Massachusetts General Hospital, Boston: Localization of sequences flanking the human hsp70 gene that are responsive to the transcriptional activation by heat shock cadmium and serum induction.

Yost, H.J., Lindquist, S.L., Dept. of Molecular Genetics and Cell Biology, University of Chicago, Illinois: Intron processing in heat-shocked cells.

SESSION 7 PROTEIN STRUCTURE AND FUNCTION

Chairperson: W. Welch, Cold Spring Harbor Laboratory

Zylicz, M.,¹ Liberek, K.,¹ Skowrya, D.,¹ Yamamoto, T.,² McIntyre, J.,² Sell, S.,² Georgopoulos, C.,² ¹Division of Biophysics, University of Gdansk, Poland; ²Dept. of Cellular, Viral and Molecular Biology, University of Utah School of Medicine, Salt Lake City: Enzymatic properties of purified *dnaK* and *dnaJ* replication heat shock proteins of *E. coli*.

Mitchell, H.K.,¹ Peterson, N.S.,² ¹Division of Biology, California Institute of Technology, Pasadena; ²Dept. of Biochemistry, University of Wyoming, Laramie: The self-destruction of hsp70K.

Chappell, T.G.,¹ Welch, W.J.,² Schlossman, D.M.,³ Schlesinger, M.J.,⁴ Rothman, J.E.,¹ ¹Dept. of Biochemistry, Stanford University Medical Center, California; ²Cold Spring Harbor Laboratory, New York; ³Division of Hema-

tology and Oncology, Duke University Medical Center, Durham, North Carolina; ⁴Dept. of Microbiology and Immunology, Washington University School of Medicine, St. Louis, Missouri: Bovine brain uncoupling ATPase is a constitutively expressed heat shock protein.

Guidon, P.T., Jr., Hightower, L.E., Dept. of Molecular and Cell Biology, University of Connecticut, Storrs: The rat 71-kD heat shock protein and its cognate are fatty acid-binding proteins.

Welch, W.J., Feramisco, J.R., Cold Spring Harbor Laboratory, New York: The mammalian stress proteins.

Pelham, H., Munro, S., Lewis, M., MRC Laboratory of Molecular Biology, Cambridge, England: Interactions and function of hsp70.

SESSION 8 PROTEIN FUNCTION AND LOCALIZATION

Chairperson: S. Lindquist, University of Chicago

Bond, U., Schlesinger, M.J., Dept. of Microbiology and Immunology, Washington University School of Medicine, St. Louis, Missouri: Ubiquitin and the stress response.

Finley, D., Ozkaynak, E., Swerdlow, P., Varshavsky, A., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Regulatory coupling between the heat shock and ubiquitin systems.

Arrigo, A.-P., Darlix, J.-L., Dept. of Molecular Biology, University of Geneva, Switzerland: Study of the cytoplasmic structures formed by the low-molecular-weight heat shock proteins in *Drosophila*.

Ornelles, D.A., Penman, S., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: The prompt heat shock proteins of *Drosophila* and HeLa cells.

Welch, W.J.,¹ Li, G.,² Feramisco, J.R.,¹ ¹Cold Spring Harbor Laboratory, New York; ²Dept. of Radiation Oncology, University of California, San Francisco: Use of the stress response to study the cytoskeleton and transformation.

Subject, J.R.,¹ Shyy, T.-T.,¹ Olson, M.,² ¹Roswell Park Memorial Institute, Buffalo, New York; ²University of Mississippi Medical Center, Jackson: Heat, growth state, actinomycin D, and serum deprivation affect the nuclear localization of the mammalian 110-kD heat shock protein.



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SESSION 9 HEAT SHOCK PROTEINS AND DEVELOPMENT

Chairperson: H. Mitchell, California Institute of Technology

Plesofsky-Vig, N., Brambi, R., Dept. of Plant Pathology, University of Minnesota, Saint Paul: Protein synthesis by *N. crassa* during heat shock.

Van der Ploeg, L.H.T., Glass, D.J., Giannini, S.H., Cantor, C.R., Dept. of Human Genetics and Development, Columbia University, New York, New York: Heat shock gene expression in insect vector-transmitted protozoa.

Petersen, N.S.,¹ Mitchell, H.K.,² ¹Dept. of Biochemistry, University of Wyoming, Laramie; ²Dept. of Biology, Cali-

fornia Institute of Technology, Caltech, Pasadena, California: Mechanism of phenocopy induction in *Drosophila*.

Chernoff, G.F., College of Veterinary Medicine, Washington State University, Pullman: Heat-induced neural tube defects in mice.

Bensaude, O.,¹ Babinet, C.,² Morange, M.,¹ ¹Génétique cellulaire, ²Génétique des mammifères, Institut Pasteur, Paris, France: Heat shock protein synthesis during early mouse embryogenesis.

Chairperson: G. Hahn, Stanford University School of Medicine

Landry, J., Lamarche, S., Laval University Cancer Research Center, Quebec, Canada: Intracellular calcium mediates heat shock induction of proteins and thermotoxicity but not thermotolerance.

Li, G.C., Dept. of Radiation Oncology, University of California, San Francisco: Possibility of using the 70-kD heat shock protein as a predictor of thermal response in tumors during fractionated hyperthermia.

Tomasovic, S.P.,¹ Updyke, T.V.,¹ Rodriguez, L.,² Armour, E.P.,³ Welch, D.R.,⁴ Depts. of ¹Tumor Biology, ²Pathology, ³Physics, University of Texas, M.D. Anderson Hospital and Tumor Institute, Houston; ⁴Cancer and Viral Diseases Research Unit, Upjohn Company, Kalamazoo, Michigan: Hyperthermic stress proteins of metastatic tumor cell subpopulations and syngeneic normal cells.

Burdon, R.H., Dept. of Bioscience and Biotechnology, Strathclyde University, Glasgow, Scotland: Modulation of protein synthesis, hsp gene expression, and thermotolerance in HeLa cells following heat shock.

Atkinson, B.G., Rodenhiser, D., Dean, R.L., Dept. of Zoology, University of Western Ontario, London, Canada: Localization and possible function for a heat shock protein in circulating (in vivo) and cultured (in vitro) blood cells from birds and mammals.

Hahn, G.M., Fisher, G., Tao, T.-W., Anderson, R., Calderwood, S.K., Dept. of Radiology, Stanford University School of Medicine, California: Are heat shock proteins necessary for heat resistance?

DNA Tumor Viruses: Control of Gene Expression and Replication

September 4 – September 8

ARRANGED BY

Michael Botchan, University of California

Terri Grodzicker, Cold Spring Harbor Laboratory

Phillip Sharp, Massachusetts Institute of Technology

190 participants

The DNA tumor viruses have served as model systems for the study of eukaryotic gene expression, DNA replication, and transformation. The third Cold Spring Harbor meeting on Cancer Cells brought together investigators who presented their latest findings in a variety of areas. The enormous growth of this field has



T. Grodzicker, B. Stillman, M. Botchan, Y. Gluzman



M. Fried, A. Smith



T. Grodzicker, R. Tijian, P. Sharp



P. Chambon



B. Sugden

led to the occurrence of specialized meetings covering the work on one or two viruses. However, the broader scope of Cancer Cells meeting included studies of the molecular biology and biochemistry of SV40, polyoma virus, adenoviruses, papilloma viruses, herpes simplex virus, and Epstein-Barr virus.

There was a great deal of progress in the development of *in vitro* systems that are now being used to dissect crucial processes such as DNA replication; RNA transcription and the interaction of proteins with promoter and enhancer elements; RNA processing, transcription termination, and poly(A) addition; and control of translation. The roles of viral *trans*-activating regulatory proteins, such as SV40 T antigen, adenovirus E1A proteins, herpesvirus immediate early proteins, and the bovine papilloma virus E2 protein, were emphasized. These proteins affect the transcriptional activity of viral and, in some cases, cellular genes, and at least for SV40 and adenoviruses, they are also transforming proteins.

During the Cancer Cell meeting, the new Sambrook Laboratory which adjoins James Laboratory was dedicated. This laboratory is named for Joe Sambrook, who over the years was responsible for stimulating much of the research on DNA tumor viruses at Cold Spring Harbor Laboratory. Joe has left his position as Assistant Director for Research to take up the chairmanship of the Biochemistry Department at the University of Texas Health Sciences Center at Dallas. The dedication remarks were given by Renato Dulbecco, and the meeting served as a



M. Yaniv, J.D. Watson



C. Anderson, R. Gesteland



U. Petterson, D. Klessig



H. Westphal, E. Winocour



B. Hirt

reunion for many of the scientists who had worked at Cold Spring Harbor and returned to present their latest results.

This meeting was supported in part by the Cold Spring Harbor Laboratory Cancer Center Grant from the National Cancer Institute, National Institutes of Health.

SESSION 1 RNA PROCESSING AND TRANSLATION

Chairperson: P. Sharp, Massachusetts Institute of Technology

Sharp, P.A., Padgett, R., Grabowski, P.J., Konarska, M.M., Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Splicing of messenger RNA precursors.

Perkins, K.K., Furneaux, H., Freyer, G.A., Arenas, J., Pick, L., Hurwitz, J., Memorial Sloan-Kettering Cancer Center, New York, New York: In vitro RNA splicing.

Solnick, D., Dept. of Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Connecticut: Pre-mRNA structural features that direct the selection of splice sites.

Manley, J.L., Noble, J., Chaudhuri, M., Ryner, L., Dept. of Biological Sciences, Columbia University, New York, New York: Processing of SV40 early pre-mRNA in vitro.

Falck-Pedersen, E.,¹ Logan, J.,² Shenk, T.,² Darnell, J.E., Jr.,³ ¹Dept. of Microbiology, Cornell Medical School, Ithaca, New York; ²Dept. of Molecular Biology, Princeton University, New Jersey; ³Dept. of Molecular and Cellular

Biology, Rockefeller University, New York, New York: Adenovirus as a model for the study of transcription termination and poly(A) site selection.

Williams, J.,¹ Karger, B.D.,¹ Ho, Y.S.,¹ Castiglia, C.L.,² Mann, T.,² Flint, S.J.,² ¹Dept. of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania; ²Dept. of Biochemical Sciences, Princeton University, New Jersey: The adenovirus E1B 495R protein is required for complete progression from the early to late phase of the viral growth cycle.

Pilder, S., Logan, J., Moore, M., Shenk, T., Dept. of Molecular Biology, Princeton University, New Jersey: The adenovirus E1B-55K polypeptide modulates transport or cytoplasmic stabilization of viral and host-cell mRNAs.

Mathews, M.B.,¹ O'Malley, B.,¹ Marino, T.M.,² Siekierka, J.,² ¹Cold Spring Harbor Laboratory, New York; ²Rochester Institute, Nutley, New Jersey: Translated control in adenovirus-infected cells.

SESSION 2 REGULATION OF TRANSCRIPTION

Chairperson: R. Gesteland, University of Utah

McKnight, J.L.C., Kristie, T.M., Mavromova-Nazos, P., Pellett, P.E., Silver, S., Roizman, B., Marjorie B. Kovler Viral Oncology Laboratories, University of Chicago, Illinois: Regulation of herpes simplex virus (HSV) genes in different chromosomal environments by *trans*-acting factors.

O'Hare, P., Mosca, J., Lieberman, P., Jeang, K.-T., Hayward, S.D., Hayward, G.S., Dept. of Pharmacology, Johns Hopkins University School of Medicine, Baltimore, Maryland:

Multiple *trans*-acting proteins involved in herpes virus early gene regulation.

Brady, J., Loeken, M., Khoury, G., NCI, National Institutes of Health, Bethesda, Maryland: *Trans*-activation of SV40 late and adenovirus E2 promoter.

Kovesdi, I., Reichel, R., Nevins, J., Rockefeller University, New York, New York: Protein interactions at an E1A-inducible promoter.



K. Dulbecco



J. Sambrook

Zajchowski, D., Boeuf, H., Jalinet, P., Goding, C., Devaux, B., Kedinger, C., LGME/CNRS, INSERM, Université de Strasbourg, France: Regulatory sequence elements in the adenovirus-2 early E1a promoter region.

Ziff, E., Cole, P., Kegler, D., Ross, D., Smith, D., Stein, R., Velcich, A., Kaplan Cancer Center and Dept. of Biochemistry, New York University Medical Center, New York, New York: Transcriptional control by the adenovirus-5 E1a proteins.

Fontana, M.R., Mora, M., Guilloyle, R., Rossini, M., Scavo Research Center, Siena, Italy: Identification of the DNA sequences in the adenovirus E2A late promoter responsive to E1A repression.

Spalholz, B.A., Yang, Y.-C., Rabson, M.S., Howley, P.M., NCI, National Institutes of Health, Bethesda, Maryland: transactivation of a BPV-1 transcriptional regulatory element by the E2 gene product.

Dedication of Joseph Sambrook Laboratory

Dedictory Remarks: **Renato Dulbecco**, Salk Institute

SESSION 3 TRANSFORMATION

Chairperson: T. Grodzicker, Cold Spring Harbor Laboratory

Glaichenhaus, N.,¹ Léopold, P.,¹ Masiakowski, P.,² Cuzin, F.,¹ ¹INSERM, Centre de Biochimie, Université de Nice; ²LGME/INSERM, Strasbourg, France: Changes in the expression of cellular genes in cells immortalized and transformed by polyoma virus.

Fried, M., Williams, T., Wilson, J., Norbury, C., Dept. of Tumor Virus Genetics, Imperial Cancer Research Fund, London, England: Organization and expression of polyoma virus DNA in transformed cells.

Vaessen, R.T.M.J.,¹ Jochemsen, A.G.,¹ Bernards, R.,¹ Israël, A.,² Oostra, B.A.,¹ van der Eb, A.J.,¹ ¹Dept. of Medical Biochemistry, Sylvius Laboratories, Leiden, The Netherlands; ²Unité de Biologie Moléculaire du gène, Institut Pasteur, Paris, France: Regulation of class I MHC expression in adenovirus-transformed cells.

Franza, R.,¹ Maruyama, K.,² Garrels, J.,¹ Ruley, E.,² ¹Cold Spring Harbor Laboratory, New York; ²Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: E1A enables REF52 cells to synthesize and stably respond to high levels of T24 Ha-ras p21.

Quinlan, M., Grodzicker, T., Cold Spring Harbor Laboratory,

New York: Production of a cell proliferation factor by baby rat kidney cells infected with AD5 12S virus.

Gallimore, P.H.,¹ Williams, J.,² Breiding, D.,² Grand, R.J.A.,¹ Byrd, P.J.,¹ ¹Dept. of Cancer Studies, University of Birmingham, England; ²Dept. of Biological Sciences, Carnegie-Mellon Institute, Pittsburgh, Pennsylvania: Studies on adenovirus E1 region—Gene expression, transformation of human and rodent cells, and malignancy.

Durham, D., McDougall, J.K., Dept. of Experimental Pathology, Fred Hutchinson Cancer Research Center, Seattle, Washington: Interactions of AD12 E1 with genes mapping in the q21-22 region of human chromosome 17.

Galloway, D.A., Buonaguro, F., Brandt, C., McDougall, J.K., Fred Hutchinson Cancer Research Center, Seattle, Washington: HSV and CMV—Unconventional DNA tumor viruses.

Kelly, F., Babinet, C.,² Mechali, F.,¹ Kellermann, O.,² ¹Institut de Recherches Scientifiques sur le Cancer, Villejuif; ²Institut Pasteur, Paris, France: Expression of SV40 oncogenes in F9 embryonal carcinoma cells, transgenic embryos, and transgenic mice.

SESSION 4 TRANSCRIPTION

Chairperson: R. Tjian, University of California, Berkeley

Tjian, R., Kadonaga, J., Jones, K., Briggs, M., Dept. of Biochemistry, University of California, Berkeley: Activation of

viral and cellular transcription by promoter-specific DNA-binding proteins.

Chambon, P., Takahashi, K., Zenke, M., Wildeman, A., Barrera, H., Schatz, C., Vigneron, M., Matthes, H., Winterlth, M., Pauly, M., Grundström, T., LGME/CNRS, INSERM, Strasbourg, France: In vivo and in vitro dissection of the SV40 early promoter.

Herr, W., Clarke, J., Ondek, B., Cold Spring Harbor Laboratory, New York: Multiple elements within the SV40 enhancer can functionally compensate for one another.

Piette, J., Cereghini, S., Kryszke, M.H., Yaniv, M., Dept. of Molecular Biology, Institut Pasteur, Paris, France: Identification of cellular proteins that interact with polyoma or SV40 enhancers.

Kern, F.G., Pellegrini, S., Basilio, C., Dept. of Pathology, New York University School of Medicine, New York: Regulation of the expression of integrated polyoma genomes.

SESSION 5 REPLICATION I

Chairperson: B. Hirt, Swiss Institute for Experimental Cancer Research

Li, J., Peden, K., Kelly, T., Dept. of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, Maryland: Replication of SV40 DNA.

Stillman, B., Dittley, J., Prelich, G., Guggenheimer, R.A., Cold Spring Harbor Laboratory, New York: Replication of adenovirus and SV40 DNA in cell-free extracts from human cells.

Murakami, Y., Wobbe, R., Weissbach, L., Dean, F., Hurwitz, J., Memorial Sloan-Kettering Cancer Center, New York, New York: In vitro replication of the DNA containing the SV40 origin.

Gluzman, Y., Gerard, R., Manos, M., McVey, D., Harper, J., Cold Spring Harbor Laboratory, New York: Properties of the SV40 T antigen produced in different expression systems.

Schneider, R., Dörper, T., Gander, I., Müller, U., Winnacker,

Prives, C., Covey, L., Michaeli, T., Dept. of Biological Sciences, Columbia University, New York, New York: SV40 gene expression in natural and unnatural host cells.

Flint, S.J., Postel, E.H., Leong, K., Dept. of Molecular Biology, Princeton University, New Jersey: Enhanced transcriptional activity of extracts of adenovirus-infected HeLa cells.

Sippel, A.E.,¹ Nowock, J.,¹ Theisen, M.,¹ Borgmeyer, U.,¹ Strech-Jurk, U.,¹ Fritton, H.P.,² Igo-Kemenes, T.,² ¹Zentrum für Molekulare Biologie der Universität Heidelberg; ²Institut für Physiologische Chemie, München, Federal Republic of Germany: DNA elements and DNA-binding proteins involved in the cell- and stage-specific expression of the chicken lysozyme gene.

E.L., Institut für Biochemie, Universität München, Federal Republic of Germany: A novel assay for DNA-binding proteins as exemplified by a functional analysis of the invertebrate terminal repetition of adenovirus DNA.

Klessig, D.F., Cleghon, V., Brough, D., Rice, S., Voelkerding, K., Dept. of Cellular, Viral and Molecular Biology, University of Utah Medical School, Salt Lake City: Studies on the adenovirus DNA binding protein.

Stow, N.D., Murray, M.D., Stow, E.C., MRC Virology Unit, Institute of Virology, Glasgow, Scotland: *cis*-acting signals involved in the replication and packaging of herpes simplex virus type-1 DNA.

Winocour, E., Dorsett, D., Vernade, D., Chitlaro, T., Dept. of Virology, Weizmann Institute of Science, Rehovot, Israel: Nonhomologous recombination in SV40-infected monkey cells—Replicative and nonreplicative pathways.

SESSION 6 TRANSFORMING PROTEINS

Chairperson: M. Botchan, University of California, Berkeley

Benjamin, T.L., Dept. of Pathology, Harvard Medical School, Boston, Massachusetts: Phosphorylations of polyoma virus middle T antigen and VP1.

Smith, A.E.,¹ Roberts, B.,¹ Cheng, S.,¹ Markland, W.,¹ Harvey, R.,¹ Paucha, E.,² Calderon, D.,³ ¹Integrated Genetics, Framingham, Massachusetts; ²Dept. of Biochemistry, Boston, Massachusetts; ³Dana-Farber Cancer Institute, Boston, Massachusetts.

Lane, D.P., Anton, I., Micklem, G., Mole, S., Simanis, V., Dept. of Biochemistry, Imperial College, London, England: Structure function studies on SV40 large T and the T-p53 complex.

Anderson, C.W.,¹ Samad, A.,² Carroll, R.B.,² ¹Dept. of Biology, Brookhaven National Laboratory, Upton, New York; ²Dept. of Pathology, New York University Medical Center, New York: Identification and characterization of sites phosphorylated in the cellular tumor antigen, p53.

Bikel, I.,¹ Agha, M.,¹ Brown, G.,² Boltax, J.,¹ Mamon, H.,¹ Brown, M.,¹ Livingston, D.M.,¹ ¹Dana-Farber Cancer

Center and Harvard Medical School, Boston; ²Genetics Institute, Cambridge, Massachusetts: Genetic and biological analyses of SV40 small t function.

Westphal, H.,¹ Krippel, B.,² Ferguson, B.,² Andriani, O.,³ Jones, N.,³ Rosenberg, M.,² ¹NICHHD, National Institutes of Health, Bethesda, Maryland; ²Dept. of Molecular Genetics, Smith Kline and French Laboratories, Philadelphia, Pennsylvania; ³Dept. of Biological Sciences, Purdue University, West Lafayette, Indiana: Mapping of domains for nuclear localization and for gene activation in adenovirus E1A proteins.

Harlow, E., Stephens, C., Whyte, P., Franza, R., Schley, C., Cold Spring Harbor Laboratory, New York: Analysis of the adenovirus E1A proteins using monoclonal antibodies.

Dunn, A.,¹ Gough, N.,¹ Stanley, E.,¹ Gonda, T.,¹ Lang, R.,¹ Metcalf, D.,² ¹Ludwig Institute for Cancer Research, Melbourne; ²Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia: Hemopoietic growth-factor gene sequences and their role in growth control.

SESSION 7 REPLICATION II

Chairperson: B. Sugden, University of Wisconsin

Countryman, J.K., Jenson, H., Grogan, E., Miller, G., Yale University School of Medicine, New Haven, Connecticut: A 2.7-kbp, rearranged EBV DNA fragment capable of disruption of latency.

Reisman, D., Yates, J., Warren, N., Sugden, B., McArdle Laboratory, University of Wisconsin, Madison: cis- and trans-acting transcriptional regulation associated with an Epstein-Barr virus-derived plasmid replicon.

Rawlins, D.R.,^{1,4} Rosenfeld, P.J.,¹ Kelly, T.J., Jr.,¹ Milman, G.,³ Shero, J.,² Hayward, S.D.,² Hayward, G.S.,² Depts. of ¹Molecular Biology and Genetics, ²Pharmacology, Johns Hopkins University School of Medicine, ³Dept. of Biochemistry, Johns Hopkins University School of Hygiene, Baltimore, Maryland; ⁴Dept. of Microbiology and Immunology, Emory University School of Medicine, Atlanta, Georgia: Sequence-specific interactions of nuclear proteins with herpes virus DNAs.

Lupton, S., Frey, A.B., Levine, A.J., Dept. of Molecular Biology, Princeton University, New Jersey: EBV-derived plasmid maintenance in EBV-positive and -negative cells.

Botchan, M., Berg, L., Naruto, M., Singh, K., Stenlund, A., Reynolds, J., Lusky, M., Dept. of Molecular Biology, University of California, Berkeley: Regulation of the BPV-1 plasmid DNA replication.

Roberts, J.M., Weintraub, H., Fred Hutchinson Cancer Research Center, Seattle, Washington: Copy number control of DNA replication.

Hassell, J.A., Muller, W.J., Mueller, C.R., Dept. of Microbiology and Immunology, McGill University, Montreal, Canada: The dual role of the polyoma virus enhancer in transcription and DNA replication.

SESSION 8 PAPILLOMA VIRUSES: TRANSFORMATION AND GENE EXPRESSION

Chairperson: U. Pettersson, University of Uppsala

Schiller, J., Androphy, E., Lowy, D., NCI, National Institutes of Health, Bethesda, Maryland: Analysis of bovine papilloma virus transformation.

Alpert, S., Lacy, M., Hanahan, D., Cold Spring Harbor Laboratory, New York: Bovine papilloma virus genome in transgenic mice.

Schwarz, E., Schneider-Gädicke, A., Institute of Virus Research, German Research Center, Heidelberg, Federal Republic of Germany: Expression of human papilloma virus type-18 DNA in cervical carcinoma cell lines.

Broker, T.R., Wolinsky, S.T., Chow, L.T., Dept. of Biochemistry, University of Rochester School of Medicine and Den-

istry, New York: Human genital papilloma virus types 6, 11, 16, 18 and 31—Specific genomic probes based on electron microscopic maps of DNA heteroduplexes.

Pettersson, U., Dept. of Medical Genetics, The Biomedical Center, Uppsala, Sweden: Plasmids from the bovine papilloma virus genome.

Chow, L.T., Pelletier, A.J., Brinckmann, U., Cheng, S., Broker, T.R., Dept. of Biochemistry, University of Rochester School of Medicine and Dentistry, New York: Electron microscopic heteroduplex analysis of families of human papilloma virus mRNAs derived from heat-shock and SV40 expression vectors.

Modern Approaches to Vaccines

September 11 – September 15

ARRANGED BY

Robert Chanock, NIAID, National Institutes of Health

Richard Lerner, Research Institute of Scripps Clinic

Fred Brown, Wellcome Biotechnology Ltd.

259 participants

The increase in the number of participants at the highly successful third meeting on Modern Approaches to Vaccines underscored the great interest in this topic. As in 1984, the program included papers on viruses, pathogenic bacteria, and parasites that cause important diseases. There were, however, two important additions to the program. First, a complete session was devoted to a single dis-

ease, AIDS. With one of the greatest microbiological problems of this century confronting scientists at this time, it was considered opportune to discuss the prospects for a vaccine against this disease. Much remains to be done on the structure of the virus itself, despite the intensive effort that has been made in many laboratories since the causative agent was identified. Clearly, there is still a need to obtain information about the antigenic sites on the viral envelope glycoprotein that are involved in protection and to determine the extent of antigenic variation in these sites among a wide variety of field isolates.

The second session included this year was on immunology. To achieve optimal effectiveness of new vaccines, it is necessary to reach a better understanding of the immunological responses that provide maximal resistance to infection and disease. The session was extremely valuable because it drew attention, quite forcibly at times, to how little is known about these aspects of the immune response. The messages from the immunologists provided a salutary lesson to those who think that the provision of an antigen in sufficient amounts is sufficient for a new vaccine.

The meeting was held within two weeks of the deaths of three Nobel Laureates, MacFarlane Burnet, John Enders, and Rodney Porter, whose contributions to the fields of virology and immunology laid the foundations of much of the work described in 1985. This meeting was dedicated to their memory.

This meeting was supported in part by the NIH-NIAID Intramural Research Program, and the Wellcome Biotechnology, Ltd.

Introduction: E. Norrby, Karolinska Institute, Stockholm, Sweden: Thoughts on the occasion of the solution of the three-dimensional structure of poliovirus.
J.M. Hogle, Research Institute of Scripps Clinic, La Jolla, California: High-resolution structure of the Mahoney strain of type-1 poliovirus.

SESSION 1 STRUCTURAL AND CHEMICAL ASPECTS OF ANTIGEN-ANTIBODY UNION I

Wright, P.E., Dyson, H.J., Houghten, R.A., Ostresh, J., Wilson, I.A., Lerner, R.A., Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California: Synthetic immunogenic peptides can adopt highly preferred conformations in water solution.

Houghten, R.A., Hoffmann, S.R., Niman, H.L., Dept. of Molecular Biology, Scripps Clinic and Research Foundation, La Jolla, California: Simultaneous multiple solid-phase peptide synthesis.

Reeke, G.N., Jr., Becker, J.W., Rockefeller University, New York, New York: Three-dimensional structure of β_2 -microglobulin and its relationships to immunoglobulins and the MHC.

Wilson, I.A., Research Institute of Scripps Clinic, La Jolla, California: Structural analysis of antipeptide antibodies against influenza virus hemagglutinin.

Stevens, V.C., Ohio State University, Columbus: Use of synthetic peptides as immunogens for developing a vaccine against human chorionic gonadotropin.

Ouldridge, E.J.,¹ Parry, N.R.,¹ Rowlands, D.J.,¹ Brown, F.,¹ Bittle, J.L.,² Houghten, R.A.,² Lerner, R.A.,² ¹Wellcome Biotechnology Limited, Pirbright, England; ²Scripps Clinic and Research Institute, La Jolla, California: Comparison of the structure of the major antigenic site of FMDV of two different serotypes.

SESSION 2 STRUCTURAL AND CHEMICAL ASPECTS OF ANTIGEN-ANTIBODY UNION

Chairperson: R. Lerner, Research Institute of Scripps Clinic

Ouldridge, E.J., Parry, N.R., Rowlands, D.J., Brown, F., Wellcome Biotechnology Ltd., Pirbright, England: FMDV variants with broad and narrow antigenic spectra.

Edmundson, A.B., Ely, K.R., Herron, J.N., Dept. of Biology, University of Utah, Salt Lake City: 3-D analyses of the binding of chemotactic and opioid peptides to the MCG light chain dimer.

Heber-Katz, E., Watari, E., Dietzschold, B., Wistar Institute, Philadelphia, Pennsylvania: Long-term protection from a lethal HSV challenge in the absence of a detectable antibody response.

Heinz, F.X., Winkler, G., Tuma, W., Mandl, C., Kunz, C., Institute of Virology, University of Vienna, Austria: Epitope mapping of a flavivirus glycoprotein—Complex network of



E. Norrby, J. Berzofsky

synergistic interactions between antibodies to structurally distinct domains.

