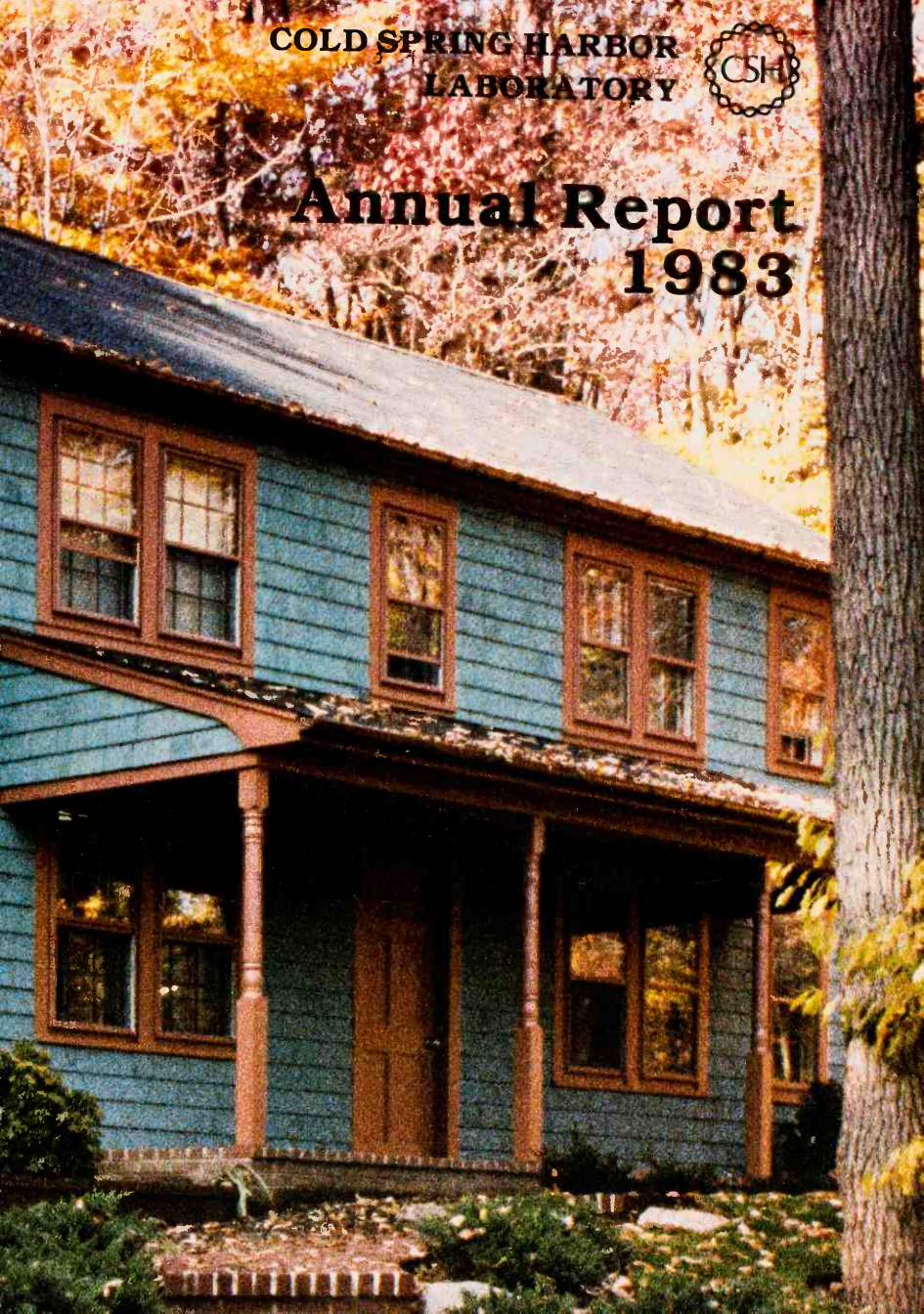
COLD SPRING HARBOR LABORATORY

Annual Report 1983



Annual Report 1983

Cold Spring Harbor Laboratory
Box 100
Cold Spring Harbor, New York 11724

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Photo editor, David Micklos

Cover photo: Urey Cottage, renovated in 1983 under the supervision of Jack Richards and the Laboratory's Buildings and Grounds staff, provides offices for the Publications Department. (Photo by Herb Parsons)

Photo page ii: Situated at the entrance to the Laboratory, Davenport House is one of seven structures on the grounds dating from the 19th century.

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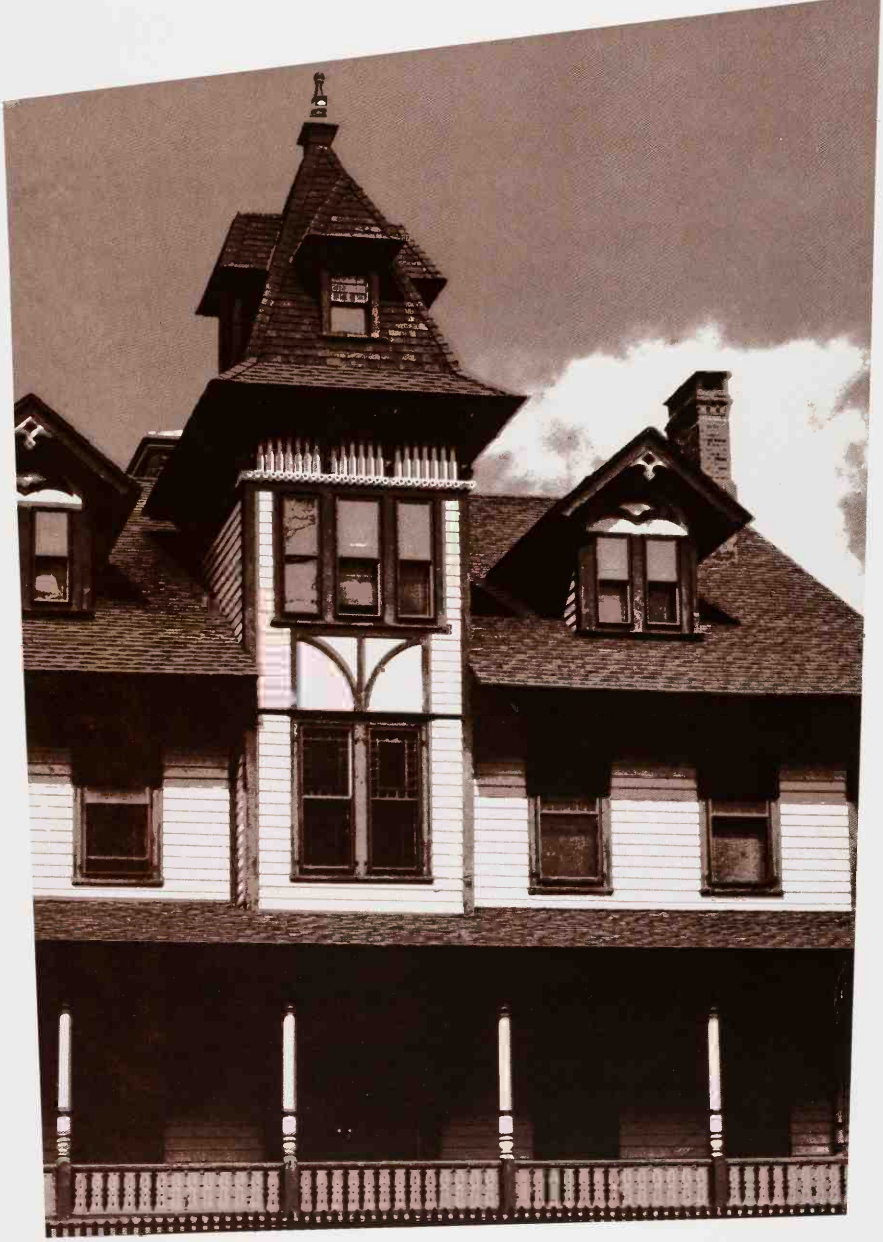

COLD SPRING HARBOR
LABORATORY

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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 members support the Laboratory through annual contributions and participation in fund drives to raise money for major construction projects.

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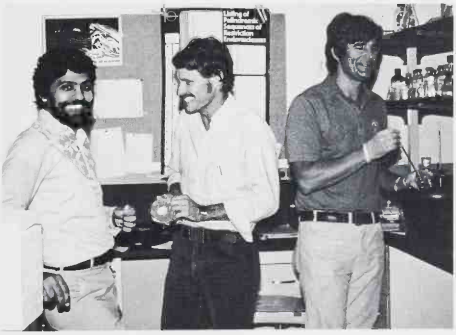

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† *We note with sorrow the death of Dr. Bukhari in November 1983.*

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DIRECTOR'S REPORT

When I first came to Cold Spring Harbor, in June of 1969, it was to a laboratory that was very different in character than it is today. The physical plant was dilapidated and only one building—Demerec—was usable as a year-round laboratory. McClintock—in those days called the Animal House—had that special air of stagnation found only in empty Victorian structures: Its musty rooms and silent corridors echoed to a visitor's footsteps like an abandoned railroad station. The lower floor of James was a grubby warren of unusably small spaces divided from one another by a series of partitions that effectively insulated workers in the building from any contact with one another; the upper floor swayed precariously, empty for all but three brief weeks of summer. Delbrück—then named Davenport—buzzed with bacteriophages in June and July. But come the fall, its water pipes were drained, its windows were shuttered, and the building hunkered down into the hillside for the cold winter. On the site of the present Hershey office building was a set of greenhouses, their cracked and grimy windows serving more effectively to screen out the sunlight than to provide protection to a few long-forgotten plants whose only source of nourishment seemed to be the still-strong smell of garden loam.

The grounds were disorderly and delightful with cover, vibrant with birds and animals. For four months of the year, Bungtown Road was little more than a leafless tunnel for the bitter north wind, but in early spring it softened into a narrow culvert whose flowered walls were a mass of head-high brambles and rhododendrons. In late evening, with the light slanting at a certain angle, it was easy to believe that this was the perfect setting for a Wagnerian opera, so overpowering was the combination of color and perfume to the senses. Of all the changes at the Laboratory, it is only the loss of those intense evenings that I regret.

Today, the vines have been tamed, the trees nursed back to health, and much of the ground has been cleared into open spaces and planted with grass. So, whereas it was difficult previously to obtain a clear view of the harbor, now the water is always in plain sight, and the land is inviting to the casual walker. As our grounds have changed over the years from bucolic to pastoral, so the individual buildings have been transformed from their rustic decay into highly functional laboratories and spacious offices. There is now the pleasure of doing science in a cultivated setting in well-equipped laboratories. It is this restoration of old buildings, together with the planning and siting of new, that has been a major interest of Jim Watson. Remarkably, he has been able to guide the Laboratory through a period of explosive growth and massive renovation without compromising the basic beauty of the land or the style of the science. His absence from the Laboratory for a sabbatical year in London gives me the opportunity to thank him on behalf of the staff for creating an environment so stimulating to the intellect and beautiful to the eye.

This year has seen the completion of a major new addition to Demerec Laboratory—chiefly to make room for the scientists associated with our joint program with Exxon Research and Engineering. The upper floor therefore houses units working on site-directed mutagenesis and prokaryotic genetics. On the lower floor, we have been at last able to achieve our ambition of a protein chemistry laboratory equipped to the best modern standards. Much more significant than our physical expansion, however, has been the infusion of new people into our scientific community: Doug Youvan from Berkeley and Mark Zoller from Vancouver, who have come here to work on site-directed mutagenesis; Pablo Scolnick, from the University of Chicago, who will continue his genetic analysis of the *nif* genes of *Rhodopseudomonas*; Steve Hinton from Purdue and Dan Lundell from Berkeley, who are studying molybdenum-iron centers in metalloenzymes; and, most recently, Clive Slaughter, an English expatriate who brings not only much-needed expertise in protein chemistry, but also a long-standing interest in mammalian cell genetics.

Despite our intensive building program, we continue to work in very crowded conditions—nowhere more so than in James Laboratory. It is therefore welcome news that the long-awaited extension to this building will be completed by midsummer of 1984. Funded in part by grants from the National Cancer Institute, the Pew Memorial Trust, the Fannie Rippel Foundation, and the Robertson Research Fund, the building will provide year-round space for the growing efforts of Ed Harlow's group to develop monoclonal antibodies against tumor-specific antigens and proteins coded by viral oncogenes.

Late in 1983, the renovations were completed to Urey Cottage, which has now become the home of our Publications Department. For the first time in its 50-year existence this building, perched on the hillside above James Laboratory, has achieved a state of elegance. Originally a small summer house and then an uncomfortable and draughty year-round residence, Urey now provides a splendid series of offices for Nancy Ford and her staff. The renovations were carried out with the efficiency and skill that have become the hallmark of our Buildings and Grounds Department under the direction of Jack Richards. For some reason, the laboratory buildings at Cold Spring Harbor seem to suffer disasters—from floods to fire—at an extraordinary rate. That we survive at all is entirely due to the work of Jack and his department. Working often under great pressure, they are infinitely cheerful and never-failingly helpful.

Dominant among our building plans for the immediate future is the new auditorium, to be sited on the west side of Bungtown Road opposite our existing Bush Lecture Hall. The final drawings by Moore, Grover, and Harper show a post-modernist structure whose architectural strength should rival the sophistication of the work presented at our annual symposia and summer meetings. Inevitably, since last year's annual report was written, the estimated costs of the building have risen (now to $3.3 million), and consequently, we have been forced to delay construction for several months. However, the building contract is finally to be signed within the next four weeks (before May 1, 1984), and we still have some hope that the building may be completed in time for next year's 50th Symposium. That the new auditorium will be realized is in large part due to the extraordinary generosity of benefactors in our local community. Year after year, the members of the Long Island Biological Association, under the continually inspired leadership of their Chairman Ed Pulling, raise very substantial sums of money to help the Laboratory achieve specific goals; 1983 was an especially successful year in that LIBA exceeded its own fund-raising target and has now pledged to contribute at least $500,000 toward the new auditorium. The magnitude of this gift makes us realize once again how fortunate we are to be surrounded by a community that is so supportive of our goals and so responsive to our needs.

A major part of the remaining money required to build the auditorium has been donated by a single family—again one of our neighbors. Although they wish for the time being to remain anonymous, we nevertheless want to take this opportunity to thank them for their magnificent gift of one million dollars. We hope that we shall soon be able to acknowledge this generosity in a more open and fitting way.

The subject of the 1983 Symposium was Molecular Neurobiology. The opening address was given by Max Cowan, who set the high intellectual plane that was to be the standard for the

subsequent seven days. Eric Kandel provided a most perceptive and balanced summary of the 91 papers that were presented. He, like many others, is optimistic about the benefits that a fusion of molecular biology and neurobiology may bring. To my mind such benefits still lie several years away—certainly the papers given at the Symposium were far more neurobiological in nature than molecular. I came away with the impression that molecular biology can do much to help define the structural properties of particular proteins that until now have been recognized on the basis of their electrophysiological behavior. Genes coding for several of the neurochemical receptor and channel proteins have already been cloned and expressed; others will rapidly follow. These studies will surely lead to a thorough understanding of the structure and function of such molecules and thence to an appreciation of one of the major properties that set nerve cells apart from other differentiated cells in the body. However, whether molecular biology can be as illuminating about the formation and selection of the complex neural networks that characterize the higher workings of the brain is a question that this Symposium left unresolved.

Our commitment to plant molecular biology has been greatly strengthened during the year. First, the Laboratory has successfully concluded negotiations with the Nature Conservancy to purchase approximately 12 acres of land at Uplands Farm, a former dairy farm located about half a mile from the Laboratory. During the summer of 1983, Barbara McClintock and Steve Dellaporta used the existing greenhouses and a large field at Uplands to carry out a series of genetic experiments with corn. With the purchase of the land, we will now be able to carry out our longer-term plans to convert the old brick garage at Uplands into laboratories and offices for six to eight scientists, and to expand and renovate the greenhouses.

Our immediate aim is to assemble a research group that will be as influential during the next ten years of plant molecular biology as our tumor virologists have been during the last decade. Central to this goal are the scientists presently working in Delbrück Laboratory. Two of them—Steve Dellaporta and Russell Malmberg—have been devoting all their efforts to plants for some time; several others, notably Jim Hicks and Jeff Strathern, have started smaller-scale projects with plants while continuing their long-standing involvement with yeast. During the last year, this group applied to the National Science Foundation for funds to renovate and equip the Uplands Farm facility. The grant of $691,000 which they were awarded—the largest ever given by the National Science Foundation for plant research—will allow us without delay to begin the expansion that is essential to the future success of the project.

Ambitious though our plans are, their scale is small compared with the magnitude of the intellectual problems that plants pose. It is a major concern, therefore, that the prospects remain bleak for secure, long-term funding of basic research into the biology of plants. Although agencies such as the National Science Foundation and the Departments of Energy and Agriculture have established programs on a small scale, the funds made available so far cannot fuel sufficient grants to have an impact on such a large area of science. Most people would agree that the major source of public funds for basic research on plants should be the Department of Agriculture. Although there are some indications that the Department of Agriculture, in response to increasing pressure from Congress, is willing to dispense a larger fraction of its research budget through a competitive granting system, it still seems likely that insufficient funds will be available from governmental sources for several years to come. We cannot wait that long. During the next year, therefore, we will need to attract support from both private individuals and large corporations who can more clearly see the economic and social benefits that will flow from research in this area.

It seems slightly incongruous that we should be worried about funds for plant research in the same year that Barbara McClintock was awarded a Nobel prize for her work on movable genetic elements in maize. This was an event that gave many forms of pleasure, because the award itself was so clearly deserved and yet so long overdue; because the work was prophetic and individual rather than, as is more common these days, the efforts of an anonymous group; and most of all, because it was a victory of radical thought over scientific orthodoxy. Barbara McClintock changed the way we think about genetics and evolution, and it is

not often that intellectual revolutionaries are honored by the establishment.

A more pleasant revolutionary than Barbara would be hard to find. On the sunny, fall day on which the award was announced, she handled the celebrations and press conferences and television interviews with great grace and wit. She made sure that the day became an occasion from which all of us could draw delight and could share in her success.

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The year brought sudden sadness as well as great pleasure. Ahmad Bukhari's death in November left emptiness in many forms—the loss of a friend and counsellor and the unresolved cadences of his interrupted science. Those of us who had known Ahmad since his arrival here in those bright early days had seen him mature scientifically in synchrony with the Laboratory. The stages of his scientific life—from postdoctoral fellow to staff investigator to tenured faculty—therefore seemed to mark the growth rings of Cold Spring Harbor itself. His death made us feel both bereft and vulnerable.

Ahmad himself would have been impatient with such feelings. He was too fascinated with curious things and derived too much joy from clever ideas to tolerate sadness for long. All of us, however, continue sorely to miss his vitality and intelligence.

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In recent years, there has been a great diversification of science at Cold Spring Harbor. For a period of 25 years following the Second World War, the work of the Laboratory was concerned almost exclusively with the genetic behavior of a small number of prokaryotic organisms, chiefly *Escherichia coli* and its temperate bacteriophages. In those days, there seemed to be no sensible direction from which molecular biologists might attack eukaryotic organisms; in any case, work on prokaryotes was taken to be prophetic and certainly was too exciting to abandon in favor of the slower eukaryotes. So it was not until 1968 that serious work began here on mammalian cells, although Phil Marcus and Gordon Sato had for several years previously taught an excellent summer course on animal viruses and cells. The choice of systems available in those days was extremely limited. The safest of a bad bunch seemed to be animal viruses, since they provided the only homogeneous pieces of eukaryotic DNA then available to the molecular biologist. So we started working on them hoping that we might be able to confirm that the truths previously discovered in prokaryotes really were universal.