- Minden, P., Houghten, R., Spear, J., Shinnick, T., Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California: Evaluation of a chemically synthesized peptide that has mycobacterial antigenic properties.
- Weijer, W.J.,¹ Kerling, K.E.T.,² Hoogerhout, P.,² Bloemhoff, W.,² Geerlings, H.J.,¹ Garretsen, H.,¹ Wilterdink, J.B.,¹ Popken-Boer, T.,¹ Slopsema, K.,¹ Welling, G.W.,¹ Welling-Wester, S.,¹ ¹Laboratorium voor Medische Microbiologie, Groningen; ²Laboratorium voor Organische Chemie, Leiden, The Netherlands: Generation and action of anti-peptide antibodies against HSV-1.
- Winther, M., Bomford, R., Allen, G., Brown, F., Wellcome Research Laboratories, Beckenham, Kent, England: Use of bacterial fusion proteins to promote immunity to small peptides.
- Kennedy, R.C., Eichberg, J.W., Dreesman, G.R., Southwest Foundation for Biomedical Research, Dept. of Virology and Immunology, San Antonio, Texas: Internal image anti-idiotypic antibodies as a vaccine for HBV.

SESSION 3 ANTIGEN PRESENTATION AND PROCESSING

Chairperson: G. Ada, Australian National University

Ada, G.L., Dept. of Microbiology, Australian National University, Canberra: Antigen presentation and enhancement of immunity—An introduction.

Allen, P., Babbitt, B., Unanue, E., Dept. of Pathology, Washington University School of Medicine, St. Louis, Missouri: Molecular events in antigen processing and presentation.

Inaba, K.,¹ Steinman, R.M.,² ¹Dept. of Zoology, Kyoto University, Japan; ²Rockefeller University, New York, New York: Function of dendritic cells in antibody responses.

Petrarca, M.,¹ Reiss, C.S.,¹ Faller, D.V.,¹ Burakoff, S.J.,¹ Huang, A.,² ¹Division of Pediatric Oncology, Dana-Farber Cancer Institute, Boston; ²Children's Hospital Medical Center, Harvard Medical School, Boston, Massachusetts:

Specificity of the IA-restricted helper-T-cell response to VSV.

Berzofsky, J.A., NCI, National Institutes of Health, Bethesda, Maryland: Antigenic structural requirements for MHC-restricted helper-T-cell recognition.

Cebra, J., Fuhrman, J., Lebman, D., London, S., Dept. of Biology, University of Pennsylvania, Philadelphia: Effective gut mucosal stimulation of IgA-committed B cells by antigen—A correlate of in vivo priming for a sIgA response.

Waldmann, T., NCI, National Institutes of Health, Bethesda, Maryland: Strategies for immunopotentialion.

SESSION 4 MALARIA

Chairperson: L.H. Miller, National Institutes of Health

Nussenzweig, R.S.,¹ Zavala, F.,¹ Nussenzweig, V.,² Depts. of ¹Medical and Molecular Parasitology, ²Pathology, New York University Medical Center, New York: Tandem repeats of the circumsporozoite protein as basis for malaria vaccine development.

Hockmeyer, W.T.,¹ Young, J.F.,² ¹Walter Reed Army Institute of Research, Washington, D.C.; ²Smith, Kline and French Laboratories, Philadelphia, Pennsylvania: Recent efforts in the development of a sporozoite malaria vaccine against *P. falciparum*.

Kumar, N., Carter, R., LPD, NIAID, National Institutes of Health, Bethesda, Maryland: Transmission-blocking im-

munity in malaria—Identification and characterization of interactions between target antigens.

Langford, C.J.,¹ Edwards, S.,² Corcoran, L.M.,¹ McIntyre, P.,¹ Moss, B.,³ Mitchell, G.F.,¹ Anders, R.F.,¹ Kemp, D.,¹ ¹Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia; ²Commonwealth Serum Laboratories, Melbourne, Australia; ³NIAID, National Institutes of Health, Bethesda, Maryland: Antigens of the asexual blood stages of *P. falciparum* and their expression in recombinant vaccinia virus.

Perlmann, P.,¹ Berzins, K.,¹ Björkman, A.,² Carlsson, J.,¹ Lundgren, K.,¹ Nguyen-Dinh, P.,^{1,3} Perlmann, H.,¹ Patar-

royo, E.,⁴ Troye-Blomberg, M.,¹ Udomsangpetch, R.,¹ Wahlgren, M.,^{1,2} Wahlin, B.,¹ ¹Dept. of Immunology, University of Stockholm, Sweden; ²Dept. of Infectious Diseases, Karolinska Institutet, Roslagstull Hospital, Stockholm, Sweden; ³Center for Disease Control, Atlanta, Georgia; ⁴Dept. of Immunobiology, National University of Colombia, Bogota: Characterization of the human immune response to Pf 155, a major antigen of the malaria parasite *P. falciparum*.

SESSION 5 MALARIA AND BACTERIAL VACCINES

Chairperson: K. Warren, Rockefeller Foundation

Cheng, K.-C.,¹ Smith, G.L.,¹ Nussenzweig, V.,² Nussenzweig, R.S.,³ Moss, B.,¹ ¹Laboratory of Viral Diseases, NIAID; ²Depts. of Biochemistry and ³Microbiology, New York University Medical Center, New York, New York: Expression of *P. falciparum* sporozoite surface antigen by infectious recombinant virus.

Wahlgren, M.,^{1,2} Berzins, K.,¹ Wåhlin, B.,¹ Perlmann, H.,¹ Carlsson, J.,¹ Åslund, L.,⁴ Franzén, L.,^{3,4} Ruangjirachuporn, V.,¹ Björkman, A.,² Pettersson, U.,⁴ Wigzell, H.,³ Perlmann, P.,¹ ¹Dept. of Immunology, University of Stockholm; Depts. of ²Infectious Diseases, ³Immunology, Karolinska Institutet, Roslagstull Hospital, Stockholm; ⁴Dept. of Medical Genetics, University of Uppsala, Sweden: A repeated octapeptide of Pf 155, a possible candidate for a synthetic vaccine against *P. falciparum* malaria.

Flint, J.E., Ardeshir, F., Richman, S., Reese, R.T. Dept. of Immunology, Research Institute of Scripps Clinic, La Jolla,

Perkins, M.E., Ravetch, J.V. Rockefeller University and Memorial Sloan-Kettering Cancer Center, New York, New York: Characterization of glycoprotein-binding proteins of *P. falciparum* merozoites.

Klotz, F.W., Hudson, D.E., Miller, L.H., LPD, NIAID, National Institutes of Health, Bethesda, Maryland: Effect of immunity to the 140-kD merozoite protein on antigen expression in chronic *P. knowlesi* malaria infections.

California: Surface antigens of *P. falciparum* merozoites cloned and expressed in *E. coli*.

Hale, T.L., Dinari, G., Oaks, E.V., Formal, S.B., Dept. of Bacterial Diseases, Walter Reed Army Institute of Research, Washington, D.C.: Immune response to *Shigella* infection and to *Shigella* vaccines.

Hewlett, E.L., Myers, G.A., Weiss, A.A., Depts. of Medicine and Pharmacology, University of Virginia School of Medicine, Charlottesville: Immunization against pertussis—Contributions of pathophysiology to vaccine development.

Steinman, L., Dept. of Pediatrics, Stanford University School of Medicine, California: Pertussis neurotoxin.

Schoolnik, G.K., O'Hanley, P., Lark, D., Schmidt, M.A., Stanford University, California: P-fimbriae—Uropathogenic role, chemical structure, antigenic topography, and vaccine efficacy.

SESSION 6 POSTER SESSION

Anderson, C.W.,¹ Samad, A.,² Carroll, R.B.,² ¹Dept. of Biology, Brookhaven National Laboratory Upton; ²Dept. of Pathology, New York University Medical Center, New York: Identification and characterization of sites phosphorylated in the cellular tumor antigen, p53.

Arora, D.J.S., Justewicz, D.M., Institut Armand-Frappier, University of Quebec, Laval, Canada: Presence of an interferon-independent mechanism of cell-mediated cytotoxicity stimulation.

Baxt, B.,¹ Morgan, D.O.,² Depts. of ¹Molecular Biology and ²Immunology, Plum Island Animal Disease Center, Greenport, New York: Foot-and-mouth disease virion-reactive antibodies elicited with anti-idiotypic antibodies.

Bittle, J.,¹ Parks, E.,¹ Francis, M.,² Brown, F.,² ¹Research Institute of Scripps Clinic, La Jolla, California; ²Wellcome Biotechnology Limited, Pirbright, England: T-cell proliferation response to FMDV type O₁ (Kaufbeuren) defined by synthetic peptides from VP1.

Blondel, B., Crainic, R., Horaud, F., Unité de Virologie Médicale, Institut Pasteur, Paris, France: Structural and functional control of an immunodominant neutralization antigenic site of poliovirus type 1 (amino acids 93 through 103 of the capsid protein VP1).

Bolwell, C., Parry, N.R., Rowlands, D.J., Brown, F., Ouldridge, E.J., Wellcome Biotechnology Limited, Pirbright, England: FMDV variants with broad and narrow antigenic spectra.

Burnette, W.N., Mar, V.L., Amgen, Thousand Oaks, California: Structural analysis of infectious bursal disease virus.

Chenciner, N.,¹ Delpeyroux, F.,¹ Lim, A.,¹ Lambert, M.,¹ Malpièce, Y.,¹ Streeck, R.E.,¹ Houssais, J.F.,² ¹Groupement de Génie Génétique, Institut Pasteur, Paris; ²CNRS, Institut Curie, Orsay, France: Effect of cell fusion with primary adult hepatocytes on the expression of the HBsAg.

Delpeyroux, F.,¹ Chenciner, N.,¹ Lim, A.,² Malpièce, Y.,¹ Blondel, B.,² Van der Werf, S.,³ Streeck, R.E.,¹ ¹Groupement de Génie Génétique, Institut Pasteur, Paris; ²Unité de Virologie Médicale; ³Unité de Virologie Moléculaire, Institut Pasteur, Paris, France: HBsAg particles carrying the sequence of a major poliovirus epitope.

De Wilde, M.,^{1,2} Harford, N.,^{1,2} Cabezon, T.,^{1,2} Rutgers, A.,^{1,2} Rosenberg, M.,² Van Wijnendaële, F.,¹ Roelants, P.,¹ Boon, B.,² Conrath, K.,¹ De Neys, R.,¹ Simoen, E.,¹ Hauser, P.,¹ Wasserman, G.,² Jeffs, P.,² Salary, A.,¹ André, F.,¹ Stephenne, J.,¹ ¹Smith-Kline-RIT, Rixensart,

- Belgium; ²Smith, Kline & French Laboratories, Sweden, Pennsylvania: Human hepatitis-B vaccine produced in yeast.
- Esposito, J.,¹ Brechling, K.,² Shaddock, J.,¹ Hoatlin, M.,³ Moss, B.,² Baer, G.,¹ ¹Centers for Disease Control, Atlanta, Georgia; ²National Institutes of Health, Bethesda, Maryland; ³Genetech, Inc., South San Francisco, California: Vaccinia virus recombinants expressing challenge virus standard rabies virus glycoprotein protects against rabies.
- Ferguson, M., Minor, P.D., Evans, D.M.A., Reed, S., Schild, G.C., National Institute for Biological Standards and Control, London, England: Reactivity of monoclonal antibodies with type-3 poliovirus and synthetic peptides containing the amino acid sequence of an immunodominant antigenic site.
- González, A., Blasco, R., Almendral, J.M., Calvo, V., Agüero, M., Talavera, A., Viñuela, E., Centro de Biología Molecular, Universidad Autónoma, Madrid, Spain: Structure and variation of ASFV DNA.
- Gould, E.A., Buckley, A., Cammack, N., Barrett, A., London School of Hygiene and Tropical Medicine, Arbovirus Research Unit, St. Albans, England: Yellow fever virus vaccines analyzed using monoclonal antibodies.
- Guiso, N.,¹ Ladant, D.,¹ Brézin, C.,² Perrin, D.,³ ¹Unité de Biochimie des Régulations Cellulaires; ²Unité d'Écologie Bactérienne; ³Unité de Programmation Moléculaire et Toxicologie Génétique, Institut Pasteur, Paris, France: Extracytoplasmic adenylate cyclase of *B. pertussis*—Regulatory aspects of its synthesis.
- Halling, S.M., Molecular Genetics, Inc., Minnetonka, Minnesota: Cloning and expression of gII pseudorabies virus epitopes in *E. coli*.
- Hosmalin, A.,¹ Carelli, C.,¹ Audibert, F.,¹ Chedid, L.,¹ Drobecq, H.,² Bernard, J.M.,³ Gras-Masse, H.,² Tartar, A.,² Lefrancier, P.,³ ¹Immunothérapie expérimentale, Institut Pasteur, Paris; ²Faculté de Pharmacie, Lille; ³Institute Choay, Paris, France: Synthesis of analytically pure LH-RH-Lys-MDP conjugates—A 2000-dalton synthetic vaccine.
- Hu, N.-T., Tseng, Y.-H., Wang, J.-T., Wang, J.-C., Chang, C.-S., Hwang, C.-J., National Chung Hsing University, Taiwan, Republic of China: Molecular approach to the vaccine against pseudorabies virus.
- Justewicz, D.M., Arora, D.J.S., Institut Armand-Frappier, University of Quebec, Canada: Influenza virus neuraminidases probably share the same cell-mediated cytotoxicity stimulation site(s).
- Kabir, S., International Centre For Diarrhoeal Disease Research, Dhaka, Bangladesh: Preparation and immunogenicity of a semisynthetic bivalent cell-surface protein-polysaccharide conjugate of *V. cholerae*.
- Katz, J.B., Van Deusen, R.A., National Veterinary Services Laboratories, Ames, Iowa: Radioimmunoassay of adjuvant-associated porcine parvovirus using a monoclonal antibody in a nitrocellulose membrane system.
- Luciw, P.A.,¹ Barr, P.J.,¹ Gibson, H.,¹ Lee-Ng, C.T.,¹ Power, M.D.,¹ Nussenzweig, V.,² Valenzuela, P.,¹ ¹Chiron Corporation, Emeryville, California; ²New York University, New York: Antigen presentation by way of HBsAg fusion proteins containing heterologous viral and parasite antigens.
- Mason, T.J., Geysen, H.M., Rodda, S.J., Dept. of Molecular Immunology, Commonwealth Serum Laboratories, Victoria, Australia: Diversity of the antibody response.
- Meloan, R.H., Puyk, W.C., Meier, D.J.A., Lankhof, H., Posthumus, W.P.A., Schaaper, W.M., Central Veterinary Institute, Lelystad, The Netherlands: Characterization of neutralizing epitopes of FMDV.
- Motz, M.,¹ Fan, J.,¹ Zeng, Y.,² Wolf, H.,¹ ¹Max von Pettenkofer-Institute, Munich, Federal Republic of Germany; ²Institute of Virology, Beijing, Republic of China: Expression of the EBV early protein p138 in *E. coli* for use as antigen in diagnostic tests.
- Newton, S.E.,¹ Mackett, M.,² Appleyard, G.,¹ Brown, F.,¹ ¹Wellcome Biotechnology, Pirbright; ²Christie Hospital and Holt Radium Institute, Manchester, England: Expression of a FMDV immunogenic site sequence in vaccinia virus.
- Nunberg, J.H., Gilbert, J.H., Cetus Corporation, Emeryville, California: Live, recombinant vaccinia virus encoding expression of the FeLV envelope protein.
- Ozaki, L.S.,¹ Mattei, D.,¹ Jendoubi, M.,¹ Druhlé, P.,² Blisnick, T.,¹ Guillotte, M.,¹ Pujalon, O.,¹ Pereira da Silva, L.,¹ ¹Institut Pasteur, Unité de Parasitologie Expérimentale; ²Hôpital de la Pitié-Salpêtrière, Paris, France: A quick method for identification of recombinant phage clones with monospecific antibodies purified directly on a lawn of plaques of the phage clone—Analysis of phage clones positive to sera against *P. falciparum* antigens.
- Plaff, E.,¹ Thiel, H.J.,² Beck, E.,¹ Kuhn, C.,¹ Leban, J.,³ Strohmaier, K.,² Schaller, H.,¹ ¹Dept. of Microbiology, ZMBH, University of Heidelberg; ²Federal Research Institute of Animal Diseases, Tübingen, Federal Republic of Germany; ³Biogen, Cambridge, Massachusetts: Studies on FMDV neutralization.
- Ramasamy, R., Reese, R.T., Dept. of Immunology, Scripps Clinic and Research Foundation, La Jolla, California: Role of carbohydrates in *P. falciparum* glycoproteins.
- Reiser, J., Germanier, R., Swiss Serum and Vaccine Institute, Berne, Switzerland: *B. pertussis* toxin and filamentous hemagglutinin genes.
- Southern, P.J., Buchmeier, M.J., Parekh, B., Singh, M.K., Francis, S.J., Oldstone, M.B.A., Dept. of Immunology, Scripps Clinic and Research Foundation, La Jolla, California: LCMV—A model system for human arenaviruses.
- Stave, J.W., Dalsgaard, K., Morgan, D.O., Moore, D.M., Plum Island Animal Disease Center, Greenport, New York: A neutralization epitope of FMDV type O₁ present on water-soluble complexes of VP1.
- Sulzinski, M.A., Wilson, M.R., Chan, A., Ubertaini, T.R., Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York: Partial sequence characterization of excreted poliovirus RNA after primary administration of oral poliovirus vaccine.
- Timmins, J.G., Marchioli, C.C., Thomsen, D.R., Yancey, R.J., Post, L.E., Upjohn Company, Kalamazoo, Michigan: Recombinant HSVs expressing pseudorabies virus glycoprotein genes.
- Welling, G.W., Weijer, W.J., van der Zee, R., Welling-Wester, S., Laboratorium voor Medische Microbiologie, Rijksuniversiteit Groningen, The Netherlands: Prediction of sequential antigenic regions in proteins.
- Wisher, M.H., Houghton Poultry Research Station, Huntingdon, England: Identification of the antigens of *Eimeria tenella*.

SESSION 7 BACTERIAL VACCINES AND PATHOGENESIS OF VIRAL DISEASE

Chairperson: M. Oldstone, Research Institute of Scripps Clinic

Oldstone, M.B.A., Dept. of Immunology, Scripps Clinic and Research Foundation, La Jolla, California: Clearance of infectious virus and viral nucleic acid sequences from persistently infected mice using immunotherapy.

Fields, P.I., Hefron, F.L., Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California: Mutants of the intracellular pathogen *S. typhimurium* that are attenuated for survival within phagocytic cells.

Maskell, D.J.,¹ Hormaeche, C.,² Sweeney, K.J.,¹ Dougan, G.,¹ ¹Wellcome Biotechnology Limited, Beckenham; ²Dept. of Pathology, University of Cambridge, England: Immune response in mice to foreign antigens carried in Aro A- *S. typhimurium*.

Patarroyo, M.E., Parra, C., Pinilla, C., del Portillo, P., Losada, D., Oramas, M.P., Torres, M.L., Clavijo, P., Ramirez, C., Fajardo, N., Cruz, N., Jiménez, C., Instituto de Immunología, Universidad Nacional, Bogota, Columbia: Immunogenic synthetic peptides against *M. tuberculosis*.

Fujinami, R.S., Oldstone, M.B.A., Dept. of Immunology, Scripps Clinic and Research Foundation, La Jolla, California: Amino acid homology and immune responses be-

tween the encephalogenic site of myelin basic protein and viruses—A mechanism for autoimmunity.

DeBorde, D.C.,¹ Donabedian, A.M.,¹ Naeve, C.W.,² Maassab, H.F.,¹ ¹Dept. of Epidemiology, School of Public Health, University of Michigan, Ann Arbor; ²St. Jude Children's Research Hospital, Memphis, Tennessee: Biological characterization and sequence analysis of type-B cold-reassortant genes in vaccine development.

Prince, G.A.,¹ Murphy, B.R.,¹ Walsh, E.E.,² Chanock, R.M.,¹ ¹LID, NIAID, National Institutes of Health, Bethesda, Maryland; ²University of Rochester School of Medicine, New York: Pulmonary pathology caused by RS virus infection in the cotton rat is enhanced by prior intramuscular inoculation of formalin-inactivated RS virus.

Ofit, P.,¹ Shaw, R.,² Blavat, G.,¹ Greenberg, H.,² ¹Wistar Institute of Anatomy and Biology and Children's Hospital of Philadelphia, Pennsylvania; ²Stanford University Medical School, Palo Alto, California: Passive protection against rotavirus-induced gastroenteritis is mediated by antibodies to VP3 and VP7.

SESSION 8 VIRAL VECTORS: BACTERIAL AND YEAST PLASMID VECTORS

Chairperson: W. Joklik, Duke University Medical Center

Bennett, D.D.,¹ Wright, S.E.,^{1,2} ¹Virology Oncology Laboratory, Veterans Administration Medical Center, ²Depts. of Medicine and Cellular, Viral and Molecular Biology, University of Utah School of Medicine, Salt Lake City: Avian retroviral recombinant expressing foreign envelope delays tumor formation of ASV-A-induced sarcoma.

Davis, A.R., Kostek, B., Mason, B.B., Hsiao, C.L., Morin, J., Dheer, S.K., Hung, P.P., Wyeth Laboratories, Microbiology Division, Philadelphia, Pennsylvania: Expression of HBsAg with a recombinant adenovirus.

Chakrabarti, S., Moss, B., NIAID, National Institutes of Health, Bethesda, Maryland: New vaccinia virus expression vectors.

Mackett, M., Arrand, J.R., Dept. of Molecular Biology, Paterson Laboratories, Christie Hospital, Manchester, England: Expression of EBV glycoprotein gp340/220 using viral vectors and production of EBV-neutralizing antibodies in animals vaccinated with a recombinant vaccinia virus.

Earl, P.L.,¹ Chesebro, B.W.,² Moss, B.,¹ ¹LVD, NIAID, National Institutes of Health, Bethesda, Maryland; ²RML, National Institute of Allergy and Infectious Diseases, Hamilton, Montana: Expression of retroviral envelope genes by vaccinia virus vectors.

Motz, M.,¹ Jilg, W.,¹ Fan, J.,² Wolf, H.,¹ ¹Max von Pettenkofer Institute, Munich, Federal Republic of Germany; ²Institute of Virology, Beijing, Republic of China: Expression of the membrane protein gp250/350 of EBV for the use as diagnostic antigen and possible vaccine.

Luciw, P.A.,¹ Parkes, D.,¹ Van Nest, G.,¹ Barr, P.J.,¹ Steimer, K.,¹ Gardner, M.B.,² ¹Chiron Corporation, Emeryville, California; ²University of California, Davis: Genetic engineering approaches to immunization for retroviral diseases—Evaluation of FeLV and AIDS-associated retroviral envelope polypeptides produced in yeast.

SESSION 9 AIDS RETROVIRUSES

Chairperson: A. Fauci, National Institutes of Health

Bolognesi, D., Dept. of Surgery, Duke University Medical Center, Durham, North Carolina: Prospects for vaccine development against human retroviruses.

Fauci, A.S., NIAID, National Institutes of Health, Bethesda, Maryland: Immunopathogenesis of AIDS.

Gallo, R., NCI, National Institutes of Health, Bethesda, Maryland: Strategy for prevention of AIDS.

Montagnier, L., Clavel, F., Krust, B., Chamaret, S., Guétard, D., Laurent, A., Galabru, J., Hovanessian, A., Unité d'Onco-cologie Virale, Institut Pasteur, Paris, France: Antigenicity



R. Lerner

of the envelope glycoprotein of lymphadenopathy-AIDS virus.

Wain-Hobson, S., Alizon, M., Sonigo, P., Institut Pasteur, Paris, France: Genome organization of the AIDS lentivirus, LAV.

Dina, D.,¹ Staben, C.,¹ Van Nest, G.,¹ Barr, P.,¹ Levy, J.,² Steimer, K.,¹ ¹Chiron Research Laboratories, Chiron Corporation, Emeryville, California; ²Cancer Research Institute, Dept. of Medicine, University of California, San Francisco: Molecular characterization of AIDS-related retroviruses.

Rabson, A.,¹ Theodore, T.,¹ Benn, S.,¹ Adachi, A.,¹ Folks, T.,¹ Lightfoote, M.,¹ Shelton, E.,² Salazar, F.,² Chan, H.,³ Martin, M.,¹ ¹NIAID, National Institutes of Health, Bethesda, Maryland; ²Institute of Bio-organic Chemistry, Syntex Research, Palo Alto, California: Structure and function of AIDS retroviral genes.

SESSION 10 HUMAN AND ANIMAL HEPADNA VIRUSES; FLAVIVIRUSES

Chairperson: R. Chanock, NIAID, National Institutes of Health

Michel, M.L.,¹ Milich, D.R.,² Chisari, F.V.,² Tiollais, P.,¹ ¹INSERM, Institut Pasteur, Paris, France; ²Dept. of Basic and Clinical Research, Scripps Clinic and Research Foundation, La Jolla, California: Synthesis of HBsAg particles containing the pre-S region expression product.

Kent, S.,¹ Neurath, A.R.,² Aebersold, R.,¹ Parker, K.,¹ Strick, N.,² ¹Division of Biology, California Institute of Technology, California; ²New York Blood Center, New York: Epitope scanning—Strategies for determining the precise location of continuous epitopes applied to the pre-S region of HBV.

Neurath, A.R.,¹ Kent, S.B.H.,² Strick, N.,¹ ¹New York Blood Center, New York; ²Division of Biology, California Institute of Technology, Pasadena: Human antibody responses to pre-S-gene-coded sequences of the HBV envelope protein.

Milich, D.R.,¹ Thornton, G.B.,² McNamara, M.K.,¹ McLachlan, A.,¹ Chisari, F.V.,¹ ¹Scripps Clinic and Research

Foundation, La Jolla; ²Johnson and Johnson, Biotechnology Center, San Diego, California: T- and B-cell recognition of native and synthetic pre-S region determinants on HBsAg.

Gerin, J.,¹ Tennant, B.,² Popper, H.,³ Tyeryar, F.,⁴ Purcell, R.,⁴ ¹Georgetown University, Rockville, Maryland; ²Cornell University, Ithaca, New York; ³Mt. Sinai School of Medicine, New York, New York; ⁴NIAID, National Institutes of Health, Bethesda, Maryland: The woodchuck model of hepadnavirus infection and disease—Vaccine studies.

Lai, C.-J.,¹ Zhao, B.,¹ Makino, Y.,¹ Mackow, E.,¹ Brandt, W.,² Burke, D.,² Chanock, R.M.,¹ ¹LID, NIAID, National Institutes of Health, Bethesda, Maryland; ²Walter Reed Army Institute of Research, Washington, D.C.: Cloning full-length DNA sequences of the dengue viral genome for use in elucidating pathogenesis and development of immunoprophylaxis.

Summary: F. Brown, Wellcome Biotechnology Institute

BANBURY CENTER



Recto: Sammis Hall at Banbury Center is a residence for meeting participants. A stunning example of postmodern architecture, it is modeled closely after the 16th century Villa Poiana in Vicenza, which was designed by the architect Palladio.

BANBURY CENTER DIRECTOR'S REPORT

The Banbury Center program of scientific conferences and publications focusing on recent biological advances that have particular relevance to environmental, regulatory, public policy, and cancer risk issues was initiated 8 years ago. Looking back over that period, a pattern of increasing program consolidation and stabilization is discernible.

The Center's first long-term commitment was received from the Alfred P. Sloan Foundation and was activated in 1980. This grant continues to support workshops in the biological sciences for either science journalists or congressional staff. Consolidation of targeted private sector support took place in 1984 with the establishment of the Corporate Sponsor Program of Cold Spring Harbor Laboratory. This enables several small meetings to be held at the Center each year in areas pertinent to gene regulation, gene expression, and developing approaches in biotechnology. With ongoing general support from corporate contributors, as listed separately at the end of this report, and grants for specific projects from both federal sources and private agencies, the Banbury Center has also been able to carry on a varied and increasingly well-recognized series of conferences and publications. Until 1985, however, this central program of conferences and publications addressing the scientific underpinnings of a variety of public health, public policy, and environmental health issues remained without a stable base of support. Thus, the awarding in 1985 of a 3-year grant in support of this core program by the James S. McDonnell Foundation must be considered the year's single most significant event with regard to the Center's present performance and future development. In addition to several future programs already being organized under this grant,

Robertson House provides housing and dining accommodations at Banbury Center



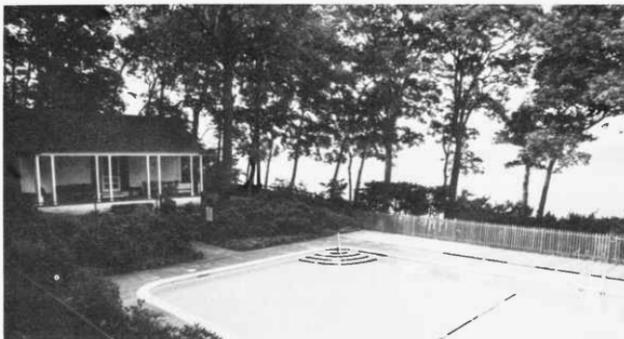
McDonnell Foundation support is facilitating the successful completion of two major present projects.

The first of these projects concerns publication of the proceedings from the April 1985 conference entitled *Origins of Female Genital Cancer*. Cervical cancer remains a major neoplastic cause of death among women worldwide. Identification of its cause would thereby present a major step toward understanding and potentially controlling this important neoplastic disease. Recent advances in molecular virology, in combination with ongoing epidemiological approaches, now seem to make this goal highly feasible. It was thus felt that bringing together the virological, epidemiological, and clinical communities concerned could be particularly useful in expediting this process. The planning for this conference with Professor Harald zur Hausen of the German Cancer Research Center in Heidelberg and Richard Peto of Oxford University began early in 1984. A subsequent federal grant application review confirmed the importance of such a project. Funding limitations, however, permitted only a small portion of meeting costs and no publication costs at all to be covered. Given the importance of the subject and the role that such a conference and subsequent publication could play, the decision was made to proceed with the project with the hope that funding to cover it would be found at a subsequent date. McDonnell Foundation support will be of tremendous assistance in the completion of this project.

Another program begun in 1985, which will also be receiving McDonnell Foundation support, is that on *New Aspects of Tobacco Carcinogenesis*. A September 1985 conference on this topic was organized through the joint efforts of Dr. Dietrich Hoffmann of the American Health Foundation and Dr. Curtis Harris of the National Cancer Institute. Representative research areas in epidemiology as well as laboratory studies in tobacco carcinogenesis were brought together with molecular and biochemical approaches concerned with mechanisms and assays of carcinogen-DNA interactions, cytogenetic lesions, and host factors that may influence susceptibility. Again, excellent grant reviews resulted in only very limited funding, and McDonnell Foundation funds will be instrumental in the 1986 publication of these proceedings in the series of *Banbury Reports*.

Meeting House, rear view





Robertson House pool

In 1985, the number of volumes in the Banbury Reports series was brought to 21 with the addition of three new titles. Two of these, *Risk Quantitation and Regulatory Policy* and *Genetic Manipulation of the Early Mammalian Embryo*, resulted from Banbury Center conferences held in 1984. The third book, *Genetically Altered Viruses and the Environment*, emanated from a late April, 1985 meeting that was developed as a cooperative agreement between the U.S. Environmental Protection Agency and the Banbury Center. Representatives from the fields of ecological virology, clinical virology, and molecular biology, were brought together to provide a broad perspective on the nature of this topic as well as to delineate key research concerns for the consideration of participants from the regulatory community.

In addition to full Banbury programs with publication in the Banbury Reports series, four workshops were also held at the Center in 1985 as part of the Corporate Sponsor Program. The first of these, held in March, concerned the role of *cis*- and *trans*-acting genetic elements in the initiation of transcription in eukaryotic cells. A related meeting in the Sponsor series was held in November, this one addressing current research in both prokaryotes and eukaryotes on the regulation of protein synthesis at the translational level. An earlier October Sponsor's meeting addressed the genetics of cell-cell interactions in plants, including the genetics of interactions with pathogens, self-incompatibility, fungal mating types, and problems in the genetics of plant sterility. The final meeting of the year in this series was held in December and was probably the most visually exciting meeting to be held at the Center—its subject being the design and use of computer graphics systems in the study of the structure and function of biological macromolecules.

The complement of regular Banbury programs in 1985 was completed with two workshops held under the Alfred P. Sloan Foundation grant described above. The first of these, held in October for congressional staff, considered scientific bases and ongoing research in the area of clinical intervention in problems of reproduction and infertility. The topic presented for journalists under the Sloan Foundation program concerned newly emerging approaches to an understanding of, and intervention in, the central nervous system and its degenerative disorders. This topic was inspired to a large extent as a result of the utilization of the Banbury Center over the past several years as a site for the regularly held high-level Cold Spring Harbor Laboratory summer courses in



Robertson House hall

neurobiology. The Center was also the site, in March of 1985, of an international workshop on the immune recognition of protein antigens and in October of a small workshop, held in conjunction with Pioneer Hi-Bred International, Inc., on the genetics of higher plants with emphasis on maize. Such programs, held at the Center in addition to Banbury-originated programs, help to broaden the dynamic and perspective of the Center, often contributing to the development of future project concepts.

1985 Support

In addition to the James S. McDonnell Foundation grant, support from the Alfred P. Sloan Foundation, and endowment funds for upkeep of the estate grounds and original structures, as provided in the original donation of Charles S. Robertson, the Center's programs were also supported in 1985 by private sector Core Contributors, by companies enrolled in the Cold Spring Harbor Laboratory Corporate Sponsor Program, and by federal grants.

The following contributed toward the general running of the Center as Core Supporters in 1985: the Bristol-Myers Fund, the Chevron Fund, the Dow Chemical Company, the Exxon Corporation, the Grace Foundation Inc., International Business Machines, Procter and Gamble, the Rockwell International Corporation Trust, and the Texaco Philanthropic Foundation Inc.

Cold Spring Harbor Laboratory Corporate Sponsors, whose funds supported four workshops at the Center in 1985, included Agrigenetics Corporation, American Cyanamid Company Amersham International plc, Becton Dickinson & Company, Biogen S.A., Cetus Corporation, Ciba-Geigy Corporation, CPC International, Inc., E.I. du Pont de Nemours & Company, Genentech, Inc., Genetics Institute, Hoffmann-La Roche Inc., Johnson & Johnson, Eli Lilly and Company, Mitsui Toatsu Chemicals, Inc., Monsanto Company, Pall Corporation, Pfizer Inc., Schering-Plough Corporation, Smith Kline & French Laboratories, and the Upjohn Company.

The 1985 conference on the Origins of Female Genital Cancer was supported in part by a grant from the National Cancer Institute with assistance from a contribution made by Merck, Sharp, and Dohme Research Laboratories. The National Cancer Institute also helped to support the conference on New Aspects of Tobacco Carcinogenesis, together with funding from the American Cancer Society and additional support from the Office on Smoking and Health. The U.S. Environmental Protection Agency joined in a cooperative agreement with the Banbury Center in the organization and funding of the conference on Genetically Altered Viruses and the Environment.

Michael Shodell

MEETINGS

The Immune Recognition of Protein Antigens

March 3–March 6

ARRANGED BY

W. G. Laver, John Curtin School of Medical Research, Canberra, Australia

G. M. Air, University of Alabama, Birmingham

SESSION 1

Chairperson: **G. M. Air**, University of Alabama, Birmingham

J. Skekel, National Institute for Medical Research, London, England: Characterization of antigenic domains on influenza virus hemagglutinin.

W. Gerhard, Wistar Institute, Philadelphia, Pennsylvania: B- and T-cell determinants on the hemagglutinin molecule of influenza virus PR8.

SESSION 2

Chairperson: **J. Skekel**, National Institute for Medical Research, London, England

I. A. Wilson, Research Institute of the Scripps Clinic, La Jolla, California: Probing the structure and antigenic determinants of influenza virus hemagglutinin using antipeptide monoclonal antibodies.

M.-J. Gething, Cold Spring Harbor Laboratory, New York: Analysis of mutant and wild-type versions of influenza virus hemagglutinin produced in different eukaryotic systems.

SESSION 3

Chairperson: **J. Sambrook**, Cold Spring Harbor Laboratory, New York

A. Caton, Wistar Institute, Philadelphia, Pennsylvania: Sequences of monoclonal antibodies to influenza virus hemagglutinin.

Pennsylvania: Antibody diversity in the immune response to influenza virus hemagglutinin.

M. Weigert, Institute for Cancer Research, Philadelphia,

M. D. Scharff, Albert Einstein College of Medicine, Bronx, New York: Somatic mutations in mouse myeloma cells.

SESSION 4

Chairperson: **T. J. Braciale**, Washington University School of Medicine, St. Louis, Missouri

C. Hackett, Wistar Institute, Philadelphia, Pennsylvania: Fine specificity of antigen recognition by influenza hemagglutinin-specific helper T cells.

J. R. Lamb, Hammersmith Hospital, London, England: Human T-cell recognition of influenza virus antigens.

SESSION 5

Chairperson: **M. Weigert**, Institute for Cancer Research, Philadelphia, Pennsylvania

S. Smith-Gill, National Cancer Institute, Bethesda, Maryland: Structure-function relationships in antibodies specific for hen egg-white lysozyme.

T. LaVoie, National Cancer Institute, Bethesda, Maryland: V-region expression of antibodies specific for hen egg-white lysozyme.

SESSION 6

Chairperson: **P. M. Colman**, CSIRO Division of Protein Chemistry, Parkville, Victoria, Australia

P. M. Colman, CSIRO Division of Protein Chemistry, Parkville, Victoria, Australia: Three-dimensional structure of an anti-neuraminidase Fab fragment.

C. Crystallographic studies of antibody-antigen complexes.

D. Davies, National Institutes of Health, Bethesda, Maryland:

R. J. Poljak, Institut Pasteur, Paris, France: Three-dimensional structure of antigen-antibody complex.

SESSION 7

Chairperson: E. Sercarz, University of California, Los Angeles

- Y. Paterson, Scripps Clinic and Research Foundation, La Jolla, California: Constraints in the recognition of horse cytochrome c by monoclonal antibodies.
- D. C. Benjamin, University of Virginia, Charlottesville: Antigenic structure of a complex protein—Serum albumin.

- E. Wimmer, State University of New York, Stony Brook: Neutralization antigenic sites. antibody binding, and peptide induction of neutralizing antibodies of poliovirus.



SESSION 8

Chairperson: R. G. Webster, St. Jude's Children's Hospital, Memphis, Tennessee

- T. J. Braciale, Washington University School of Medicine, St. Louis, Missouri: Viral antigen recognition by cytolytic T lymphocytes of different subsets.
- A. Townsend, John Radcliffe Hospital, Oxford, England: CTL recognition of the influenza virus nucleoprotein molecule.
- C. S. Reiss, Dana-Farber Cancer Institute, Boston, Massachusetts: Localization of restricting elements on class I MHC molecules using antiviral CTLs.
- E. Huber-Katz, Wistar Institute, Philadelphia, Pennsylvania: T-cell responses to glycoprotein D of herpes simplex virus.

SESSION 9

Chairperson: P. Colman, CSIRO Division of Protein Chemistry, Parkville, Victoria, Australia

- G. M. Air, University of Alabama, Birmingham: Antigenic structure of influenza virus neuraminidase.
- W. G. Laver, John Curtin School of Medical Research, Canberra, Australia: Preparation of crystalline influenza virus neuraminidase-antibody complexes.

- P. Tulloch, CSIRO Division of Protein Chemistry, Parkville, Victoria, Australia: Electron diffraction and imaging of influenza virus neuraminidase antibody complexes.

SESSION 10

Chairperson: D. Davies, National Institutes of Health, Bethesda, Maryland

- J. A. Berzofsky, National Cancer Institute, Bethesda, Maryland: Structural and conformational requirements for myoglobin epitope recognition by T-cell clones—A contrast with monoclonal antibodies.
- J. Rothbard, Stanford University, Palo Alto, California: Antigenic epitopes—Comparison of those elicited by

peptides corresponding to V_H regions with those generated by the intact immunoglobulin.

- J. E. Johnson, Purdue University, West Lafayette, Indiana: Antibody binding to cowpea mosaic virus in the crystal-line state.

SESSION 11

Chairperson: I. A. Wilson, Research Institute of the Scripps Clinic, La Jolla, California

- A. C. Bloomer, MRC Laboratory of Molecular Biology, Cambridge, England: Segmental mobility correlates with the location of epitopes in proteins.
- M. H. V. van Rogenmortel, Institut de biologie moleculaire et cellulaire CNRS, Strasbourg, France: Segmental mobility in proteins and the epitopes of tobacco mosaic virus.

- D. Jackson, University of Melbourne, Australia: B- and T-lymphocyte responses to influenza viruses, protein, and peptides.
- R. F. Anders, Royal Melbourne Hospital, Australia: Immune recognition of tandemly repeated sequences in asexual blood-stage antigens of *Plasmodium falciparum*.

The Role of *cis*- and *trans*-acting Elements in the Initiation of Eukaryotic Transcription

March 24–March 27

ARRANGED BY

Y. Gluzman, Cold Spring Harbor Laboratory, New York

SESSION 1 VIRAL ENHANCERS

- G. Khoury, National Cancer Institute, Bethesda, Maryland: Viruses as models for eukaryotic gene regulation.
- P. Hearing, Rockefeller University, New York, New York: Adenovirus enhancer elements.
- M. Botchan, University of California, Berkeley: BPV enhancer and interaction with different promoters.
- J. A. Hassell, McGill University, Montreal, Canada: Dual role of the Py virus enhancer in transcription and DNA replication.

- W. Herr, Cold Spring Harbor Laboratory, New York: Sequence duplications that restore activity to mutated SV40 enhancers.
- A. Wildeman, Institut de Chimie Biologique, Strasbourg, France: SV40 enhancer mutants.
- P. Johnson, Carnegie Institution of Washington, Baltimore, Maryland: Interactions between cellular components and viral enhancers.

SESSION 2 CELLULAR PROMOTERS I

- W. Schaffner, University of Zurich, Switzerland: Constitutive and inducible enhancer elements.
- R. Palmiter, Howard Hughes Medical Institute, Seattle, Washington: Metal regulatory elements of mouse metallothionein gene.
- P. Chambon, Institut de Chimie Biologique, Strasbourg, France: Control of transcription by steroid hormones.
- D. DeFranco, University of California, San Francisco:

- Regulation of transcription by the glucocorticoid receptor.
- M. Walker, University of California, San Francisco: Cell-specific expression of insulin and pancreatic acinar cell genes.
- M. Fried, Imperial Cancer Research Fund Laboratories, London, England: Use of expression selection for the isolation of enhancer sequences.

SESSION 3 TRANSGENIC ORGANISMS

- K. Arndt, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: *Cis*- and *trans*-acting elements in *His4* gene expression in yeast.
- L. Guarente, Massachusetts Institute of Technology, Cambridge: Regulation of yeast cytochrome genes.
- M. Ptashne, Harvard University, Cambridge, Massachusetts: Regulation of the *gal* promoter by specific binding of *gal-4* protein, bacterial *lex* protein, and a *lex-gal-4* fusion protein.
- J. Hicks, Cold Spring Harbor Laboratory, New York: Reverse enhancer controls silent mating-type cassettes.

- K. Nasmyth, MRC Laboratory of Molecular Biology, Cambridge, England: The control of HO transcription in yeast.
- E. Meyerowitz, California Institute of Technology, Pasadena: *Cis*- and *trans*-acting factors in expression of the *Drosophila* 68C glue genes.
- T. Maniatis, Harvard University, Cambridge, Massachusetts: Identification of DNA sequences required for the development and tissue-specific regulation of *Drosophila* ADH gene expression.

SESSION 4 IN VITRO SYSTEMS

- K. Jones, University of California, Berkeley: Gene-specific RNA polymerase II transcription factors.
- A. Berk, University of California, Berkeley: EtA protein activation of transcription factor IIC.
- A. Ephrussi, Massachusetts Institute of Technology, Cambridge: Footprints of the immunoglobulin enhancer in living cells and in nuclei.
- R. Sen, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: DNA elements affecting immunoglobulin gene transcription in vitro.
- P. Gruss, University of Heidelberg, Federal Republic of Germany: In vitro systems to study cell-specific enhancers.



T. Maniatis, E. Ziff, W. Schaffner, P. Chambon

SESSION 5 CELLULAR PROMOTERS II

- K. Zinn, Harvard University, Cambridge, Massachusetts: Human β -interferon gene expression is regulated by an inducible enhancer element.
- S. McKnight, Carnegie Institution of Washington, Baltimore, Maryland: Properties of the herpes virus Tk promoter.
- M. Yaniv, Institut Pasteur, Paris: Identification of proteins that bind to enhancers and upstream activation sites.
- N. Heintz, Rockefeller University, New York, New York: Human histone gene regulation.
- R. Treisman, MRC Laboratory of Molecular Biology, Cambridge, England: Regulation of the human *c-fos* gene.
- E. Ziff, New York University Medical School, New York, New York: Growth factor control of gene expression.

Origins of Female Genital Cancer: Virological and Epidemiological Aspects

April 14–April 17

ARRANGED BY

H. zur Hausen, German Cancer Research Center, Heidelberg, FRG, R. Peto, University of Oxford, England

SESSION 1 DESCRIPTIVE AND ANALYTIC EPIDEMIOLOGY OF CERVICAL CARCINOMA

Chairperson: L. A. Brinton, National Cancer Institute, Bethesda, Maryland

- R. Peto, University of Oxford, England: Descriptive epidemiology: Geographic correlates and major trends.
- I. I. Kessler, University of Maryland School of Medicine, Baltimore: Social and sexual correlates.
- M. P. Vessey, University of Oxford, England: Hormonal factors, tobacco, and occupation.
- M. Hakama, Finnish Cancer Registry, Helsinki, Finland: Efficacy of screening for cervical cancer.
- L. A. Brinton, National Cancer Institute, Bethesda, Maryland: Current epidemiological studies: Emerging hypotheses.

SESSION 2 DYSPLASTIC AND PRENEOPLASTIC LESIONS

Chairperson: L. G. Koss, Montefiore Medical Center, Bronx, New York

- E. Brughardt, Geburtshilflich-gynakologische Universitätsklinik, Graz, Austria: Classification and natural history of cervical lesions.
- R. Reid, Sinai Hospital of Detroit, Michigan: Is there a morphological spectrum linking condylomas to cancer?
- A. Meisels, Saint-Sacrement Hospital, Sainte-Foy, Quebec, Canada: Cytology in the assessment of natural history of cervical lesions.
- W. W. Franke, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Intermediate filaments.
- G. Gross, University of Freiburg, Federal Republic of Germany: Bowenoid papillosis: A venereally transmitted disease as reservoir for HPV16.
- K. Syrjänen, Finnish Cancer Society, Kuopio, Finland: Prospective follow-up in assessment of the biological behavior of cervical HPV-associated dysplastic lesions.
- S. Franceschi, Mario Negri Institute for Pharmacological Research, Milan, Italy: Correlations of cervical neoplasia with sexual factors, including specific venereal diseases.

SESSION 3 EVIDENCE CONNECTING SPECIFIC VIRUSES TO GENITAL CANCER

Chairman: W. E. Rawls, McMaster University, Hamilton, Ontario, Canada

- W. E. Rawls, McMaster University, Hamilton, Ontario, Canada: Seroepidemiological evidence about HSV involvement.
- J. K. McDougall, Fred Hutchinson Cancer Research Center, Seattle, Washington: The enigma of viral nucleic acids in cervical neoplasia.
- J. R. Schlehofer, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Interactions of herpes simplex virus infections with host cell DNA.
- L. Aurelian, University of Maryland School of Medicine, Baltimore: General discussion.
- G. Orth, Institut Pasteur, Paris, France: Plurality of human papilloma viruses, and their involvement in skin cancer.
- L. Gissmann, German Cancer Research Center, Heidelberg, Federal Republic of Germany: HPV DNA in preneoplastic and neoplastic genital lesions.
- D. J. McCance, Guy's Hospital Medical School, London Bridge, England; A. Lorincz, Bethesda Research Laboratories, Inc., Gaithersburg, Maryland: General discussion.
- E.-I. Grussendorf-Conen, Technischen Hochschule, Aachen, Federal Republic of Germany: In situ hybridization with papilloma virus DNA in genital lesions.
- R. S. Ostrow, University of Minnesota Medical School, Minneapolis: General discussion.

SESSION 4 MOLECULAR MECHANISMS OF HUMAN PAPILLOMA VIRUS INTERACTIONS WITH HOST CELLS

Chairperson: P. M. Howley, National Cancer Institute, Bethesda, Maryland

- P. M. Howley, National Cancer Institute, Bethesda, Maryland: Molecular cloning of papilloma virus DNA.
- M. Duerst, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Integration and persistence of HPV DNA.
- E. Schwarz, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Expression of HPV DNA in cervical cancer biopsies and in tissue culture.
- G. H. Sato, W. Alton Jones Cell Science Center, Inc., Lake Placid, New York: Immune response to specific cervical carcinoma antigens.
- K. V. Shah, Johns Hopkins University, Baltimore, Maryland: Detection of papilloma virus antigen and DNA in cells and tissues.
- T. Broker, University of Rochester, New York: Genetic organization and expression of human papilloma viruses.



G. Orth, K. Shah

SESSION 5 PAPILLOMAVIRUS ASSOCIATIONS AND EPIDEMIOLOGICAL PERSPECTIVE

Chairperson: J. Cairns, Harvard University School of Public Health, Boston, Massachusetts

- Y. S. Fu, UCLA Center for the Health Sciences, Los Angeles, California: Stemline evolution in preneoplastic and neoplastic genital lesions.
- N. B. Atkin, Mount Vernon Hospital, Northwood, Middlesex, England: Chromosome changes in preneoplastic and neoplastic genital lesions.
- R. Doll, Imperial Cancer Research Fund, Oxford, England: Implications of epidemiological evidence for future progress.
- J. Cairns, Harvard University School of Public Health, Boston, Massachusetts: How many important causes of cervical cancer might we expect?
- H. zur Hausen, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Review of what is presently established and what remains speculative in the role of viruses in human genital cancer: Prospects for progress.

Genetically Altered Viruses and the Environment

April 28–May 1

ARRANGED BY

- D. Kamely, U.S. Environmental Protection Agency, Washington, DC.
- B. N. Fields, Harvard Medical School, Boston, Massachusetts
- M. A. Martin, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland

SESSION 1 LEGISLATIVE & REGULATORY FRAMEWORK

Chairperson: A. H. Teich, American Association for the Advancement of Science, Washington, D.C.

- P. B. Hutt, Covington & Burling, Washington, DC: Existing regulatory authority to control the products of biotechnology.
- J. G. Perpich, Meloy Laboratories, Inc., Springfield, Virginia: The biotechnology industry: Federal regulation of product development.
- E. L. Anderson, Environmental Protection Agency, Washington, DC: Risk assessment/risk management of environmental agents.
- S. Schatzow, Environmental Protection Agency, Washington, DC: The role of the Environmental Protection Agency.



R. Chanock, G. Khoury

SESSION 2 ENVIRONMENTAL VIROLOGY

Chairperson: R. L. Dixon, Environmental Protection Agency, Washington, D.C.

- R. E. Shope, Yale University, New Haven, Connecticut: Viral spread between hosts.
- V. Knight, Baylor College of Medicine, Houston, Texas: Airborne transmission of viral infections.
- T. G. Metcalf, Baylor College of Medicine, Houston, Texas: Distribution of viruses in the water environment.
- E. D. Kilbourne, Mt. Sinai School of Medicine, New York, New York: Epidemiology of viruses genetically altered by man: Predictive principles.
- A. P. Kendal, Centers for Disease Control, Atlanta, Georgia: The effects of influenza virus genetic alteration on disease in man and animals.

SESSION 3 TROPISMS

Chairperson: M. A. Martin, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland

- R. L. Crowell, Hahnemann University, Philadelphia, Pennsylvania: Cellular receptors as determinants of viral tropism.
- B. Fields, Harvard Medical School, Boston, Massachusetts: Effect of genetic manipulation on viral-receptor interactions.
- G. Khoury, National Cancer Institute, Bethesda, Maryland: Enhancers and tissue specificity.
- N. Hopkins, Massachusetts Institute of Technology, Cambridge, Massachusetts: Tropism and pathogenicity of retroviruses.
- A. Helenius, Yale University School of Medicine, New Haven, Connecticut: Membrane proteins in viral tropism and pathogenicity.

SESSION 4 HOST INTERACTIONS

Chairperson: T. C. Merigan, Stanford University School of Medicine, California

- T. C. Merigan, Stanford University School of Medicine, California: Variation in viral disease manifestation in humans related to host defenses.
- R. Ahmed, University of California School of Medicine, Los Angeles: Viral persistence—Role of viral variants and T-cell responses.
- R. C. Gallo, National Cancer Institute, Bethesda, Maryland: Human T-lymphotropic retroviruses.
- B. Roizman, University of Chicago, Illinois: Genetic engineering of herpes simplex virus genomes—Virulence and latency.
- R. M. Chanock, National Institutes of Health, Bethesda, Maryland: Human host responses to genetically altered viruses.

SESSION 5 VIRAL VECTORS

Chairperson: B. Fields, Harvard Medical School, Boston, Massachusetts

- B. Moss, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland: Vaccinia virus vectors.
- R. C. Mulligan, Massachusetts Institute of Technology, Cambridge: Introduction of genes into cultured cells and whole animals using retroviral vectors.
- P. M. Howley, National Cancer Institute, Bethesda, Maryland: Functions controlling papilloma virus gene expression.
- J. Logan, Princeton University, New Jersey: The use of adenovirus recombinants to study the regulation of viral gene expression.
- M. D. Summers, Texas A&M University, College Station, Texas: The polyhedrin gene and baculovirus expression vectors.

New Aspects of Tobacco Carcinogenesis

September 8–September 11

ARRANGED BY

D. Hoffmann, American Health Foundation, Valhalla, New York

C. C. Harris, National Cancer Institute, Bethesda, Maryland

SESSION 1 LABORATORY-EPIDEMIOLOGY STUDIES (A)

Chairperson: C. C. Harris, National Cancer Institute, Bethesda, Maryland

N. J. Haley, American Health Foundation, Valhalla, New York: Uptake of smoke components.

A. H. Conney, Hoffmann-La Roche Inc., Nutley, New Jersey: Studies on the urinary excretion of nitrosamines.

- G. Becher, National Institute of Public Health, Oslo, Norway: Determination of exposure to PAH by analysis of urine samples.
- H. Bartsch, International Agency for Research on Cancer,

- Lyon, France: Modifiers of endogenous carcinogen formation: Studies on in vivo nitrosation in tobacco users.
- S. R. Tannenbaum, Massachusetts Institute of Technology, Cambridge: Detection of carcinogen-protein adducts.

SESSION 2 LABORATORY-EPIDEMIOLOGY STUDIES (B)

Chairperson: D. Hoffmann, American Health Foundation, Valhalla, New York

- K. Randerath, Baylor College of Medicine, Houston, Texas: Detection of carcinogen-DNA adducts.
- M. P. Rosin (for H. F. Stich), University of British Columbia, Vancouver, Canada: Micronucleus test—Application to tobacco uses.
- P. Correa, Louisiana State University Medical Center, New Orleans: Validation of smoke exposure with the

- micronuclei test.
- E. J. LaVoie, American Health Foundation, Valhalla, New York: Mutagens in the urine of cigarette smokers.
- G. Scherer, Forschungsgesellschaft Rauchen und Gesundheit MBH, Hamburg, Federal Republic of Germany: Endogenous formation of *N*-nitrosoproline in smokers and nonsmokers.

SESSION 3 NEW ASPECTS OF TOBACCO CARCINOGENESIS

Chairperson: P. N. Magee, Temple University School of Medicine, Philadelphia, Pennsylvania

- M. B. Wise, Oak Ridge National Laboratory, Tennessee: Chemical analysis of the major constituents in clove cigarette smoke.
- C. R. Enzell, Swedish Tobacco Company, Stockholm: Isoprenoid flavor components of tobacco and their formation.
- H. Tjalve, Swedish University of Agricultural Sciences, Uppsala: Perinatal metabolism and activation of tobacco

- carcinogens.
- K. D. Brunneemann, American Health Foundation, Valhalla, New York: Laboratory studies on oral cancer and smokeless tobacco.
- H. Heck, Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina: The formation of DNA-protein cross-links by aldehyde present in tobacco smoke.

SESSION 4 NEW ASSOCIATIONS OF TOBACCO USE AND CANCER RISK

Chairperson: W. Winkelstein, Jr., University of California, Berkeley

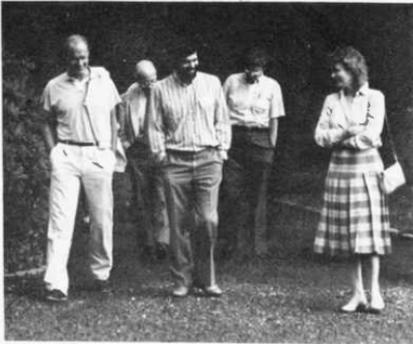
- W. Winkelstein, Jr., University of California, Berkeley: Cigarette smoking and cancer of the uterine cervix.
- P. Correa, Louisiana State University Medical Center, New Orleans: The passive smoking-cancer controversy.
- D. M. Winn, National Cancer Institute, Bethesda, Maryland:

- Snuff dipping and cancer.
- S. D. Stellman, American Cancer Society, New York, New York: Interactions of tobacco with occupational and dietary risk factors.

SESSION 5 BIOCHEMICAL, CELLULAR, AND MOLECULAR STUDIES ON HUMAN TISSUES AND CELLS

Chairperson: A. H. Conney, Hoffmann-La Roche Inc., Nutley, New Jersey

- I. Parsa, Downstate Medical Center, Brooklyn, New York: Transformation of human cells by *N*-nitroso compounds.
- S. S. Hecht, American Health Foundation, Valhalla, New York: Carcinogenic nitrosamines—Recent studies on metabolic activation of tobacco-specific nitrosamines—Prospects for dosimetry in humans.
- H. N. Autrup, University of Copenhagen, Denmark: Carcinogenic PAH—Metabolism and DNA binding.
- R. Grafstrom, Karolinska Institute, Stockholm, Sweden: Effects of tobacco-smoke-related aldehydes on DNA, DNA repair, and *N*-nitroso compound-induced mutagenesis.
- C. C. Harris, National Cancer Institute: Role of oncogenes in human respiratory carcinogenesis.
- A. E. Pegg, Pennsylvania State University: Factors influencing activity of *O*⁶-alkylguanine-DNA alkyltransferase.
- F. A. Beland, National Center for Toxicological Research: Aromatic amines and tobacco carcinogenesis.