Of course, our preconceptions about eukaryotes fortunately were wrong. But our esprit as a Laboratory in those early years was not merely the reflection of an excitement generated by the unexpected results that eukaryotes provided, but also had to do with the way we organized ourselves to attack the problems at hand. Virtually all of the Laboratory was then working on one aspect or another of gene organization and expression in adenoviruses and SV40. Although each individual group might bring different ideas and techniques to bear on the various aspects of this problem, there was a strong sense of shared purpose that came naturally from the fact that much of our scientific attention was focused toward a common end. If the science in retrospect seems highly descriptive, it was nevertheless exhilarating at the time.

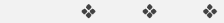
How long such a group effort could have remained coherent is hard to say. Before very long, however, two scientific events occurred that led to its premature dissolution: the discovery of splicing and the advent of molecular cloning. The intellectual ferment caused by the first of these and the technical facility granted by the second combined to form forces for change that we were unable to resist. Suddenly, any eukaryotic gene became as interesting and amenable to structural analysis as viral genomes had been just a short time previously. Freed from the confines of technical limitations we, like many other laboratories, began to spread laterally into many other systems. This urge to diversify led us to study the wealth of topics that are discussed in the body of this annual report.

My guess is that the tendency to radiate outward into other systems soon will come to an end. Should it continue, we would have less and less to say to one another aside from the latest technical tricks in molecular cloning. And in any case, it now seems unlikely that we will be able easily to achieve a satisfactory understanding of more than a small fraction of the systems currently under study. We are in an era in which large numbers of genes are

identified each year, isolated from the organism of choice, and quickly reduced to their component DNA sequences. In time, wild-type and mutant versions of many of these genes will be inserted into the appropriate vectors and expressed in the parental organism. This area of detailed structural and functional analysis of individual genes—the Great Middle Ground of molecular biology—will undoubtedly continue to yield huge quantities of valuable information for many years to come. Major among the foreseeable benefits is a thorough analysis of the structure of eukaryotic regulatory elements, particularly promoters and enhancers. This is likely to lead to an understanding of the mechanisms that determine tissue-specific expression of mammalian genes. We can also expect over a longer term to learn a great deal about the evolution of gene families and to decipher the rules that determine the relationship between the domains of proteins and the location of introns within the corresponding genes.

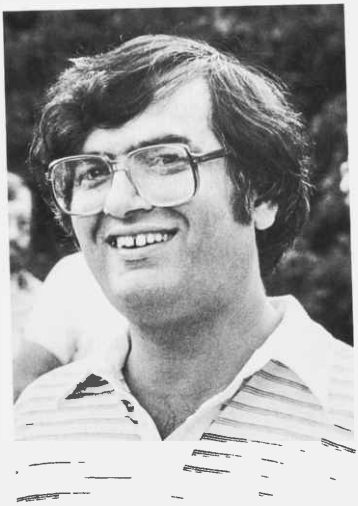
In the excitement of finding out all that we need to know about the organization of eukaryotic genes, it is sobering to remember the limitations of the techniques that are currently so dominant. Even when the Great Middle Ground has been completely explored, we will know comparatively little about the proteins that are coded by those genes we have mapped. Unfortunately, the sequence of nucleotides in a gene presently makes no predictions about the tertiary structure or function of the corresponding protein. Not even the power and elegance of site-directed mutagenesis can completely solve this problem. For example, the gene coding for SV40 large T antigen has been under intensive study for some 20 years; its complete nucleotide sequence is known and its transcriptional pattern has been established. Hardly a single base pair of the gene has been left unmutated, and the biological properties of most of the resulting mutants have been cataloged. Molecular biologists can fairly claim to have carried out the best possible analysis of the gene. Yet I am sure none of them would believe that they have anything but the most superficial understanding of the structure and function of the T-antigen protein itself. We will soon arrive at a similar situation with many other mammalian genes.

Because there is not likely to be a general solution to this paradox for several years to come, there is at present little choice but to determine individually the structure of each of the proteins in which we are interested. To what level of resolution we pursue each protein is an open question, although to my mind, nothing short of the atomic level will be satisfactory in the last analysis. While the idea of bringing large numbers of proteins to the point where we can derive their structures by X-ray crystallography is clearly far beyond our current capacity, it now seems imperative that we do so, at least for those proteins in which we already have considerable intellectual investment. At Cold Spring Harbor, the natural choice would include proteins such as those encoded by the *ras*-gene family, early region 1 of adenoviruses, and SV40 large T antigen. Our desire to work effectively on the chemistry of these proteins may well prompt a partial return to the sort of group effort that was so productive previously. However, the Laboratory is now much larger than it was ten years ago and it is inconceivable that we could, or would wish to, return to an essentially monolithic system. Clearly, we need to find a balanced position that will allow us quickly to develop critical mass in areas new to us, such as protein chemistry and protein structure, without unnecessarily sacrificing the catholic nature of the topics now under study here.



During this year as Acting Director, I have drawn help and encouragement from many sources. I owe particular thanks to three groups of people: to the scientific staff, who have been uncommonly tolerant and supportive; to Bill Udry and his administrative staff, who have been unfailingly cooperative and innovative; and finally, to the Board of Trustees of the Laboratory, whose counsel, both individual and collective, has been excellently wise.

March 18, 1984 *Joe Sambrook*



Ahmad Iqbal Bukhari
1943–1983

On November 19, 1983, Dr. Ahmad Bukhari died suddenly of a heart attack. Dr. Bukhari came to Cold Spring Harbor Laboratory in 1970 as a postdoctoral fellow. In 1972 he was appointed to the staff and conducted research involving genetic rearrangements of the bacteriophage Mu.

A native of Pakistan, he entered the University of Karachi at age 15 and earned his master's degree by age 20. He came to the U.S. in 1964 as a Fulbright Hays scholar at Brown University and received his Ph.D. in 1970 from the University of Colorado.

Although a U.S. citizen, Dr. Bukhari maintained scientific liasons with Pakistan and other third-world countries. He served as an advisor to the United Nations International Development Organization and helped to establish an International Center for Genetic Engineering and Biotechnology, whose aim is to increase the flow of biotechnology to developing countries. He was also a scientific advisor to UNESCO and played a key role in establishing a national biotechnology center for Pakistan.

His friends and colleagues respected him as an outstanding scientist and humanitarian. They also enjoyed his enthusiasm for sports and games. Dr. Bukhari was Captain of the Laboratory's cricket team and was a challenging player at tennis and chess. An avid reader of poetry, he found an outlet for his own creative talents in writing short stories.

Through his work, personality, and philosophy, Dr. Bukhari enhanced the lives of the scientists, students, and co-workers with whom he interacted. He is survived by his wife Christine, and two children, Yousaf Ali and Jaffer Ahmad.

DEPARTMENTAL REPORTS

Recto: *Carnegie Library. Designed as a laboratory in 1905, this building now houses the Library, Marketing Department, Book Store, and Office of Information Services.*

ADMINISTRATION

As discussed in this year's Director's Report, we have managed to protect the Laboratory's charming environment in the face of rapid changes and growth. This has certainly pleased our neighbors, and the small community structure thus maintained we believe has allowed ideas to move quickly and easily throughout the organization. In a similar vein, we have also eschewed a highly formalized bureaucracy. While both these policies result in a certain inefficiency, they also make it possible for us to respond flexibly and rapidly.

Continuing growth is, however, leading us in the administrative sense to rely less on the spoken word and memory and move towards the embryonic stirrings of memos, forms and "guidelines." And as frustrations build over the lack of a place to put one's car, or a desk for a new secretary, or a needed computer attachment, we are beginning to accept that planning may be more than a "bugaboo" or an infringement on academic freedom. Thus, in 1983, we began, under the leadership of a special committee of our Board of Trustees, a detailed analysis of the Laboratory's infrastructure of physical and personnel resources and of the directions in which our research potentially might lead us. While the "business" considerations will be of continuing importance for our guidance in the future, the studies most interestingly provided the framework for a dramatic expression of consensus by the Board for a serious commitment to plant genetics.

The studies also provided us with the opportunity to present our future plans to a combined meeting of the officials of the Village of Laurel Hollow. Once again, we received their expression of appreciation and support.

As we faced the necessity of upgrading our support facilities, the major expressions of which are the new auditorium and the new additions to James and Demerec, we sought out more creative financing arrangements which would relieve some of the strain on our capital and operating funds. With the cooperation of the Nassau County Industrial Development Agency, we received approval for an $8 million dollar development bond issue which was immediately subscribed. The funds derived at low, tax exempt rates of interest will be of immense benefit to the Laboratory's building program and by costing far less than otherwise possible, will result in substantial savings to the Laboratory.

As the Laboratory, and indeed the academic research community in general, continued to explore its relationships with industry, the question moved from the basic challenges of "should we" to "how do we best" handle such relationships. These include financial support of our ongoing research projects, equity and consultant arrangements between individual scientists and commercial firms, collaborative projects, and licensing. Again a special committee of our Board of Trustees was appointed to investigate these questions. As its study progressed, a consensus developed that it is indeed in the Laboratory's interest, which is reinforced by federal government policy, to encourage the transfer of findings from our basic research to the general public and that many times a variety of relationships with commercial organizations is the best or only way to accomplish that objective. Accordingly, during 1983, the Laboratory licensed its discoveries and know-how in the two-dimensional gel techniques developed by Dr. James Garrels to a new corporation beginning operations on Long Island (Protein Databases, Inc.). The company hopes to develop these basic techniques to the extent needed to make them viable as a clinical diagnostic tool of major importance.

We also licensed the H-*ras* oncogene isolated in Mike Wigler's lab to the Actagen Corporation of New Haven, Connecticut. We are now conducting negotiations with a number of firms for use of the various *ras* oncogenes as potential probes for the diagnosis of cancer.

The year's end marked the completion of a collaborative research project among the Laboratory, Baxter-Travenol, and Genetics Institute. The project successfully obtained, by recombinant DNA techniques, a plasminogen activator substance of human origin which may be useful as a therapeutic medicine for treating thromboembolism in humans. The Wellcome Foundation in Great Britain has now taken over the project and will endeavor to scale-up the process of making the substance and will do the testing and experiments necessary to bring it to the public at the earliest possible time.

Retiring from our Board after completing the six-year limit in our By-Laws were Charles Cantor, Institutional Trustee from Columbia University, where he was Professor of Biology, who provided us much appreciated advice on both scientific and administrative matters, and Walter Frank, Jr., whose keen insight on financial markets served us so well while he was a member of the Finance and Investment Committees. We accepted the resignation of John Carr after his move to Washington, D.C., to head the Grumman Corporation office, which removed him from the possibility of continuing his invaluable help in a variety of administrative areas.

Dr. Eric Kandel, University Professor at Colum-

bia University Medical Center, was elected as an Institutional Trustee, making even more official his long-time association with the Laboratory.

Mr. Taggart Whipple, partner in the law firm of Davis, Polk, and Wardwell, and John Klingenstein, partner in Wertheim Company, were newly elected as Individual Trustees.

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After an extensive search to fill the post of Personnel Director, we found an ideal replacement in Bill Putnam who brought to us many years of successful experience in personnel management, most recently at the Celanese Corporation.

We were also greatly pleased to appoint Susan Schultz as Grants Manager. Susan had previously served for two years as Grants Assistant and her great helpfulness and most pleasing personality are well-known to the staff.

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Two festive occasions, which were orchestrated by David Micklos, highlighted our otherwise too intensive summer of 1983. In June, the lawn at Airslie, under the direction of Elizabeth Watson, was bedecked in splendor for an Appreciation Dinner at which over 200 friends and neighbors honored the combined forty years of service to the Laboratory by Walter Hines Page and Edward Pulling. For twenty-five years Mr. Page has served on the Boards of the Long Island Biological Association and of the Laboratory, mostly as President or Chairman. Mr. Pulling has for the past fifteen years been Chairman of the Long Island Biological Association and a member of the Board of Trustees of the Laboratory.

Margaret M. Heckler, Secretary of Health and Human Services

In September, the Laboratory joined the American Cancer Society in a tribute to Mary Lasker for her contribution to the advancement of scientific research on the causes and cure of cancer. In conjunction with a Laboratory conference on "The Cancer Cell," which was dedicated to Mrs. Lasker, a dinner was held in her honor at the Piping Rock Club. Speakers included Secretary of Health and Human Services, the Honorable Margaret Heckler; Director of the National Institutes of Health, Vincent DeVita, Jr.; President of the American Cancer Society, Lane Adams; and Chairman of the National Advisory Cancer Council, Benno Schmidt. J. Michael Bishop was Master of Ceremonies and Jim Watson welcomed the guests. Unfortunately, an untimely illness prevented Mrs. Lasker's attendance, but the speeches, so laudatory to her activities, were recorded and presented to her.

William R. Udry

Edward Pulling

Walter H. Page

INFORMATION SERVICES

The Information Services Department was created in 1983 to perform three major functions: (1) public relations, (2) fundraising for special projects, and (3) media relations.

In the broadest sense, the department seeks to explain the Laboratory to various interest groups. Toward this end, two publications were initiated in 1983. A multipurpose brochure, "Cold Spring Harbor Laboratory: At the Horizon of Molecular Biology," explains the history and current research efforts of the Laboratory in language understandable to educated laymen. "Harbor Transcript" is a quarterly internal newsletter designed to keep employees up-to-date on current events at the Laboratory.

During the year, the department coordinated several special events held in conjunction with the Long Island Biological Association (LIBA). Also, an educational outreach program was begun to provide tours and presentations for school and civic groups.

The department coordinated successful efforts to find funding for the Undergraduate Research Training Program and the Neurobiology Training Program. A major grant was also secured from the Rippel Foundation to help purchase equipment for the Monoclonal Antibody Extension to James Laboratory.

A long-standing objective to formalize and strengthen the Laboratory's relationships with companies that have expertise in genetic engineering/biotechnology was also realized. In 1983, fifteen companies were recruited as charter members of the 1984 Corporate Sponsor Program—Agrigenetics Corporation; Biogen, Inc.; CPC International, Inc.; E.I. du Pont de Nemours & Company; Genentech, Inc.; Genetics Institute; Hoffman-La Roche, Inc.; Johnson & Johnson; Eli Lilly & Company; Molecular Genetics, Inc.; Monsanto Company; Pall Corporation; Pfizer, Inc.; Schering Corporation; and Upjohn Company.

Throughout the year, an effort was made to keep media outlets apprised of the activities of the Laboratory. The department acted as liaison to schedule interviews and filming sessions with print and broadcast journalists. A press conference held the day of the announcement of the Nobel prize to Barbara McClintock was attended by media representatives from around the world.

David Micklos

TWELVE YEARS OF LIBRARY SERVICE

The 1972 Annual Report states that the Laboratory's library collection numbered almost 25,000 volumes. Interestingly enough, at the end of 1983 our collection numbered only 25,895. In 1973, approximately 360 journal titles comprising 10,800 bound volumes were placed on permanent loan with The State University of New York at Stony Brook. Placements such as this, and a program of continuous weeding, account for the relative stability in total volume count. This is the only library statistic that has *not* significantly increased in the past 12 years.

In the areas of patron services and collection development, the story is quite different. In these 12 years there has been a 75% increase in journal subscriptions; the addition of two branch libraries, one for Neurobiology, the other for the Banbury Center; the processing of 2100 bound books and journals per year; and the addition of several computer-based systems for business application and for access to highly sophisticated scientific information networks. All of this has contributed to the 161% increase in materials being received.

Patron services, including manual and computerized scientific searches, ready reference, interlibrary loan, circulation, archives, and photocopying, have increased an overall 246% to meet the needs of the 183% increase in the Laboratory's staff.

In 1972, a staff of two provided access to a standard card catalog of books, 261 subscriptions, and ten tedious manual searches from two complicated scientific sources for 56 scientists. Today, 5.5 staff members provide services that range from highly technical on-line literature searches using over 170 databases covering all disciplines, through citation verification for grant applications and our Publications Department, to the acquisition of obscure historical materials for 165 scientists.

These sizeable increases in the numbers of patrons, materials, and personnel make space the major priority of the library's long-range planning. It is essential to look at both renovation of the present facility and off-grounds storage to provide adequate reader facilities. We look with pleasure to the completion in 1985 of the new auditorium which will allow our present tenants, the computer center, Information Services, and audio-visual services, a place of their own.

Susan Gensel

PUBLICATIONS

In 1983 I began my 11th year at the Laboratory and it was an exciting year indeed. Most exciting was the move of the Publications offices to Urey cottage, which was completely renovated and enlarged to meet the needs of the editorial, production, and order fulfillment functions. In 1983 we also saw our all-time best-seller, *Molecular Cloning*, reach cumulative sales of more than 22,500 copies. This was not an unmixed blessing, however, since the unexpected continuing strong demand for this title necessitated three rapid reprintings during the year.

Net sales of all publications for 1983 were almost $1,600,000 (with more than 35,000 units sold), giving us a year second only to 1982 when *Molecular Cloning* was first released. Among the titles published in 1983 were: a two-book set on *Structures of DNA* as volume 47 of the Symposia; *Teratocarcinoma Stem Cells*, volume 10 in the series Cold Spring Harbor Conferences on Cell Proliferation; three monographs—*Nucleases*, *Molecular Biology of the Yeast Saccharomyces: Metabolism and Gene Expression*, and *Lambda II;* a collection of reprint articles entitled *Readings in Tumor Virology; Muscle Development;* and *Genetic Maps* volume 2. The Publications Department was also responsible for the production of two Strain Kits to accompany the lab manuals *Experiments in Molecular Genetics* and *Advanced Bacterial Genetics*, as well as the Annual Report and ten different Abstract booklets for the Meetings Program.

Although scheduled to appear in 1983, volume 48 of the Symposia, *Molecular Neurobiology*, did not make its appearance until early 1984, due to delays at the printers. When published, however, it marked a radical departure in policy, since it appeared simultaneously both in paperback and in its traditional cloth binding. It is still too early to tell whether our aim of increasing unit sales of the Symposia to their previously higher levels will be achieved by offering the volume in the lower-priced paperback format.

We also began a new series in 1983, Current Communications in Molecular Biology. This series, composed of paperback editions of extended abstracts, makes possible the rapid dissemination of information exchanged at small meetings focused on rapidly expanding areas of research. The first two titles in the series, *Plant Infectious Agents* and *Enhancers and Eukaryotic Gene Expression*, appeared less than five months after their respective meetings.

Work begun almost two years ago to establish codes and procedures to enable direct photocomposition from our on-grounds computer also yielded results in 1983 with the publication of *Nucleases* and both volumes of our Current Communications series. Results to date indicate that savings of 30–40% on initial composition costs can be attained by keying and coding manuscripts on our own system. Now with the space available in Urey, we intend to expand our capabilities in this direction.

The Urey facility has also made it possible to provide individual offices for the editors, which has resulted in a level of efficiency not previously possible when they shared a communal office. The new building has allowed computerization not only of our own typesetting, but also of word processing and financial and scheduling management, as planned for implementation in 1984, and the establishment of a true production group.

With the number of new titles steadily increasing, the importance of our Marketing Department, headed by Susan Gensel, becomes increasingly evident. In 1983, the Marketing Department achieved high visibility for our publications by exhibiting our titles at five major scientific conferences. They also prepared eight direct mailings (consisting of some 16 flyers) that reached a total of 306,500 scientists, libraries, and universities.

Additional exposure is achieved through our on-grounds bookstore, operated by the Marketing Department. In 1983, bookstore sales amounted to $84,836 with 2994 books sold. Through both the bookstore and attendance at conferences, we are able to gather important information from our customers so that we can better serve their needs.