The Genetics of Plant Cell/Cell Interactions

October 1-October 4

ARRANGED BY

R. L. Malmberg, University of Georgia, Athens

SESSION 1 SELF-INCOMPATIBILITY

- M. L. Crouch, Indiana University, Bloomington: Introduction.
D. J. Ockendon, National Vegetable Research Station, Warwick, England: Genetics and physiology of self-incompatibility in *Brassica*.
H. G. Dickinson, University of Reading, England: The cytophysiological basis of the sporophytically controlled cell-incompatibility mechanism operating in *Brassica*.
S. Brown, Indiana University, Bloomington: Self-incompatibility in the evening primrose, *Oenothera organensis*.

SESSION 2 MATING TYPES

- R. L. Malmberg, University of Georgia, Athens: Introduction.
P. Collin-Osdoby, Washington University, St. Louis, Missouri: *Chlamydomonas reinhardtii* mating-type-specific agglutinins.
R. C. Ullrich, University of Vermont, Burlington: Mating type in the basidiomycete *Schizophyllum commune*.

SESSION 3 CELL INTERACTIONS AND DEVELOPMENT

- I. Sussex, Yale University, New Haven, Connecticut: Graft chimeras and the analysis of positional differentiation in plants.
D. Walker, University of California, Los Angeles: The control of positional cell differentiation.

SESSION 4 HOST/PARASITE INTERACTIONS

- A. H. Ellingboe, University of Wisconsin, Madison: Introduction to genetic patterns in host-parasite interactions.
A. P. Roelofs, University of Minnesota, St. Paul: The *Puccinia graminis-triticum* sp. pathogen host interaction.
N. Panopoulos, University of California, Berkeley: Clustering and conservation of genes controlling the interactions of *Pseudomonas syringae* pathogens with plants.
M. J. Daniels, John Innes Institute, Norwich, England: Molecular genetic analysis of the pathogenicity of *Xanthomonas campestris*.
W. R. Bushnell, University of Minnesota, St. Paul: The role of the haustorium-host interface in host-parasite recognition.
V. M. Morales, University of Wisconsin, Madison: Genetics of avirulence in *Pseudomonas solanacearum*.
D. Mills, Oregon State University, Corvallis: Cloning and characterization of pathogenicity determinants from phytopathogenic pseudomonads.
D. W. Gabriel, University of Florida, Gainesville: Specific avirulence genes from *Xanthomonas malvacearum*.

- D. Mulcahy, University of Massachusetts, Amherst: Pollen-style interaction.
J. Mascarenhas, National Science Foundation, Washington, D.C.: Genes expressed during pollen development.
M. Anderson, University of Melbourne, Australia: Molecular cloning cDNA for a stylar glycoprotein associated with expression of self-incompatibility in *Nicotiana glauca*.
J. B. Nasrallah, Cornell University, Ithaca, New York: S-gene expression in pollen and stigma of *Brassica*.



M. Anderson, A.E. Clarke, R. Malmberg

- B. Staskawicz, University of California, Berkeley: Molecular genetics of race-specific avirulence genes in *Pseudomonas syringae* pv. *glycinea*.
A. Kerr, University of Adelaide, Australia: A possible method to clone a plant gene for disease resistance.
T. T. Egelfhoff, Stanford University, California: *Rhizobium* modulation gene products and gene regulation.
M. P. Gordon, University of Washington, Seattle: Control of expression of foreign genes in plants.
O. C. Yoder, Cornell University, Ithaca, New York: Molecular technology for studying fungus/plant interactions.
N. T. Keen, University of California, Riverside: Pectic enzymes, a role in specificity?
M. Essenberg, Oklahoma State University, Stillwater: Sesquiterpenoid phytoalexins and response of cotton to *Xanthomonas campestris* pv. *malvacearum*.
R. Rohringer, Agriculture Canada Research Station, Winnipeg: Surface macromolecules in the intercellular space of stem rust-infected wheat leaves.

Congressional Workshop on 'New Reproductive Technologies'

October 9–October 11

ARRANGED BY

M. Shodell, Banbury Center, Cold Spring Harbor Laboratory, New York

SESSION 1

F. Naftolin, Yale University School of Medicine, New Haven, Connecticut: Overview of reproductive physiology and development.

A. DeCherney, Yale University School of Medicine, New Haven, Connecticut: In vitro fertilization.

SESSION 2

J. E. Buster, L. A. Country Harbor/UCLA Medical Center, Torrance, California: Embryo transfer.

C. T. Caskey, Baylor College of Medicine, Houston, Texas: Antenatal monitoring.

SESSION 3

G. D. Hodgen, Eastern Virginia Medical School, Norfolk: Research approaches.

K. J. Ryan, Brigham and Women's Hospital, Boston, Massachusetts: Research goals.



Translational Control

November 3–November 6

ARRANGED BY

M. B. Mathews, Cold Spring Harbor Laboratory, New York

SESSION 1 INITIATION FACTORS

Chairperson: W. C. Merrick, Case Western Reserve University, Cleveland, Ohio

W. C. Merrick, Case Western Reserve University, Cleveland, Ohio: Binding of initiation factors to mRNA.

M. J. Clemens, St. George's Hospital Medical School, London, England: The role of phosphorylation of eIF-2 α in translational regulation in nonerythroid cells.

R. L. Matts, Massachusetts Institute of Technology, Cambridge, Massachusetts: The phosphorylation of eIF-2 α and the role of the 60S subunit in translational control.

R. Kaempfer, Hebrew University-Hadassah Medical School,

Jerusalem: Energy metabolism and eIF-2 activity.
R. Panniers, University of Rochester Cancer Center, New York: Regulation of translation through modulation of energy charge, intracellular calcium level, eIF-2 phosphorylation, and eIF-4F activity.

J. W. B. Hershey, University of California, Davis: The role of initiation factor covalent modifications in translational control—Variable phosphorylation of eIF-2, eIF-4B, and eIF-4F.

SESSION 2 METABOLIC EFFECTS

Chairperson: J. W. B. Hershey, University of California, Davis

R. J. Jackson, University of Cambridge, England: Heat shock and hypertonic shock of L cells—Properties of the

cell-free systems, and rescue by reticulocyte lysate components.

- C. Baglioni, State University of New York, Albany: Heat-shock proteins and interferon.
- S. Lindquist, University of Chicago, Illinois: Translational control in the heat-shock response.
- A. Colman, University of Warwick, Coventry, England: Translational control of heat-shock expression in *Xenopus* oocytes—Factors influencing the translation of synthetic

- mRNAs in *Xenopus* oocytes.
- J. D. Richter, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: RNA binding proteins in *Xenopus* oocytes.
- R. E. Thach, Washington University, St. Louis, Missouri: Effect of secondary structure on the translation of eukaryotic mRNA and its unwinding by initiation factors.

SESSION 3 RNA EFFECTS

Chairperson: A. J. Shatkin, Roche Institute of Molecular Biology, Nutley, New Jersey

- M. Kozak, University of Pittsburgh, Pennsylvania: Selection of translational start sites in eukaryotic mRNAs.
- F. Sherman, University of Rochester School of Medicine and Dentistry, New York: Rules of translation in yeast—Studies with mutant forms of the *CYC1* gene.
- H. DeBoer, Genentech, Inc., South San Francisco, California: Manipulating the ribosome and its mRNA binding site in *E. coli*—Redirecting ribosomes to a single mRNA species.
- P. Green, Rockefeller University, New York, New York: Use of artificial micRNA for regulating a specific gene.
- S. Sarkar, Boston Biomedical Research Institute, Massachusetts: A novel cytoplasmic translation inhibitory RNA of chick embryonic muscle—Possible role in myogenesis as antimessenger RNA.
- P. Walter, University of California, San Francisco: Elongation control by signal recognition particle.

SESSION 4 VIRAL SYSTEMS

Chairperson: M. B. Mathews, Cold Spring Harbor Laboratory, New York

- J. Siekierka, Merck, Sharp & Dohme Research Laboratories, Rahway, New Jersey: Translational control by adenovirus—VA RNA₁ prevents activation of host double-stranded RNA activated protein kinase during viral infection.
- R. J. Schneider, New York University Medical Center, New York: Function of adenovirus VA RNAs.
- R. M. Krug, Memorial Sloan-Kettering Cancer Center, New York, New York: Translational control by influenza virus.
- E. Ehrenfeld, University of Utah School of Medicine, Salt Lake City: Control of protein synthesis in poliovirus-infected cells.
- N. Sonenberg, Whitehead Institute, Cambridge, Massachusetts: Involvement of eukaryotic cap binding protein complex in translational control in uninfected cells and cells infected with poliovirus.
- J. M. Lucas-Lenard, University of Connecticut, Storrs: Control of protein synthesis in virus-infected cells.

SESSION 5 COMPLEX AND DEVELOPMENTAL SYSTEMS

Chairperson: T. Hunt, University of Cambridge, England

- T. Hunt, University of Cambridge, England: Control of protein synthesis at fertilization of marine invertebrate eggs and oocytes.
- M. Nomura, University of California, Irvine: Translational control of ribosomal protein synthesis in *Escherichia coli*.
- A. G. Hinnebusch, National Institute of Child Health and Human Development, Bethesda, Maryland: Translational control of the positive regulator of amino acid biosynthetic genes in yeast.
- J. L. Maller, University of Colorado School of Medicine, Denver: Regulation of phosphorylation of ribosomal protein S6.
- D. F. Klessig, Rutgers University, Piscataway, New Jersey: Translational regulation of light-induced ribulose biphosphate carboxylase gene expression in amaranth.
- D. L. Kirk, Washington University, St. Louis, Missouri: Translational regulation of protein synthesis and the onset of cytodifferentiation in *Volvox*.



M. Mathews, R. Matts

Journalists' Workshop on Research and Clinical Perspectives in the Central Nervous System

December 5–December 7

ARRANGED BY

M. Shodell, Banbury Center, Cold Spring Harbor Laboratory, New York

SESSION 1

I. Black, Cornell University Medical College, New York, New York: An introduction to the nervous system and its diseases.

SESSION 2

- L. Olson, Karolinska Institute, Stockholm, Sweden: Transplantation of brain tissue.
A. J. Aguayo, Montreal General Hospital, Canada: The regenerative powers of the nervous system.

SESSION 3

- M. Mishkin, National Institutes of Health, Bethesda, Maryland: Experimental approaches to the biological bases of thought and memory.
Roundtable Discussion: Clinical pressures and research perspectives in brain function.



Computer Graphics and Molecular Modelling

December 10–December 13

ARRANGED BY

M. Zoller, Cold Spring Harbor Laboratory, New York
R. Fletterick, University of California, San Francisco, School of Medicine

SESSION 1

- R. Fletterick, University of California, San Francisco, School of Medicine: Opening remarks.
J. Greer, Abbott Laboratories, Abbott Park, Illinois: Comparative modeling of blood complement proteins.
C. Chothia, MRC Laboratory of Molecular Biology, Cambridge, England: The use of sequence homologies to predict protein structures.

- B. W. Erickson, Rockefeller University, New York, New York: Betabellin—An engineered protein.
J. E. Anderson, Harvard University, Cambridge, Massachusetts: Fitting 434 repressor-operator complex with FRODO.

SESSION 2

- R. Langridge, University of California, San Francisco: The future of computer graphics.
T. Ferrin, University of California, San Francisco: Hardware and software status report from the UCSF Computer Graphics Laboratory.

- R. E. Hubbard, University of York, England: Hydra—Current and future development.
M. E. Pique, University of North Carolina, Chapel Hill: Technical trends in molecular graphics.

SESSION 3

- C.-I. Branden, University of Uppsala Biomedical Center, Sweden: Structural principles of active sites in protein domains.
- D. Eisenberg, University of California, Los Angeles: Hydrophobic moments and solvation energy in protein folding.
- P. Argos, European Molecular Biology Laboratory,

Heidelberg, Federal Republic of Germany: Searching for weak sequence homologies and testing for their significance.

- W. F. DeGrado, E. I. du Pont de Nemours & Company, Wilmington, Delaware: The molecular basis for the fusogenic activity of influenza hemagglutinin.

SESSION 4

- D. Tronrud, University of Oregon, Eugene: Computer graphics and its application to the structure and function of biological macromolecules.
- R. J. Feldmann, National Institutes of Health: Thoughts on the use of parallel computers in molecular graphics.
- H. Dayringer, Monsanto Company, St. Louis, Missouri: Proteus—Graphics software for proteins.
- W. Taylor, Birkbeck College, London, England: Prediction of protein structure from amino acid sequence using computer graphics and data bases.
- T. A. Jones, University of Uppsala Biomedical Center, Sweden: On making use of known protein structures in macromolecular modeling.



M. Zoller, C.-I. Branden, J. Anderson

SESSION 5

- P. S. Stern, Weizmann Institute of Science, Rehovot, Israel: Normal-mode dynamics—Energy calculations, interactive graphics, and movies.
- M. Karplus, Harvard University, Cambridge, Massachusetts:

Dynamics of macromolecules.

- A. Hagler, Agouron Institute, La Jolla, California: Conformationally based design of gonadotropin-releasing hormone and antagonists.

EDUCATIONAL ACTIVITIES



Recto: Under the tutelage of staff scientist Steve Dellaporta (left), undergraduate researcher Mark Allenito learns the subtleties of maize transposable elements.

Postgraduate Courses

The summer program of Postgraduate Courses at Cold Spring Harbor Laboratory is aimed at meeting the special need for training in interdisciplinary subjects that are either so new or so specialized that they are not adequately treated by universities. Our aim is to provide intensive study in the most recent developments and techniques in these subjects and to prepare students to enter directly into research in a particular area. To ensure up-to-date coverage of current research work, we bring together a workshop staff from many laboratories around the world and supplement this staff with a series of seminar speakers.

Molecular Neurobiology of Human Disease

June 7-June 17

INSTRUCTORS

Breakefield, Xandra O., Ph.D., E.K. Shriver Center and Harvard Medical School, Boston, Massachusetts

Black, Ira B., M.D., Cornell University Medical College, New York, New York

Gusella, James F., Ph.D., Massachusetts General Hospital and Harvard Medical School, Boston

This intensive seminar course explored the molecular and cellular basis of abnormal neural function. It focused on basic scientific studies that have provided insight into the etiology and pathogenesis and neurologic and psychiatric diseases. Topics included: (1) molecular pathology of neurotransmitter derangement; (2) developmental plasticity and choice of neurotransmitter phenotype; (3) synthesis and regulation of neuropeptides; (4) cellular events in neural regeneration and brain transplantation; (5) neural pathways involved in pain syndromes; (6) genetic linkage analysis using DNA polymorphisms; (7) defects in DNA repair and activation of onc genes; (8) biochemistry of the lipidoses; (9) autoimmune diseases; (10) brain imaging and metabolism; (11) epilepsy and seizure disorders; (12) cell death in degenerative disorders; (13) viral infections of the nervous system; and (14) experimental models of learning and memory.

PARTICIPANTS

Anthony, Donna T., B.S., Rockefeller University, New York, New York

Bissette, Garth, Ph.D., Duke University, Durham, North Carolina

Blum, Andrew, B.A., Rockefeller University, New York, New York

Buck, Charles R., B.S., Cornell University, Ithaca, New York

Chu, Dorothy C., B.S., University of Michigan, Ann Arbor

Denney, Richard M., Ph.D., University of Texas, Galveston

Donnerfeld, Hyman, M.D., St. Vincent's Hospital, New York, New York

Farrer, Lindsay A., Ph.D., Riley Hospital, Indiana University, Indianapolis

Goodman, Gay, Ph.D., E.K. Shriver Center, Boston, Massachusetts

Gorevic, Peter, State University of New York, Stony Brook
Gray, Patrick W., Ph.D., Genetech, Inc., South San Francisco, California

Hishinuma, Akira, M.D., Columbia University, New York, New York

Kaufman, Daniel L., B.S., University of California, Los Angeles

Knopf, John L., Ph.D., Genetics Institute, Cambridge, Massachusetts



Lark, Lisa A., B.A., University of Illinois, Urbana
 Lee, James E., M.D., Duke University, Durham, North Carolina
 Lyster, Timothy A., Ph.D., Clark University, Worcester, Massachusetts
 MacDonald, Marcy, Ph.D., Massachusetts General Hospital, Boston
 Powell, John F., Ph.D., CNRS, Paris, France
 Rimvall, Karin, M.D., Zurich University, Switzerland
 Taylor, Anne J., B.S., Boston University Medical School, Massachusetts
 Tresley, Richard M., University of Chicago, Illinois
 Trumper, Maria J., Yale School of Medicine, New Haven, Connecticut

Benedict, Children's Hospital, Los Angeles. Oncogenes in neural tumors.
 McNamará, J., Duke University, and P. Schwartzkroin, University of Washington Medical School. Epilepsy and seizure disorders.
 Caskey, T., Baylor College of Medicine. Molecular lesions in the Lesch-Nyhan syndrome.
 Breakfield, X., Shriver Center and Harvard Medical School. Deficiencies in DNA repair and metal ion transport that affect the nervous system.
 Raminsky, M., Montreal General Hospital. Neural regeneration.
 Raichle, M., Washington University School of Medicine. Brain metabolism and imaging.
 Wexler, N., Columbia University School of Medicine. Huntington's disease in Venezuela.
 Black, I., Cornell University Medical School. Development, plasticity, and transmitter phenotype.
 Mishkin, M., National Institute of Mental Health. Learning and memory: Primate models.
 Gage, F., University of California, San Diego. Brain transplantation.
 Scheller, R., Stanford University. Genes, polyproteins, and behavior.
 Fields, H., University of California, San Francisco, and A. Light, University of North Carolina. Pain and neuropeptides.
 Lindstrom, J., Salk Institute. Autoimmune diseases: Myasthenia gravis.
 Haase, A., University of Minnesota, and K. Typer, Harvard Medical School. Viral infections of the nervous system.
 Gajdusek, C., National Institutes of Health. Slow viruses and their molecular pathology.

SEMINARS

Black, I., Cornell University Medical School. Pathology of neurotransmitter disorders.
 Breakfield, X., Shriver Center and Harvard Medical School. Molecular genetic approaches to neurologic diseases.
 Gusella, J., Massachusetts General Hospital and Harvard Medical School. Linkage analysis using recombinant DNA technology in Huntington's disease.
 Breakfield, X., Shriver Center and Harvard Medical School. Molecular genetic studies of familial amyloid polyneuropathies.
 Ginns, E., National Institutes of Health, and E. Kolodny, Shriver Center and Harvard Medical School. Molecular approaches to the lipidosis.
 Schwab, M., University of California, San Francisco and W.

Immunoglobulins: Molecular Probes of the Nervous System

June 7-June 27

INSTRUCTORS

Hockfield, Susan, Ph.D., Cold Spring Harbor Laboratory, New York
 Silberstein, Laura, Ph.D., Stanford University, California
 Evans, Christopher, Ph.D., Stanford University, California

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 explored immunochemical and immunohistochemical techniques in detail and the application of these techniques to current issues in neurobiology.

The laboratory work included: 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; light and electron microscopic immunohistochemistry using peroxidase, fluorescence, and biotin-avidin techniques; and methods for the colocalization of multiple antibodies.

A series of lectures by invited speakers covered: structure and function of immunoglobulins; molecular genetics of antibody diversity; cellular regulation of the immune response; hybridoma technology; studies using synthetic peptides; immunological characterization of the acetylcholine receptor and the neuromuscular junction; and immunological characterization of cellular diversity in vertebrate and invertebrate nervous systems and in cell culture.

PARTICIPANTS

Balak, Kenneth J., Ph.D., University of Utah, Salt Lake City
Barres, Barbara A., M.D., Harvard Medical School, Boston,
Massachusetts

Bug, William J., B.S., Columbia University, New York, New
York

Burd, Gail D., Ph.D., Rockefeller University, New York, New
York

Fels, Gregor, Ph.D., Max-Planck Institut, Munich, Federal
Republic of Germany

Grothe, Claudia, Ph.D., Philipps-University Marburg, Federal
Republic of Germany

Gumaraes, Aurea, Ph.D., Universidade Federal Fluminense,
Rio de Janeiro, Brazil

Mobbs, Charles Vernon, Ph.D., Rockefeller University, New
York, New York

Sagen, Jacqueline, Ph.D., University of Illinois, Urbana
Shiurba, Robert, Ph.D., Stanford University, California

SEMINARS

Steiner, L. Massachusetts Institute of Technology. Antibodies
and the immunoglobulin super family.

Woodland, R., University of Massachusetts Medical School.
Cellular immunology.



———. B-cell function.
Raulat, D., University of Massachusetts Medical School.
T-cell biology and specificity.
Giorgi, J., University of Massachusetts. Flow cytometry and cell sorting.
Evans, C., University of California, Stanford, RIA.
Pintar, J., Columbia University. Gene expression and peptide processing during pituitary development.
Lindstrom, J., Salk Institute. Use of monoclonal antibodies to study acetylcholine receptors and myasthenia gravis.
Matthew, B., Harvard Medical School. Immunological studies of nerve regeneration.

Kindt, T., National Institutes of Health. Molecular biology of immunoglobulin synthesis.
Alt, F., Columbia University. Molecular biology of antibody diversity.
Helfman, D., Cold Spring Harbor Laboratory. Antibodies in molecular cloning.
Rosenberg, L., Stanford Medical School. Immunocytochemical localization of single and dual antigens in brain.
Davies, D., National Institutes of Health. Three-dimensional structure of immunoglobulins.
Scharff, M., Albert Einstein College of Medicine. Somatic mutation in antibody-producing cell lines.

Advanced Techniques in Molecular Cloning

June 7–June 27

INSTRUCTORS

Mark Zoller, Ph.D., Cold Spring Harbor Laboratory, New York
Atkinson, Tom, B.S., University of British Columbia, Vancouver, Canada
Brosius, Jurgen, Ph.D., Columbia University, New York, New York
MacDonald, Ray, Ph.D., University of Texas Health Science Center, Dallas

ASSISTANTS

Williamson, Vicki, Imperial College, London, England
Swift, Galvin, University of Texas Health Science Center, Dallas

This was a laboratory and lecture course on advanced aspects of molecular cloning designed for scientists who are familiar with basic recombinant DNA techniques. It included the chemical synthesis of oligodeoxyribonucleotides of defined sequence and their characterization by the Maxam-Gilbert sequencing method. These oligonucleotides were used in various ways, including their use as probes for the isolation of specific genes from libraries and as reagents for directing site-specific *in vitro* mutagenesis. Specific mutants were isolated, and the sequence of the altered genes was determined. Other methods of *in vitro* mutagenesis such as nucleotide misincorporation into DNA, sodium bisulfate treatment of DNA, and linker insertion were covered in both laboratory exercises and lectures.

Guest lectures and demonstrations covered the application of these techniques to analysis of various cloned genes, as well as the use of alternate methods of mutagenesis, expression, and analysis of cloned genes by immunological screening.

PARTICIPANTS

Birnbaum, Morris J., M.D., Ph.D., Memorial Sloan-Kettering Cancer Center, New York, New York
Freund, Erwin, Ph.D., Roche Institute, Nutley, New Jersey
Knight, Regina, M.S., National Institutes of Health, Bethesda, Maryland
Kolakowski, Lee Franklin, Jr., B.S., University of Pennsylvania, Philadelphia

Nagel, Julianne, Ph.D., University of Kentucky, Lexington
O'Halloran, Thomas V., Ph.D., Massachusetts Institute of Technology, Cambridge
Ohlrogge, John B., Ph.D., USDA Northern Regional Research Center, Peoria, Illinois
Paddock, Mark L., M.S., University of California, San Diego
Reed, John C., B.A., University of Pennsylvania, Philadelphia
Risuleo, Gianfranco M., Ph.D., Karolinska Institute, Stockholm, Sweden



Schulman, LaDonne, Ph.D., Albert Einstein School of
Medicine, Bronx, New York
Shears, Bradley T., Ph.D., Massachusetts Institute of
Technology, Cambridge
Siminovich, Katherine Anne, M.D., Toronto Western Hospital,
Canada
Vernick, Kenneth D., M.S., National Institutes of Health,
Bethesda, Maryland

SEMINARS

Urdea, M., Chiron Corp. Gene synthesis.
Fiddes, J., California Biotechnology, Inc. Structure and
expression of atrial *natriuretic* factor genes.
Russell, D., University of Texas Health Science Center. The
LDL receptor gene: A mosaic of exons assembled from
other genes.
Sutcliffe, J. G., Scripps Clinic and Research Institute. Cloning
messenger RNA from the brain.

Ullrich, A., Genetech. cDNA approaches.
Noller, H., University of California, Santa Cruz. Probing the
structure and function of large ribosomal RNAs.
Young, R., Massachusetts Institute of Technology.
Immunoscreening of λ 6III recombinant DNA expression
libraries.
Wharton, R., Harvard University. Defining the DNA-binding
surface of a repressor.
Marians, K., Memorial Sloan-Kettering Cancer Center.
Mutational analysis reveals the structure of an origin of
DNA replication.
Kawasaki, G., Zymogenetics. Expression of protein in yeast.
Schatzman, A., Smith, Kline & French. Strategies for the
overproduction of heterologous gene production: Search
for the perfect *E. coli* system.
Smith, M., University of British Columbia. The use of
oligonucleotides as primers and probes.

Molecular Biology of Plants

June 7-June 27

INSTRUCTORS

Sussex, Ian, Ph.D., Yale University, New Haven, Connecticut
Messing, Joachim, Ph.D., University of Minnesota, St. Paul
Horsch, Robert, Monsanto Company, St. Louis, Missouri

ASSISTANTS

Kerk, Nancy, B.A., Yale University, New Haven, Connecticut
Ludwig, Steven, B.A., University of Minnesota, St. Paul
Kirihara, Julie, B.A., University of Minnesota, Saint Paul
Fry, Joyce, B.A., Monsanto Company, St. Louis, Missouri
Hauptmann, Randy, Ph.D., Monsanto Company, St. Louis, Missouri



This course will provide an introduction to current techniques for the manipulation of plant material as applied to experiments in genetics and molecular biology. It is designed primarily for scientists working in other areas of molecular biology who wish to pursue research in plants. It is assumed that applicants will have a working knowledge of molecular techniques, but no previous experience with plants is required. Laboratory experiments will include: plant anatomy and development, nucleic acid isolation and manipulation from plant materials, crown gall tumorigenesis, cell and protoplast culture techniques, photosynthesis, genetic and cytogenetic analysis of maize. Guest lecturers will cover both current molecular genetic research, and also special topics in classical botany and systems unique to plants.

PARTICIPANTS

Cannon, Maura C., Ph.D., Biotechnica International,
Cambridge, Massachusetts
 Chu, Lily L., M.S., Molecular Genetics Inc., Boston,
Massachusetts
 Cuzzo, Maria A., M.S., Albert Einstein College of Medicine,
Bronx, New York
 DeLisle, Alice J., Ph.D., Indiana University, Bloomington
 Garcarrubio, Alejandro, M.S., Nitrogen Fixation Center,
Mexico City, Mexico
 Gheysen, Godelieve, M.S., University of Gent, Belgium
 Golden, James W., Ph.D., University of Chicago, Illinois
 Kjellbom, Per, M.S., University of Lund, Sweden
 Nierzwicki-Bauer, Sandra, Ph.D., University of Chicago,
Illinois
 Pang, Patty P., Ph.D., Massachusetts Institute of Technology,
Cambridge
 Pedersen, Henrik, Ph.D., Rutgers University, New Brunswick,
New Jersey
 Shotwell, Mark A., Ph.D., Hospital for Sick Children, Toronto,
Canada

Singh, Arjun, Ph.D., Genetech, Inc., South San Francisco,
California
 Staneloni, Roberto, Ph.D., McMaster University, Ontario,
Canada
 Suzuki, Akira, Ph.D., McMaster University, Ontario, Canada
 Toennissen, Gary, Ph.D., Rockefeller University, New York,
New York

SEMINARS

Horsch, R., Monsanto Company. Tissue culture and
transformation.
 Sussex, I., Yale University. Organization and development of
plants.
 Gengenbach, B., University of Minnesota. Tissue-culture
systems in maize.
 Phillips, R., University of Minnesota. Maize cytogenetics.
 Theologis, S., Washington University. Hormone-inducible
genes.
 Messing, J., University of Minnesota. Plant gene structure.
 Bohnert, H., University of Arizona. Molecular biology of the
chloroplast.

- Verma, D. P., McGill University. Nodulation genes in legumes.
- Shah, D., Monsanto Company. Molecular biology of herbicide resistance.
- McIntosh, L., Michigan State University. Light-inducible genes.
- Crouch, M., Indiana University. Molecular biology of seed development.
- Maliga, P., Advanced Genetic Sciences Co. Organellar genetics.
- Levings, S., North Carolina State University. Plant mitochondrial genome structure and function.
- Binns, A., University of Pennsylvania. Biology of *Agrobacterium tumefaciens*.
- Dooner, H., Advanced Genetic Sciences Co. Controlling elements in maize.
- Shepherd, R., University of Kentucky. Plant DNA viruses.
- Meyerowitz, E., California Institute of Technology. Molecular genetics of *Arabidopsis*.
- Miles, D., University of Missouri. Photosynthetic mutants in maize.
- Cashmore, T., Rockefeller University. Expression of chimeric genes in plants.
- Staskowicz, B., University of California, Berkeley. Molecular genetics of host-pathogen interactions.
- Robertson, H., Rockefeller University. Plant viroids.
- Meinke, D., Oklahoma State University. *Arabidopsis* development.
- Wang, A., Sangene Co. Cytogenetics
- Dellaportia, S., Cold Spring Harbor Laboratory. Genetics.

Computational Neuroscience

June 20–July 3

INSTRUCTORS

Atkeson, Christopher G., M.S., Massachusetts Institute of Technology, Cambridge
Blizi, Emilio, M.D., Massachusetts Institute of Technology, Cambridge
Hildreth, Ellen C., Ph.D., Massachusetts Institute of Technology, Cambridge
Movshon, J. Anthony, Ph.D., New York University, New York

Computational approaches to problems in neuroscience have produced important advances in our understanding of neural processing. The most prominent successes have come in areas where strong inputs from neurobiological behavioral and computational approaches can interact. This intensive lecture course examined two areas: the visual processing of motion information, and motor control. The theme was that an understanding of the computational problems, the constraints on solutions to these problems, and the range of possible solutions can help guide research in neuroscience.

The first half of the course was devoted to studies of visual motion processing. Topics considered included 1) the anatomical and physiological organization of motion-sensitive portions of the visual pathway; 2) the construction of motion detectors and the directional selectivity of visual neurons; 3) analysis of the motion of complex objects in two and three dimensions; 4) processing of optic flow information. The second half focused on computational approaches to the study of motor control, and their interactions with motor control neuroscience. Examples of computational approaches were taken from 1) single- and multi-articular arm movements; 2) body posture and locomotion and 3) grasping and utilization of tactile information. Areas addressed were movement planning, kinematics, dynamics, control and actuation.

PARTICIPANTS

Barash, Shablai, B.S., Hebrew University Medical School,
 Jerusalem, Israel
 Brown, Richard, B.S., University of California, San Francisco

Chipalkatti, Renu, M.S., University of Massachusetts,
 Amherst
 Cole, Kelly J., Ph.D., University of Wisconsin, Madison
 Dehaene, Stanislas, M.S., École Normale Supérieure, Paris,
 France



Dornay, Menashe, M.S., Weizmann Institute, Rehovot, Israel
 Dupoux, Emmanuel, M.S., École Normale Supérieure, Paris, France
 Ewer, John, M.S., Brandeis University, Waltham, Massachusetts
 Iberall, Althea, M.S., University of Massachusetts, Amherst
 Kefer, Joyce, B.S., University of Wisconsin, Madison
 Kruse, Margaret, B.A., University of Minnesota, Duluth
 Lackner, Klaus, Ph.D., Los Alamos National Laboratory, New Mexico
 Letelier, Juan-Carlos, M.S., City College of New York, New York
 Liestol, Knut, Ph.D., University of Oslo, Norway
 Miller, Kenneth, M.S., Stanford University, California
 Minor, Lloyd, M.S., University of Chicago, Illinois
 Morris, Edward, B.A., University of California, San Francisco
 Ohrbach, Harry, Ph.D., California Institute of Technology, Pasadena
 Reid, Robert Clay, B.S., Rockefeller University, New York, New York
 Stepnoski, R. Aaron, M.S., University of Philadelphia, Pennsylvania
 Szuiborski, Robert, B.A., University of Philadelphia, Pennsylvania
 Unnikrishnan, K. P., M.S., Syracuse University, Ithaca, New York

SEMINARS

Movshon, J. A., New York University. Historical overview of experimental work on motion analysis.
 ———. Organization of subcortical visual pathways.
 ———. Physiological studies of the aperture problem.
 Hildreth, E. C., Massachusetts Institute of Technology. The computational study of vision.
 ———. Computation of the two-dimensional velocity field.
 ———. Computational models of structure from motion.
 Adelson, E. H., RCA Laboratories. Spatiotemporal energy models for motion detection.
 ———. Psychophysical studies of the aperture problem.
 Andersen, R., Salk Institute. Motion analysis in the posterior parietal cortex.
 Ballard, D., University of Rochester. Computational models of motion correspondence.
 ———. Connectionism and motion processing.
 Maunsell, J., Massachusetts Institute of Technology. Separate cortical pathways for the analysis of form and motion.
 Morgan, M. J., University College London. Short-range and long-range motion processes.
 ———. Psychophysical tests of computational models.
 Nakayama, K., Smith-Kettlewell Institute of Visual Sciences. Psychophysical isolation of motion-sensitive mechanisms.
 ———. The spatial integration of motion information.
 ———. The extraction of higher-order information from the optic flow field.
 Newsome, W., State University of New York, Stony Brook. Responses of cortical neurons to sampled motion.
 Pasternak, T., University of Rochester. The role of cortical directionality in visual behavior.
 Poggio, T., Massachusetts Institute of Technology. Theoretical properties of motion detection models.
 ———. Biophysical mechanisms for direction selectivity.
 ———. Neurobiological tests of computational models.
 Sekuler, R., Northwestern University. Psychophysical studies of direction-specific mechanisms.
 ———. Integration of stochastic local motions.
 Simpson, J. I., New York University School of Medicine. Integration of visual and vestibular motion signals.
 Sperling, G., New York University. Theory and experiments regarding generalized Reichardt models.
 ———. The role of time and distance in apparent motion.
 ———. Perceptual studies of the recovery of structure from motion.
 Thompson, W., University of Minnesota. Multi-resolution analysis of time-varying imagery.
 ———. Computational models of discontinuity detection and analysis.
 Van Essen, D., California Institute of Technology. Organization of cortical visual pathways.

- Wurtz, R., National Institutes of Health. Visual motion processing for control of pursuit eye movements.
- Bizzi, E., Massachusetts Institute of Technology. Single joint studies: Muscle properties, the equilibrium position hypothesis and the reference trajectory hypothesis.
- Atkeson, C. G., Massachusetts Institute of Technology. What is computational neuroscience? A motor control viewpoint.
- . Multi-joint kinematics.
- . Multi-joint dynamics.
- . Review of control theory.
- . Improving motor performance with experience.
- Hunter, I., McGill University. Muscle and joint mechanical dynamics.
- Hogan, N., Massachusetts Institute of Technology. Using optimal control theory to model behavior: Minimum jerk movements.
- . Multi-joint arm mechanics and control.
- Mussa-Ivaldi, F., Massachusetts Institute of Technology. Multi-joint arm studies.
- McCollum, G., Good Samaritan Hospital and Medical Center. Theoretical approaches to sensorimotor control of posture.
- McMahon, T., Harvard University. The mechanics of terrestrial locomotion.
- Raibert, M., Carnegie-Mellon University. The dynamics of running in robots.
- Loeb, G., National Institutes of Health. Muscle spindle: A biological solution to a classical dilemma in signal theory.
- Abbs, J., University of Wisconsin. Coordination of multi-articulate speech and finger movement.
- Hollerbach, J., Massachusetts Institute of Technology. Tactile sensing and hand control.

Neurobiology of *Drosophila*

June 30–July 20

INSTRUCTORS

- Jan, Lily Yeh, Ph.D., University of California, San Francisco
- Jan, Yuh Nung, Ph.D., University of California, San Francisco
- O'Farrell, Patrick H., Ph.D., University of California, San Francisco
- Greenspan, Ralph J., Ph.D., Princeton University, New Jersey

ASSISTANT

- Timpe, Leslie, University of California, San Francisco

This laboratory/lecture course was designed for people who may want to use *Drosophila* as an experimental system for studying function or development of the nervous system. One major aim was to introduce students to the various genetic, molecular, and physiological techniques that are currently available in *Drosophila* research and that make it distinctive.

The course began with a crash course on *Drosophila* genetics (a series of lectures supplemented with laboratory demonstrations and exercises) to familiarize the students with classical genetics of *Drosophila*, strategies of mutant isolation, methods used to analyze newly isolated mutations, and mosaic analysis. This was followed by workshops and laboratory projects on molecular genetics and electrophysiology. In the area of molecular genetics topics covered were strategies and techniques for cloning genes, in situ hybridization to polytene chromosomes, PM hybrid dysgenesis, and DNA-mediated transformation, while the electrophysiology section covered neuromuscular transmission and voltage clamp analysis, as applied to mutants with altered ion channels. Additionally, a series of lectures by invited speakers illustrated the application of these techniques to current research. The research topics included: molecular and functional analysis of mutants with distinctive ion channels, establishment of neuronal pathways in the developing embryo as well as in imaginal discs, analysis of behavioral mutants, maternal and zygotic mutations that alter polarity and segmentation, homeotic mutations, and the use of DNA transformation for the study of gene expression.

PARTICIPANTS

- Blochlinger, Karen, B.S., Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland
- Davies, Jane A., Ph.D., Australian National University, Canberra
- Desplan, Claude R., Ph.D., University of California, San Francisco
- Gil, Daniel W., Ph.D., University of Pennsylvania, Philadelphia
- Isacoff, Ehud Y., Ph.D., McGill University, Montreal, Canada
- Johansen, Kristen M., M.A., Yale University, New Haven, Connecticut
- Nichols, Ruthann, Ph.D., Purdue University, Lafayette, Indiana
- Rabin, Bruce A., M.S., Albert Einstein College of Medicine, Bronx, New York
- Schmidt, Carl J., B.A., National Institutes of Health, Bethesda, Maryland
- Siwicki, Kathleen K., Harvard Medical School, Boston, Massachusetts

SEMINARS

- Greenspan, R. J., Princeton University. Bean bag genetics.
- . *Drosophila* genetics
- . Construction and use of stocks.
- . Generating and isolating mutations.
- . Analyzing mutations.
- . Advanced chromosomesmanship
- Jan, L. Y., University of California, San Francisco. Introduction to behavioral mutants.

- . Mutations affecting synaptic transmission.
- Quinn, W. G., Massachusetts Institute of Technology. Learning mutants.
- Greenspan, R. J., Princeton University. The varieties of mutation experience.
- . Complex loci.
- Wieschaus, E., Princeton University. Early embryogenesis.
- . Segmentation mutants.
- . Maternal effects.
- O'Farrell, P., University of California, San Francisco. General recombinant DNA technology.
- Pirrota, V., Baylor College of Medicine. Microdissection; various cloning vectors.
- Spradling, A., Carnegie Institution of Washington. Hybrid dysgenesis and p element transformation.
- . Use of transformation to dissect junctional elements; chorion amplification.
- O'Farrell, P., University of California, San Francisco. Cloning DNA sequences coding known and unknown products.
- Levine, M., Columbia University. In situ localization of transcripts.
- . Engrailed and homeotic loci.
- Pardue, M. L., Massachusetts Institute of Technology. Cytogenetics.
- Bender, W., Harvard Medical School. Molecular biology of mutation.
- . Combining classical genetics with molecular biology; homeotic mutations.
- Bate, M., University of Cambridge. Development of the nervous system.
- . Pathfinding.





Molecular Embryology of the Mouse

June 30–July 20

INSTRUCTORS

Rossant, Janet, Ph.D., Brock University, St. Catharines, Ontario, Canada

Pedersen, Roger, Ph.D., University of California, San Francisco

ASSISTANTS

Vijh, Marian, M.S., Brock University, St. Catharines, Ontario, Canada

Fox, Niles, Ph.D., Wistar Institute, Philadelphia, Pennsylvania

Robertson, Liz, Ph.D., University of Cambridge, England

This course was designed for biochemists, molecular biologists, and cell biologists interested in applying their expertise to the study of mouse development. In particular, the genetic manipulation of the mouse through the introduction of foreign genes and cells in early embryos was stressed. Through laboratory exercises and lectures, the participants were introduced to the following procedures and their possible applications: the isolation and *in vitro* culture of germ cells and preimplantation and early postimplantation embryos, the microinjection of DNA into fertilized eggs, nuclear transplantation, the formation of chimeras, dissection of germlayers, the localization of antigens and mRNAs in embryonic tissue sections, and basic mouse handling and breeding techniques. Guest speakers discussed current research in related fields.

PARTICIPANTS

Begemann, Martin, M.D., Columbia University, New York, New York

Calof, Anne L., Ph.D., University of California School of Medicine, San Francisco

Carey, Frederick J., Ph.D., University of Michigan, Ann Arbor

Ebihara, Lisa, M.D., Ph.D., University of Colorado, Boulder

Goodwin, Maureen M., Ph.D., Memorial Sloan-Kettering Cancer Center, New York, New York

Leavitt, John C., Ph.D., Linus Pauling Institute, Menlo Park, California

Odom, Daniel P., B.A., Case Western Reserve University, Cleveland, Ohio

Parada, Luis F., Ph.D., Massachusetts Institute of Technology, Cambridge

Sapienza, Caren, Ph.D., McGill University, Montreal, Canada
Sequin, Carl, Ph.D., National Institutes of Health, Bethesda, Maryland
Skowronski, Jacek, Ph.D., National Institutes of Health, Bethesda, Maryland
Surh, Linda C., M.D., Baylor College of Medicine, Waco, Texas
Trounson, Alan, Ph.D., Monash University, Clayton, Australia
Varmuza, Sue L., M.S., McMaster University, Hamilton, Canada

SEMINARS

Palmiter, R., University of Washington. Transgenic mice.
Copp, A., Stanford University. Primordial germ cells.
Schatter, G., Florida State University. Cytoskeleton in eggs and embryos.
Papaioannou, V. E., Tufts University. Mouse mutants in development.
Damsky, C., Wistar Institute. The importance of cell adhesion molecules in early development.

Lawson, K., Hubrecht Laboratory. Fate mapping the endoderm.
Angerer, L., University of Rochester. In situ hybridization in sea urchin embryos.
Mahon, K., National Institutes of Health. In situ hybridization in transgenic mice.
Solter, D., Wistar Institute. Nuclear transfer.
Chapman, V., Roswell Park Memorial Institute. X-inactivation.
Hanahan, D., Cold Spring Harbor Laboratory. Transgenic mice and oncogenes.
Lacy, L., Memorial Sloan-Kettering Cancer Center. Insertional mutations affecting mammalian development.
Linney, E., Duke University. Retroviral enhancers and teratocarcinoma cells.
Schultz, G., University of Calgary. Gene expression in early embryos.
McGinnis, W., Yale University. Homeo boxes.
Jaenisch, R., Whitehead Institute. Retroviruses and development.

Advanced Bacterial Genetics

June 30-July 20

INSTRUCTORS

Silhavy, Thomas J., Ph.D., Princeton University, New Jersey
Berman, Michael L., Ph.D., Litton Institute of Applied Biotechnology, Rockville, Maryland
Enquist, Lynn W., Ph.D., E.I. du Pont de Nemours & Co., Wilmington, Delaware

ASSISTANTS

Miessner, Scott, Ph.D., Litton Institute of Applied Biotechnology, Rockville, Maryland
Trun, Nancy, B.S., Princeton University, New Jersey
Robbins, Alan, Ph.D., E.I. du Pont de Nemours & Co., Wilmington, Delaware



This course demonstrated the use of gene fusions, transposable elements, and recombinant DNA for genetic analysis in *Escherichia coli*. Students learned to construct gene fusions both in vivo and in vitro. Subsequent experiments stressed the use of these fusions for monitoring gene expression and, in conjunction with transposable elements, for obtaining defined mutations (nonsense, deletion, and insertion) either in the target gene or in regulatory genes. Recombinant DNA was applied to clone and define physically the target gene, the regulatory genes, and the sites of action of the regulatory proteins at the target gene. In addition, the cloned DNA and the defined mutations were used to analyze the genes and the regulatory system genetically. For the sake of clarity, the course focused on a particular region; however, the experimental techniques presented are sufficiently general to be applicable to any gene in *E. coli* for which there exists a mutation conferring a recognized phenotype.

PARTICIPANTS

Beaucage, Serge L., Ph.D., Beckman Instruments, Palo Alto, California
Berg, Howard C., Ph.D., California Institute of Technology, Pasadena
Champeny, W. Scott, Ph.D., East Tennessee State University College of Medicine, Johnson City
Davidson, John P., Ph.D., Tuskegee Institute, Alabama
Fikes, John D., B.S., University of North Carolina, Chapel Hill
Kramer, Tina J., B.S., California Institute of Technology, Pasadena
Machlin, Sara M., B.A., Gray Freshwater Biological Institute, Navarre, Minnesota
Puaoli, Deidre L., M.S., University of Kentucky, Lexington
Ray, Catherine C., B.S., Emory University, Atlanta, Georgia
Ruger, Barbara, Ph.D., University of Erlangen, Federal Republic of Germany
Saber, Diane L., Gray Freshwater Biological Institute, Navarre, Minnesota
Sacerdot, Christine C., Ph.D., Institut de Biologie Physico-Chimique, Paris, France
Sanders, May Ellen, Ph.D., Miles Laboratories, Elkhart, Indiana

Spitznagel, John K., M.D., Emory University, Atlanta, Georgia
Tully, Raymond E., Louisiana State University, Baton Rouge
Valenzuela, Dario, M.S., Albert Einstein College of Medicine, Bronx, New York

SEMINARS

Menzel, R., E.I. du Pont de Nemours & Co. The regulation of *E. coli* DNA gyrase: A new twist to transcriptional control.
Irwin, N., Harvard University. Positive control of transcription in *E. coli*.
Brown, S., University of Massachusetts. Mutational analysis of 45S RNA of *E. coli*.
Mauer, R., Case Western Reserve University. Interaction of DNA polymerase III genes in *Salmonella typhimurium*.
Birgit, P., University of Texas Medical School. Transposon-assisted fine structure genetic mapping.
Zagursky, B., E.I. du Pont de Nemours & Co. Advances in rapidly sequencing double-stranded DNA.
Maloy, S., University of Illinois. Autogenous regulation by a membrane-bound enzyme.

Cellular and Molecular Biology of Behavior

July 12–July 26

INSTRUCTORS

Byrne, Jack, Ph.D., University of Texas Medical School, Houston
Kandel, Eric, M.D., Columbia University College of Physicians & Surgeons, New York, New York
Pearson, Keir, Ph.D., University of Alberta School of Medicine, Edmonton, Canada

This lecture course provides an introduction to cellular and molecular approaches used in the study of behavior and behavioral modifications. It is well suited for both graduate students in neurobiology and research workers in other disciplines who are interested in obtaining an introduction to cellular neurobiology. The



course covered a wide variety of topics ranging from gene regulation in the nervous system to cellular aspects of complex behaviors and learning. Rather than being an extensive survey, however, the lecture provided an intensive coverage of four selected areas: 1) introduction to the cell biology of neurons including membrane biophysics, synaptic transmission and regulation of gene expression; 2) cellular and subcellular mechanisms underlying neuronal plasticity and their relationship to simple behavioral modifications; 3) cellular organization and modifiability of complex behaviors such as locomotion and the control of movement; and 4) neural approaches to the study of higher forms of behavior such as communication and language. General principles were illustrated with a variety of model systems in which modern cell biological approaches (including the application of recombinant DNA technology) have been applied. To put the cellular work into perspective, selected examples were also taken from human behavior and its abnormalities.

PARTICIPANTS

Astrand, Per, M.S., Karolinska Institute, Stockholm, Sweden
Barash, Shabtai, B.S., Hebrew University, Jerusalem, Israel
Brilliant, Murray, Ph.D., Tufts University, Medford,

Massachusetts

Brodin, Lennart, M.S., Karolinska Institute, Stockholm,
Sweden

Colwill, Ruth, Ph.D., Columbia University, New York, New
York

Fujita, Ichiro, Ph.D., National Institute for Physiological
Sciences, Okazaki, Japan

Gilbert, David, B.A., Stanford University, California

Goodman, Gay, Ph.D., E.K. Shriver Center, Boston,
Massachusetts

Gynther, Ian, B.S., University of Alberta, Edmonton, Canada

Haynes, Lynne, Ph.D., Stanford University, California

Johnson, Stephen, M.S., University of Iowa, Iowa City

Kasai, Haruo, M.D., University of Tokyo, Japan
Knapp, Michael, Ph.D., Genetica, Paris, France
Lackner, Klaus, Ph.D., Los Alamos National Laboratory, New
Mexico

Lindstrom, Anders, M.S., Center for Neurobiology/Behavior
Research, New York, New York

Manier, Edward, Ph.D., University of Notre Dame, Indiana

McAfee, Donald, Ph.D., Beckman Research Institute, Duarte,
California

Pettersson, Lars-Gunner, B.S., University of Goteborg,
Sweden

Raskovsky, Sonia, B.S., University of Buenos Aires, Argentina

Schaeffer, Eric, B.A., Albert Einstein College of Medicine,
Bronx, New York

Sonetti, Dario, B.S., University of Modena, Italy

Tang, Andrew, Ph.D., Upjohn Company, Kalamazoo,
Michigan

Tse, Frederick, M.S., University of Toronto, Canada

SEMINARS

- Kandel, E., Columbia University College of Physicians & Surgeons. Introduction to the cellular study of behavior.
- Quinn, C., Massachusetts Institute of Technology. Introduction to the study of genes and behavior.
- Adler, J., University of Wisconsin. Genes and the behavior of bacteria.
- King, C., University of Wisconsin. Genes and the behavior of paramecium.
- Byrne, J., University of Texas Medical School. Cellular and biophysical determinants of elementary behavioral acts I.
- . Cellular and biophysical determinants of elementary behavioral acts II.
- Wyman, R., Yale University. Genetic approaches to the analysis of simple behaviors.
- Byrne, J., University of Texas Medical School. Synaptic plasticity and neural modulation.
- Kandel, E., Columbia University College of Physicians & Surgeons. Learning I. Habituation.
- . Learning II. Sensitization.
- . Learning III. Classical conditioning.
- Quinn, C., Massachusetts Institute of Technology. Genetic approaches to learning.
- Scheller, R., Stanford University. Genes, peptides, and behavior.
- Weeks, J., University of California, Berkeley. Hormones and behavior.
- Kupfermann, I., Columbia University. Motivation.
- Zigmond, M., University of Pittsburgh. Biochemical regulation of synaptic function.
- Kaplan, B., University of Pittsburgh. Genetic regulation of synaptic function.
- Zigmond, M., University of Pittsburgh. Role of neurotransmitters in behavioral abnormalities.
- Pearson, K., University of Alberta School of Medicine. Introduction to motor sequences.
- Marder, E., Brandeis University. Rhythm generation in invertebrate motor systems.
- Pearson, K., University of Alberta School of Medicine. Central and reflex control of movements.
- . Locomotion in vertebrates.
- Lisberger, S., University of California. Adaptive regulation in the oculomotor system.
- Hollerbach, J., Massachusetts Institute of Technology. Strategies for the control of voluntary movement.
- Delong, M., Johns Hopkins University. Functional organization of the basal ganglia.
- Wyatt, R., National Institute of Mental Health. Brain grafting in animals and man.
- Pearson, K., University of Alberta School of Medicine. Introduction to communication.
- Suga, N., Washington University. Brain pathways for sound processing in bats.
- Ojemann, G., University of Washington. Exploration of language areas of the brain by electrical stimulation.
- Ross, E., Southwestern Medical School. Language functions of the human brain and their localization.
- Nottebohm, F., Rockefeller University. Brain pathways for vocal song control in birds.
- . Hormonal regulation of synaptogenesis and neurogenesis.

Single Channel Methods: Expression, Reconstitution, and Recording

July 22-August 11

INSTRUCTORS

- Dionne, Vincent E.**, Ph.D., University of California, San Diego
- White, Michael M.**, Ph.D., California Institute of Technology, Pasadena
- Coronado, Roberto**, Ph.D., University of North Carolina, Chapel Hill
- Yellen, Gary**, Ph.D., Brandeis University, Waltham, Massachusetts
- Bean, Bruce**, Ph.D., Harvard University, Cambridge, Massachusetts

ASSISTANTS

- Stevens, Meg**, Harvard University, Cambridge, Massachusetts
- Perry, Dave**, Wesleyan University, Middletown, Connecticut

The technologies of patch-clamping, reconstitution and molecular biology promise major revisions and novel approaches for the examination of many neurobiological problems. The application of these methods to study the physiology and biophysics of ion channels was taught in this intensive laboratory and lecture course. Students concentrated initially on single-channel recording using the patch clamp technique. Several practical aspects were covered: fabrication of patch

electrodes, giga-seal formation, and methods for cell-attached, cell-free and whole-cell recording. The design and implementation of recording equipment, and the theory and analysis of single channel currents were discussed during lectures. The course included two workshops. During one the methods of handling and injecting foreign mRNA into *Xenopus* oocytes for expression and study of both voltage-gated and ligand-gated ion channels were covered. Newly expressed channels in the oocytes were studied with the two electrode voltage clamp. The second workshop covered the techniques for reconstitution of functional ion channels into artificial lipid bilayers. Ion channels from several sources were incorporated into synthetic membranes on microelectrodes and into large planar films and studied by single channel methods.

PARTICIPANTS

Braun, Maike, B.S., University of Cambridge, England
Cox, Thomas, Ph.D., Southern Illinois University, Carbondale
Ferguson, James, M.S., University of California, Davis
Goldsmith, Mary, Ph.D., Yale University, New Haven, Connecticut
Greenfield, John, B.A., University of Virginia, Charlottesville
Hill, Joseph, Jr., B.S., Duke University Medical Center, Durham, North Carolina
Kinnamon, Sue, Ph.D., University of Colorado, Boulder
Sahley, Christie, Ph.D., Yale University, New Haven, Connecticut
Swanson, Richard, Ph.D., Massachusetts Institute of Technology, Cambridge
Weiss, David, B.A., Baylor College of Medicine, Waco, Texas

SEMINARS

Siegelbaum, S., Columbia University College of Physicians & Surgeons. The role of protein phosphorylation in the modulation of a K⁺ channel in *Aplysia* neurons.
Claudio, T., Yale University. Stable expression of AChR cDNA genes in tissue cultured cells.
French, R. J., University of Maryland. Modulation and modification of single Na channels in planar lipid bilayers.
Sachs, F., State University of New York, Stony Brook. Mechano-reception by stretch-activated channels.
Stevens, C. F., Yale University. Kinetic behavior of Na channels in cultured cells.
Gruol, D. L., Research Institute of Scripps Clinic. Mechanisms of inter- and intracellular communications in the vertebrate CNS.





Molecular Cloning of Eukaryotic Genes

July 22-August 11

INSTRUCTORS

Bothwell, Al, Ph.D., Yale University, New Haven, Connecticut

Alt, Fred, Ph.D., Columbia University College of Physicians & Surgeons, New York, New York

Lehrach, Hans, EMBL, Heidelberg, Federal Republic of Germany

ASSISTANTS

Bleir, Peter, B.A., Yale University, New Haven, Connecticut

Zimmerman, Kathy, B.A., Columbia University, New York, New York

Poustka, Anna Marie, M.T.R., EMBL, 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 were discussed. Guest lectures discussed the application of molecular cloning procedures to the study of specific eukaryotic gene systems.

PARTICIPANTS

Al-Awqati, Qais, M.B., Ch.B., Columbia University, New York, New York

Baekkeskov, Steinunn, Ph.D., Hagedorn Research Laboratory, Denmark

Beguino, Laura, M.D., National Cancer Institute, Bethesda, Maryland

Carlstedt-Duke, Jan, Ph.D., Karolinska Institut, Stockholm, Sweden

Dvorak, Harold, M.D., Beth Israel Hospital, New York, New York

Feldman, David, M.D., Stanford University, California
Hockfield, Susan, Ph.D., Cold Spring Harbor Laboratory,
New York
Hoy, Marjorie, Ph.D., University of California, Berkeley
Hughey, Rebecca, Ph.D., University of Pittsburgh,
Pennsylvania
Kalsheker, Noor, M.D., University of Wales, Cardiff
Morgan, Robin, Ph.D., University of California, Berkeley
Nairn, Roderick, Ph.D., University of Michigan, Ann Arbor
Nissley, S. Peter, M.D., National Cancer Institute, Bethesda,
Maryland
Springer, Timothy, Ph.D., Dana Farber Cancer Institute,
Boston, Massachusetts
Unanue, Emil, M.D., Washington University School of
Medicine, St. Louis, Missouri

SEMINARS

Schimke, R., Stanford University. Gene amplification.
Myers, R., Harvard University. Detection of single base
mismatches in eukaryotic DNA.
Goff, S., Columbia University College of Physicians &
Surgeons. In vitro mutagenesis of retrovirus genes.
Yancopoulos, G., Columbia University College of Physicians
& Surgeons. Gene transfer into eukaryotic cells.
Blackwell, K., Columbia University College of Physicians &
Surgeons. Gene transfer into eukaryotic cells.

McKnight, S., Fred Hutchinson Cancer Center. Regulation of
transcription.
Hood, L., California Institute of Technology. The genes
encoding the T-cell receptor.
Fink, G., Whitehead Institute. Ty element as disguised
retroviruses.
Miyada, G., Beckman Research Institute of the City of Hope.
The use of oligonucleotides in screening DNA.
Gluzman, Y., Cold Spring Harbor Laboratory. Eukaryotic viral
vectors.
Kellems, R., University of Texas, Houston. Gene
amplification.
Gething, M.-J., Cold Spring Harbor Laboratory. Studies of
the cellular transport of Fla hemagglutinin mutants.
Hanahan, D., Cold Spring Harbor Laboratory. Transformation
in transgenic mice.
Murray, N., University of Edinburgh. Lambda genetics.
Synder, M., Stanford University. Chromosome segregation in
yeast and the λ gt11 expression system.
Lehrach, H., EMBL. The murine T locus and chromosome
walking.
Bothwell, A., Yale Medical School. The tissue-specific
expression of Ig genes and the development of somatic
variants.
Alt, F., Columbia University. The family of *myc* genes.