An aggressive program to increase our foreign sales resulted in 1983 in overseas sales constituting 32% of our net sales, a 42% increase over our 19% in foreign sales in 1979.

To provide better service to our expanding foreign market and our individual customers, Charlaine Apsel, our Fulfillment Manager, instituted in 1982 services which address their special needs. The first allows foreign customers to opt for a shipping service which provides 2–4-week airmail delivery overseas at less than standard airmail costs. The second provides our individual customers with the convenience of having their orders billed to any of three major credit cards.

Both of these services have greatly increased the number of shipments made from the Laboratory, the end result being a 42% increase in direct shipments in 1983 over 1982. This has sorely strained our on-grounds storage capacity, and the acquisition of sufficient local warehousing is just one of our goals for 1984.

Nancy Ford

RESEARCH

Barbara McClintock
Nobel Prize in Medicine, 1983

MAMMALIAN CELLS

STRUCTURAL STUDIES

This past year of research on the structural and functional organization of cells focused on molecular details and on integration of cell biological and biochemical approaches. As we delve deeper into the individual components responsible for cell architecture, we find that we have to return at some point to the whole cell in order to understand more fully the functional significance of a given molecular component. More and more, as proteins are identified as interesting spots on two-dimensional gels, purified by some appropriate method, used to elicit antibodies in rabbits and mice, and obtained in the form of molecular DNA clones, the conversation and ideas turn to attempts to study the protein as an active part of the living cell. Thus, we have pressed to advance our capabilities in the microinjection techniques and the subsequent "micro" cell biological assays that need to be done following injections. These types of experiments, which bring together virtually all of the various individuals working here in the field of cell structure, will most likely continue to be an important tool for the foreseeable future.

The QUEST System for Two-dimensional Gel Analysis

J.I. Garrels

The QUEST software package for two-dimensional gel analysis has been further developed for the PDP-11/60 computer by the addition of several new matching programs. Matching of patterns containing up to 1500 spots is now completely automatic, and very little manual editing or checking of the matched patterns is required.

A typical experiment will generate about 40 gels, representing ten samples run on four different types of two-dimensional gels (differing in pH ranges and slab-gel concentrations). Each gel is exposed to film three times (on the average) to detect the full range of spot intensities. Each film pattern is scanned into the computer, and spot detection and integration take place automatically. To match the group of ten gels of a given pH range and acrylamide concentration to their respective standard, the following protocol is now used.

One of the darkest films is selected as a model for the experiment. The model is matched carefully to the standard pattern. (The standard is the collection of all spots detected so far on this type of gel. The broad pH range, 10% standard currently contains over 2600 spots.) The match of the model to the standard is done by automatic procedures similar to those described below, and the accuracy of this match is carefully checked by interactive graphics. Next, each of the other dark films is matched first to the model and then to the standard, by completely automatic procedures.

Three automatic steps are involved in the match of a typical film to a standard. The first step is the selection of landmark matches between the film and the model. (Until recently, this was done manually.) The second step is the automatic match of the film to the model. The automatic matching process begins at the landmark spots and uses spot-neighbor relationships to match the rest of the pattern. Because the spots of the model have standard spot numbers (through its match to the standard), the spots of each film can also be assigned standard spot numbers as they are matched to the model. In the third step of automatic matching, each spot having a standard spot number becomes a landmark to initiate a complete match to the standard. In this match, any spots on the film that were not present in the model have a chance to be assigned a standard spot number by direct match to the standard. Matching the spots of a film to those of a previous matched (model) film from the same experiment is much more accurate than matching directly to the standard. Patterns from the same experiment are usually quite similar, whereas the standard pattern is a much more complicated distribution of spots derived from many experiments.

When all the dark films have been matched by the above procedure, a cross-matching program is run to improve the match quality further. Each film of the group is automatically matched to each other film of the group (using spots with identical standard spot numbers as landmarks), and the results are

checked for internal consistency. If, during the above matching procedures, any spots have failed to match or have been matched incorrectly due to gel distortion, the error will usually be corrected by the cross-match. Discrepancies that cannot be conclusively resolved by the automatic program are flagged for operator inspection. Usually fewer than 10 out of 1000 spots are flagged, and not all of these are actually in error.

After the darkest films have been matched, the next lightest exposure of each film is matched automatically. This group does not use one model film; instead, the darker exposure of each gel serves as the model for the lighter exposure. The same automatic procedure, followed by a cross-match, is used to complete the match of the lighter films. The very lightest films (used to quantitate actins, tubulins, and a handful of other major proteins) usually require manual intervention because there are too few spots for the operation of the automatic programs.

This procedure is sufficiently accurate that we no longer need to routinely check and edit each of the matches. However, all FILMSPOTS (spot description) files and MATCHLIST (match status) files are preserved so that a graphic display of the match of any film to another film or to a standard can be obtained at any future time. Manual editing, or further automatic cross-matching, can be used to improve the matches further at a later date, taking into account new spots that may have been added to the standard in the meantime.

Rat Protein Data Base

J.I. Garrels, B.R. Franza, Jr.

The gel-analysis software runs around the clock, processing data from our large backlog of experiments. Quantitative data from each film are matched to standard patterns, as described in the preceding report, and the standardized data are entered into the rat protein data base. Part of our data base consists of background information necessary for the interpretation of other studies, and part of it is composed of data from experiments of great immediate interest. The background part of the data base that has already been established includes data from five clones of REF52 cells, data from six SV40 transformants of REF52 cells selected by focus formation in monolayer culture, data from REF52 cells labeled for differing lengths of time, and data from a large experiment in which normal REF52 cells and nine virus-transformed REF52 derivatives were each labeled at five time points during their growth to confluence. The latter experiment includes data from two of the SV40 transformants selected by focus formation, two SV40 transformants further selected for growth in soft agar, and two SV40 transformants yet further selected for tumor formation followed by return to culture. All SV40 transformants were isolated by McClure et al. (*Cold Spring Harbor Conf. Cell Proliferation 9:* 345 [1982]). The other lines studied include a Kirsten murine sarcoma virus (Ki-MSV) transformant of REF52 (from B. Ozanne, University of Texas Health Science Center, Dallas) and two adenovirus-5 (Ad5) transformants of REF52 (from J. Logan and T. Shenk, SUNY, Stony Brook). For a typical sample, approximately 1500 proteins are quantitated, matched, and entered into the data base.

A few general conclusions can be made from the baseline data already in the system. In independent clones of REF52 cells, approximately 6% of the proteins differ in intensity by more than twofold, compared to approximately 4% when duplicate samples of the same clone are analyzed. REF52 cells do not change their protein composition rapidly during logarithmic growth; samples labeled 1 day apart are as similar as duplicate samples labeled on the same day. However, when confluent cells are compared with rapidly proliferating cells, approximately 15% of the proteins are found to differ by more than twofold in rate of synthesis.

In comparisons of normal and virus-transformed REF52 cells, we found that the series of focus-selected SV40 transformants are highly reproducible. The same proteins are altered in each line relative to the control, although there are differences in the degree of alteration. Comparison of a typical SV40-transformed line with normal REF52 cells reveals that about 20% of the proteins have been altered by twofold or more, but comparison of SV40-transformed lines with one another reveals that 5–8% of proteins differ by twofold or more. When the proteins altered due to transformation were compared with the proteins that differ in proliferating versus confluent cells, no overall correlation was found. This statistical comparison does not exclude some proteins that are induced both in proliferating cells and in transformed cells, but it does show that a transformed cell is not just a normal cell held in a highly proliferative state.

The adenovirus-transformed lines are altered to a much greater extent relative to REF52 than are the SV40-transformed lines. More than 30% of the total proteins are altered by more than twofold in each of our two adenovirus-transformed lines. Interestingly, many of the same proteins are affected by SV40 and by adenovirus, but the magnitude of the alteration is greater for adenovirus transformation.

One of our recent interests has been the proteins that respond to serum stimulation in normal and transformed cells. Normal, SV40-transformed, and Ad5-transformed REF52 cells were held in low serum until proliferation had stopped (in REF52) or slowed significantly (in the transformants). Cells were labeled at several times after the addition of

fresh serum. Data for about 1000 spots have been quantitated and entered into the data base. Both early and delayed responses to refeeding have been identified. One of the most interesting delayed responses is the protein PCNA (proliferating cell nuclear antigen), or cyclin. This protein is identified on a section of the standard map in Figure 1. Each of the graphic inserts plots the relative intensities of PCNA for each sample in one of the experiments from the data base. (Users of the graphics workstation can obtain such graphs for every experiment analyzed and for every spot on the standard map.)

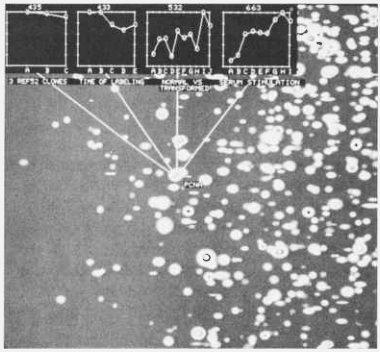


Figure 1
Display of data-base information for the protein PCNA. A region of the standard rat-protein map was displayed on the graphics monitor, and the PCNA spot was selected (at white cross-hair). Four experiments from the data base were selected (experiment numbers are at the top of each graph), and the results for the PCNA spot from each experiment were plotted on the screen. Each graph shows the relative intensity of the spot for each of the samples generated in the experiment, normalized so that the highest intensity is at full scale. (Quantitative data, expressed as spot dpm divided by total incorporated dpm, are simultaneously displayed on another terminal.) In experiment 435, each sample (*A–C*) represents a different clone of normal REF52 cells. In experiment 433, the samples were labeled with [^{35}S]methionine for 30 min (*A*), 2 hr (*B*), 24 hr (*C*), 24 hr followed by 2-hr chase (*D*), and 24 hr followed by 48-hr chase (*E*). In experiment 532, normal and transformed REF52 cells were labeled in log phase growth for 2 hr. The samples are normal REF52 cells (*A*), focus-selected SV40 transformants (*B,E*), agar-selected SV40 transformants (*C,F*), tumor-selected SV40 transformants (*D,G*), Ki-MSV transformant (*H*), and Ad5 transformants (*I,J*). The latter Ad5 transformant does not produce the E1B 57K viral protein. In experiment 663, normal REF52 cells (*A–C*), focus-selected SV40-transformed cells (*D–F*), and Ad5-transformed cells (*G–I*) were deprived of serum for 60 hr, followed by addition of fresh serum. Samples *A*, *D*, and *G* represent the serum-deprived cells; samples *B*, *E*, and *H* represent cells labeled 1 hr after refeeding; and samples *C*, *F*, and *I* represent cells labeled 21 hr after refeeding. All labelings were done for 3 hr. Note that the user of the graphics workstation can obtain such graphs for any experiment in the data base and for any spot on the standard map. Equipment for recording the graphic data on instant Polaroid prints and slides is now available.

Experiment 663 (Fig. 1, fourth graph) shows the response of normal cells at 0, 1, and 21 hours after refeeding (samples A–C), of SV40-transformed cells at the same time points (samples D–F), and of Ad5-transformed cells at the same time points (samples G–I). Other experiments (not shown) confirm that PCNA synthesis in normal REF52 cells is stimulated between 8 and 16 hours after refeeding. Each of the transformants maintains an elevated level of PCNA synthesis even during serum deprivation, and this level is not further induced by serum stimulation. The relative levels of PCNA in this experiment are consistent with the levels found in experiment 532, in which normal REF52 and the nine transformed lines were compared (for further details, see Fig. 1).

Proteins of L6 skeletal-muscle cells have been analyzed throughout the period of differentiation in culture. L6 cells labeled for long and short periods of time have been analyzed to measure turnover rates, and phosphoprotein patterns have been obtained. A group of proteins, identified as actin-filament-binding proteins by the monoclonal antibody studies of F. Matsumura, S. Yamashiro-Matsumura, and J. Lin (this section), was found to be induced during muscle differentiation. Each of these becomes fully phosphorylated over a period of 4 hours after synthesis.

Future entries into the rat protein data base will consist of many more transformed lines, especially lines or REF52 cells transformed by introduction of cloned oncogenes (see report by B.R. Franza, Oncogene Section). New oncogene protein products are being identified as monoclonal antibodies become available, and the expression and modification of each of these proteins will be examined for each of the transformed lines. Studies of other cell types, including muscle cells, NRK cells, primary rat kidney cells (in collaboration with H.E. Ruley, Tumor Virus Section), and PC12 pheochromocytoma cells (in collaboration with W. Huse) are extending the data base to more types of rat cells. We hope in the future to expand the capacity of our computer facility and to make more graphic workstations available so that more users can interact with the protein data bases.

Microfilaments in Normal and Transformed Cells: Changes in the Multiple Forms of Tropomyosin

F. Matsumura, J.J.-C. Lin, S. Yamashiro-Matsumura

As described in last year's annual report, we have found changes in tropomyosin patterns of microfilaments between normal and virus-transformed rat cultured cells. To examine whether these changes in tropomyosin patterns are a common phenomenon with transformation, we have extended similar

studies to human and mouse cells with different types of transformation. These cells included NIH-3T3 cells transformed by transfection with tumor DNA, BALB/c-3T3 cells transformed with chemical carcinogen or by UV-irradiation, human fibroblasts transformed with chemical carcinogen, and naturally occurring tumor cells from human. Like the viral transformation of rat cultured cells, we have found that the level of one or two of the major tropomyosin forms with a higher molecular weight is decreased or missing, and the level of one of the tropomyosin forms with a lower molecular weight is increased in all types of transformed cells so far examined. Because the changes in tropomyosin patterns are well correlated with morphological transformation, we suggest that differential expression of tropomyosin forms in microfilaments may be responsible for the rearrangement of actin cables in transformed cells.

Molecular Analysis of Microtubule Components in Normal and Transformed Cells

S.H. Blose, G. Blose, F. Matsumura

Two important properties of cancer cells are their ability to grow by cell division and to move in the invasion of tissue. The microtubule system of the cytoskeleton is intimately involved in both properties: (1) It sets up the mitotic spindle apparatus responsible for the movement of chromosomes into daughter cells as part of cell growth; (2) microtubule depolymerization has been shown to regulate the initiation of cellular DNA synthesis; and (3) the polarity of the system indicates the direction of movement a cell is taking. Several studies in the late 1970s investigated the tubulin content (the major constituent protein of microtubules) of normal and transformed cells and found virtually no difference in the total chemical amount between both types of cells. However, several studies proposed that the pool of disassembled tubulin was increased in transformed cells. The regulation of microtubule assembly has been attributed in part to the *m*icrotubule-*a*ssociated *p*roteins, MAP-1 (M_r = 330,000) and MAP-2 (M_r = 300,000), and a MAP called tau (four proteins with M_r between 50,000 and 68,000). In addition to these, several new MAPs of cultured cells with unknown function have been described. Therefore, the expression, modification, and behavior of the MAPs may regulate the microtubule assembly dynamics that are ultimately linked to growth and movement. We initiated pilot studies to isolate the intact microtubule complex from normal and transformed cells to determine whether any molecular component of the microtubules such as the MAPs was altered. We felt that this might lead to an understanding of what controls the assembly and reorganization of the microtubules in response to transformation.

We have modified a method adapted from Matsumura et al. (*J. Biol. Chem. 285:* 6636 [1983]) in which a mouse monoclonal IgM antibody that binds both α- and β-tubulin subunits, SBMAP-D3 (here called D3), was used to harvest cellular microtubules. This was selected over the classic method of purification by reversible temperature-dependent assembly of microtubules because the concentration of tubulin in cultured cells was low and microtubules are very labile. After several experiments, we determined optimal conditions to stabilize cytoplasmic microtubules: We found that brief exposure of the cell lysate to the plant antitumor drug taxol was necessary. Taxol, a drug that stabilizes microtubules in cells, used in combination with D3 antibody allowed us to remove intact microtubules from the cytoplasm for subsequent analysis without prior disassembly.

When a lysate is obtained by gentle disruption of the cells followed by low-speed spin (12,500*g* for 15 min) to remove nuclei, large membrane fragments, and insoluble 10-nm filaments, the dispersed assembled microtubules will not sediment. To this supernatant (total protein concentration of 6–8 mg/ml) was added 0.3 volume of D3 monoclonal antibody ($\sim$7 mg/ml), and the mixture was incubated 30–45 minutes at 37°C to allow D3 to aggregate the microtubules. The mixture was spun at 12,500*g* for 7 minutes, and the pellet was then washed three times with Buffer B. The final pellet was then analyzed by negative-stain electron microscopy and one- and two-dimensional SDS gel electrophoresis. Electron microscopy confirmed that the isolated microtubules were in their native-assembled state aggregated and decorated with the antibody (Fig. 2) versus a denatured precipitated aggregate. The major constituent proteins consisted of α- and β-tubulin plus several other associated proteins (Figs. 3 and 4). Autoradiographs were made from dried Coomassie-blue-stained two-dimensional gels, and the α- and β-tubulin spots were cut out of gels obtained from the total lysate and the antibody precipitate. We found that this method enables us to recover 60–70% of the cell's tubulin within 2–3 hours from modest numbers of cells (as compared with conventional cycling methods, which recover $\sim$10% in a longer time from many liters of cells). One of the proteins that coisolates with the microtubules was the M_r 73,000 heat-shock protein (hsp73) (Fig. 4, spot 1). Approximately 10% of the cell's hsp73 was recovered associated with the microtubules. This protein has been postulated to associate with 10-nm filaments and/or microtubules, and we propose to determine whether it is a cross-linking protein. By counting the radioactive spots, we can quantitate the amount of putative MAPs that are removed from the total cell protein. We will use this information

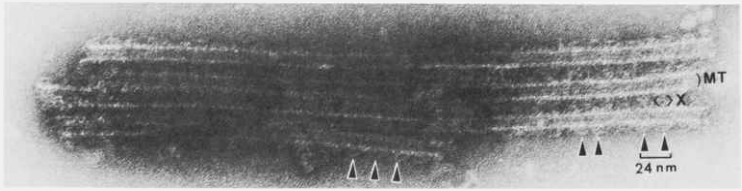


Figure 2
Electron micrograph of D3-antibody-induced microtubule aggregate from NRK-1570 cells negatively stained with 2% aqueous uranyl acetate. Arrowheads indicate positions of the antibody (IgM) decorating the microtubules (MT) with a periodicity of ~24 nm. The antibody cross-linked (X) adjacent microtubules.