Yeast Genetics

July 22-August 11

INSTRUCTORS

Sherman, Fred, Ph.D., University of Rochester, New York
Fink, Gerald, Ph.D., Massachusetts Institute of Technology, Cambridge, Massachusetts
Hicks, James, Ph.D., Cold Spring Harbor Laboratory, New York

ASSISTANTS

Moerschell, Rick, B.S., University of Rochester, New York
Morfeld, Ramona, B.S., Cold Spring Harbor Laboratory

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

Breviaro, Diego, Ph.D., National Institutes of Health,
Bethesda, Maryland

Haltiner, Michele, B.S., University of California, Berkeley
Herscovics, Annette, Ph.D., McGill Cancer Centre, Montreal,
Canada
Lavi, Sara, Ph.D., Tel Aviv University, Israel



Lazarow, Paul, Ph.D., Rockefeller University, New York
 Lew, Daniel, B.A., Rockefeller University, New York
 Ljungdahl, Per, B.A., Dartmouth Medical School, Hanover,
 New Hampshire
 Nelsky, Bradley, B.A., Cornell University, Ithaca, New York
 Neilsen, Ole, Ph.D., University of Aarhus, Denmark
 Olempska-Beer, Zofia, Ph.D., National Institutes of Health,
 Bethesda, Maryland
 Rothman, James, Ph.D., Stanford University, California
 Scordilis, Gail, Ph.D., University of Massachusetts, Amherst
 Shen, Nancy, B.S., Rutgers University, New Brunswick, New
 Jersey
 Thevelein, Johan, Ph.D., Katholieke Universiteit, Louvain,
 Belgium
 White, Terry, Ph.D., University of Texas Medical School,
 Houston
 Wright, Tony, Ph.D., Imperial College of Science and
 Technology, London, England

SEMINARS

Plashne, M., Harvard University. Regulatory proteins in
 yeast.
 Pringle, J., University of Michigan. Cellular morphogenesis in
 the yeast cell cycle.
 Prakash, L., University of Rochester. Structure and function
 of DNA repair genes.
 Broach, J., Princeton University. Control of plasmid propaga-
 tion in yeast.

Fink, G., Massachusetts Institute of Technology. Reverse
 transcription in yeast.
 Hicks, J., Cold Spring Harbor Laboratory. Regulation and
 interconversion of mating types.
 Lindquist, S., University of Chicago. Sporulation-specific
 gene expression in yeast.
 Sherman, F., University of Rochester. Structure and
 expression of iso-1-cytochrome c.
 Fitzgerald-Hayes, M., University of Massachusetts. Mutations
 of the centromere affecting chromosome segregation.
 Petes, T., University of Chicago. Mechanisms that alter yeast
 genome structure
 Silver, P., Harvard University. Localization of proteins to the
 yeast nucleus.
 Sternglanz, R., State University of New York, Stony Brook.
 Yeast DNA topoisomerases.
 Garrard, W., University of Texas, Dallas. Chromatin structure
 of a yeast heat-shock locus.
 Periman, P., Ohio State University. Mitochondrial introns:
 Maturases and self-splicing.
 Wigler, M., Cold Spring Harbor Laboratory. Function of yeast
 RAS genes.
 Guthrie, C., University of California, San Francisco. RNA
 splicing: Genetic approaches in yeast.
 Warner, J., Albert Einstein College of Medicine. Ribosomal
 genes and their regulation.
 Thorner, J., University of California, Berkeley. Synthesis and
 action of the mating pheromones.

Molecular Biology of the Nervous System

July 29-August 11

INSTRUCTORS

Kelly, Regis, Ph.D., University of California, San Francisco
 McKay, Ronald D., Ph.D., Massachusetts Institute of Technology

The technologies of patch-clamping and immunochemistry have revolutionized molecular neurobiology. Increasingly, the tools of recombinant DNA technology and molecular genetics are being used to identify and characterize molecules involved in neuronal function and development. The aim of this lecture course was to familiarize the participants with recent developments in molecular biology and to discuss their application to neurobiological problems.

PARTICIPANTS

Bandlow, Christine, M.S., Max-Planck Institute, Martinsried,
Federal Republic of Germany
Benveniste, Eity, Ph.D., University of California, Los Angeles
Bodary, Sarah, Ph.D., Institut Swiss de Recherches
Expérimentales sur le Cancer, Epalinges, Switzerland
Brar, Anoop, Ph.D., Clinical Research Institute of Montreal,
Canada
Brookhart, Gery, M.S., Purdue University, West Lafayette,
Indiana
Cambi, Franca, M.D., E.K. Shriver Center, Waltham,
Massachusetts
Chalmers, Alison, Ph.D., University of California, Riverside
Foote, Alison, Ph.D., E.K. Shriver Center, Waltham,
Massachusetts
Fox, Susan, Ph.D., Rocketteller University, New York, New
York
Hanecak, Ronnis, Ph.D., University of California, Irvine
Johnson, Jeffrey, M.S., University of Texas, Houston
Kashara, Noriyuki, Tokyo Medical and Dental University,
Japan
Liu-Chen, Lee-Yuan, Ph.D., E.I. du Pont de Nemours & Co.,
Wilmington, Delaware

Lowndes, Catherine, B.S., Institute of Neurology, London,
England
McCobb, David, M.S., University of Iowa, Iowa City
Methlessel, Christoph, Ph.D., Max-Planck Institute, Göttingen,
Federal Republic of Germany
Ramaswami, Mani, M.S., California Institute of Technology,
Pasadena
Rayburn, Helen, Ph.D., California Institute of Technology,
Pasadena
Schmidek, Henry, M.D., University of Vermont, Burlington
Vogel, Michael, Ph.D., Yale University, New Haven,
Connecticut
Wennogle, Lawrence, Ph.D., Ciba-Geigy, Corp. Summit, New
Jersey
Wentzel, Daniel, Ph.D., Cornell University, Ithaca, New York
Wu, Hen Ming, Yale University, New Haven, Connecticut

SEMINARS

Anderson, D., Columbia University College of Physicians &
Surgeons, and L. Zipursky, California Institute of Techno-
logy. Cloning strategies.
———. Sequencing.
———. Nucleic acid synthesis and in situ hybridization.



- . Review of gene structure, control, and relationship to protein structure.
- Stuhlmann, H., Massachusetts Institute of Technology. Expression systems.
- Zipursky, L., California Institute of Technology. Retinal development in *Drosophila*.
- Kelly, R., University of California, San Francisco. Protein synthesis and targeting.
- Yamamoto, K., University of California, San Francisco. Gene regulation.
- Varshavsky, A., Massachusetts Institute of Technology. Chromosome structure.
- Sharp, P., Massachusetts Institute of Technology. RNA processing.
- Evans, R., Salk Institute. Protein expression in transgenic mice.
- Burden, S., Massachusetts Institute of Technology. Structure of the neuromuscular junction.
- Getfer, M., Massachusetts Institute of Technology. Antibody generation I.
- . Antibody generation II.
- Quinn, W., Massachusetts Institute of Technology. Neurobiology of *Drosophila*.
- Rouley, E., Massachusetts Institute of Technology. Oncogenes.
- Struhl, G., Harvard University. Development.
- Chalfie, M., Columbia University. Neurobiology of nematodes.
- Wold, B., California Institute of Technology. Antisense message.
- Melton, D., Harvard University. Frog oocytes.
- Sheetz, M., University of Connecticut. Organelle transport of cytoskeletons.
- Hynes, R., Massachusetts Institute of Technology. Extracellular matrix.
- Kelly, R., University of California, San Francisco. Membrane traffic in neurons.
- Bange, R., Washington University School of Medicine. Neuron-glia interaction.
- Merlie, J., Washington University School of Medicine. Biogenesis of the AChR.
- Reichardt, L., University of California, San Francisco. Growth factors.
- McKay, R., Massachusetts Institute of Technology. Everything else.

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.

1984-1985

August

Alexander Varshavsky, Massachusetts Institute of Technology, Cambridge: Heterochromatin-specific proteins and the problem of nucleosome positioning.

September

Hartmut Land, Whitehead Institute, Cambridge, Massachusetts: Cooperation between oncogenes in cellular transformation.

Rodrigo Bravo, European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: Effect of growth factors on cyclin and oncogene expression.

October

William Jamieson, Grumman Aerospace Corporation, Bethpage, New York: Scientific utilization of the space shuttle.

Ronald Guggenheimer, Memorial Sloan-Kettering Cancer Center, New York, New York: Enzyme and template requirements for adenovirus DNA replication in vitro.

George Fey, Scripps Clinic, La Jolla, California: The compliment C3-alpha 2 macroglobulin gene family—cDNA structure and RNA expression during acute inflammations.

Karl Riabwol, University of Arkansas, Fayetteville: Cloning of extrachromosomal covalently closed, circular DNA in human fibroblasts.

November

Asao Fujiyama, University of Osaka Medical School, Japan: Hepatitis B virus genes—Their identification and their expression in yeast cells.

Monica Luskey, University of California, Berkeley: Genetic analysis of the bovine papilloma virus replicon.

Michael Fried, Imperial Cancer Research Fund, London, England: Viral and cellular gene expression.

Yoram Groner, Weizmann Institute of Science, Rehovot, Israel: Molecular structure and expression of the gene locus on human chromosome 21 encoding the Cu/Zn superoxide dismutase and its relevance to Down's syndrome.

Dale Dorset, Weizmann Institute of Science, Rehovot, Israel: Illegitimate recombination of circular and linear SV40 DNA.

Susan Kilkowski, University of California, San Francisco: Pattern recognition and the structural determination of proteins.

Rodrigo Bravo, European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: Induction of cyclin and oncogenes by growth factors in 3T3 cells.

Oliver Bensaou, Institut Pasteur, Paris, France: Heat-shock protein synthesis during mouse early embryogenesis.

Marilyn Anderson, University of Melbourne, Australia: A single gene system that prevents self-pollination in the ornamental tobacco *Nicotiana glauca*.

Marc Yves Fiszman, Institut Pasteur, Paris, France: Smooth muscle and skeletal muscle tropomyosin—From the protein to the gene.

John Northrup, University of Calgary, Canada: GTP binding proteins in hormone action—Implication for *ras*.

Jim Shapiro, University of Chicago, Illinois: Sociobacteriology.

Bill Dynan, University of California, Berkeley: A mammalian transcription factor that binds to and activates selected viral and cellular promoters.

December

Michael Botchan, University of California, Berkeley: Trans-activation of SV40 enhancer.

Jeff D. Boeke, Whitehead Institute, Cambridge, Massachusetts: Transposition of Ty elements in yeast.

Danny Reinberg, Rockefeller University, New York, New York: In vitro RNA polymerase transcription system—A biochemical approach.

Irwin Greenblatt, University of Connecticut, Storrs: Transposition of Ac.

George Church, Biogen, Cambridge, Massachusetts: Tissue-specific contacts to activate immunoglobulin enhancers.

January

Dan Klüssig, University of Utah, Salt Lake City: Posttranscriptional regulation of plant gene expression.

Philip Cofino, University of California, San Francisco: Regulation of ornithine decarboxylase.

Robert Horsch, Monsanto Company, St. Louis, Missouri: Expression of foreign genes in plants.

February

- Mike Levine, Columbia University, New York, New York: Spatial regulation of homeotic gene expression in *Drosophila*.
- John Bednarczyk, University of Texas Health Science Center, Dallas. The effects of ionophores on the processing of the third component of mouse complement.
- Josef Aloni, Weizmann Institute of Science, Rehovot, Israel, and Princeton University, New Jersey: Attenuation in SV40, Adeno and MVM. I. A mechanism that regulates gene expression and II. A mechanism of transcription termination.
- Paul Fisher, State University of New York, Stony Brook: The molecular architecture of the *Drosophila* nucleus.

March

- David Spector, Baylor College of Medicine, Houston, Texas: The nucleoplasmid reticulum—A new organelle?
- David Schwartz, Columbia University, New York, New York: Post-field gradient gel electrophoresis—Its use for analyzing eukaryotic genomes.
- David Solter, Wistar Institute, Philadelphia, Pennsylvania: Transnuclear mice.
- Thomas Wilke, University of Washington, Seattle: Mosaicism and insertional mutagenesis in transgenic mice.
- Paul Modrich, Duke University, Durham, North Carolina: Mismatch repair in vitro.
- Andrew Rice, ICRF, London, England: Purification and characterization of vaccinia virus growth factor.
- Richard Palmiter, University of Washington, Seattle: Expression of genes introduced into mice.

April

- Paul Young, Queens University, Kingston, Canada: Control of cell division in fission yeast.
- Thomas Alber, University of Oregon, Eugene: Protein thermostability—Genetic and structural studies of phage T4 lysozyme.
- John Hassell, McGill University, Montreal, Canada: Dual role of the polyoma virus enhancer in transcription and DNA replication.
- Loren Field, Roswell Park Memorial Institute, Buffalo, New York: Structure and expression of mouse renin genes.
- Janet Rossant, Brock University, St. Catharines, Canada: Cellular and molecular aspects of lineage development in the mouse embryo.
- Dona Chikaraishi, Tufts University, Medford, Massachusetts: Gene expression in mammalian brain.

May

- Roger Brent, Harvard University, Cambridge, Massachusetts: Turning eukaryotic genes on and off with a prokaryotic repressor.
- Alain Nicolas, University of Paris, France, and Harvard Medical School, Cambridge, Massachusetts: Genetics of genetic recombination in *Ascombolus*.
- Tom Sims, University of California, Los Angeles: Organization and expression of organ-specific genes in soybean and tobacco.
- John Dunn, Brookhaven National Laboratories, Upton, New York: Development of high-expression vectors based on bacterial phage P7.
- Robert Haselkorn, University of Chicago, Illinois: Gene rearrangement during development in the cyanobacterium *Anabaena* sp.
- Harold Neil Bramson, Rockefeller University, New York, New York: Synthetic peptides in enzymology and immunology.
- Thomas Curran, Hoffmann-La Roche Inc., Nutley, New Jersey: The *fos* oncogene—Transformation, differentiation, and proliferation.

June

- Daniel Marshak, NIMH, National Institutes of Health, Bethesda, Maryland: Structural analysis of proteins from the nervous system.
- Gilbert Morris, Florida State University, Tallahassee: U1 RNA synthesis in sea urchin embryo extracts.
- Kathryn Ely, University of Utah, Salt Lake City: X-ray crystallography as a probe of conformational flexibility.

July

- Gerry Both, CSIRO, Sydney, Australia: Expression of the rotavirus VP7 gene—Construction of secreted variants.
- Adrian Kramer, Harvard University, Cambridge, Massachusetts: In vitro splicing.
- Denise Roberts, Harvard University, Cambridge, Massachusetts: IS70 transposition is regulated by adenine methylation.

August

- Ted Chrisman, Vanderbilt University, Nashville, Tennessee: Regulation of hepatic phosphorylase kinase activity.
- Frances Jurnak, University of California, Riverside: Biochemical gene crystallography studies of the elongated factor Tu-GDP.
- Alex McPherson, University of California, Riverside: Crystal structures of protein single-stranded DNA complexes.

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, 275 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 Metropolitan Life Foundation, Burroughs Wellcome Fund, and Alfred D. Sloan Foundation.

Mark R. Alfenito, Cornell University
Research Advisor: **Stephen Dellaporta**
Studies of a controlling element and complex locus in maize.

Pedram Argani, Princeton University
Research Advisor: **Yasha Gluzman**
Expression of SV40 T antigen by E1 deleted adenovirus type 5 vectors.

Todd A. Brown, Pittsburg State University
Research Advisor: **Douglas Youvan**
Isolation of the *Rhodopseudomonas capsulata* reaction center and development of transformation protocols.

Gabrielle Costello, Harvard University
Research Advisor: **Robert Franza**
Cell cycle study of the fission yeast *Schizosaccharomyces pombe*.

Lisa Griffin, Notre Dame University
Research Advisor: **David Kurtz**
Isolation of a transcriptional factor in the hormonal control of α_2u globulin.

Nicholas A. Hanchak, University of Scranton
Research Advisor: **Douglas Hanahan**
Transformation efficiency in nucleoside transport mutants of *Escherichia coli* K12 and the establishment of beta cell lines from transgenic mice.

Stuart A. MacNeill, University of Glasgow
Research Advisor: **Bruce Stillman**
Simian virus 40 replication in vitro.

Susan M. McEvoy, University of Wisconsin
Research Advisor: **Richard J. Roberts**
Establishing E1A-producing cell lines and analysis of restriction endonucleases.

Kevin S. Murphy, Massachusetts Institute of Technology
Research Advisor: **James B. Hicks**
Transformation of *Chlamydomonas reinhardtii*.

Robert Paul Ray, University of California, Berkeley
Research Advisor: **Ronald Guggenheimer**
SV40 replication in vitro.

Geraldine Seydoux, University of Maine
Research Advisor: **David Beach**
Genetic suppressors of the genes *ran1* and *mei3* in *Schizosaccharomyces pombe*.

Alyssa Shepard, University of California, Riverside
Research Advisor: **Winship Herr**
Cell-type specificities of SV40 enhancer elements.

Henry Stapp, Hampshire College
Research Advisor: **Pablo A. Scolnik**
Location of carotenoid D gene product in *Rhodopseudomonas capsulata*.

Sheila S. Wong, Yale University
Research Advisor: **Fevzi Daldal**
Cytochromes and the photosynthetic pathway of *Rhodopseudomonas capsulata*.

Olney Fellow
Andrew Mirhej, Columbia University
Research Advisor: **Clive Slaughter**
The use of hydrophobic interaction chromatography as a new method for the typing of ALP.

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 1985 a total of 410 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.

PROGRAM DIRECTOR

William M. Payoski, M.A., Adjunct Professor,
Nassau Community College

INSTRUCTORS

Kathryn Bott, M.S., former science teacher, Friends Academy
Ruth Burgess, B.A., naturalist, Nassau County BOCES
Steven Lander, M.S., science coordinator, Harborfields School District
Fred Maasch, M.S., science teacher, Islip High School
Margret Nathanson, B.A., naturalist, Nassau County BOCES
Linda Payoski, B.A., science teacher, Uniondale High School

COURSES

Nature Bugs	Frogs, Flippers, and Fins	Seashore Life
Nature Detectives	Pebble Pups	Invertebrate Biology
Advanced Nature Study	Bird Study	Marine Biology
Introduction to Ecology	Fresh Water Life	Nature Photography

COLD SPRING HARBOR CURRICULUM STUDY

Genetic engineering, recombinant DNA, cloning, and gene splicing are buzzwords for the DNA revolution that has brought scientists to the very brink of understanding the molecular basis of life. Upon this explicit knowledge is based a burgeoning biotechnology industry which, like the explosive growth of computer technology, promises to profoundly influence American culture.

Although the DNA revolution has been science fact for more than a decade, to the general public it still largely belongs to the realm of science fiction. Adequate knowledge of DNA science has not filtered far beyond the doors of academia. This state of public ignorance threatens the nation's ability to make informed policy decisions about issues generated by biotechnical enterprise.

Time to Clean a Cluttered Closet

Most critical, there has not yet been a coordinated effort nor national commitment to update science teaching to take into account the recent, dramatic developments in biological science. We are in the infancy of a scientific revolution of monumental proportions, but our children don't know it. We are failing miserably to prepare young people for the technological world they must inhabit.

Accumulated in the closet of science teaching are anachronistic concepts and methods that bear little relevance to the real science of discovery. At a time when biologists are on the verge of understanding cancer and engineering plants to quell world hunger, biology students are required to memorize terms and definitions of an historical science no longer practiced at the lab bench.

Peering into a closet cluttered with the accumulated minutiae of bygone days, one can understand why science teaching fails to capture the excitement of modern biology. It is time to sweep clean the closet of earthworm anatomy and frog physiology to make room for gene therapy and molecular engineering.

Addressing the Problems of Science Education

The Cold Spring Harbor Curriculum Study was initiated in January 1985 to bridge the gap between science and society, and to bring modern molecular biology down to a level appropriate for precollege and freshman college students. Its goal is to design novel teaching materials and to train teachers in their use.

The program directly addresses the major problems associated with integrating up-to-date ideas into science courses at the precollege and freshman college level: 1) outdated syllabi, 2) lack of teacher retraining programs, 3) lack of teacher motivation, 4) lack of interactive and lab-oriented teaching materials, and 5) lack of modern lab equipment. By affecting change at the top of the education pyramid—at the level of board members, administrators, science chairpersons, teachers, and professors—we hope that benefits will filter down to the largest number of students.

Recombinant DNA for Beginners

The pivotal achievement of the Curriculum Study has been the development and testing of an integrated series of laboratory exercises that give students hands-on experience in molecular biology. With strong technical help from Dr. Greg Freyer,



Instructors Greg Freyer and David Micklos observe Northport H.S. student Robert Kehn.

now a research associate at Memorial Sloan-Kettering Cancer Center, we have successfully adapted protocols and techniques used by practicing researchers so that they can be safely used in the classroom. These experiments at once excite imagination and give insight into the process of scientific discovery. When published in late 1986, these experiments will be the first lab/text available for this level of instruction.

Another important achievement was the development of *Recombinant DNA for Beginners*. Telescoping instruction equivalent to a three-credit college course, this five-day course gives teachers hands-on experience with the elegant tools of biotechnology. Using equipment identical to that found in research laboratories, teachers perform nine experiments that culminate in the production and analysis of recombinant-DNA molecules, including: microbial culture, gel electrophoresis, DNA restriction analysis, DNA ligation, plasmid transformation of *E. coli*, and purification of plasmid DNA.

Morning lectures introduce major concepts and theories behind the experimental techniques. Afternoon seminars presented by practicing researchers illustrate the application of molecular approaches to solve biological problems.

Facilitating Classroom Implementation

In addition to bringing teachers up-to-the-minute with research in molecular biology, this course is designed to help participants transfer what is learned into improved classroom methods. Post-lab discussions alert educators to variables that can influence classroom results and show how to "troubleshoot" when something goes wrong. One session introduces a small-group approach for analyzing the personal, social, and biological implications of recombinant DNA. Another session focuses on practical aspects of starting a laboratory teaching program, including how to build consensus and support, finding funds for equipment, how to schedule labs around time constraints, lab safety, and proper disposal of biologicals.



University of Connecticut Professor Irwin Greenblatt explains maize genetics at Honors Colloquium for students from 19 local school districts.



Greg Freyer explains subtleties of bacterial transformation to local high school teachers at the summer workshop: Steve Sporenberg and Lois Miller, Syosset; and Henrietta Dold, Wheatley.

An Expanding Role

Only one year after its founding by the Laboratory and eight local school districts, the Curriculum Study has grown into a consortium of 19 school districts on Long Island and in Westchester County. The largest program of its kind, the Curriculum Study serves as a national model for retraining teachers and retooling classrooms for laboratory-based learning about molecular biology.

During its first year of operation, the Curriculum Study provided approximately 100 hours of advanced instruction to both students and teachers—equivalent to three college courses. Approximately 40 teachers and 300 students have attended Curriculum Study seminars, workshops, and training sessions. The number of students who, in turn, have benefited from the retraining provided to their teachers can be conservatively estimated at 4,000. This number will compound each year.

In 1986 we are initiating two new projects to expand significantly the scope of the Curriculum Study:

Vector Mobile DNA Laboratory. In molecular biology, the term *vector* describes bacterial plasmids or viruses that are used to ferry new DNA fragments into a host cell. In the same way, the Vector Mobile DNA Laboratory will carry new knowledge and teaching methods to educators around the country. We have purchased and equipped a utility van as a mobile laboratory to transport all necessary equipment for regional workshops held for up to 36 teachers and/or students. During summer 1986, the Vector Laboratory will travel to workshop sites around the country, including: Huntington and Westchester County, New York; Boston, Massachusetts; Concord, New Hampshire, Milwaukee, Wisconsin; Chicago, Illinois; and Davis, California.

DNA Education Center and Museum. Serving as a national clearinghouse for up-to-the-minute information on biotechnology and DNA science, the DNA Center will be unique among educational institutions in the United States. A Science Media Resource Unit, located within the Center, will explore innovative methods and technologies for presenting science concepts in an exciting and stimulating fashion. Through teacher-training courses administered in the teaching laboratory at Cold Spring Harbor and several mobile laboratories that travel to regional workshops, the Museum will make its materials and resources available to educators throughout the country.



Cold Spring Harbor H.S.
student Alexandra Murray
pipets plasmid DNA
for electrophoresis.

Broad-based Support

The broad-based support for the Curriculum Study attests to growing awareness of its leadership role in modern biology education. Local core programs are supported through appropriations from each of the 19 participating school districts. The Vector Workshop Program is supported through grants from Citibank, N.A., the J.M. Foundation, and New England Biolabs Foundation. Private companies, including Fotodyne, Inc. and Amersham Corporation, support regional training workshops.

A variety of educational associations and institutions are now being drawn into participation, including: New York State Bureau of Science Education, Science Teachers Association of New York State, Science Supervisors Association, National Science Teachers Association, New York State Board of Cooperative Extension Services, State University of New York at Stony Brook, University of California at Davis, Marquette University, and Argonne National Laboratory.

David Micklos, Program Director

1985-1986 Curriculum Study Membership

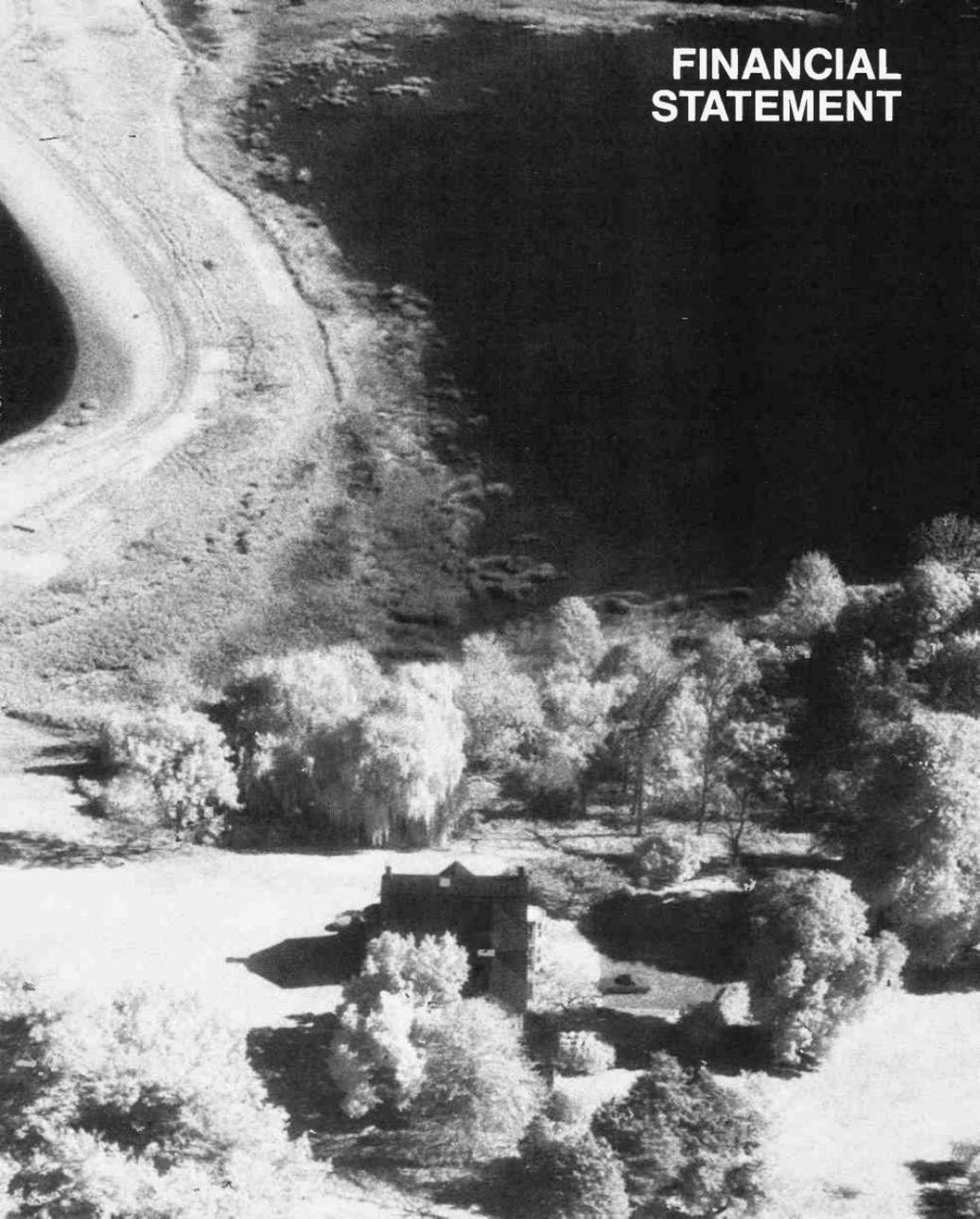
FOUNDING MEMBERS: Cold Spring Harbor Central School District; East Williston Union Free School District; Great Neck Union Free School District; Herricks Union Free School District; Jericho Union Free School District; Northport—East Northport Union Free School District; Oyster Bay—East Norwich Central School District; Syosset Central School District

NEW MEMBERS: Commack Union Free School District; Half Hollow Hills Central School District; Huntington Union Free School District; Irvington Union Free School District; Lawrence Union Free School District; Manhasset Union Free School District; North Shore Central School District; Plainview-Old Bethpage Central School District; Portledge School; Port Washington Union Free School District; Sachem Central School District at Holbrook

INSTITUTIONAL MEMBERS: Citibank, N.A.; J.M. Foundation; New England Biolabs Foundation; Fotodyne, Inc.; Amersham Corporation



Elementary school teacher Richard Micklos and high school student Leslie Gould prepare bacterial cells to receive foreign DNA.