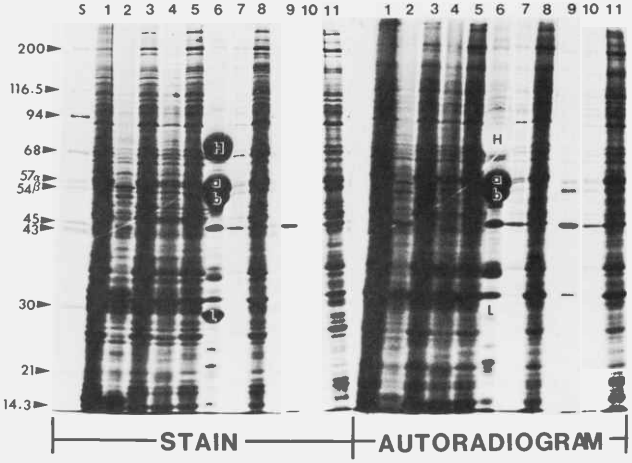


Figure 3
One-dimensional SDS gel (11.5% acrylamide; 0.1% *bis*-acrylamide) stained with Coomassie blue (STAIN), dried, and exposed as an autoradiogram for 15.5 hr at 20°C. NRK-49F (NRK-1570) cells were grown to near confluence in three 24.5-cm² dishes, and cells at the same density in one 100-mm-diameter dish were metabolically labeled with [^{35}S]methionine (750 μCi [^{35}S]methionine, 16 hr, in 2.5% calf serum and media lacking methionine). All cells were then combined and processed for rapid isolation of microtubules as described. (Lane *1*) Total cell homogenate. (Lane 2) First pellet (P_1) obtained from the 12,500*g* spin for 15 min. P_1 contains the 10-nm filaments (vimentin), the nuclei, and other large sedimentable particles. (Lane *3*) First supernatant (S_1) from the low-speed spin. (Lane *4*) Material that sediments (12,500*g*, for 15 min) after the S_1 is incubated for 30 min at 37°C. Microtubules do not pellet at this stage. (Lane *5*) Supernatant (S_1-S) obtained after incubation of S_1 at 37°C and centrifugation. To S_1-S was added the monoclonal antibody D3 (anti-α/β tubulin); the mixture was incubated for 40 min at 37°C to aggregate the microtubules and then centrifuged at 12,500*g* for 7 min to sediment the antibody-induced aggregated microtubules (P_2) from the supernatant (S_2). (Lane *6*) P_2 after being washed three times each in 5–10 volumes of buffer by suspension and sedimentation. In the stained gel, the heavy (H) and light (L) chains of D3 (IgM) antibody are seen, as well as α-tubulin (a) and β-tubulin (b) of the harvested microtubules. In the corresponding autoradiograph, both tubulin subunits are heavily labeled with [^{35}S]methionine. The displacement of radioactive peptides occurred due to the unlabeled heavy (H) and light (L) chains. This P_2 is analyzed in lane *6*. (Lane 7) Pooled supernatants obtained from the three washes of P_2. They contain very little tubulin. (Lane *8*) S_2 does not have any remaining antibody. All of the antibody is found in the P_2. (Lanes *9–11*) Control in which buffer is substituted for antibody. (Lane 9) Control P_2 showing only trace amounts of the tubulin subunits. (Lane *10*) Pooled wash supernatants of the control P_2. (Lane *11*) S_2 from the control. (Lanes *1–5*, *7*, *8*, *10*, *11*) Isovolumetric loads relative to the total homogenate (10 μl per lane); (lanes *6*,9) P_2s were suspended in 50 μl of sample buffer, and 10 μl was loaded per lane. Lane S contains the M_r standards expressed as $M_r \times 10^{-3}$; actin (M_r = 45,000), α-tubulin (M_r = 57,000), and β-tubulin (M_r = 54,000) are included as standards.

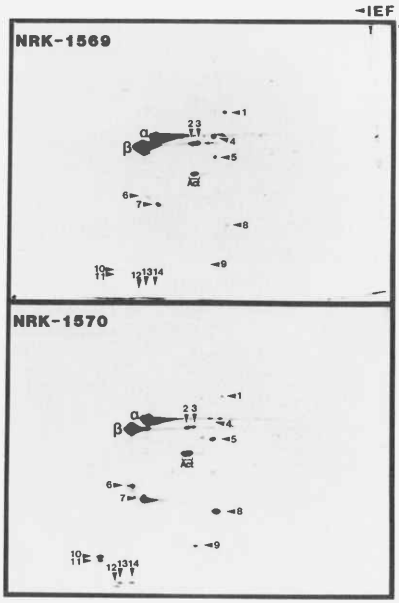


Figure 4
Autoradiographs of two-dimensional gels of D3-antibody-harvested microtubules obtained from KNRK cells (NRK-1569) and NRK-49F cells (NRK-1570) metabolically labeled with [^{35}S]methionine. Alpha and beta indicate the positions of the tubulin subunits. Proteins numbered 1–14 (arrowheads) were found in association with the microtubule complexes. In the KNRK complex, proteins 1 and 4 were found to be increased; 2, 3, and 5–11 were decreased; and 12–14 were absent when compared with NRK-49F. Protein 4 was not detected in NRK-49F. Act indicates the position of actin. Protein 1 is identified as the heat-shock protein hsp73, and proteins 2 and 3 appear to be related to mitochondrial protein 24 of Bravo et al. (*Cell Biol. Int. Rep. 5:* 93 [1981]). The differences in spot intensities appear to be related to the cell phenotype and growth characteristics: NRK-49F cells grow slowly and are flat; KNRK cells are very round and rapidly proliferate.

as a guide to establish what is an associated protein versus a contaminant derived from coprecipitation. For instance, in Figure 4, actin (Act) was found with the microtubule complexes. Although it is thought that actin may bind to MAPs such as MAP-1, it is thought not to be a MAP. The spot was counted and found to represent approximately 0.26% of the cell's total actin, indicating a possible contaminant. When microtubule complexes were compared between normal NRK (1570) cells and NRK (1569) cells transformed by Kirsten sarcoma virus, several differences in associated proteins were observed. Spot 1, the M_r 73,000 heat-shock protein (hsp73), was elevated in the transformed cells (NRK-1569), and spot 4 was only observed in the transformant. The normal cells exhibited several proteins (spots 5–14) that were expressed in very low amounts in the transformed cells. We are currently evaluating the relationship of these proteins to microtubule stability and proteins associated with the mitotic spindle.

Purification and Characterization of Multiple Forms of Tropomyosin in Normal and Transformed Rat Cultured Cells

S. Yamashiro-Matsumura, F. Matsumura

Rat cultured cells have five forms of tropomyosin—TM-1 (M_r = 40,000), TM-2 (M_r = 36,500), TM-3 (M_r = 35,000), TM-4 (M_r = 32,400), and TM-5 (M_r = 32,000). Of these, three (TM-1, TM-2, and TM-4) are major tropomyosins (normal-cell tropomyosin) in normal cells, and two (TM-3 and TM-5) are major tropomyosins (transformed-cell tropomyosin) in transformed cells. We have purified and partially separated normal- and transformed-cell tropomyosins to examine whether these multiple forms of tropomyosin play different roles in the organization of microfilaments between normal and transformed cells.

By hydroxyapatite column chromatography, a mixture of multiple forms of tropomyosin was separated into four fractions, i.e., pure TM-4, pure TM-5, a mixture of TM-3 and TM-1, and a mixture of TM-2 and TM-1. Using these fractions, we have examined some biochemical properties. Like muscle tropomyosin, TM-1, TM-2, and TM-3 had higher apparent molecular weights on SDS gels and showed a higher actin affinity ($\sim 10^5$) than that ($\sim 10^4$) of TM-4 and TM-5 with lower molecular weights. Moreover, polyclonal rabbit antiserum against smooth-muscle tropomyosin also showed much higher affinity to high-molecular-weight tropomyosins (TM-1, TM-2, and TM-3) than to low-molecular-weight tropomyosins (TM-4 and TM-5). These results suggest that high-molecular-weight tropomyosins may be similar to muscle tropomyosins. We have also examined whether these five forms of tropomyosin are homo- or heterodimers. Although TM-3 did not make an S-S dimer with the oxidization of cysteine residues, all of the other forms of tropomyosin were found to form homodimers. Because the formation of homodimers appears to be more than 90% in each form of tropomyosin, it is likely that all five forms of tropomyosin are present as homodimers.

Amino acid compositions of these five forms of tropomyosin were examined. Normal-cell tropomyosins (TM-1, TM-2, and TM-4) showed compositions very similar to those of muscle tropomyosins. Interestingly, however, the amino acid compositions of transformed-cell tropomyosins (TM-3 and TM-5) are considerably different, although their amino acid compositions are very similar to each

other. The contents of glutamic acid, lysin, arginine, and leucine residues in transformed-cell tropomyosin were two times less than those in normal-cell tropomyosin, whereas the contents of serine, glycine, and phenylalanine in transformed-cell tropomyosin were increased 2–3 times. In spite of these differences in amino acid compositions, both normal- and transformed-cell tropomyosins are recognized with either monoclonal or polyclonal antibody against tropomyosin, suggesting that these two types of tropomyosins share a stretch of homologous sequence.

Tropomyosin Isoforms in Normal and RSV-transformed CEF Cells

J.J.-C. Lin

We have identified seven polypeptides in chicken embryo fibroblasts (CEF) as tropomyosins (Lin et al., *J. Cell Biol. 98:* 116 [1984]). Of these, spots 1 (M_r = 38,000), 2 (M_r = 36,500), 3a (M_r = 32,800), and 3b (M_r = 32,800) were major forms, whereas spots a (M_r = 45,000), b (M_r = 43,000), and c (M_r = 38,000) were relatively minor. Spots 3a and 3b, as well as spots 1 and c, had a slight difference in their pI values. All of these polypeptides were found to be associated with microfilaments. These tropomyosin isoforms have also been purified from CEF by the combination of heat treatment, ammonium sulfate fractionation, and DEAE chromatography. The purified tropomyosins (particularly, 1, 2, and 3) showed different binding to F-actin under conditions of 100 mM KCl and no Mg^{++}. Other biochemical and biophysical properties of these isoforms of tropomyosin are under investigation.

Using the microfilament isolation method that we developed (Lin, et al., *J. Biol. Chem. 258:* 6636 [1983]), we have compared the protein patterns of the microfilaments from normal and RSV-transformed CEF cells. In addition to the previous finding that tropomyosins 1 and 2 were greatly reduced in transformed cells (Hendricks and Weintraub, *Proc. Natl. Acad. Sci. 78:* 5633 [1981]), we have found that the relative amount of tropomyosin 3 was increased. Because of the different actin-binding properties observed for tropomyosins, the change in tropomyosin isoform expression may be responsible for the reduction of actin cables and the alteration of cell shape found in transformed cells.

Purification and Characterization of 55K Actin-bundling Protein from HeLa Cells

S. Yamashiro-Matsumura, F. Matsumura

It is believed that actin-binding proteins regulate the organization of actin cables in cultured cells. However, little is known regarding how actin-binding proteins organize actin cables in cultured cells and what role the proteins play in the disorganization of actin cables upon cell transformation. As the first step in addressing these questions, we have purified some actin-binding proteins using a method utilizing actin affinity. Briefly, muscle actin was mixed with the supernatant of HeLa cell homogenates, and the resultant actin gel was precipitated by a low-speed centrifugation. The actin gel was depolymerized, and proteins bound to actin were separated on a DEAE-cellulose column. By this simple and rapid method, we could prepare filamin and α-actinin and a yet unknown actin-binding protein with a relative molecular weight of 55,000. The 55K protein is a monomer with a native molecular weight of 55,000. It has a Stokes' radius of 32 A and a sedimentation coefficient ($S_{20,w}$) of 4.35. The protein bound to actin at a stoichiometry of four actin molecules to one 55K protein. The protein caused F-actin to aggregate side by side into bundles as do other F-actin-bundling proteins such as fimbrin (M_r = 68,000) and fascin (M_r = 55,000). Although a proteolytic fragment of fimbrin had the same molecular weight (M_r = 55,000), the 55K protein did not comigrate with this proteolytic fragment on two-dimensional gels. Antiserum against either fimbrin or fascin did not cross-react with this 55K protein. It is not likely that the 55K protein is a proteolytic fragment because the same 55K protein is found in freshly prepared total cell lysates. Further characterization, including the localization in cultured cells and the differences between normal and transformed cells, is in progress.

Production of Monoclonal Antibodies to Tropomyosin Isoforms

C.-S. Chou, J.L.-C. Lin, J.J.-C. Lin

Multiple forms of tropomyosin have recently been observed to be present in a single cell type. During muscle differentiation, tropomyosin switched from one isoform to another. Moreover, in many transformed cells, tropomyosin isoforms with higher apparent relative molecular weights tended to decrease or disappear, whereas another form of tropomyosin with a lower apparent relative molecular weight was increased. The intracellular localization and functional properties of these tropomyosin isoforms are not understood. To address these questions, we have used in vivo and in vitro immunization methods to generate many monoclonal antibodies (mAbs) against specific isoforms of tropomyosin from chick cells. For example, mAb CL2 reacted strongly with a striated muscle form of tropomyosin, whereas no reactivity or poor reactivity was detected for the smooth-muscle form or fibro-

blastic form, respectively, of tropomyosin. The reactivity for the β form of skeletal-muscle tropomyosin was threefold stronger than that for the α form. mAb CH1 recognized both α and β forms of skeletal-muscle tropomyosin equally well but did not react with either the smooth-muscle or the fibroblastic form. mAb CG1, obtained from the fusion with smooth-muscle tropomyosin as immunogen, reacted preferentially to the α form of smooth-muscle tropomyosin. By Western blot analysis, CG1 also reacted with CEF tropomyosin; however, by indirect immunofluorescence, only some populations of CEF showed staining with this antibody. The significance of these results is currently under investigation.

Expression of Tropomyosin in Normal and Transformed Cells

D. Helfman, Y. Kataoka

Transformation of cells by DNA and RNA tumor viruses has profound effects on the organization of the cytoskeleton, including disruption of the microfilament organization. However, the molecular, biochemical, and structural bases for these alterations of the actin cables are poorly understood. Recent studies have suggested that alterations in the pattern of tropomyosin expression may be involved in the rearrangement of stress fibers and that this may be responsible for morphological alterations seen in transformed cells (see Matsumura and coworkers, this section). We have begun to study the nonmuscle (rat embryonic fibroblast) forms of tropomyosin and the genes that encode these proteins. We have constructed a cDNA expression library of approximately 100,000 members from rat embryonic fibroblast RNA using the plasmid expression vectors pUC8 and pUC9. Using both an immunological screening procedure and ^{32}P-labeled cDNAs, we have identified and isolated at least three different classes of clones. One set of clones has been identified by hybrid-selection translation to contain sequences complementary to tropomyosin 1 (m.w. 40,000). Overlapping clones containing the entire coding region and the entire 3′-untranslated region for tropomyosin 1 have been identified and are being sequenced to determine the primary structure of the protein. Northern blot analysis reveals a transcript of approximately 1.2 kb. Furthermore, this transcript is absent in adenovirus-transformed REF52 cell lines (Ad5D.1A and Ad5D.4A). These two cell lines were previously shown not to contain tropomyosin 1 (see Matsumura and co-workers, this section). Thus, it would appear that transcription of tropomyosin 1 is shut off in these cells. We are continuing to isolate and characterize other cDNAs and hope to obtain clones to all five forms of fibroblast tropomyosin. The cloned cDNAs will be used to study the structure and organization of these genes, as well as their expression in normal and transformed cells.

Isolation of Cytoskeletal Genes

S. Hughes, J. Feramisco, D. Helfman, N. Maihle, T. Kost, Y. Kataoka, E. Kosik

The long-range goal is to develop a system in which it is possible to study and to alter the patterns of expression of gene families that have tissue-specific gene products (e.g., the cytoskeletal proteins). We wish to ask questions about the functional differences between the various gene family members expressed in specific cell types. We are developing retroviral vectors appropriate for use in primary cell culture and ultimately for use in vivo. Such vectors will be used to cause the expression of particular genes in cells (or tissues) where they are not normally expressed. These experiments can be used to look for subtle functional differences between proteins encoded by the various members of a gene family. In addition, the vectors can be used to analyze a variety of mutant genes, either naturally occurring or created in vitro, and to test the functions of recombinant genes made between different members of particular gene families.

The chicken was chosen as the experimental animal because chick embryos are a simple and inexpensive source of material for the isolation of cell-type-specific RNA and for the preparation of a variety of different types of primary cultured cells. In addition, Rous sarcoma virus appears to offer significant advantages as a prototype vector for these experiments. Our efforts have been focused on two convergent pathways: (1) development of the retroviral vectors and (2) development of procedures for the rapid identification of genes that express low to moderate levels of RNA. These techniques have made it a relatively simple procedure to identify several cytoskeletal genes in the chicken. Although the analysis of these genes is far from complete (see below), a reasonable start has been made.

We believe that sufficient progress has been made on both projects so that the initial attempts to reintroduce specific cytoskeletal genes with the retroviral vectors will come in the next 6 months.

We initially screened genomic libraries directly for cytoskeletal genes, and although we were able to isolate and identify clones that encode the chicken β-actin gene, the protocols are inefficient. To facilitate the rapid identification of genes, we have developed a method for the immunological screening of cDNA expression libraries (Helfman et al., *Proc. Natl. Acad. Sci. 80:* 31 [1983]). With

these techniques, cDNA clones from rare to moderately abundant mRNAs may be readily identified. To test the general utility of the procedures we use, we have collaborated with several laboratories to identify particular cDNA clones. The initial experiments have been done with a cDNA library containing approximately 100,000 members made from chick smooth-muscle mRNA. Two sets of expression clones from the library have been characterized by DNA sequence and hybrid selection-translation.

The first set of cDNA clones that were isolated specify smooth-muscle tropomyosin. A full-length cDNA clone (1100 bp) has been completely sequenced (Helfman et al., *J. Biol. Chem.* [in prep.]), and overlapping genomic clones have been isolated and partially mapped. Northern analysis reveals tissue-specific transcripts. The second set of cDNA clones specify smooth-muscle tropomyosin. A partial cDNA clone has been partially sequenced, and genomic clones have been isolated but not fully characterized. Northern analysis reveals tissue-specific transcripts.

Several other clones have been isolated, usually as collaborations. These are less well characterized, most having only been identified as antibody-positive clones, and they include α-actinin, 36K protein (the major *src* tyrosine phosphorylation target), myosin light-chain kinase, Na^+/K^+ ATPase, 70K heat-shock protein, and spectrin. To complete the isolation and characterization of tissue-specific genes, other cDNA expression libraries are being prepared and screened. Either complete or in preparation are the following cDNA libraries:

1. Rat fibroblast cDNA library. This library is complete and has been screened both with DNA probes and with antibodies to tropomyosin.
2. Chick fibroblast cDNA library. The cDNA has been prepared, ligated to plasmid, and tested by transformation on a small scale. The cDNA is ready for large-scale transformation.
3. Chick striated-muscle cDNA library. The cDNA has been prepared, ligated to plasmid, and tested by transformation on a small scale. The cDNA is ready for a large-scale transformation.

The chicken β-actin gene. The nucleotide sequence of the chicken β-actin gene has been determined. The gene contains five introns: four introns interrupt the translated region at codons 41/42, 121/122, 267, and 327/328 and a large intron occurs in the 5′-untranslated region. The gene has a 97-nucleotide 5′-untranslated region and a 594-nucleotide 3′-untranslated region. A slight heterogeneity in the position of the poly(A)-addition site exists; polyadenylation can occur at either of two positions two nucleotides apart. The gene codes for an mRNA of 1814 or 1816 nucleotides, excluding the poly(A) tail. In contrast to the chick skeletal-muscle actin gene, the β-actin gene lacks the cysteine codon between the initiator ATG and the codon for the aminoterminal amino acid of the mature protein. In the 5′-flanking DNA, 15 nucleotides downstream from the CCAAT sequence, there is a tract of 25 nucleotides that is highly homologous to the sequence found in the same region of the rat β-actin gene.

α- and β-Tropomyosin cDNA clones from smooth muscle. Two cDNA clones for α-tropomyosin were isolated from a small (~10,000 members) cDNA library prepared from smooth-muscle RNA. Subsequently, a larger smooth-muscle library (~100,000 members) was rescreened for tropomyosin clones with both cDNA and immunological reagents. In all cases the cDNA was inserted via *Eco*RI and *Sal*I linkers. The order in which the linkers were added determines the orientation of the insert; the cDNAs were matched to the expression plasmids pUC8 and pUC9 such that the cDNA was appropriately oriented for expression in *Escherichia coli*. The pUC9 portion of the library contained 13 clones that could be detected with the DNA probe, two of which reacted with anti-tropomyosin antisera. The pUC8 portion of the library contained 39 clones that could be detected with cDNA and 13 clones that reacted with anti-tropomyosin antisera. Two of the clones from the pUC8 library reacted with antisera were not detected with α-tropomyosin cDNA. These two clones have been shown by hybrization/translation to contain β-tropomyosin cDNA.

All three clones from the pUC8 library that must be checked by DNA sequencing have identical 3′ termini. None of these clones contain the poly(A) tail or the sequence normally associated with polyadenylation. The simplest explanation is that there is a natural *Sal*I at the 3′ end of the message for α-tropomyosin. This would also explain why there are relatively fewer clones in the pUC9 library and why only a small percentage of the pUC9 clones can express a tropomyosin-related protein.

The clones that express α-tropomyosin in *E. coli* contain cDNAs that encode anywhere from the carboxyterminal third of the gene up to the entire coding region. When lysates of the clones were analyzed on SDS-acrylamide gels, the tropomyosin-related proteins could be seen by staining. Despite the fact that all the proteins are made from the LacZ promoter/AUG, there were considerable differences in the steady-state level of tropomyosin-related protein present in the lysates. These differences did not correlate with the sizes of the fragments. Furthermore, clones that contained different-size cDNAs contained common carboxyterminal protein fragments, which are probably produced by proteolysis.

These observations suggest two things. First, a reasonable percentage of the clones that contain an authentic insert can be expected to produce an immunologically detectable protein in *E. coli*; second, at least for tropomyosin, certain "domains" exist that are reasonably stable in *E. coli*. Since the cloning protocol in effect scans through the gene, the immunological screening procedure has a diverse set of clones to search through for those that produce proteins that are relatively stable in *E. coli*.

The smooth-muscle α-tropomyosin sequence. Two of the cDNA clones contained the entire α-tropomyosin-coding region; one of these clones was sequenced. Despite the fact that the clone clearly selects the RNA for α-tropomyosin in hybridization/translation experiments, the sequence is much closer to the rabbit skeletal-muscle β-tropomyosin than to rabbit skeletal-muscle α-tropomyosin. However, the α-β designation is based on the position of migration of SDS-polyacrylamide gels, and since all three proteins contain 284 amino acids, the α-β designation may be somewhat arbitrary.

What is more interesting is that the major differences between the chicken smooth-muscle α-tropomyosin and the rabbit skeletal-muscle α- and β-tropomyosins are in the carboxyterminal portion of the proteins. Skeletal-muscle tropomyosins interact with troponin in vivo; nonmuscle tropomyosins apparently do not. The site of interaction with troponin is in the carboxyl terminus of the skeletal-muscle tropomyosins.

What reinforces the interpretation that these differences have functional significance is the observation that the carboxyterminal portion of the chicken smooth-muscle α-tropomyosin is closer in sequence to equine platelet tropomyosin than it is to either of the skeletal-muscle tropomyosins.

Elevation of Tubulin Levels by Microinjection Suppresses New Tubulin Synthesis

J.R. Feramisco [in collaboration with D.W. Cleveland and M.F. Pittenger, Johns Hopkins University School of Medicine]

Most eukaryotic cells rapidly and specifically depress synthesis of α- and β-tubulin polypeptides in response to microtubule inhibitors that cause microtubule depolymerization and presumably increase the intracellular concentration of free subunits. Other drugs that interfere with microtubule function but lead to a decrease in the subunit pool size have little effect on the rate of new tubulin synthesis. These findings have previously been interpreted to indicate that cultured cells synthesize tubulin constitutively unless the subunit pool rises above a specified level. At this point an autoregulatory control mechanism is triggered that suppresses new tubulin synthesis through specific loss of tubulin mRNAs. That tubulin RNA levels are dramatically lowered by microtubule depolymerizing drugs is unquestionably correct; that fluctuations in the depolymerized tubulin pool size are responsible for altered RNA levels rests, however, entirely on the presumptive effects of different microtubule drugs. This caveat is not trivial, as these drugs induce gross morphological alterations, and the specificities and detailed mechanisms of action of such drugs remain poorly understood. To investigate the effect of altered levels of tubulin subunits on the rate of new tubulin synthesis in mammalian cells, we have microinjected purified tubulin subunits into cells in culture and analyzed the synthesized proteins. We have found that tubulin synthesis is rapidly and specifically suppressed by injection of an amount of tubulin roughly equivalent to 25–50% of the amount initially present in the cell, thus indicating the presence of a eukaryotic, autoregulatory control mechanism that specifies tubulin content in a cultured mammalian cell line.

Role of the Cytoskeleton in Gene Expression Induced by Heat Shock

W.J. Welch, S.H. Blose, J.R. Feramisco

Although there has been considerable interest and speculation concerning the function of the cytoskeleton in gene expression and signal transduction into the nucleus, few direct tests of these possible cytoskeletal functions have been made. We have used the gene-expression changes induced by heat shock as one model to examine the necessity (or lack thereof) of cytoskeletal integrity for appropriate gene switching to occur. As heat shock induces the synthesis of several discrete mRNAs, which are translated into readily identifiable protein products, and induces the translocation of one of the heat-shock proteins into the nucleus and nucleoli, several different types of effects of the heat-shock response were tested in these studies. The drugs cytochalasin D and Colcemid were used to disassemble the actin microfilaments or microtubules and to disrupt the normal, splayed intermediate-filament structures in living fibroblasts. Combinations of the drugs, coupled with variations in the time of treatment of the cells with the drugs, allowed for the individual disruption of the three cytoskeleton networks, as well as for the disruption of the three simultaneously. The cells were subsequently heat-shocked and analyzed for the induction of the heat-

shock proteins (in quantity, quality, and kinetics) and for the migration of hsp72 into the nucleus. The results of these analyses indicated that none of the cytoskeleton systems were required for the heat-shock response, nor for the migration of hsp72 into its appropriate locale in the cell. These results, although very much limited at the moment to the heat-shock system, pose several interesting questions concerning the importance of the cytoskeleton in cellular events other than cell motility, shape, and adhesion; we are currently examining other gene-switching systems in this regard.

Toward the Intracellular Localization and Function of Protein Kinase C

K. Matlack, J.R. Feramisco

The response of cells to external stimuli may sometimes involve the stimulation of intracellular protein kinases. The last several years have seen the discovery and characterization of a new type of widely distributed protein kinase whose properties suggest that it may play a role in signal transduction into the cell. This kinase, called protein kinase C and discovered and first purified by Y. Nishizuka and co-workers (Kobe University of Medicine, Japan), is dependent on divalent calcium ion and phospholipid and is stimulated by diacylglycerol. Diacylglycerol is not a common constituent of eukaryotic membranes. It is present in them only transiently and is the result of the breakdown of inositol phospholipids. Inositol phospholipids are broken down in response to external stimuli, which can produce either short-term responses or long-term proliferative effects in the target cells. That this protein kinase is stimulated by a compound intimately involved in signal transduction suggests that the kinase may be involved either in mediating the early phases of the cellular response or in modulating it. The evidence for its mediation of the response of platelets to thrombin is good. The recent discovery of protein kinase C as a receptor for tumor-promoting phorbol esters also suggests that it may play an important role in the coordination of cellular activity.

To date, the biochemistry of protein kinase C leaves unclear how it may function within the cell. The same molecular species can apparently be isolated from both the particulate and soluble fractions of rat brain, yet detergent is required for the isolation from the particulate fraction. Treatment of intact cells with tumor-promoting phorbol esters results in an increase of the fraction associated with the plasma membrane. In extracts, this kinase is capable of phosphorylating perhaps 30 or more proteins. Highly purified protein kinase C can phosphorylate several purified proteins at some of the same sites at which they are found phosphorylated in vivo. These proteins include vinculin, myosin light chain, and the EGF receptor. The myosin light chain is not known to be closely associated with membranes.

To clarify the role of protein kinase C, and of protein kinases in general, in regulating cellular responses, we would like to determine its distribution in vivo and to develop a means of specifically inhibiting its activity in living cells. We have therefore purified protein kinase C and are trying to produce monoclonal antibodies that inhibit the activity of the kinase; these will then be used for microinjection. By doing this, we hope to be able to implicate the kinase directly in a number of cellular responses and to begin to get an idea of the part it may play in cellular responses to external signals.

Detection of Hemopexin (a Heme-binding Plasma β-Glycoprotein) in the Lysosomes of Cultured Cells Using a Monoclonal Antibody Against Hemopexin

G.-Y. Cai, J. Suhan, G.A. Blose, S.H. Blose

A mouse monoclonal antibody, SBV22 (IgG1), was generated against bovine hemopexin, a heme-binding plasma glycoprotein made by the liver. Western immunoblot (Fig. 5) analysis demonstrated that SBV22 recognized the hemopexins found in a variety of mammalian sera, including human serum. A variety of cultured cells grown in calf serum and then stained with SBV22 revealed intensely fluorescent perinuclear granules. These granules were coincident with structures that specifically concentrated the supravital dye acridine orange, indicating that hemopexin was concentrated in lysosomes (Fig. 6). Immunoelectron microscopy using SBV22 confirmed that the hemopexin was contained in the lysosomes of the perinuclear region as well as the peripheral cytoplasm (Fig. 7). The lysosomal accumulation of hemopexin could be enhanced by incubating the cells with chloroquine, a lysosomal trophic drug. This drug inhibited lysosomal degradation of hemopexin, causing the observed hemopexin accumulation in lysosomes. When the cells were fed fetal calf serum (10% v/v), no hemopexin staining was observed, consistent with the fact that fetal serum has only trace amounts of hemopexin. These results indicate that the cells in culture can specifically ingest hemopexin from the ambient media for lysosome-mediated degradation and is the first demonstration that hemopexin is taken up by nonliver cells. This might in part serve as a source for intracellular iron. Current studies are directed at determining whether hemopexin uptake is facilitated by receptor-mediated endocytosis.

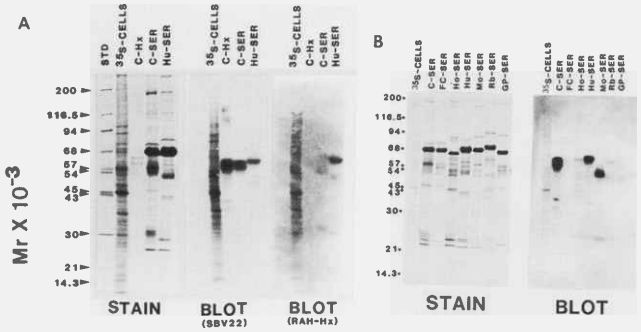


Figure 5

Western immunoblots analyzing the specificity of SBV22 monoclonal antibody (IgG1), an antihemopexin. (*A*) Comparison of the binding of SBV22 and a polyclonal rabbit anti-human hemopexin (RAH-Hx) (from Calbiochem-Behring Corp.) to partially purified calf serum hemopexin (C-Hx), calf serum (C-SER), and human serum (Hu-SER). SBV22 recognized calf hemopexin as a doublet ($\sim M_r$ = 60,000 and 62,000) in the partially purified protein (C-Hx) and calf serum (C-SER) and as a single band ($\sim M_r$ = 63,000) in human serum. The polyclonal anti-human hemopexin (RAH-Hx) on Western blots only detected hemopexin in human serum (Hu-SER). (*B*) SBV22 was used to probe sera from various mammalian species. SBV22 bound strongly to hemopexin in C-SER (M_r = 60,000 and 62,000), Hu-SER (M_r = 63,000), and mouse serum (Mo-SER, M_r = 54,000). It bound weakly to hemopexin in horse serum (Ho-SER, M_r = 62,800) and rabbit serum (Rb-SER, M_r = 61,000). It did not detect the protein in fetal calf serum (Fc-SER) or guinea pig serum (Gp-SER). (STD) Molecular mass standards; (^{35}S-CELLS) [^{35}S]methionine-labeled cell protein for internal M_r standard that is transferred to the blot (BLOT) and exposes the autoradiogram.

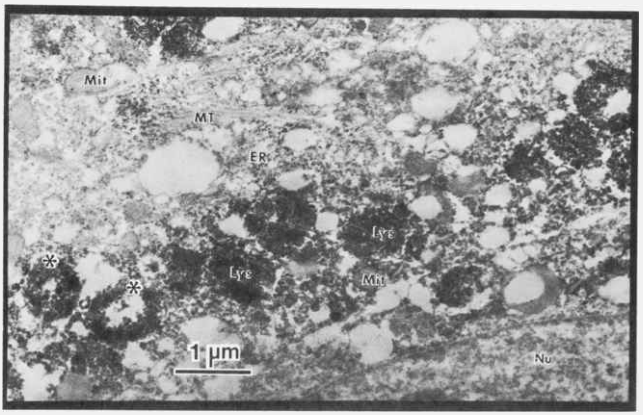
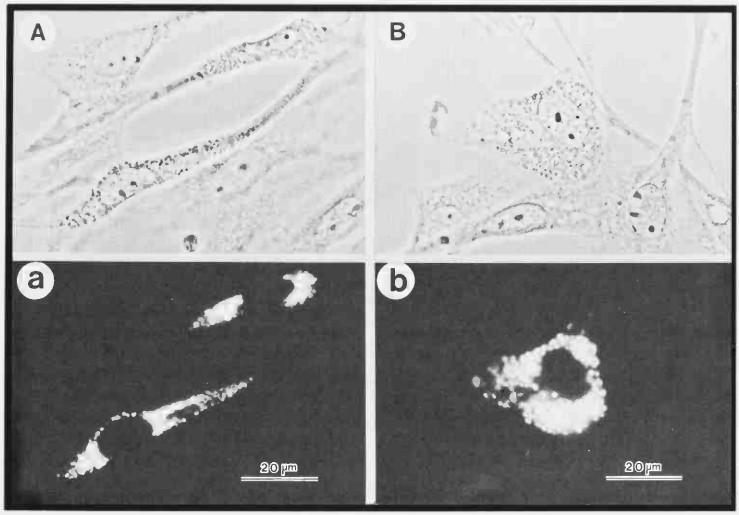


Figure 7

Immunoelectron micrograph of a BHK-21 cell stained with SBV22 followed by peroxidase-labeled goat anti-mouse. The lysosomes (Lys) were stained intensely by the peroxidase reaction product, thus confirming at the ultrastructural level that hemopexin was in the lysosomes. Asterisks mark lysosomes in which the centers were open, similar to the observations made at the light level by immunofluorescence. (Nu) Nucleus; (Mit) mitochondria; (MT) microtubules; (ER) endoplasmic reticulum.



LYSOSOME STAINING OF BHK-21 CELLS WITH SBV22(A,a) AND RAH-HPXN(B,b)

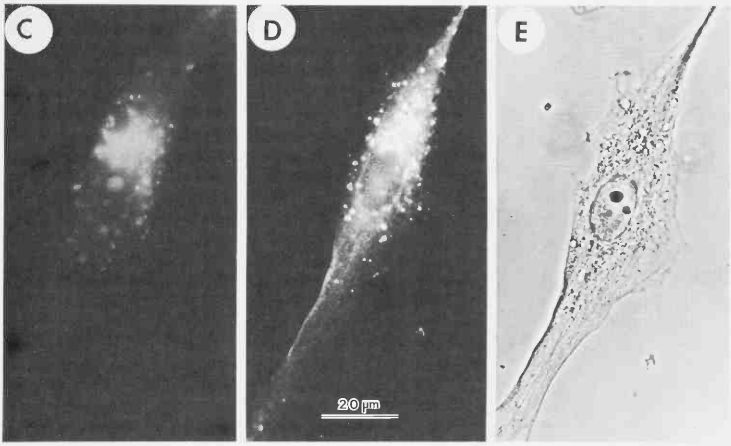


Figure 6

Phase (*A,B,E*) and fluorescent (*a,b,C,D*) micrographs of BHK-21 cells used to detect the intracellular distribution of hemopexin. BHK-21 cells grown in nutrient media supplemented with calf serum (containing hemopexin) for 3 days show that approximately 30–40% of the cells at any one time contain hemopexin as detected by indirect immunofluorescence using the monoclonal antibody SBV22 (*a*) or the rabbit polyclonal anti-hemopexin (RAH-HPXN) (*b*). These antibodies intensely stained granules in the perinuclear region. The individual granules had an intensely stained outer region with a hole in the center. Cells supravitally stained with acridine orange (*C*) were then stained with SBV22 (*D*). The antibody staining was coincident with acridine-orange-positive granules, indicating that hemopexin is in lysosomes. (*E*) Phase micrograph of the same cell in *C* and *D*.

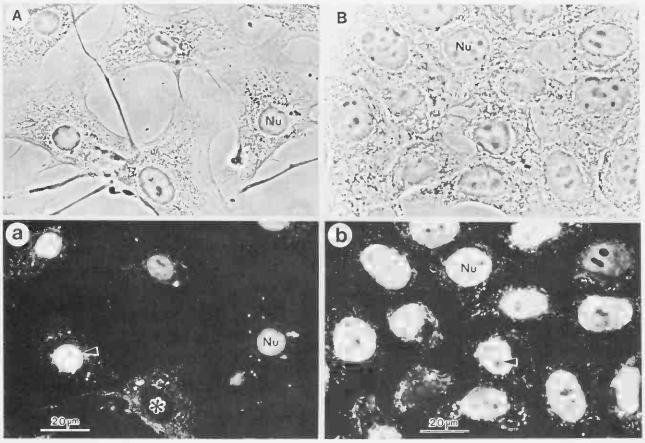


Figure 8
Phase (*A*,*B*) and fluorescence (*a*,*b*) micrographs of gerbil fibroma cells (*A*,*a*) and HeLa cells (*B*,*b*) stained with SBC1. Some nuclei were stained intensely (arrowhead, *a*) and others were not stained (asterisk, *a*). Nucleoli were not stained (arrowhead, *b*) by SBC1. Fluorescent dots in the cytoplasm are the mitochondria stained by SBC1. (Nu) Nucleus.

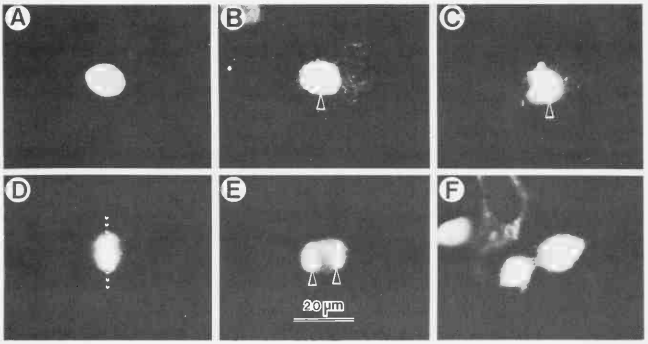


Figure 9
Fluorescent micrographs of gerbil fibroma cells at various stages of mitosis stained with SBC1. (*A*) Early prophase; (*B*) prophase; (*C*) prometaphase; (D) metaphase (dotted line indicates the equatorial plate); (*E*) anaphase; (*F*) telophase. Arrowheads indicate the positions of the chromosomes.