An aerial black and white photograph of a rural landscape. In the upper left, a winding road or path curves through a field. The middle ground is dominated by a dense cluster of trees, including a large, prominent weeping willow tree. In the lower center, a large, dark, rectangular building, possibly a barn or farmhouse, is visible, surrounded by more trees and a grassy area. The overall scene is captured from a high angle, showing the textures of the land and the layout of the structures and vegetation.

**FINANCIAL
STATEMENT**

FINANCIAL STATEMENT

BALANCE SHEET

year ended December 31, 1985

with comparative figures for year ended December 31, 1984

		ASSETS	
		1985	1984
COLD SPRING HARBOR LABORATORY	CURRENT FUNDS		
	<i>Unrestricted</i>		
	Cash and Short-term investments	\$11,567,359	\$10,963,401
	Accounts Receivable	286,511	317,217
	Prepaid expenses and other assets	480,909	679,516
	Inventory of books	218,043	209,329
	Due from restricted fund	—	351,938
	Total unrestricted	<u>12,552,822</u>	<u>12,521,401</u>
	<i>Restricted</i>		
	Marketable securities (quoted market 1985 — \$850,000; 1984 — \$287,500)	212,500	212,500
	Grants and contracts receivable	6,648,896	4,433,529
	Due from unrestricted fund	501,239	—
	Total restricted	<u>7,362,635</u>	<u>4,646,029</u>
	Total current funds	<u>\$19,915,457</u>	<u>\$17,167,430</u>
	ENDOWMENT FUNDS		
	<i>Robertson Research Fund</i>		
	Cash	231,649	3,308,119
	Marketable securities (quoted market 1985 — \$22,240,523; 1984 — \$14,435,055)	18,393,860	12,772,766
	Total Robertson Research Fund	<u>18,625,509</u>	<u>16,080,885</u>
	<i>Olney Memorial Fund</i>		
Cash	1,138	1,693	
Marketable Securities (quoted market 1985 — \$41,928; 1984 — \$33,247)	32,101	29,418	
Total Olney Memorial Fund	<u>33,239</u>	<u>31,111</u>	
Total endowment funds	<u>\$18,658,748</u>	<u>\$16,111,996</u>	
PLANT FUNDS			
Investments	879,871	759,318	
Due from unrestricted fund	3,594,835	6,608,932	
Land and improvements	1,340,375	1,129,875	
Buildings	16,374,820	11,318,113	
Furniture, fixtures and equipment	3,846,120	3,490,933	
Books and periodicals	365,630	365,630	
Construction in progress	406,541	1,461,489	
	26,808,192	25,134,290	
Less allowance for depreciation and amortization	<u>5,896,757</u>	<u>4,861,975</u>	
Total plant funds	<u>\$20,911,435</u>	<u>\$20,272,315</u>	

LIABILITIES AND FUND BALANCES

	1985	1984
CURRENT FUNDS		
<i>Unrestricted</i>		
Accounts payable	\$ 854,619	\$ 747,261
Deferred income	150,000	120,000
Due to Banbury Center	148,944	74,580
Due to plant fund	3,594,835	6,608,932
Due to restricted fund	501,239	—
Fund balance	<u>7,303,185</u>	<u>4,970,628</u>
Total unrestricted	<u>12,552,822</u>	<u>12,521,401</u>
<i>Restricted</i>		
Accounts payable	275,171	65,792
Due to Banbury Center	—	8,952
Due to unrestricted fund	—	351,938
Fund balance	<u>7,087,464</u>	<u>4,219,347</u>
Total restricted	<u>7,362,635</u>	<u>4,646,029</u>
Total current funds	<u>\$19,915,457</u>	<u>\$17,167,430</u>
ENDOWMENT FUNDS		
Fund balance	<u>\$18,658,748</u>	<u>\$16,111,996</u>
PLANT FUNDS		
Accounts payable	—	121,055
Loan payable	8,000,000	8,000,000
Fund balance	<u>12,911,435</u>	<u>12,151,260</u>
Total Plant Funds	<u>\$20,911,435</u>	<u>\$20,272,315</u>

ASSETS

		<u>1985</u>	<u>1984</u>
BANBURY CENTER	CURRENT FUNDS		
	<i>Unrestricted</i>		
	Cash	\$ 700	\$ 700
	Prepaid and deferred expenses	5,726	19,184
	Inventory of books	56,299	46,763
	Due from CSHL unrestricted fund	<u>103,322</u>	<u>16,497</u>
	Total unrestricted	<u>166,047</u>	<u>83,144</u>
	<i>Restricted</i>		
	Grants and contracts receivable	99,141	111,450
	Due from CSHL unrestricted fund	45,622	58,083
	Due from CSHL restricted fund	<u>—</u>	<u>8,952</u>
	Total restricted	<u>144,763</u>	<u>178,485</u>
	Total current funds	<u>\$ 310,810</u>	<u>\$ 261,629</u>
	ENDOWMENT FUNDS		
	<i>Robertson Maintenance Fund</i>		
	Cash	10,707	597,661
	Marketable securities		
	(quoted market 1985—\$3,637,079;		
	1984—\$2,347,644)	<u>3,013,692</u>	<u>2,077,778</u>
	Total endowment funds	<u>3,024,399</u>	<u>2,675,439</u>
	PLANT FUNDS		
	Land	772,500	772,500
	Buildings	846,028	846,028
	Furniture, fixtures and equipment	191,383	180,383
	Construction in progress	<u>—</u>	<u>1,385</u>
		1,809,911	1,800,296
	Less allowance for depreciation	<u>387,505</u>	<u>357,019</u>
	Total plant funds	<u>\$ 1,422,406</u>	<u>\$ 1,443,277</u>
	Total Banbury Center	<u>\$ 4,757,615</u>	<u>\$ 4,380,345</u>
	Total—All funds	<u>\$64,243,255</u>	<u>\$57,932,086</u>

LIABILITIES AND FUND BALANCES

	1985	1984
CURRENT FUNDS		
<i>Unrestricted</i>		
Accounts payable	\$ 8,142	\$ 18,466
Fund balance	<u>157,905</u>	<u>64,678</u>
Total unrestricted	<u>166,047</u>	<u>83,144</u>
<i>Restricted</i>		
Accounts payable	4,577	1,394
Fund balance	<u>140,186</u>	<u>177,091</u>
Total restricted	<u>144,763</u>	<u>178,485</u>
Total current funds	<u>\$ 310,810</u>	<u>\$ 261,629</u>
ENDOWMENT FUNDS		
Fund balance	<u>\$ 3,024,399</u>	<u>\$ 2,675,439</u>
PLANT FUNDS		
Fund balance	<u>1,422,406</u>	<u>1,443,277</u>
Total Banbury Center	<u>\$ 4,757,615</u>	<u>\$ 4,380,345</u>
Total—All funds	<u>\$64,243,255</u>	<u>\$57,932,086</u>

CURRENT REVENUES, EXPENSES AND TRANSFERS
year ended December 31, 1985
with comparative figures for year ended December 31, 1984

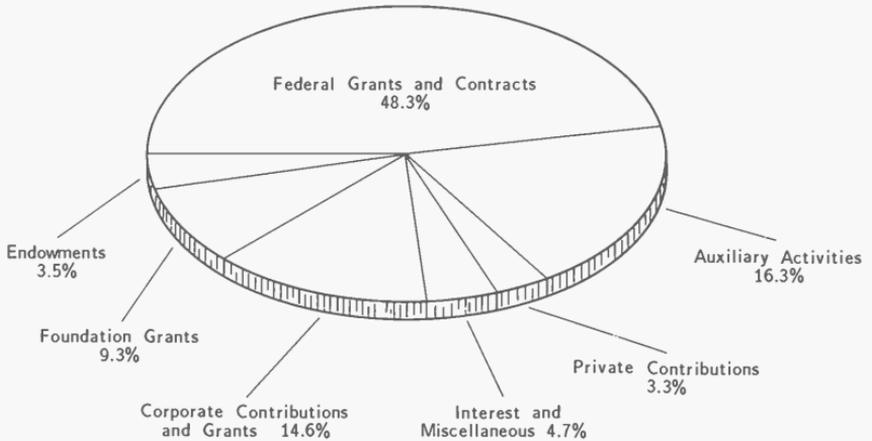
		<u>1985</u>	<u>1984</u>	
COLD SPRING HARBOR LABORATORY	REVENUES			
	Grants and contracts	\$15,869,438	\$12,258,740	
	Indirect cost allowances on grants and contracts	4,406,047	4,141,453	
	Contributions			
	Unrestricted	332,423	154,046	
	Restricted and capital	278,483	421,918	
	Long Island Biological Association	100,000	515,252	
	Robertson Research Fund Distribution	575,000	475,000	
	Royalty and licensing fees	197,000	160,000	
	Summer programs	553,395	487,966	
	Laboratory rental	20,732	20,732	
	Marina rental	61,278	58,528	
	Investment income	903,132	775,231	
	Publications sales	1,432,527	1,512,667	
	Dining Hall	638,690	556,931	
	Rooms and apartments	248,854	309,924	
	Other sources	22,968	56,998	
		<u>25,639,967</u>	<u>21,905,386</u>	
		Total revenues		
		EXPENSES		
		Research *	11,710,764	10,628,482
		Summer and training programs *	1,660,496	1,388,942
		Publications sales *	1,029,060	1,276,015
		Dining hall *	769,574	735,044
		Research support	400,341	289,493
		Library	267,341	236,255
		Operation and maintenance of plant	2,413,788	2,179,543
		General and Administrative	1,965,647	1,805,567
		Depreciation	1,046,630	848,205
			<u>21,263,641</u>	<u>19,387,546</u>
		Total expenses		
		TRANSFERS		
		Capital building projects	3,805,100	2,003,456
		Banbury Center	-	80,420
			<u>3,805,100</u>	<u>2,083,876</u>
		Total transfers		
		Total expenses and transfers	<u>25,068,741</u>	<u>21,471,422</u>
		Excess of revenues over expenses and transfers	<u>\$ 571,226</u>	<u>\$ 433,964</u>

* Reported exclusive of an allocation for research support, operation and maintenance of plant, general and administrative, library, and depreciation expenses

BANBURY CENTER		<u>1985</u>	<u>1984</u>
REVENUES			
Endowment income	\$	112,000	\$ 106,000
Grants & contributions		335,210	407,806
Indirect cost allowances on grants and contracts		28,445	2,060
Rooms and apartments		97,026	65,757
Publications		133,164	102,410
Conference fees		22,086	14,697
Dining Hall		42,314	34,884
Transfer from Cold Spring Harbor Laboratory		—	80,420
Total revenues		<u>770,245</u>	<u>814,034</u>
EXPENSES			
Conferences		188,559	171,095
Publications		124,563	128,181
Operation and maintenance of plant		124,760	120,103
Program administration		222,715	196,244
Depreciation		30,486	36,569
Capital plant		11,000	4,125
Total expenses		<u>702,083</u>	<u>656,317</u>
Excess of revenues over expenses	\$	<u>68,162</u>	\$ <u>157,717</u>

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, 1985



The background of the page is an abstract composition of overlapping, semi-transparent geometric shapes, primarily rectangles and trapezoids, in various shades of gray. The shapes create a sense of depth and perspective, with some appearing to be in the foreground and others receding into the background. The overall effect is a complex, layered pattern of light and dark tones.

**GRANTS AND
CONTRIBUTIONS**

Recto: The intricate play of light and shadow creates abstract art forms in the foyer of the Oliver and Lorraine Grace Auditorium.

GRANTS

January 1, 1985–December 31, 1985

COLD SPRING HARBOR LABORATORY

<i>Grantor</i>	<i>Program/Principal Investigator</i>	<i>Duration of Grant</i>	<i>Total Award</i>
FEDERAL GRANTS			
NATIONAL INSTITUTES OF HEALTH			
<i>Program Projects</i>	Cancer Research Center, Drs. Watson, Sambrook, Roberts and Mathews	11/77–12/86	18,604,426
	Gene Organization, Drs. Watson and Sambrook	4/81– 3/86	5,626,997
<i>Research Support</i>	Dr. Beach	12/84–11/87	361,511
	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. Gething	2/83– 8/85	490,114
	Dr. Hellman	9/85– 8/88	572,760*
	Dr. Hicks	7/81– 6/87	1,710,283
	Dr. Hockfield	2/82– 1/85	282,400
	Dr. Klar	7/81– 6/86	1,572,978
	Dr. Kurtz	4/80– 3/86	606,389
	Dr. Mathews	4/80– 3/86	729,283
	Dr. Matsumura	7/83–10/85	306,641
	Dr. Stillman	7/83– 6/86	677,384
	Dr. Watson	4/84– 3/85	103,479
	Dr. Watson	4/85– 3/86	146,986*
	Dr. Watts	9/84– 1/85	9,557
	Dr. Welch	4/84– 3/86	325,239
	Dr. Wigler	7/85– 6/92	8,426,929*
	Dr. Zoller	9/85– 8/88	545,752*
<i>Fellowships</i>	Dr. Bar Sagi	9/85– 8/87	47,000*
	Dr. Bautch	9/83– 8/86	57,244
	Dr. Flanagan	9/83– 9/85	36,204
	Dr. Gerard	5/84– 4/86	38,204
	Dr. Krangel	10/82– 9/85	54,584
	Dr. Livi	10/83–10/86	57,244
	Dr. McLeod	2/85– 1/88	63,996*
	Dr. Quinlan	6/84– 5/87	57,244
	Dr. Roth	9/83– 8/85	36,776
	Dr. Potashkin	10/85– 9/88	64,886*
<i>Training</i>	Institutional, Dr. Grodzicker	7/78– 8/89	1,291,646
<i>Course Support</i>	Advanced Bacterial Genetics, Dr. Hicks	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 1985

<i>Grantor</i>	<i>Program/Principal Investigator</i>	<i>Duration of Grant</i>	<i>Total Award</i>
<i>Meeting Support</i>	50th Symposium: Molecular Biology of Development	1985	23,320*
	Hepatitis B Virus	1985	7,000*
	C. Elegans	1985	23,433*
NATIONAL SCIENCE FOUNDATION			
<i>Research Support</i>	Dr. Malmberg	4/85– 8/85	22,000*
	Dr. Malmberg, Dr. Hicks	7/82–12/85	510,832
	Dr. Lewis	11/83– 5/85	120,908
	Dr. Roberts	3/83– 8/86	40,592
	Dr. Roberts	1/83– 6/86	240,000
	Dr. Roberts	7/83–12/86	252,000
<i>Course Support</i>	Dr. Hockfield	3/85– 8/85	40,000*
	Plant Molecular Biology, Dr. Malmberg	5/84– 4/87	121,676
<i>Meeting Support</i>	Chromosome Structure & Expression	1985	5,000*
	Hepatitis B Virus	1985	3,000*
	C. Elegans	1985	6,000*
	Heat Shock	1985	3,500*
	50th Symposium—Molecular Biology of Development	1985	4,000*
<i>Construction</i>	Plant Genetics Facility, Dr. Watson	12/83–11/86	406,350
DEPARTMENT OF AGRICULTURE			
<i>Research Support</i>	Dr. Dellaporta	9/85– 8/87	124,000*
	Dr. Dellaporta	9/84– 8/86	100,000
DEPARTMENT OF ENERGY			
<i>Research Support</i>	Dr. Dellaporta	9/85– 8/88	217,000*
<i>Meeting Support</i>	Symposium: Molecular Biology of Development	1985	9,000*
NONFEDERAL GRANTS			
<i>Research Support</i>			
A.B.C. Foundation	Dr. Wigler	5/82– 4/87	600,000
Rita Allen Foundation	Dr. Herr	9/85– 8/86	30,000*
Rita Allen Foundation	Dr. Stillman	1/83–12/87	150,000
American Cancer Society	Dr. Beach	1/85–12/86	70,000*
	Dr. Gething	1/85–12/86	145,000*
Amersham International plc	Drs. Stillman, Gluzman—Institutional Award	7/82– 6/86	115,000
	Dr. Wigler	7/84– 6/86	200,000
Anonymous	Dr. Welch	10/85– 9/87	50,000*
Avon Products	General Research	1985	2,000
Foundation, Inc.	General Research	1985	70
Cancer Research Institute	Dr. Garreis	7/83– 6/85	72,000
Cetus Corporation	Dr. Feramisco	1985	5,000*
Russell and Janet			
Doubleday Fund	General Support	1985	16,750

<i>Grantor</i>	<i>Program/Principal Investigator</i>	<i>Duration of Grant</i>	<i>Total Award</i>
Exxon Research and Engineering Company	Cooperative Research	1/82-12/86	7,500,000
Grumman Corporation	General Research	1985	5,000
Hoffmann-La Roche Inc.	Dr. Franza	1985	15,000*
International Foundation for Cancer Research	Dr. Hanahan	10/84- 9/86	50,000
McKnight Foundation	Dr. Malmberg	3/83- 2/86	105,000
Monsanto Company	Cooperative Research	10/84- 9/89	2,060,252
Muscular Dystrophy Association	Dr. Mathews	7/83- 6/86	78,776
New England Biolabs	Dr. Roberts	1985	21,000
New York State Science and Technology Foundation	Dr. Garrels	10/84- 9/85	25,000
Norden Systems, Inc.	General Research	1985	1,000
Pioneer Hi-Bred International, Inc.	Cooperative Research	8/85- 1/91	2,500,000*
Pfizer Inc.	Dr. Wigler	1985-1990	500,000*
Protein Databases Inc.	General Research	1985	100,000
Marie Robertson Memorial Fund	Neurobiology	1/85-12/85	125,000*
<i>Fellowships</i>			
American Cancer Society	Dr. Young	8/84- 7/85	16,500
Anna Fuller Fund	Dr. Field	10/85- 9/86	18,000*
Cancer Research Institute	Dr. Cone	10/85- 9/86	21,500*
	Dr. Young	8/85- 8/86	19,500*
Leukemia Society of America	Dr. Hanahan	7/84- 6/86	44,280
	Dr. Powers	12/83-12/85	34,000
	Dr. Sadaie	7/84- 8/85	20,702
Muscular Dystrophy Association	Dr. Helfman	7/83- 6/85	37,000
Robert P. Olney Memorial Cancer Fund	Undergraduate Research	1/85-12/85	2,013*
Rockefeller Foundation	Dr. Hicks	9/84- 8/85	25,900
Damon Runyon-Walter Winchell Cancer Fund	Dr. Broek	6/84- 5/86	38,000
	Dr. Gallagher	7/84- 9/85	26,000
	Dr. Hearing	9/84- 8/86	38,000
	Dr. Hiatt	8/84- 7/86	38,000
	Dr. Sass	9/84- 8/86	38,000
	Dr. White	11/83-10/85	35,000
<i>Training</i>			
Burroughs Wellcome Fund	Undergraduate Research Program	1985	11,100*
Citibank, N.A.	Curriculum Study	1985	5,000
Grass Foundation	Neurobiology Scholarships	1980-1985	81,190
Lucille P. Markey Charitable Trust	Scholarship Support	1985-1988	150,000*
Metropolitan Life Foundation	Undergraduate Research Program	1985	10,000*
Rockefeller Foundation	Scholarship Support	1985	16,000*
Alfred P. Sloan Foundation	Undergraduate Research Program	1985-1988	74,000*

<i>Grantor</i>	<i>Program/Principal Investigator</i>	<i>Duration of Grant</i>	<i>Total Award</i>
<i>Course Support</i>			
The Esther A. and Joseph Klingenstein Fund, Inc.	Neurobiology Support	1982-1988	300,000
Alfred P. Sloan Foundation	Computational Neuroscience Course	1985	26,000*
<i>Meeting Support</i>			
Anheuser-Busch, Incorporated	Yeast Meeting	1985	500*
Gist Brocades	Yeast Meeting	1985	500*
Miller Brewery	Yeast Meeting	1985	1,000*
Smith Kline & French Laboratories	Yeast Meeting	1985	500*
Zymo Genetics, Inc.	Yeast Meeting	1985	1,000*
Wellcome Biotechnical Ltd.	Modern Approaches to Vaccines	1985	5,000*
<i>Equipment</i>			
Chemapec, Inc.	14-liter laboratory fermenter		
Samuel Freeman Charitable Trust	Computer Facility	1985-1986	450,000*
McKnight Foundation	Neurobiology Course	1984-1985	110,000
Pandex Laboratories, Inc.	Screen machine system		
<i>Construction</i>			
Anonymous	Plant Molecular Biology Facility	1985	25,000
Charles E. Culpepper Foundation	Plant Molecular Biology Facility	1985-1987	75,000
Mary Livingston Griggs and Mary Griggs Burke Foundation	Plant Molecular Biology Facility	1985	10,000

BANBURY CENTER

<i>Grantor</i>	<i>Program/Principal Investigator</i>	<i>Duration of Grant</i>	<i>Total Award</i>
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FEDERAL GRANTS

NATIONAL INSTITUTES OF HEALTH

<i>Meeting Support</i>	New Aspects of Tobacco Carcinogenesis	1985	15,000*
	Origins of Female Genital Cancer	1985	10,000*

DEPARTMENT OF ENERGY

Environmental Protection Agency

<i>Meeting Support</i>	Genetically Altered Mammalian Viruses	1/85-12/85	47,500*
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NONFEDERAL GRANTS

Meeting Support

American Cancer Society	New Aspects of Tobacco Carcinogenesis	1985	10,000*
James S. McDonnell Foundation	Conference Support	1985-1987	300,000*
Merck Sharp & Dohme	Origins of Female Genital Cancer	1985	1,000*
Alfred P. Sloan Foundation	Journalists' Workshop	1984-1986	162,000

Corporate Sponsor Program

Since the founding of the annual Symposium in 1933, Cold Spring Harbor Laboratory has played a special role in American science as a clearinghouse for high-level information on quantitative biology. In 1985, more than 3,500 scientists attended 20 professional conferences and 13 advanced-training courses held at the Laboratory and its allied Banbury Center. Among the participants were 280 researchers representing 75 companies.

This prominence as a gathering place for researchers from many disciplines—in addition to a distinguished heritage of pioneering research—makes Cold Spring Harbor Laboratory an ideal window on the world of molecular biology and its applications to medicine and industry. The Corporate Sponsor Program was established in 1984 to offer a select group of companies an opportunity to share this unique vantage point.

At the same time, the yearly commitment of \$15,000 by each Sponsor ensures a stable base of support for our meetings program, allowing us to devote more time to planning timely conferences, rather than searching for funding. With the mandatory budget cuts of the Gramm-Rudman law looming over the National Institutes of Health and the National Science Foundation, we can only anticipate that federal support for scientific meetings will virtually dry up over the next several years. By helping strengthen Cold Spring Harbor's role as a conduit for high-level information, our Corporate Sponsors send a clear message to the research community that they care about maintaining the quality of American science.

Additionally, proceeds from the Corporate Sponsor Program entirely support a series of Special Banbury Conferences that deal with technical aspects of genetic engineering especially relevant to industry. Combining high-level science with a restful country setting conducive to informal exchange, these meetings are unique in science. The 1985 meetings were "Structure and Function of Eukaryotic Enhancers, Promoters and Trans-Acting Elements," "Genetics of Plant Cell/Cell Interactions," "Translation Regulation of Gene Expression," and "Computer Graphics and Protein Modeling."

Benefits to Sponsor companies include gratis attendance 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.

Since its inception, Corporate Sponsor membership has expanded from 15 to 21 companies. The renewal rate for participating companies is 93 percent. We take this as a good report card on a program worthwhile to industry. Perhaps the best recommendation for the Corporate Sponsor Program, however, is its members—world leaders in the application of biotechnology to health care, agriculture, and manufacturing:

1985 Members

Agrigenetics Corporation
American Cyanamid Company
Amersham International plc
Becton Dickinson and Company
Biogen S.A.
Cetus Corporation
Ciba-Geigy Corporation

CPC International Inc.
E.I. du Pont de Nemours & Company
Genentech, Inc.
Genetics Institute
Hoffmann-La Roche Inc.
Johnson & Johnson
Eli Lilly and Company

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Pall Corporation
Pfizer Inc.
Schering-Plough Corporation
Smith Kline & French Laboratories
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Bill, Joan, Robert and Michael Weisberg
Dr. Charles A. Werner
Dr. Heiner Westphal
Mrs. Susan Platkin Wexler
Mr. William F. Whitman, Jr.
Mr. Robert J. Zindler

In memory of . . .

Mr. Boyd E. Alford
Mrs. Marie W. Carmody
Mrs. Mary Cium
Mr. Charles Eckelkamp
Mr. Edmund Entin
Mr. William J. Ferguson, Sr.
Mrs. Florence H. Fink
Mrs. Bertha Greenberg

Mrs. Anita Kasten
Mr. Aaron Kihn
Mr. Jimmy Klecar
Ms. Janet Sonya Psipsikas
Mr. Frank Quinn
Mrs. Sara Delano Redmond
Mr. Joseph Reisig
Mrs. Joyce Royce

40th anniversary tribute . . .

Dr. and Mrs. Monroe Levin

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.

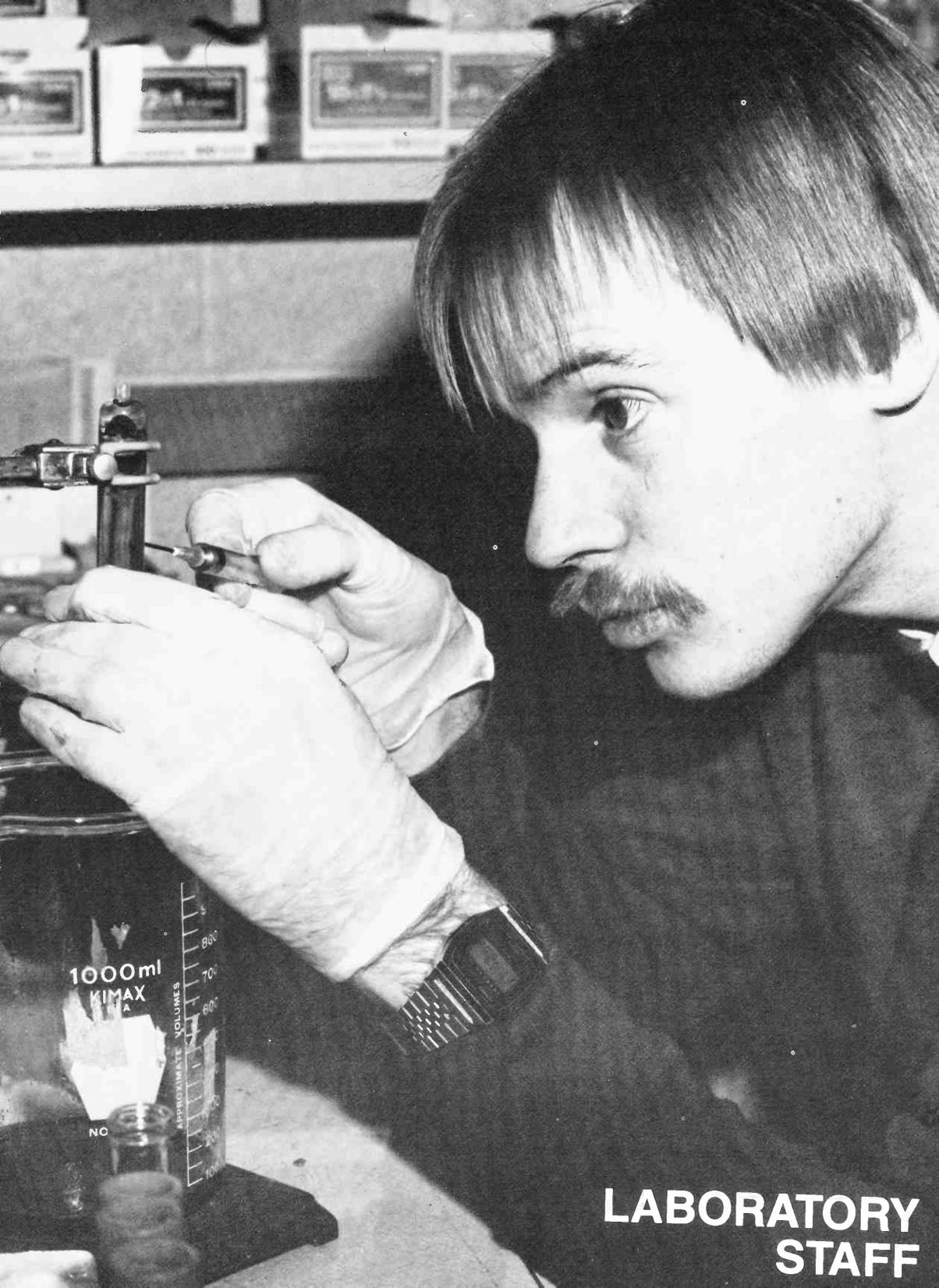
Bequests Probably most wills need to be updated. Designating Cold Spring Harbor Laboratory as beneficiary ensures that a bequest will be utilized as specified for continuing good.

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 Development and Public Affairs, Cold Spring Harbor Laboratory, Box 100, Cold Spring Harbor, N.Y. 11724, or call 516-367-8455.



**LABORATORY
STAFF**

Recto: Postdoctoral Fellow Claude Dery draws off plasmid DNA that has been separated through density-gradient centrifugation.