Monoclonal Antibody to DNA

G.-Y. Cai, J. Suhan, G.A. Blose, S.H. Blose

Immunological approaches developed recently in several laboratories have been used to probe structural and conformational domains of DNA in vitro. We have cloned a mouse monoclonal antibody SBC1 (IgM) that binds DNA. When this antibody was used to stain cultured cells by immunofluorescence, three patterns were observed: (1) Only some of the nuclei stained, (2) the mitochondria were stained in a beaded pattern, and (3) the chromosomes were intensely stained. Nuclear fluorescence varied from very intense to absence of staining; and in the case where the nuclei were stained, the nucleoli were unstained (Fig. 8). The intensely stained nuclei appeared to be related to the cell cycle, since prophase nuclei (Fig. 9) were very intensely stained, as were the chromosomes (Fig. 9). Immunoelectron microscopy demonstrated that the antibody stained over both euchromatin and the heterochromatin next to the nuclear envelope (Fig. 10) but not the nucleolus. In mitotic cells, the chromosomes were also stained (Fig. 11). By light immunofluorescence (Fig. 12) and immunoelectron microscopy (Fig. 13), the antibody stained the mitochondria in a beaded pattern. By solid-phase radioimmunoassay, the SBC1 antibody bound nick-translated λ DNA (a gift from O. Fasano, this section) and nick-translated p-alpha 176 DNA (a gift from J. MacInnes, this section). When SBC1 was absorbed with total rat liver DNA (a gift from J. MacInnes), fluorescence staining of the cell's nucleus and mitochondria was abolished. When the DNA was first digested with DNase and then used to absorb SBC1, antibody staining was not abolished. When cultured cells were treated with DNase (0.2 mg/ml, 30 min) prior to staining with SBC1, the fluorescent staining of both nuclei and mitochondria was abolished. These results indicate that SBC1 was directed against DNA. Current studies in progress are aimed at determining the specificity of SBC1 binding to DNA structure/conformation and sequence. We are also investigating the possibility that the intense nuclear staining is an indication of DNA synthesis and/or conformational changes that occur in the S and M phases of the cell cycle.

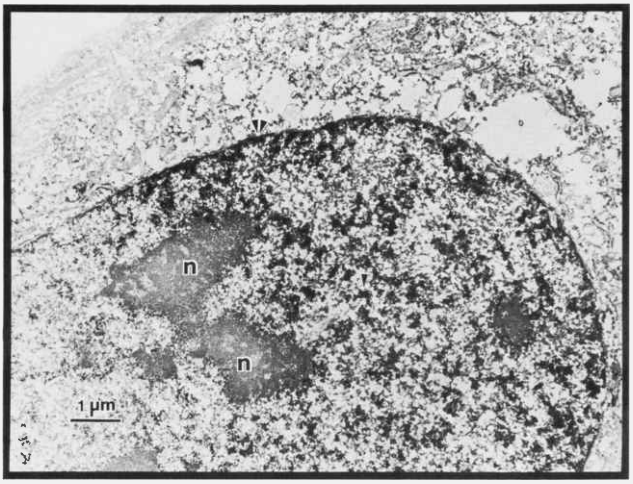


Figure 10
Immunoelectron micrograph of a cell stained by SBC1, followed by peroxidase-labeled goat anti-mouse. The antibody stained the chromatin in the nucleoplasm (single arrowhead) and near the nuclear envelope (double arrowhead). The nucleoli (n) were not stained.

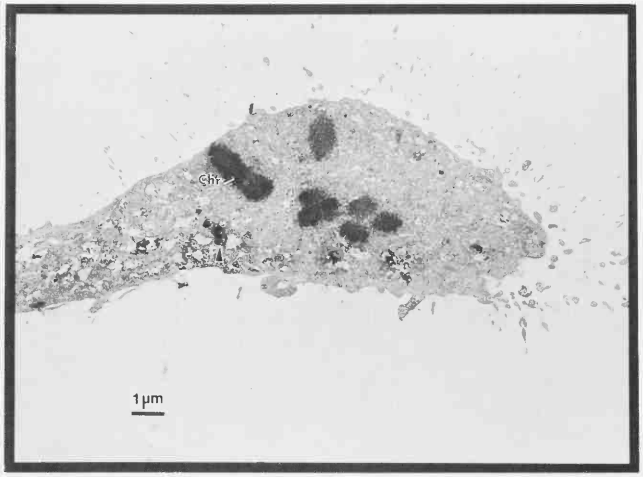


Figure 11
Immunoelectron micrograph of a gerbil fibroma cell in metaphase stained with SBC1, followed by peroxidase-labeled goat anti-mouse. The chromosomes (Chr) were stained. Arrowhead indicates a stained mitochondrion.

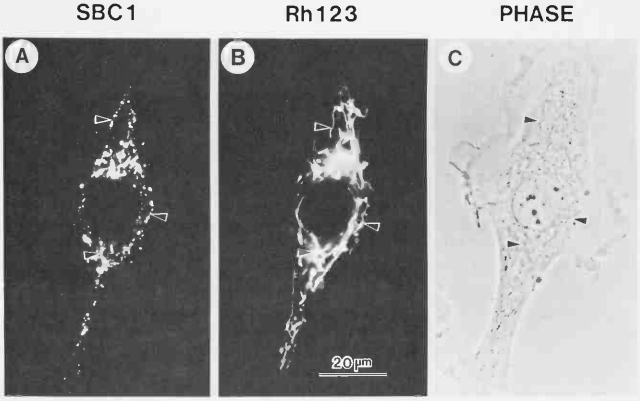


Figure 12
Fluorescent (*A*,*B*) and phase (*C*) micrographs of a gerbil fibroma cell stained supravitally with rhodamine 123 (Rh123) and SBC1. The living cell was seen in *B* and *C* with rhodamine 123 labeling the living mitochondria. This cell was then rapidly fixed and stained with SBC1. (*A*) SBC1 stained the mitochondria in a beaded pattern. Some translocation of the mitochondria was observed when comparing *A* with *B* due to fixation shrinkage and natural movement of the mitochondria during the time it took to fix. Arrowheads indicate mitochondria positions.

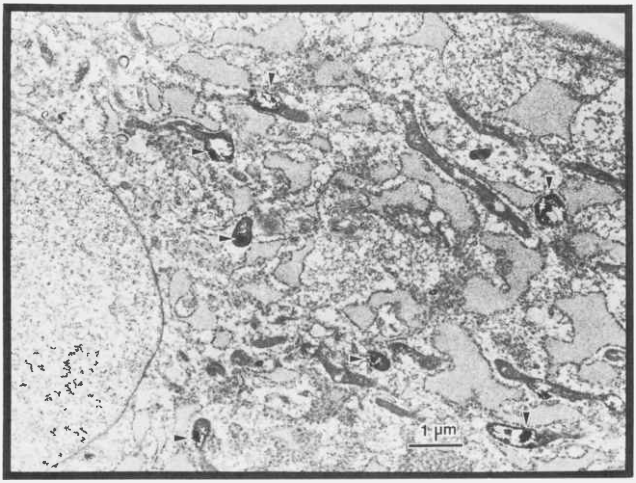


Figure 13
Immunoelectron micrograph of a cell stained with SBC1, followed by peroxidase-labeled goat anti-mouse. The antibody stained discrete regions of the mitochondria (arrowheads).

TRANSPORT AND SECRETION OF EUKARYOTIC GLYCOPROTEINS

During the past year, we have continued our studies on the molecular genetics of membrane and secretory proteins. Our two major interests are (1) to correlate structure with function by identifying and analyzing the protein domains or epitopes involved in receptor recognition, enzyme activity, and antigenicity and (2) to understand the mechanisms that determine the route of transport and final destination of nascent glycoproteins in eukaryotic cells. Most of our work has involved the hemagglutinin of influenza virus, whose biosynthesis utilizes host-cell enzymes and processes for translation, membrane transport, glycosylation, and maturation. The hemagglutinin provides an ideal system for our studies because it is the best characterized of all integral membrane proteins: Its three-dimensional structure is known, and the location of its major antigenic sites, the points at which it is glycosylated, its organization into trimeric structures, and its orientation with respect to the membrane have been defined. More recently, we have begun to study tissue-type plasminogen activator, a serine protease secreted from human cells. The genes encoding both of these glycoproteins have been cloned and expressed in eukaryotic cells using recombinant vectors. This provides the opportunity to study the expression of the wild-type genes in a number of different cell types and to introduce site-directed mutations into the cDNAs and analyze the structure, function, and transport of the altered glycoproteins.

Development and Analysis of Continuous Cell Lines That Express Influenza Hemagglutinin

J. Sambrook, L. Rogers, M.-J. Gething

In recent years, we have used the hemagglutinin (HA) of influenza virus as a model to study the biosynthesis, modification, and transport of cellular, integral membrane proteins. The groundwork for this approach was laid when it was shown that the cloned gene for HA could be expressed with very high efficiency in mammalian cells and that the newly synthesized HA was translocated through the endoplasmic reticulum to the Golgi apparatus and then to the cell surface along a route similar to, if not identical with, that believed to be taken by the majority of authentic cellular membrane proteins (Gething and Sambrook, *Nature 293:* 620 [1981]). Virtually all of this work has been carried out using vectors based on SV40 that express extremely large

quantities of HA during the course of a lytic cycle of viral growth in permissive simian cells. However, it would also be advantageous to study the biosynthesis and expression of HA in mammalian cells that are not destined to die as a consequence of viral infection.

The first continuous cell lines that were developed contained the HA gene integrated into the chromosome of murine LMtk$^-$ or human 143tk$^-$ cells. The HA gene was introduced into these cells in a recombinant plasmid containing (1) the β-lactamase gene, (2) the chicken thymidine kinase (*tk*) gene, and (3) the HA gene inserted between the SV40 early promoter and SV40 early sequences containing RNA processing signals. *tk*$^+$ clones were obtained that expressed up to 10^5 molecules/cell. Evidence that HA is expressed at the surfaces of these cells was provided by H2-restricted lysis of the cells by Japan HA-specific cytotoxic T lymphocytes (CTLs) (Braciale et al., *J. Exp. Med. 159:* 341 [1984]) (in collaboration with T. Braciale, Washington University, St. Louis). CTLs that occur in both mice and humans in response to type-A influenza virus show an unusually high degree of cross-reactivity for target cells infected with serologically distinct influenza virus strains. We have shown that the HA molecule, expressed in the absence of any other influenza virus gene products, can serve as target antigen for both the subtype-specific and cross-reactive subpopulations of influenza-specific CTLs. Thus, it is possible that HAs of different subtypes of type-A influenza carry a conserved epitope (or epitopes) that is immunodominant for CTL recognition/activation but very poorly recognized by the humoral response. These findings now open the way for mapping specific CTL epitopes on the HA molecule by site-directed mutagenesis.

To obtain cell lines that constitutively express higher levels of HA, murine cells (C127, NIH-3T3, or BALB MME) were transformed with bovine papilloma virus (BPV)-HA vectors in which the HA gene was under the control of either the SV40 early promoter or the murine metallothionein promoter. Transformed cell lines were obtained that contained 50–200 copies of the BPV-HA genome maintained extrachromosomally as episomes. The HA protein is expressed continuously at levels of up to approximately 10^7 molecules/cell per 24 hours. The production of HA was higher when the gene was under the control of the metallothionein promoter than when the SV40 early promoter was utilized. The addition of zinc to the medium resulted in an approximately two- to tenfold increase in expression of HA from the recombinants containing the metallothionein promoter. Cell-surface expression was demonstrated by indirect immunofluorescence and hemadsorption of guinea pig erythrocytes to the cell monolayer. These cell lines combine the advantage of high-level production with the convenience of constitutive and continuous expression.

Signal Sequences Can Be Exchanged between Eukaryotic Glycoproteins

S. Sharma, J. Brandsma, J. Sambrook, M.-J. Gething

The precursors of secretory and integral membrane proteins of eukaryotic cells contain at or near their amino termini a tract of hydrophobic amino acids (the signal peptide) that functions in the translocation of the nascent polypeptide across the lipid bilayer of the endoplasmic reticulum. Analyses of these peptides from a large number of proteins have revealed a remarkable diversity of amino acid sequence, although hydrophobicity is always maintained. The question arises as to whether these signal peptides can be functionally exchanged between different glycoproteins and, furthermore, whether the attachment of a signal peptide to a normally intracellular protein can cause its export from the cytoplasm.

We have constructed chimeric genes that fuse the nucleotide sequence coding for the signal peptides of calf preprochymosin and the *env* glycoprotein of Rous sarcoma virus (in collaboration with E. Hunter, University of Alabama) in front of the sequences coding for the hemagglutinin (HA) glycoprotein of influenza virus. Alternatively, we have placed the sequence coding for the HA signal in front of those coding for the *env* glycoprotein or for SV40 large T antigen. The chimeric genes were inserted into recombinant genomes based on SV40 or adenovirus, and the chimeric proteins were expressed in eukaryotic cells. We have demonstrated that when the signal peptides are exchanged between glycoproteins, the chimeric proteins are efficiently translocated across the endoplasmic reticulum (ER) membrane. Subsequent cleavage of the signal sequence and transport of the mature protein to the cell surface appear to depend on how close to the cleavage site the fusion has been placed and whether the chimeric protein can fold correctly. When the HA signal peptide is attached to large T antigen, the chimeric protein molecules are at least partially translocated across the ER membrane. The signal sequence is cleaved from these molecules, which also undergo glycosylation at a normally cryptic canonical site (Asn_{153}.Arg.Thr).

Recognition Signals for Sorting Surface Proteins

M. Roth, C. Doyle, M.-J. Gething

Eukaryotic cells maintain a complex, directed flow of proteins between their various organelles, including their surface membranes. The mechanisms

by which cells control this intracellular traffic are at present poorly understood. Polarized epithelial cells that maintain two separate apical and basolateral surface domains provide a promising system for the study of cellular sorting mechanisms. These surface domains, each containing distinct complements of proteins, are separated by the *zonula occludentes*, belts of tight junctions between adjacent cells that serve as barriers to the diffusion of macromolecules. Previous work has shown that viral glycoproteins are concentrated to either the apical or basolateral surface domain of such cells (Rodriguez-Boulan and Pendergast, *Cell 20:* 45 [1980]; Roth et al., *Cell 33:* 435 [1983]).

To determine which topological features of proteins are recognized by cellular sorting mechanisms, we are investigating the surface expression of cloned glycoprotein genes in polarized epithelial cells. In the past year, in collaboration with E. Rodriguez-Boulan of Cornell Medical School, we have characterized a polarized cell line that supports the growth of SV40 expression vectors containing a cloned gene for the hemagglutinin (HA) of influenza virus. In this cell line, MA104 rhesus kidney cells, the HA glycoprotein is preferentially expressed at the apical domain. To determine whether features of HA important for sorting reside on the large external domain of the protein or on the transmembrane or cytoplasmic domains, we are investigating the expression of a secreted form of HA in MA104 cells infected with an SV40-HA recombinant virus. We have also constructed several genes coding for chimeric glycoproteins in which the transmembrane and/or cytoplasmic domain of HA has been replaced by that of a different viral glycoprotein. These chimeric molecules appear to retain all of the functional activities of wild-type HA and are efficiently expressed on the cell surface in MA104 cells. We are in the process of quantitating the surface location of the wild-type and chimeric HA molecules using electron microscopy and are developing an in situ radioimmunoassay for differentiating between apical and basal HA expression.

Expression and Intracellular Transport of Mutant Hemagglutinins with Altered, Deleted, or Extended Cytoplasmic Domains

C. Doyle, M. Roth, M.-J. Gething

Many cell-surface glycoproteins contain at their carboxyl termini a short stretch of hydrophilic amino acids that follow a hydrophobic domain spanning the lipid bilayer of the plasma membrane. The carboxyterminal hydrophilic region of the influenza virus hemagglutinin (HA) extends for only ten amino acids, with four of the final five residues being absolutely conserved among HA subtypes. The terminal ten amino acids are likely to be exposed on the cytoplasmic face of cellular membranes during transport of HA to the cell surface. This location could allow for specific interactions between HA and molecules involved in intracellular transport.

To investigate the possibility that the "cytoplasmic tail" contains recognition signals important in its transport through the cell, we have mutated portions of the cloned A/Japan/305/57 HA gene that code for this region of the molecule. Several types of altered HA genes were constructed: (1) a group of mutants in which sequences coding for the carboxyterminal amino acids were changed or deleted, (2) two mutants in which the termination codon was altered and the coding sequences extended for either 16 or 21 codons, and (3) one mutant in which the sequences coding for the cytoplasmic tail of HA is replaced by nucleotides encoding the cytoplasmic tail of the envelope glycoprotein of Rous sarcoma virus. These genes were inserted into SV40 vectors and expressed in CV-1 cells as mutant HA molecules in which the cytoplasmic tail is either altered, truncated, extended, or changed to that of a different viral glycoprotein. The rate of biosynthesis and transport, the cellular localization, and the biological activity of the altered gene products have been analyzed. Several of the mutants displayed abnormalities in the pathway of transport from the endoplasmic reticulum (ER) to the cell surface. The HA mutant whose tail was extended by 16 amino acids is retarded in the ER and does not reach the cell surface. Another mutant whose tail was replaced by 16 nonhomologous amino acids is blocked in transport from the Golgi apparatus to the surface. Other mutants are delayed in reaching the Golgi apparatus after core glycosylation is completed in the ER, although their subsequent transport to the surface appears normal. However, the mutant in which the conserved amino acids at the carboxyl terminus have been altered cannot be distinguished from the wild type in the kinetics or pathway of transport. We conclude that although some changes in the cytoplasmic tail drastically influence cell-surface expression and the rate of glycoprotein assembly and transport, the conservation of wild-type sequences coding for this region of the molecule is not absolutely required for maturation and efficient expression of a biologically active HA on the surfaces of infected cells.

Site-directed Mutagenesis of the Fusion Peptide of Influenza Hemagglutinin

M.-J. Gething, D. York

Enveloped animal viruses enter and infect cells by a process involving fusion of the viral membrane with a cellular membrane. In some cases (e.g., Sendai virus), the fusion event occurs at the plasma membrane; for many other viruses including influ-

enza, entry occurs through the membranes of intracellular vesicles such as endosomes or lysosomes, where the fusion is triggered by the endogenous low pH. Fusion is a function of the viral surface glycoproteins and occurs at a threshold pH that is characteristic of each viral species and strain. Experimentally, this activity can be manifested as cell-cell fusion when monolayers of cells displaying these glycoproteins on their plasma membranes are subjected to transient low pH. We have previously shown, using cells expressing influenza virus hemagglutinin (HA) from a cloned copy of the HA gene inserted into a recombinant SV40 vector, that the HA molecule displays fusion activity in the absence of any other influenza-virus-coded components (White et al., *Nature 300:* 658 [1982]). This fusion activity requires a posttranslational proteolytic cleavage of the HA precursor, HA_0, into the active form of the molecule, HA, which consists of two disulfide-bonded subunits, HA_1 and HA_2. A new amino terminus, which has been implicated in the fusion activity and which becomes exposed at low pH, is generated on the HA_2 subunit. The first ten amino acids of this aminoterminal "fusion peptide" are hydrophobic in nature and are highly conserved between HAs from different influenza virus strains.

To confirm the function of the fusion peptide and to identify the role of individual amino acids in the fusion activity, we have used oligonucleotide-directed, site-specific mutagenesis to alter the nucleotide sequence coding for selected residues at the amino terminus of HA_2. Four mutants have been constructed that contain single base alterations that result in nonconservative amino acid changes in the fusion peptide. These changes have included interrupting the hydrophobic stretch with charged amino acids and extending the hydrophobic sequence to 18 amino acids by replacing a hydrophilic residue. The altered sequences were used to replace wild-type sequences in an SV40-HA recombinant vector, and the mutant HAs were expressed in CV-1 cells. Immunofluorescence, red-cell binding, and cell-fusion assays (in collaboration with J. White, Yale University) have been used to establish the phenotypes of the mutants. So far we have defined two positions at which amino acid replacements can abolish the fusion activity and one position where introduction of a charged residue causes a change in the pH at which fusion occurs.

Expression of Tissue-type Plasminogen Activator Using Recombinant Viral Vectors

M.-J. Gething, D. Hanahan, J. Sambrook

Many normal and abnormal physiological processes requiring extracellular proteolysis are thought to be mediated by plasminogen activators (PAs) that cleave plasminogen to the active protease plasmin. These processes generally involve fibrinolysis, tissue remodeling, and cell migration. Two immunologically and biochemically distinct types of PAs have been identified as urokinase (uPA; found in urine) and tissue PA (tPA; found in tissue extracts). tPA is probably the physiological thrombolytic agent, since it not only binds to and requires fibrin for its activity, but also is released from the vascular wall into the blood by stimuli such as physical exercise, venous occlusion, vasoactive drugs, and a variety of clinical states. Recently, much attention has been focused on the role of PAs in tumor formation and metastasis. Levels of PA activity in numerous transformed cell lines are enhanced, compared with levels in normal-cell counterparts. A recent study suggests that it is the level of tPA rather than uPA that correlates with the malignancy of human myeloid leukemic cells (Wilson et al., *Blood 61:* 568 [1983]).

In collaboration with G. Larsen, R. Kay, R. Hewick, E. Fritch, G. Brown, and R. Kaufmann (Genetics Institute) and D. Rifkin (New York University), we have cloned a cDNA copy of the tPA gene from a human melanoma cell line (Bowes). Determination of its nucleotide sequence of 2350 bp showed that the polypeptide backbone of tPA is 562 amino acids in length, including a hydrophobic signal sequence of 20–23 amino acids and a hydrophilic "pro" sequence of 12–15 amino acids. The mature polypeptide (527 amino acids) has 35 cysteine residues capable of forming disulfide linkages and four potential glycosylation sites. Variations in the structure of the oligosaccharide side chains may account for the two forms of PA that are secreted from cells and that run as a doublet on SDS-PAGE (65K and 63K species).

If the full-length tPA clone were inserted into an SV40 vector between the *Hpa*II and *Bam*HI sites (as used for HA expression), the resulting recombinant genome would be too large to fulfill the size constraint for packaging into SV40 virions. Consequently, a truncated cDNA containing all of the coding sequences but lacking the majority of the 3′-nontranslated sequences was inserted into the SV40 vector, and high-titer recombinant viral stocks were generated as described for SV40-HA vectors (Gething and Sambrook, *Nature 293:* 620 [1981]). At late times after infection with SV40-PA, simian CV-1 cells secrete high levels of tPA into the medium (10 μg/ml of protein; 500 units/ml of fibrinolytic activity). This corresponds to about 50-fold higher levels per cell than are produced in the Bowes melanoma cells from which the cDNA was cloned. Metabolic labeling of infected cells, followed by immunoprecipitation and SDS-PAGE, indicated that the tPA protein produced from the recombinant vector is essentially identical in size to that secreted from Bowes cells. The tPA cDNA has also been

inserted into a bovine papilloma virus vector under the control of the murine metallothionein promoter. Transformation of NIH-3T3 cells with the recombinant genome has yielded cloned lines that secrete high levels of tPA (50 units/ml).

HEAT-SHOCK AND STRESS RESPONSES

A seemingly general and highly conserved response to sudden adverse conditions is the so-called "heat-shock" or "stress" response. It is found throughout nature at all levels and is minimally characterized by the induction of a specific and limited suite of proteins, the "stress proteins" of as yet undefined function. These proteins are related to proteins that are expressed under normal conditions and are by several criteria related among evolutionarily distant organisms; the general features of their induction also appear similar. Interest in the stress response itself and the functions of these proteins has grown quite remarkably, as judged from the number of concerned publications, and work here at Cold Spring Harbor has largely addressed two broad aspects of the stress response displayed by human and mammalian cells: (1) purification and detailed characterization of the stress proteins and (2) analysis of the genes that encode them and the means by which their induction is effected.

Purification and Characterization of the Mammalian Stress Proteins

W.J. Welch, G. Binns, J.R. Feramisco

In our efforts to understand the biology of the stressed cell, we have continued to focus our attention on the structure, function, and intracellular location of the individual stress proteins themselves. As we reported last year, approximately half a dozen polypeptides (M_r = 28,000, 72,000, 73,000, 80,000, 90,000, 100,000, and 110,000) are synthesized at elevated levels in all mammalian cells incubated under certain adverse conditions. Purification of the 72K, 73K, 80K, 90K, and 100K proteins has been accomplished (Welch and Feramisco, *J. Biol. Chem. 257:* 14949 [1982] and unpubl.). Currently, we are in the process of purifying the remaining two proteins, the 28K and 110K species. Using cell-fractionation techniques and metabolic labeling studies, we have found that both the 28K and 110K proteins localize, in part, to the nucleus following stress and are heavily phosphorylated. The 28K stress protein, composed of at least four related isoforms, may prove to be most interesting since its phosphorylation state appears to be affected by a number of other treatments to cells (in collaboration with B.R. Franza, this section). As indicated last year, the major induced mammalian heat-shock protein of 72K localizes to the nucleus and nucleolus. The remaining heat-shock protein to be purified, the 110K protein, similarly localizes to the nucleolus and appears to correspond to the C-23 nucleolar protein described during the past few years by other laboratories. Purification of both the 28K and 110K proteins is being accomplished using a combination of cell fractionation and ion-exchange and gel-filtration chromatography.

We are also continuing our efforts in the production of both polyclonal and monoclonal antibodies against the various stress proteins. As reported last year, polyclonal antibodies specific for the 72K, 80K, 90K, and 100K proteins have been raised and used to determine their intracellular localization in the cell (Welch et al., *J. Biol. Chem. 258:* 7102 [1983]; Welch and Feramisco, *J. Biol. Chem. 259:* 4501 [1984]). More recently, we have been concentrating much effort on the production of monoclonal antibodies against all of the stress proteins. In this context, we have just obtained one monoclonal antibody that appears to be specific for the 73K stress protein. Biochemical fractionation, as well as indirect immunofluorescence analysis, using this antibody has shown that a portion of the 73K protein is associated with cytoskeletal elements, specifically microtubules and/or intermediate filaments (in collaboration with S. Blose, this section).

Our battery of antibodies against the various stress proteins is also proving useful in studies aimed at dissecting the function of the individual stress proteins. We have begun a systematic study in which the various antibodies are introduced into living cells via microinjection, and the effects are assayed both morphologically and biochemically. Using this approach, we have found that introduction into the cell of anti-72K antibodies and subsequent heat treatment of the cells results in accelerated cell death. Hence, although it had been established previously that the stress proteins collectively afford the cell protection during heat-shock treatment, this result is suggestive of at least the 72K protein being directly involved in thermal protection. We are currently examining whether the

anti-72K antibodies injected into the cytoplasm prevent the migration of newly synthesized 72K protein into the nucleus/nucleolus of the heat-treated cells.

Comparison of the Stress Response in Normal and Transformed Cells

W.J. Welch, B.R. Franza, J.R. Feramisco

Although it has long been recognized that transformed cells in vitro, as well as tumorigenic tissue in vivo, show increased thermal sensitivity as compared with normal cells, the biochemical basis for this difference is not understood. Consequently, we have been examining and comparing a number of properties of the stress response in both normal and transformed cells grown in vitro. Whereas the transformed cells do show a typical stress response at the protein synthetic level, there appear to be differences in the location of some of the stress proteins in the transformed cell as compared with their normal cell counterparts. Specifically, we have found that although the major induced 72K stress protein localizes to the nucleus and nucleolus in normal cells, the protein localizes to only the nucleus and not the nucleolus in the heat-treated transformed cells. Similarly, the 110K stress protein, present in the nucleolus of both normal and transformed cells grown at 37°C, shows a diminished nucleolar locale in only the heat-treated transformed cells. In the case of the 72K protein, the failure to localize in the nucleolus of the transformed cell appears to reflect differences in the cells and not in the protein itself. For example, we have purified the 72K protein from transformed HeLa cells (which show no nucleolar distribution of 72K at high temperatures) and conjugated the protein with the fluorescent tag, rhodamine isothiocyanate. The rhodamine-coupled 72K protein was then microinjected back into the cytoplasm of normal rat fibroblasts, SV40-transformed fibroblasts, or HeLa cells, and the cells were heat-treated. Only in the normal cells (and not in the transformed cells) was the 72K protein observed to redistribute into the nucleolus. It would appear then that the state of the cell, be it normal or transformed, somehow exerts an influence on the intracellular distribution of the 72K protein following heat-shock treatment.

Energy Metabolism and the Stress Response

W.J. Welch

Over 20 years ago, Ferruccio Ritossa observed that in addition to heat-shock treatment, a number of agents that interfere with oxidative phosphorylation can similarly induce the heat-shock response. In the ensuing years, a large variety of treatments, many of which affect general energy metabolism, have been shown to be effective inducers of the heat-shock proteins, prompting us and others to refer to the response in more general terms as the "stress response." Consequently, I have begun examining the apparent relationship between cellular metabolism and the ability of cells to undergo and survive heat-shock treatment. In this light, we have shown that the synthesis of three of the stress proteins (80K, 90K, and 100K) can also be affected by altering the extracellular levels of glucose in the culture medium of various mammalian cells (Welch et al., *J. Biol. Chem. 258:* 7102 [1983]). In addition, although many of the oxidative phosphorylation inhibitors apparently induce the stress proteins in excised *Drosophila* salivary glands, these same agents have surprisingly little effect on protein synthesis patterns in mammalian tissue-culture cells. However, a profound effect is observed when the mammalian cells are first treated with these inhibitors and subsequently heat-treated. In this case, the cells' ability to synthesize the stress proteins as well as survive the heat treatment is very much diminished as compared with heat-treated cells not exposed to the drugs. Similarly, glucose deprivation of normal cells followed by heat-shock treatment again results in accelerated cell death. It is also interesting to note that highly glycolytic transformed cells also deplete their extracellular glucose quite rapidly and, as mentioned above, show increased thermal sensitivity. Hence, it appears that by affecting the manner by which cells generate their energy pools, one can similarly affect their ability to respond and survive heat-shock treatment. Although these studies are still at a preliminary stage, it is hoped that this approach may shed light on how the cells recognize the environmental insult at hand and how they subsequently react.

Mechanisms of Translational Control in the Stress Response

G.P. Thomas, D.D. Pascucci, M.B. Mathews

In addition to induced synthesis of the stress proteins, the synthesis of the normal spectrum of cellular polypeptides is markedly depressed, and the two processes, although separable, are part and parcel of the response. For our studies on the mechanisms involved in stress-protein induction, we have used as inducer an amino acid analog that is incorporated into newly made proteins, resulting in aberrant polypeptides. It is easily shown that incorporation is necessary, and we assume that aberrant polypeptides are at least the initial signal for induction in this situation. RNA synthesis is also neces-

sary and there are two requirements: The first, as could be expected, is for the production of high levels of the mRNAs for the stress proteins, and the second is more general in that protein synthesis as a whole requires continuous synthesis of RNA. When mRNA production was examined, it was found that the stress-protein mRNAs are the only species produced shortly after stress is imposed. The patterns of protein synthesis also simplify with time so that the stress proteins become the only proteins made. Such parallelism could be mechanistically based and would result if only newly made mRNAs were translated.

Although the normal mRNAs are not used for protein synthesis in stressed cells, they remain intact and are associated with polysomes, the site of protein synthesis. As RNA does not exist in a naked state within cells, one means by which mRNAs could be distinguished is through protein associations. We have analyzed the distribution of normal and stress-protein mRNAs in normal and stressed cells as a function of their density, a measure of the protein content of structures containing mRNAs. We have found that polysomes of stressed cells contain two classes of mRNA-containing particles: One is the same as the particles found in normal cells, but the second is found only in the polysomes of stressed cells. To date, we have characterized this fraction as follows: (1) It contains a large proportion of the newly synthesized stress-protein mRNA made over a given period; (2) it contains both of the subunits that comprise normal ribosomes, leading us to suspect its functional significance; (3) mRNAs for three major normal cell proteins cannot be detected easily in this polysome class, in contrast to the fraction at normal density; and (4) the novel peak has a greater ratio of protein to RNA than has normal polysomal mRNA. We are tempted to speculate that this second polysome fraction is the active vehicle of protein synthesis in stressed cells, and experiments to prove or disprove such a hypothesis are under way.

Human Stress-response Protein Genes

G.P. Thomas, D.D. Pascucci

For many of the experiments aimed at monitoring transcription and translation of both normal and induced genes, specific probes are essential. We have isolated genomic clones for the two major proteins, 90K and 72K, as well as what appears to be a 72K-related gene that is expressed under normal growth conditions yet is not induced by stress. When these clones are used as probes in genomic DNA blots, there are many more bands than would be expected for a single gene; therefore, we presume that there are families of 90K and 73/72K genes and/or that these hybridizing species represent pseudogenes. On the basis of S_1 protection experiments, the clones in hand represent expressed copies, rather than pseudogenes, although nucleotide sequence determination of the complete genes will presumably be required before we can be sure. Nevertheless, they are of utility as probes in the experiments described above.

The 72K genes are currently being sequenced to discover common and divergent regions and hopefully uncover sequences implicated in inducibility. The availability of the genes, the proteins, and the antibodies directed against the proteins will greatly facilitate direct analyses of the expression of the genes, particularly of autoregulation, for which there is circumstantial evidence. In addition, through comparison with the corresponding stress-protein genes of other organisms that have been sequenced to date, it will provide a measure of the evolutionary conservation of these gene products revealed by immunological cross-reactivity.

ONCOGENES

Structure and Activation of Mammalian *ras* Genes

K. Shimizu, M. Goldfarb, O. Fasano, E. Taparowsky, D. Birnbaum, M. Ruley, M. Wigler

DNA-transfer experiments have led to the observation that many human tumor cells contain genes capable of the tumorigenic transformation of NIH-3T3 cells. Most of these so-called "transforming genes" are members of the *ras*-gene family, a highly conserved group of genes that were first identified by their presence in the Harvey and Kirsten sarcoma viruses. We have cloned these genes from tumor cells and have been studying their nucleotide sequences, their intron/exon structures, and the predicted amino acid sequences of the proteins they encode. Additionally, we have determined the nature of the mutations that activate the transforming potential of the normal cellular genes. Further studies, utilizing techniques of in vitro mutagenesis, have provided information about the functional domains of the *ras* proteins. Finally, we have been analyzing the *ras* proteins synthesized by cells to understand the complex posttranslational processing events accompanying *ras* protein maturation.

Structure and activation of human Ki-ras *genes.* We have completed the cloning of the human Ki-*ras* gene and the sequencing of its coding regions. This large gene encompasses 45 kbp and has the potential to encode two *ras*-gene products, one of 189 amino acids homologous to the viral Ki-*ras* gene, and one of 188 amino acids. These two proteins can be encoded by mRNA that utilizes "exon choice": There are two alternate fourth coding exons. Similarly, the vestige of an "alternate" fourth coding exon is found in the 3′-untranslated sequences of the viral Ki-*ras* gene, suggesting that the rat Ki-*ras* gene also has two fourth coding exons. Except for the alternate fourth coding exon, the Ha-*ras* and the Ki-*ras* genes have a similar intron/exon structure, indicating that they have evolved from a common spliced ancestral gene. Exon-shuffling experiments show that when either alternate fourth coding exon of Ki-*ras* replaces the fourth exon of Ha-*ras*, the resulting chimeric gene is still functional. Chimeric gene constructions also show that the Ki-*ras* gene of Calu-1 lung carcinoma cells is activated by a single point mutation, resulting in the substitution of cysteine for glycine at the 12th amino acid position.

Structure and activation of the human N-ras *gene.* We have now completed the cloning and sequencing of the N-*ras* gene. Like the Ha- and Ki-*ras* genes, a functional protein of 189 amino acids is encoded by four coding exons. This gene has the same exonic structure as the Ha- and Ki-*ras* genes. We have found no evidence for an alternate fourth coding exon. Comparison of the amino acid sequences of the three human *ras* genes reveals a remarkable sequence conservation: All three are identical for the first 86 amino acids. Thereafter, until position 165, they are very similar (>80% homology). After this there is almost complete divergence, in a region we call the variable region, until again there is homology in the carboxyterminal sequence Cys-AAX, where *A* is an aliphatic amino acid and *X* is the terminal amino acid.

Comparison of the N-*ras* genes of SK-N-SH neuroblastoma cells and human placenta indicates that the former contains a mutation that activates the N-*ras* transforming potential. Chimeric gene construction, transformation assays, and nucleic acid sequence analysis reveal that this is due to a single point mutation that results in the substitution of lysine for glutamine at the 61st amino acid position. Position 12 of the transforming and wild-type N-*ras* genes is glycine.

Processing of the ras *proteins.* Analysis of the predicted amino acid structure of the *ras*-gene products suggests that the carboxyterminal variable domain is the candidate determinant for physiologic specificity of the *ras* proteins. However, it was shown several years ago that the *ras* proteins undergo processing in the carboxyterminal two thirds of the molecule. This processing is visualized by a marked increase in the electrophoretic mobility of mature *ras* protein, suggestive of protein cleavage at the carboxyl terminus. This putative cleavage event would remove the variable region, making it unlikely that it could have more than a transient function. We therefore prepared two-dimensional tryptic cleavage maps of the processed and unprocessed Ha-*ras* proteins to resolve this

question. These studies indicate that the processing event is not the result of proteolytic cleavage and that the variable region is present on mature processed *ras* proteins. Work is continuing to determine the nature and location of the processing event. Preliminary data indicate that the same processing event or a similar one occurs when the mammalian Ha-*ras* proteins are expressed in yeast cells (see below).

Activating mutations of the Ha-ras *gene.* All of the activating mutations of the *ras* genes that occur in vivo appear to be point mutations resulting in amino acid substitutions at the 12th or 61st codons. There are at least nine examples of these reported by our laboratory and others. However, they are not necessarily the only mutations that can activate the transforming potential of the *ras* genes. To test this, we randomly mutagenized a wild-type Ha-*ras* gene by the bisulfite method, introducing C→T transitional mutations. By screening the resulting mutants in NIH-3T3 transformation assays and sequencing the transforming mutants, we found that point mutations leading to amino acid substitutions at positions 13, 59, and 63 can also activate the transforming potential of the *ras* genes. Not all mutants are equally effective in transformation. Mutants encoding aspartic acid for glycine at position 13 are perhaps one-fifth as potent as those encoding valine for glycine at position 12. Mutants encoding serine for glycine at position 13 are perhaps 1/100 as potent. Mutants encoding threonine for alanine at position 59 are particularly potent. This is puzzling, since both Ha-*ras* and Ki-*ras* viral genes have at least two transforming mutations: one encoding substitutions at position 12 and one encoding threonine at position 59. Furthermore, it is no longer clear why only position-12 and position-61 mutants are found in vivo.

The clustering of activating mutations has two other important implications. First, these results suggest that activating mutations disrupt some critical *ras* protein function by disrupting protein structure. We have argued that these mutations occur about the guanine-nucleotide-binding site of the *ras* protein. From this, we have further argued that activating mutations alter the conformational changes induced by guanine nucleotide binding. Second, it may now be easier to screen tumor DNAs to determine whether they contain activating mutations using methods of hybridization.