Laboratory Staff

James D. Watson, Director
G. Morgan Browne, Administrative Director
John Maroney, Assistant Administrative Director
William Keen, Comptroller
Jack Richards, Director Buildings & Grounds
Michael Shodell, Banbury Center Director

Research Staff

David Beach
Stephen Blöse
Fevzi Daldal
Stephen Dellaporta
James Feramisco
Robert Franza
James Garrels
Yakov Gluzman
Terri Grodzicker
Douglas Hanahan
Edward Harlow
David Helfman
Winship Herr
James Hicks
Stephen Hinton
Tohru Kalaoka
Amar Klar
David Kurtz
Michael Mathews
Andrew Rice
Richard Roberts
Pablo Scolnik
Clive Staughter
David Spector
Bruce Stillman
Paul Thomas
William Welch
Michael Wigler
Douglas Youvan
Mark Zoller

Postdoctoral Research Fellows

William Addison
Maran Avudaappan
Dalna Bar-Sagi
Victoria Bautch
Ashok Bhagwat
Carmen Birmeier
Graeme Bolger
Daniel Broek
Robert Cafferkey
Steven Cheley
Jychian Chen
Roger Cone
Edgar Davidson
Claude Dery

John Dittley
Shimon Elrat
Jeffrey Field
Howard Fox
Robert Gerard
Giovanni Giuliano
Joan Harper
Janet Hearing
Andrew Hiatt
Tohru Kamala
Yuriko Kalaoka
Antal Kiss
Richard Kostriken
Jeremy Lamb
Michael Lambert
John Langstaff
Patsy Lin
George Livi
Brenda Lowe
Maureen McLeod
Elizabeth Moran
Judy Potashkin
Scott Powers
Karl Riabowol
Philip Sass
Petra Schurmann
Margaret Sharma
Neil Sullivan
Takashi Toda
Eileen White
Marie Wooten
Dailan Young
Bradley Zerler

Genetics Research Unit Carnegie Institution of Washington

Alfred D. Hershey
Barbara McClintock

Visiting Scientists

Diane Esposito
Seth Grant
Mark Homonoff
Esa Kuusmanen
Junichi Nakawa

Massimo Romani
Marcello Siniscalco
Kryslyna Slaska-Kiss

Support Staff

Patricia Bird
Louisa Dalesandro
Marilyn Goodwin
Mary Hannaford
James Kos
Laurie Lowman
Karen Otto
Philip Renna
Joseph Suhan
Madeline Szadkowski
Barbara Weinkauff

Graduate Students

Susan Alpert
Robert Booher
Leonardo Brizuela
Karen Buchkovich
Edward Bylina
Scott Cameron
Mary Chapman
Paul Chomet
Christine Herrmann
Margaret Kelly
Lonny Levin
Duncan McVey
Kenneth Mellits
Christine Mitchell
Robert O'Malley
Brian Ondek
Gregory Preich
Nora Sarvelnick
Sreenath Sharma
Carmella Stephens
Peter Whyte

Laboratory Technicians

Barbara Ahrens
Mark Allenlo
Susan Allan
Susan Bader

Carmelita Baulista
Georgia Binns
Gladys Blose
Cecile Chang
Dolly Chao
Jennifer Clarke
Joan Cossetti
Debra Danna
Michael Delanno
Barbara Faha
Linda Finn
Thomas Fischer
Jeffrey Hager
Debra Hartly
Patricia Hinton
Sajida Ismail
Stephen Johann
Karen Johnson
Nancy Kronenberg
Mark Lacey
Barbara MacDonald
Pamela Mailloux
Lisa Manche
Krystyna Marcinczak
Robert McGuiirk
Jean McIndoo
Lisa Miglio
Lisa Miranda
Ian Mohr
Carolyn Moomaw
Phyllis Myers
Paul Newman
Diane Pascucci
Daniel Pollock
Linda Rodgers
Susan Rook
Janet Ross
Heidi Sacco
Susan Scheib
Carol Schley
Lisa Schoenherr
Patricia Scolnik
Alyssa Shepard
Monika Stein
Sachiko Toda
Gayle Waitches
Patricia Wendel
Jeanne Wiggins
Jonathan Wood

Laboratory Aides

Shinta Cheng
Rodney Chisum
Robert Gensel
Connie Hallaran
Sunhee Lee
John Lillie
Sharon Losee
Eleanor Mathews
William Packer

Sandra Penzi
Steven Simonte
Gloria Wilson

Medium Makers

Patricia Barnwell
William Eisner
Margaret Falkowski
Mary Freedman
Marie Hallaran
Ronnie Packer
Suzanne Vavrinec
Margaret Wallace

Safety and Core Facilities Services

Arthur Brings

Safety
Marlene Rubino

Computing Center
Michael Balamuth
Christopher Keller

Harris Animal Facility
Diane Biedermann

Lena DiLacio
Carolyn O'Loughlin

Art Department
Michael Ockler

David Greene
Jean Michaelis

Technical Services
Robert Borruso
August Dulis
Peter Markiewicz
Clifford Sutkevich

Publications

Nancy Ford
Annette Kirk
Dorothy Brown
Judith Cuddihy
Joan Ebert
Martha McMaster
Douglas Owen
Elizabeth Ritcey
Marie Sullivan
Pauline Tanenholz

Library/Marketing

Susan Gensel Cooper
Library
Petra Durnin
Genemary Falvey

Wanda Felker
Cathy Garg
Laura Hyman
Melissa Hyman
Laurie Kennedy
Carol O'Shea

Marketing
Elaine Gaveglia
Amy Gibson
Maureen Spirn

Accounting

Accounting
Guy Cozza
Fran Gerard
Mary Ellen Goldstein
Dorrine Lambert
Fred Lukas
Carlos Mendez
Robert Pace
June Polistina

Fulfillment
Charlaiane Apstel

Ann Felten
Barbara Skelly
Barbara Terry

Grants
Susan Schultz
Patricia Kurfless
Patricia Maroney

Development and Public Affairs

David Micklos
Herb Parsons
Ellen Skaggs
Susan Zehl

Personnel and Meetings

William Putnam
Personnel
Catherine Erb
Merilyn Simkins
Jacqueline Terrence

Meetings
Maureen Berejka
Chris Brett
Gladys Kist
Michela McBride
Barbara Ward

Purchasing

Peter Rice
Deon Baker
Bruce Fahibusch
Steven Horly
Graceann Lovell
Kevin O'Rourke
James Sabin
Elissa Troisi
Nancy Wysong

Central Administration

Roberta Salant
Andrea Stephenson

Buildings and Grounds

Administration

Charles Schneider
Barbara Cuff

Carpentry and Painting

Diamond Scarduzio

John Chilcott
Lawrence Daw
John Meyer
Stephen O'Connell
Joseph Pirnak
Paul Thomas

Custodial

Willie Gardner

John Berejka
Robert Brymer
George Leedham
Brian Leite
Daniel Jusino
Alfred Nickel
Donald Rose
Steven Tang
Peter Tierney

Banbury Grounds and Maintenance

Peter Stahl
Daniel Miller

Electrical and Mechanical Maintenance

Robert Collins
Frank Messina
Jerry Reichert
Owen Stewart

Grounds

Hans Trede
Vincent Carey
Joseph Ellis
John Robert Fuchs
Robert Iwanejko
Christopher McEvoy
Harry Wozniak
Anthony Zaroni

Housekeeping

Dorothy Youngs

Rita Abrams
Carol Caldarelli
Turid Mikalsen
Barbara Robinson
Cherie Thurn

Plumbing

Douglas Haskett
Lane Smith

Security

Paul Anzalone
Frank Carberry

Banbury Center

Judith Blum
Dessie Carter
Katya Davey
Beatrice Toliver

Scientific Staff Departures during 1985

Tenured Scientist
Joseph Sambrook

Senior Staff Investigators

Mary-Jane Gething
Susan Hockfield
Russell Malmberg
Fuyu Tamanoi

Staff Investigators

Ronald Guggenheimer
John Lewis
Fumio Matsumura

Visiting Scientists

Daniel Birnbaum
Ottavio Fasano
Asao Fujiyama
Eric Hunter
Ling-Pai Ting

Postdoctoral Fellows

Timothy Adams
Philip Bird
Patricia Cotton
Carolyn Doyle
Thomas Flanagan
Howard Fox
Greg Freyer
Beth Friedman
Patricia Gallagher
John Ivy

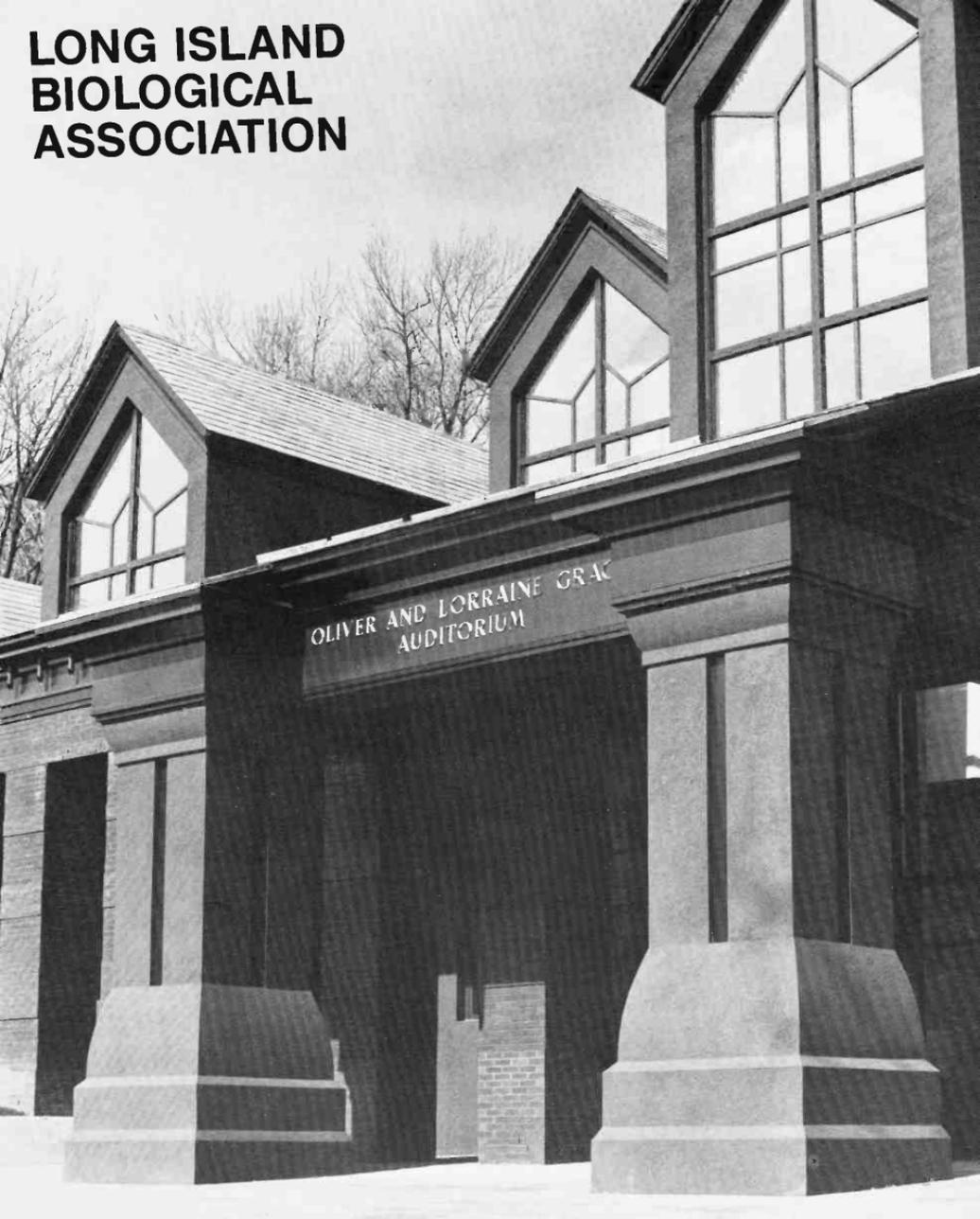
Michael Krangel
Janet MacInnes
Shigeko Matsumura
Michael Roth
Reza Sadaie
Kevin Van Doren
Susan Watts
Masao Yamada
Sam Zarembo

Graduate Students

Ramaninder Bhasin
William Costello
Madan Rao

LONG ISLAND BIOLOGICAL ASSOCIATION

OLIVER AND LORRAINE GRAC
AUDITORIUM

A black and white photograph of a large, classical-style building. The building features a prominent portico supported by thick, square columns. The columns are topped with decorative, rounded capitals. Above the columns, a wide entablature is visible. On the front face of the entablature, the words "OLIVER AND LORRAINE GRAC" are inscribed in a serif font, with "AUDITORIUM" centered below it. The building has a gabled roof with several large, multi-paned windows set in gables. The windows have a grid pattern. In the foreground, there is a flat, light-colored surface, possibly snow or a paved plaza. The background shows some bare trees and a clear sky.

Recto: The massive dormers of the Oliver and Lorraine Grace Auditorium echo the Victorian architecture prevalent on Long Island. Seating 360, the auditorium is the hub of activity for scientific meetings held during the summer.

Officers

Mr. George W. Cutting, Jr., Chairman
Mr. George J. Hossfeld, Jr., Vice-Chairman
Mrs. James A. Eisenman, Treasurer
Mrs. John P. Campbell, Secretary
Mr. G. Morgan Browne, Asst. Secretary-Treasurer

Executive Committee

The Officers, *also*
Mr. Samuel R. Callaway
Mrs. Duncan Elder
Mrs. Sinclair Hatch

Directors

Mrs. Donald Arthur
Dr. Alfred A. Azzoni
Mrs. Gilbert A. Ball
Mr. Samuel R. Callaway
Mrs. John P. Campbell
Mrs. Miner D. Crary, Jr.
Mr. Arthur M. Crocker
Mr. Roderick H. Cushman
Mr. George W. Cutting, Jr.
Mr. Lawrence L. Davis

Mr. Joseph C. Dey, Jr.
Mr. Charles Dolan
Mr. James A. Eisenman
Mrs. Duncan Elder
Mrs. Henry U. Harris, Jr.
Mrs. Sinclair Hatch
Mr. George J. Hossfeld, Jr.
Mr. Edward M. Lamont
Mrs. George N. Lindsay
Mr. Arthur C. Merrill

Mr. William F. Payson, Jr.
Mrs. Richardson Pratt
Mr. Edward Pulling
Mr. John R. Reese
Mr. Harvey E. Sampson
Mr. Charles R. Thompson
Dr. James D. Watson
Mrs. Bradford Weekes III

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 Laboratory'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. At least twice a year LIBA members are invited to bring their friends to a lecture or an open house at the Lab.

Membership in LIBA requires a minimum annual contribution (tax deductible) of \$25 for a husband and wife, \$15 for a single adult, \$5 for a junior member (under 21). 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) 367-8486.

CHAIRMAN'S REPORT

The most important event for LIBA in 1985 was the annual meeting of our members. This is customarily held in December but this year was postponed to January 12th, 1986. The postponement allowed time for the completion of the Oliver and Lorraine Grace Auditorium and also of a video documentary depicting the history and the current functioning of the Laboratory. As our members know, the financing of the auditorium (to the extent of \$600,000) and of the documentary (in full) were LIBA's most recent projects undertaken in behalf of the Lab.

The overflow crowd of members and our guests who attended the meeting were thrilled by both the auditorium and the documentary. They greatly admired the auditorium for its aesthetic qualities, its comfortable seats, and especially for its remarkable acoustics. Everybody agreed it is exceptionally well suited for its two purposes. One is to provide an adequate meeting place for the Lab's summer conferences. Some 3,000 scientists from all over the world come to Cold Spring Harbor each year for the exchange and distribution of the latest discoveries in molecular biology. The auditorium's other purpose is to enable the Lab to offer to its neighboring communities the use of the building for concerts and other appropriate meetings when it is not needed for the Lab's own functioning.

At the Annual Meeting there was a strong approval given also for the documentary. We hope it will serve effectively to widen and deepen interest in the vital research which has brought world-wide prestige to the Laboratory. The value of the documentary will be enhanced by its availability in half-inch tapes to institutions and individuals either on loan or by purchase for \$25.

I think we LIBA members can be justly proud of the success of both these projects as well as the previous ones accomplished in recent years.

Speaking of our projects, I was pleased to announce that our current one, to help finance the Lab's new Plant Genetics Program, is going very well. I hope that the generosity of members who have not yet sent in their contributions for this year will permit us to increase the amount of \$110,000 we originally set as our goal for this most important project. The Lab needs, and will greatly appreciate,



A record 430 people attended the LIBA Annual Meeting in the new Grace Auditorium



Mr. George W. Cutting, Jr.
and Dr. James D. Watson at
the LIBA Annual Meeting

whatever additional help we can give to it. The chance of increasing our contribution is enhanced by the fact that the documentary is costing less than anticipated because the technical equipment of Cablevision was made available to us through the greatly appreciated courtesy of Mr. and Mrs. Charles Dolan.

At the meeting I announced my retirement as LIBA chairman and expressed my gratitude for the wonderful cooperation my fellow members have given me during my many very happy years in office. Then I had the pleasure of introducing as my successor Mr. George W. Cutting, Jr., who had been enthusiastically elected by the directors at their autumn meeting. He is eminently qualified for the job and I am fully confident that LIBA's functioning will become increasingly effective under his leadership. Also, I know that he can count on maximum support by our directors and members.

In September Mr. Walter Page, chairman of the Laboratory trustees, appointed a Special Committee comprised of representatives of the Lab's trustees, of its administrative staff and of LIBA directors. Its purpose was to discuss the basic question as to whether LIBA should continue to function autonomously in its fund-raising efforts in behalf of the Lab or preferably should become integrated in some way with the Lab's own fund-raising procedures.

The unanimous opinion of the Committee was that LIBA should retain its autonomy, which has proved to be so effective not only in fund raising but also (and more important in the opinion of the Lab's representatives on the Committee) in extending and deepening the interest of the community in the Lab. However, it offered two suggestions for the consideration of our directors regarding ways in which LIBA might cooperate with the Lab in the major appeal planned for the occasion of its forthcoming Centenary. One was that LIBA undertake a project in the next few years which would not only appeal to its membership but also relate to the Lab's overall campaign. The other suggestion was that our membership list be studied carefully to identify potentially important donors who might want to contribute substantially to a Centenary project.

The Committee also commented that repeated re-education of our members is needed in regard to LIBA's relationship with the Lab (which seems to be ambiguous in some people's minds) and also in regard to the procedure for obtaining matching gifts. I have taken advantage of both these suggestions in my recent letters to the membership and Mr. Cutting intends to follow suit in his communications.

We were fortunate at the Annual Dorcas Cummings Memorial Lecture in May to have as our speaker Mr. S. Dillon Ripley II, the distinguished retired head of The Smithsonian Institution. Mr. Ripley gave us a most interesting talk on "Environmental Degradation in the Tropics."

Again in 1985, as in past years, the Symposium dinner parties given by our members in June for visiting scientists and members of the Lab's permanent scientific staff were a great success, enjoyed equally by the hosts and hostesses and their guests. Dinners were given this year by

Mrs. Donald Arthur
Mr. & Mrs. Norris Darrell, Jr.
Mrs. Eugene DuBois
Mr. & Mrs. Mario Fog
Mr. & Mrs. Oliver R. Grace
Mr. & Mrs. Henry Upham Harris, Jr.
Mr. & Mrs. Sinclair Hatch

Mr. & Mrs. Walter H. Page
Mrs. Francis T. P. Plimpton
Mr. & Mrs. E. E. Post
Mr. & Mrs. Martin B. Travis, Jr.
Mr. & Mrs. Bronson Trevor, Jr.
Mrs. Ethelbert Warfield
Mrs. Alexander M. White

At the annual meeting, Mrs. John P. Campbell, chairman of the Nominating Committee, proposed the following for election to the Board of Directors:

For election to the class of 1986

Mrs. George N. Lindsay, Jr.

For election to the class of 1989

Mrs. Bradford G. Weekes

Mrs. Sinclair Hatch

Mr. William F. Payson, Jr.

Mr. Charles R. Thompson

For re-election to the class of 1989

Mr. Samuel R. Callaway

Mr. James A. Eisenman

Mr. Arthur M. Crocker

There being no further nominations from the floor, the above were duly elected. Then thanks were extended to the members above whose term had expired, Messrs. Edmund Bartlett, and Stanley S. Trotman, Jr., and also to Mr. Grinnell Morris who regretfully found it necessary to resign. Very special thanks were expressed to one retiring secretary, Mrs. James J. Pirtle, Jr., for her twelve years of most loyal and efficient service.

Edward Pulling, Ex-Chairman

Members of the Long Island Biological Association

- Mr. & Mrs. Thomas Ackerman
 Mr. Amyas Ames
 Mrs. Charles E. Ames
 Dr. & Mrs. Hoyt Armidion
 Miss Barbara Amott
 Drs. Harold & Shirley Andersen
 Mr. Robert W. Anthony
 Mr. & Mrs. Silas Reed Anthony
 Mr. & Mrs. S. Arbonies
 Mr. & Mrs. J. S. Armentrout
 Mrs. Donald Arthur
 Mr. & Mrs. Henry Austin III
 Mrs. Robert W. Ayer
 Dr. & Mrs. Allred A. Azzoni
 Mr. & Mrs. Henry D. Babcock, Jr.
 Mr. & Mrs. Benjamin H. Balkind
 Mrs. Gilbert A. Ball
 Mr. & Mrs. William M. Bannard
 Mrs. George C. Barclay
 Dr. & Mrs. Henry H. Bard
 Mr. & Mrs. Edmund Bartlett
 Miss Linda Bennetta
 Mr. & Mrs. Loren C. Berry
 Mr. & Mrs. Robert P. Beuerlein
 Mr. & Mrs. Nicholas Biddle, Jr.
 Mrs. William Binnian
 Mr. F. Roberts Blair
 Mrs. Mary Lenore Blair
 Mr. & Mrs. Bache Bleecker
 Mrs. Margery Blumenthal
 Mr. & Mrs. Elito Bongarzone
 Mr. & Mrs. A. L. Boorstein
 Mr. & Mrs. Murray Borson
 Mr. & Mrs. William Braden
 Mr. & Mrs. William Brigham
 Dr. & Mrs. Arik Brissenden
 Mrs. Horace Brock
 Mr. & Mrs. F. Sedgwick Browne
 Mr. & Mrs. G. Morgan Browne
 Mr. & Mrs. Frank Bruder
 Mr. & Mrs. James Bryan, Jr.
 Mr. & Mrs. Julian Buckley
 Mrs. R. P. Burr
 Mr. & Mrs. John Busby
 Mr. & Mrs. Albert Bush-Brown
 Miss Grace T. Caidin
 Mr. & Mrs. T. J. Calabrese, Jr.
 Miss Monique Calabro
 Mr. & Mrs. Samuel R. Callaway
 Mr. & Mrs. John P. Campbell
 Mr. & Mrs. Ward C. Campbell
 Miss Martha Worth Carder
 Mr. John Carr
 Mr. & Mrs. T. C. Cattrall, Jr.
 Centerbrook
 Mr. & Mrs. Lionel Chaikin
 Mr. & Mrs. Gilbert W. Chapman, Jr.
 Mr. & Mrs. Harry Charlston
 Dr. Carlton Chen
 Mrs. Helen Chenery
 Mr. & Mrs. Beverly C. Chew
 Mr. Jan S. Chock
 Mrs. Charles M. Clark
 Mr. & Mrs. David C. Clark
 Dr. & Mrs. Bayard Clarkson
 Mrs. Robert L. Clarkson, Jr.
 Mr. & Mrs. John P. Cleary
 Mr. & Mrs. Thomas N. Cochran
 Dr. & Mrs. Peter Cohn
 Mrs. C. Payson Coleman
 Mr. & Mrs. Francis X. Coleman, Jr.
 Mr. & Mrs. John H. Coleman
 Mrs. John K. Colgate
 Mr. & Mrs. Emilio G. Collado
 Mr. & Mrs. Bradley Collins
 Mr. & Mrs. Patrick Collins
 Mr. & Mrs. Kingsley Colton
 Mrs. Ralph C. Colyer
 Dr. & Mrs. Joseph B. Conolly, Jr.
 Mrs. Crispin Cooke
 Mrs. Howard Corning, Jr.
 Mr. Duncan B. Cox
 Mr. & Mrs. Charles L. Craig
 Mr. & Mrs. Miner D. Cray, Jr.
 Mr. & Mrs. Arthur M. Crocker
 Mr. & Mrs. Robert Cuddeback
 Mr. & Mrs. Robert L. Cummings
 Mr. Robert L. Cummings III
 Mr. & Mrs. Richard T. Cunniif
 Dr. & Mrs. Paul Cushman, Jr.
 Mr. & Mrs. Roderick H. Cushman
 Mr. & Mrs. A. Neville Cutting
 Mr. & Mrs. George W. Cutting, Jr.
 Miss Lucy Cutting
 Mrs. Howard Dana
 Mr. Theodore H. Danlorth
 Mr. & Mrs. Norris Darrell, Jr.
 Miss Katya Davey
 Mr. & Mrs. Lawrence L. Davis
 Mrs. F. Trubee Davison
 Mr. & Mrs. Donald C. Death
 Mr. & Mrs. Jay DeBow
 Mr. & Mrs. Raymond DeClairville
 Mr. & Mrs. Donald L. Deming
 Mr. & Mrs. Douglas Despard, Jr.
 Mr. & Mrs. Joseph C. Dey, Jr.
 Mr. & Mrs. Charles F. Dolan
 Russell & Janet Doubleday Fund
 Mrs. Joseph P. Downer
 Mr. & Mrs. Clark M. Drasher, Jr.
 Dr. & Mrs. Arthur Drazan
 Dr. & Mrs. Lester Dubnick
 Mrs. Eugene DuBois
 Mr. & Mrs. James C. Dudley
 Dr. & Mrs. John L. Duffy
 Mr. Anthony Drexel Duke
 Mr. & Mrs. Edgar P. Dunlaevy
 Mr. & Mrs. William T. Dunn, Jr.
 Dr. & Mrs. Gerard L. Eastman
 Dr. & Mrs. James D. Ebert
 Mr. & Mrs. Paul S. Eckhoff
 Mr. & Mrs. James A. Eisenman
 Mrs. Fred J. Eissler
 Mr. & Mrs. Duncan Elder
 Mr. & Mrs. Ashton G. Eldredge
 Mr. & Mrs. Martin Elias
 Mr. & Mrs. John Evans
 Mr. & Mrs. William Everdell
 Mr. Henri Eyl
 Mr. & Mrs. Raymond Z. Fahs
 Mr. & Mrs. Joel M. Fairman
 Mr. & Mrs. Harold L. Fates
 Mr. John R. Fell, Jr.
 Mrs. Allston Flagg
 Mr. & Mrs. Mario Fog
 Mr. & Mrs. Joseph G. Fogg III
 Mr. & Mrs. Henry L. Fox
 Mr. Walter N. Frank, Jr.
 Mr. & Mrs. George S. Franklin
 Mrs. Mary Freedman
 Mr. & Mrs. Joel B. Friedman
 Mr. Louis M. Fullier
 Mr. Stephen D. Fuller
 Mr. D. Kent Gale
 Mr. & Mrs. Clarence E. Galston
 Mr. & Mrs. John W. Galston
 Mrs. James E. Gardner, Jr.
 Mr. & Mrs. Robert Gardner, Jr.
 Mr. & Mrs. Charles S. Gay
 Mr. & Mrs. Robert A. Gay
 Mrs. Louis F. Geisler, Jr.
 Miss Vera C. Gibbons
 Mr. & Mrs. Harry Giffords
 Mr. & Mrs. Stephen E. Gihuley
 Mrs. Ruth Reed Gillespie
 Mr. & Mrs. Robert Gilmor, Jr.
 Dr. & Mrs. H. Bentley Glass
 Mrs. J. Wooderson Glenn
 Mr. & Mrs. Bernard Gloisten
 Mr. & Mrs. E. Rawson Godfrey
 Mr. C. F. Gordon
 Mr. & Mrs. Kilbourn Gordon, Jr.
 Mr. & Mrs. Hank Grabowski
 Mr. & Mrs. Oliver R. Grace
 Mr. & Mrs. William R. Grant
 Mr. & Mrs. Austen T. Gray
 Mr. & Mrs. Craig P. Greason
 Mr. & Mrs. Alfred T. Gregory

Mr. & Mrs. George Haberer
Mr. & Mrs. Sidney Hack
Mrs. Joanna Hadden
Mr. & Mrs. John W. B. Hadley
Mr. & Mrs. Lawrence Hahn
The Rev. Linda Peyton Hancock
Dr. Elizabeth Hansen
Mrs. Beverly Hanson
Mr. & Mrs. Gordon Hargraves
Mr. & Mrs. Henry U. Harris
Mr. & Mrs. Henry U. Harris, Jr.
Miss Maxine Harrison
Dr. & Mrs. C. Hartenstein
Mr. & Mrs. Rolf D. Hartmann
Mr. & Mrs. Sinclair Hatch
Mr. & Mrs. Horace Havemeyer, Jr.
Mr. Ralph F. Hayden
Mr. & Mrs. T. M. Hearn
Mr. Huyler C. Held
Mr. & Mrs. James E. Hellier
Mr. & Mrs. W. P. Henderson
Dr. & Mrs. William W. Heroy
Mr. & Mrs. Charles L. Hewitt
Mrs. Thomas L. Higginson
Mr. & Mrs. Charles R. Holcomb
Mr. & Mrs. Karl Holtzschue
Mr. & Mrs. George J. Hossfeld
Mr. & Mrs. George J. Hossfeld, Jr.
Mrs. George Howard
Mrs. J. Taylor Howell
Mr. & Mrs. Charles J. Hubbard
Mr. & Mrs. Philip G. Hull
Mrs. John P. Humes
Mr. Waldo Hutchins III
Mr. & Mrs. Warren Hutchins
Mr. & Mrs. J. A. V. Hyatt
Mrs. Elmira S. Ingersoll
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