Expression of the Human Ha-*ras* Gene in *Escherichia coli* and Yeast

S. Powers, M. Goldfarb, O. Fasano, M. Wigler

Biochemical study of the *ras* proteins requires a source from which a functional *ras* protein can be purified in abundance. In collaboration with M. Rosenberg, R. Sweet, and M. Gross at Smith, Kline and French Laboratories and J. Feramisco and T. Kamata at Cold Spring Harbor, we have utilized an efficient λ promoter cloned into a pBR322 derivative to express clones of normal and mutant Ha-*ras* cDNAs. *Escherichia coli* harboring these vectors express Ha-*ras* in great abundance. This protein comigrates with the unprocessed Ha-*ras* protein made in animal cells and also efficiently binds guanine nucleotides. Ha-*ras* synthesized and purified from *E. coli* can now be used to study the effects of mutation on *ras* protein structure, to raise monoclonal antibodies, and to study *ras* function by microinjection. Since *ras* made in *E. coli* is unprocessed, we have examined the processing of Ha-*ras* expressed in yeast. In collaboration with J. Broach (SUNY, Stony Brook), we constructed a galactose-inducible expressor of the Ha-*ras* cDNA. *Saccharomyces cerevisiae* containing this vector can be induced to express an Ha-*ras* protein that undergoes a time-dependent processing event similar to, but not identical with, the processing events in animal cells. Thus, yeast expression systems may be used to purify large amounts of the processed Ha-*ras* protein for biochemical studies and also to study the signals for protein processing.

Yeast *ras* Genes

S. Powers, T. Kataoka, O. Fasano, M. Goldfarb, M. Wigler

Since the *ras* genes are so well conserved in evolution, being present even in *Drosophila*, we looked for their existence in *Saccharomyces cerevisiae*, a simple eukaryotic organism that can be subjected to powerful genetic analysis. We have found two homologous genes in yeast by Southern blot analysis, have cloned both from plasmid libraries, and have completed DNA sequence analysis of the coding regions. The predicted amino acid sequence was compared with that of the human *ras* genes, with striking results. Both yeast genes show almost 90% homology with the first 80-amino-acid positions of the mammalian *ras* and 50% homology with the next 80. Although the yeast *ras* genes encode much larger proteins (315 amino acids for yeast *RAS1* and 322 amino acids for *RAS2*), they both terminate with the sequence Cys-AAX, common to mammalian *ras*. Thus, there are clear domains of conservation that we think reflect functional domains of the encoded proteins. Although the two yeast genes are far closer to each other than to the mammalian *ras*, they diverge radically from each other at precisely the point where the mammalian variable region begins. We have hypothesized that the first 80 amino acids comprise the effector domain of the *ras* proteins and that the variable region forms a domain that functions as a physiologic receptor.

The availability of powerful genetic tools in yeast has enabled us to begin a series of experiments probing the function of yeast *ras* genes. This work was carried out with the collaboration of J. Strathern (Yeast Section) and J. Broach (SUNY, Stony Brook). We have found that yeast cells with a deletion of either yeast *RAS1* or *RAS2* are viable; deletion of both genes is lethal. By site-directed mutagenesis, we have introduced the activating valine for glycine substitution in *RAS2* at the position homologous to the mammalian *ras* position 12. Cells containing this mutant have a drastically altered phenotype: They grow poorly on certain media, are flocculant, and do not appear to arrest properly in G_1. Diploids with this mutation do not sporulate. This phenotype is dominant.

These experiments indicate that it will be possible to do detailed genetic studies of the yeast *ras* genes and that they may have cellular functions that are homologous to their mammalian counterparts. In particular, failure to arrest properly in G_1 is a hallmark of transformed animal cells.

Tumorigenicity Assay for Transforming Genes

O. Fasano, D. Birnbaum, C. Birchmeier, L. Edlund, M. Wigler

All of the transforming genes we have detected in tumor-cell DNA using the NIH-3T3 focus assay have been members of the *ras*-gene family. All of these genes have had mutations that introduced structural alterations into the *ras* proteins. We therefore suspected that the NIH-3T3 focus assay was biased in its sensitivity to structurally mutated *ras* genes. In collaboration with J. Fogh (Sloan-Kettering Institute, Rye, New York), we have developed and applied an alternative assay for transforming genes present in NIH-3T3 cells after DNA transfer: tumorigenicity in nude mice. NIH-3T3 cells are cotransfected with a selectable marker in the presence of tumor DNA and then assayed for tumorigenicity. We found that many more DNAs are positive in this assay than in the focus assay. We have identified and cloned three transforming genes in the DNA of the human breast carcinoma cell line, MCF-7, which did not induce transformed foci of NIH-3T3 cells in our hands. One of these genes is N-*ras*, and we find N-*ras* to be amplified in MCF-7 cells. The other two genes have no apparent relationship to previously known viral or cellular oncogenes. We have not yet established whether these human genes are genetically altered in MCF-7. These results raise anew the question of the number of potential oncogenes in our genome and indicate that some (perhaps most) tumor cells may contain a multiplicity of weakly transforming genes.

Cooperation among Oncogenes

D. Birnbaum, L. Edlund, M. Wigler

Recent observations by E. Ruley at Cold Spring Harbor and H. Land and others at the Massachusetts Institute of Technology indicate that certain oncogenes can cooperate in the morphologic and tumorigenic transformation of primary embryo cells. In particular, a mutant *ras* gene appears to be insufficient to induce full transformation but requires in addition the presence either of high-level expression of *myc* or adenoviral E1A or of another suitable viral gene. We have confirmed these observations concerning the cooperation of *myc* and *ras* and, in particular, have shown that high-level expression from a normal human *myc* gene is sufficient to complement a mutant *ras* gene in the tumorigenic transformation of primary rat embryo fibroblasts. As in the work of Land, we observed that mutant *ras* alone is sufficient to induce morphological changes in primary embryo cells, but that such cells have only a very limited life span. Indeed, we observed that primary embryo fibroblasts containing mutated *ras* genes have a greatly reduced life span compared with normal embryo fibroblasts, and we interpret this to mean that, in the absence of high-level expression of *myc* function, mutant *ras* genes are actually toxic to these primary cells. We have also tested the cooperation between *myc* and various other viral oncogenes using the primary rat embryo fibroblast transformation system. So far, we have observed cooperative interactions between *sarc* and *myc* and, surprisingly, none between *myc* and a variety of other tyrosine kinases or between *myc* and *mos*. We also observed a cooperative interaction between *myc* and *fos*, an oncogene encoding a nuclear protein.

Biochemistry of *ras* and *ras*-related Proteins

F. Tamanoi, M. Walsh, M. Rao

Mutations in *ras* genes are known that are responsible for the alteration of a normal protein to a form active in transformation of NIH-3T3 cells. An intriguing question is, what biochemical activities are affected by these mutations? To obtain an answer, it is necessary to carry out a detailed enzymological study, as well as a conformational and crystallographic study, of the protein. One of the most promising ways to obtain pure *ras* protein for such a study is to express the protein in *Escherichia coli*. Therefore, during the past year we have concentrated on expressing *ras* protein in *E. coli* using various expression vectors.

First, we have attempted to express mammalian *ras* protein using the *lac* promoter. Using a unique *Mst*I site five nucleotides upstream of ATG, we inserted the *ras* gene downstream from the *lac* pro-

moter in M13mp10. The recombinant phage directed the production of a 23K fusion protein that was immunoprecipitable with a monoclonal antibody against the mammalian *ras* protein. The level of expression could be increased to 5–10% of the total protein by transferring into a pUC vector. The protein made in *E. coli* is active in binding GDP. Recently, a *ras* expression system that utilizes the λ P_L promoter has been constructed by M. Wigler (this section) in collaboration with M. Rosenberg (Smith, Kline and French Laboratories) and J. Feramisco. This system has the advantage that an intact protein is expressed. Characterization of this intact protein is discussed by J. Feramisco et al. (this section).

Recently, two yeast genes (*RAS1* and *RAS2*) that exhibit remarkable homology with the mammalian *ras* gene have been isolated (see M. Wigler, this section). To investigate the biochemical activity of these gene products, we have started to express the gene products in *E. coli*. The *RAS2* gene contains a unique *Hpa*I site just upstream of ATG that could be used to insert the gene into expression vector pUC. The recombinant plasmid produced two proteins, 46K and 35K, that could be immunoprecipitated by the monoclonal antibody against the mammalian *ras* protein. The relationship between the two proteins is currently being investigated. With the *RAS1* protein, no restriction sites suitable for cloning were found around ATG. Therefore, we have created a *Sph*I restriction site (GCATGC) at the ATG by a single base change. This was possible by cloning the *RAS1* gene into bacteriophage M13 and by using a synthetic oligonucleotide. Using this *Sph*I site, we are inserting the *RAS1* gene into vectors that contain P_R or P_L promoters of bacteriophage λ. Our immediate goal is to see whether yeast *ras* proteins exhibit any GTP-binding activity and, if they do, to compare the activities between the two yeast proteins, as well as to the mammalian protein.

Yeast provides an attractive system to carry out biochemical analyses of *ras* proteins. Powerful yeast genetics could lead to the identification of proteins that interact with the *ras* proteins (see M. Wigler, this section). These interacting proteins could be expressed in *E. coli* to carry out studies on the protein-protein interaction. Mutation of *ras* protein to a form active in transformation might involve alteration of such a protein-protein interaction.

Microinjection of Cloned Genes to Study the Mechanisms of Transformation

B.R. Franza, Jr., H.E. Ruley, J.I. Garrels

We are studying the effects of cloned oncogenes introduced into defined populations of cells utilizing the techniques of microinjection, photomicroscopy, and computer analyzable two-dimensional gel electrophoresis. Initial studies have focused on the cloned Ha-*ras*-1 (pT24) transforming gene from the human bladder carcinoma line T24 (Fasano et al., *J. Mol. Appl. Genet. 2:* 173 [1983]). We found that this gene will not transform REF52 cells (a rat-embryo-derived line; McClure et al., *Cold Spring Harbor Conf. Cell Proliferation 9:* 345 [1982]) unless other genes such as the Ad5 E1A region are cointroduced into the cell either by calcium phosphate transfection or by microinjection. Despite the failure of the T24 gene product to transform, it is abundantly expressed in microinjected REF52 cells. At 72 hours postinjection, the protein product is easily detected on two-dimensional gels (Fig. 1); however, no major alteration of cell morphology is apparent.

Another cloned oncogene is capable of rapid and dramatic morphological transformation of REF52 cells. Less than 24 hours after injection of the cloned *c-sis* gene—cloned from HUT-102 cells, a T-cell lymphoblastoid cell line that produces a human T-cell leukemia virus (Poiesz et al., *Proc. Natl. Acad. Sci. 77:* 7415 [1980]), and kindly provided, as part of an ongoing collaboration, by S. Josephs, F. Wong-Staal, and R. Gallo (National Cancer Institute)—many cells have changed their morphology and their growth rate, leading to formation of a focus within 72 hours of the time the gene was injected (Fig. 2). We are intrigued by the response of REF52 cells to the *c-sis*-gene product given the fact that considerable homology exists between the protein product of *v-sis* (the simian sarcoma virus *onc* gene) and a platelet-derived growth factor (PDGF) (Deuel et al., *Science 221:* 1348 [1983]; Doolittle et al., *Science 221:* 275 [1983]); and yet PDGF will not support growth of normal REF52 cells.

We intend to continue such studies as these and utilize the resolution capacity of the two-dimensional gel and QUEST computer analysis (Garrels et al., in *Two-dimensional Gel Electrophoresis of Proteins*, Academic Press, New York [1984]) of the protein patterns to begin defining the early events that occur in changes of protein synthesis and turnover as cells respond to the products of genes known either to transform or to assist in the transformation process.

Production of Monoclonal Antibodies to the *ras* Proteins

T. Kamata, C. Fraser, G. Binns, K. Ramirez, J.R. Feramisco

In a collaborative effort utilizing the human *c*-Ha-*ras* genes cloned in M. Wigler's laboratory, an efficient protein expression system of M. Rosenberg and colleagues (at Smith, Kline and Beckman Laboratories), and the combined use of ours and Smith, Kline and Beckman's protein-purification techniques and facilities, we have obtained homoge-

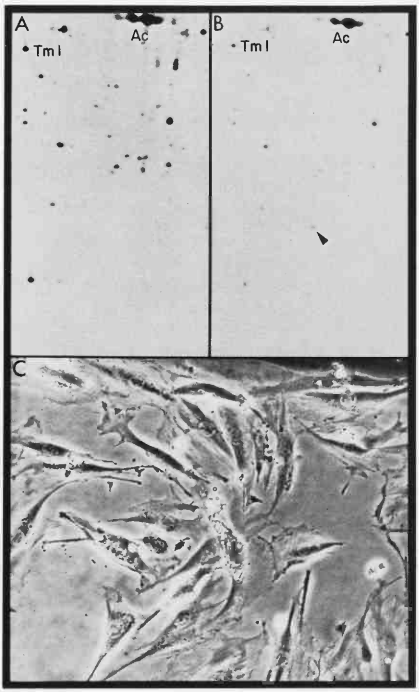


Figure 1
Expression of pT24 gene in REF52 cells. Subconfluent REF52 cells were labeled for 2 hr with [^{35}S]methionine, 72 hr after introduction of the pT24 gene by microinjection. (*A*) Proteins resolved from control, noninjected cells; (*B*) proteins resolved from cells injected with pT24; (*C*) phase photomicrograph of pT24-injected cells just prior to preparation for two-dimensional gel analysis. (Ac) Actin; (Tm1) tropomyosin 1. Arrow indicates the synthesis of the T24 gene product by the injected cells.

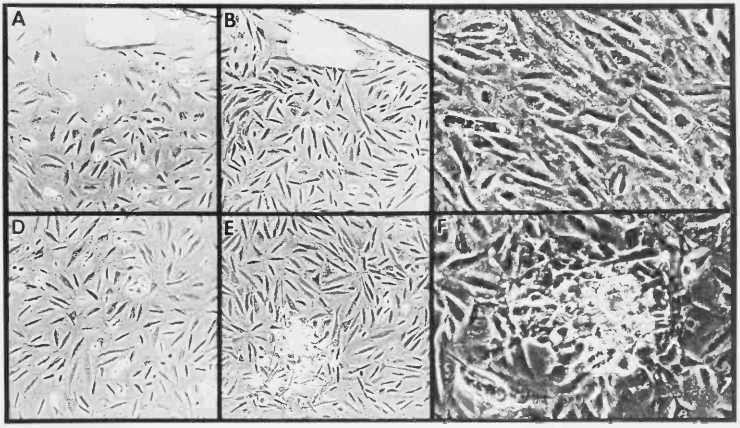


Figure 2
Morphological transformation of REF52 by the *c-sis* oncogene. Confluent (stringent condition for inducing transformation) REF52 cells were injected with the plasmid containing the *sis* gene (pSM1) and photographed at 24 hr and 72 hr after microinjection. (*A–C*) Noninjected controls for each time point. Cells microinjected with the vector minus the *c-sis*-gene insert are indistinguishable from noninjected cells at each time point. (*D–F*) Phase photomicrographs of the pSM1-injected cells at 0, 24, and 72 hr, respectively. Note that *C* and *F* are photographed at higher magnification.

neous, biochemically active preparations of several protein forms of the human *ras* proto-oncogenes and oncogenes, including the "activated" *c*-Ha-*ras* product, the "wild-type" *c*-Ha-*ras* product, and a carboxyterminal truncate of the activated *c*-Ha-*ras* protein lacking 19 amino acids.

Using in the first case a readily obtainable aminoterminal truncate (−20 amino acids) as an antigen, we have prepared several hundred mouse hybridoma cell lines secreting antibodies that recognize the antigen. Of these, approximately seven have been cloned through soft agar to homogeneity. At least five of these cloned lines produce IgGs that are capable of immunoprecipitating the *ras* protein in the presence or absence of denaturants (SDS) and are being characterized as to their relative specificities toward the other protein members of the *ras* family and to their effects on the guanine-nucleotide-binding reaction characteristic of the *ras* proteins.

In addition, we produced two 18-residue synthetic peptides corresponding to the two aminoterminal sequences that differ by one residue between the activated and proto-oncogenic forms of the human Ha-*ras* proteins. Smith, Kline and Beckman have provided us with a 22-residue peptide that corresponds to the carboxyl terminus of the human *c*-Ha-*ras* proteins. All of these peptides will be used as antigens and, with luck, might provide us with some very useful antibodies.

Microinjection of the Purified Human Forms of the Activated and Normal Cellular *ras* Proteins into Living Cells

J.R. Feramisco, T. Kamata, G. Binns

As mentioned above, we have made homogeneous preparations of the full-length forms of the human

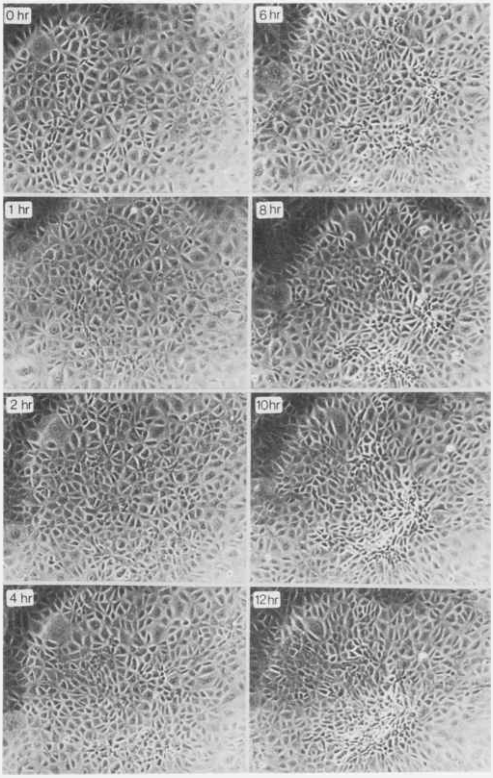


Figure 3
Approximately 30 NRK cells in the center of the field were each injected with approximately 10^6 molecules of the human activated Ha-*ras* protein (full length) purified after expression in *E. coli*. Photographs of the area were taken right after injection (0 hr) and as indicated in the frames.

Ha-*ras* proteins (both the wild-type or proto-oncogenic form and the activated or oncogenic form), as well as an activated protein lacking the carboxy-terminal 20 or 50 amino acids. All of these proteins seem to be "native" and are capable of binding guanine nucleotides (to at least the 30% level) by several different types of assays. M. Gross, S. Yokoyama, R. Sweet, M. Rosenberg, and colleagues (Smith, Kline, and Beckman Laboratories) are in the process of quantitating the binding properties of these proteins more exactly.

We have begun studies utilizing these purified proteins and microinjection into living, normal cells in order to gain more insight into the function of the human *ras* oncogenes. The initial results of these experiments show some very promising features. As shown in Figure 3, within several hours after injection of the oncogenic form of the human Ha-*ras* protein into confluent NRK cells, dramatic morphological changes in the cells can be detected. Microinjection of high levels of the normal, proto-oncogenic form of the *ras* protein has a slight effect on the cells, but nothing as obvious as the effect of the activated protein. The carboxyterminal truncate of the activated protein appears to have no effect on the cells. Video time-lapse recording of the cells after microinjection of the activated *ras* protein indicated numerous cell divisions and incredibly active cell movements such as blebbing and ruffling. Other experiments indicate that the effect of the *ras* protein requires entry into the cells, is temporary, does not require the presence of serum, and is inhibited by cycloheximide or actinomycin D. We are in the process of trying to understand this effect in molecular detail.

Is the *ras* Oncogene Protein a Component of the Epidermal Growth Factor Receptor System?

T. Kamata, G. Binns, J.R. Feramisco

A number of human cellular oncogenes have been identified in part by transfection of DNA from tumor-cell lines and tumor tissues into NIH-3T3 cells. Several of these oncogenes have been classified as members of the *ras*-gene family because of their relatedness to the viral oncogenes of Harvey (*v*-Ha-*ras*) or Kirsten (*v*-Ki-*ras*) murine sarcoma virus (Ha-MSV or Ki-MSV). The human genes have been put into at least three classes, *c*-Ha-*ras*, *c*-Ki-*ras*, or *c*-N-*ras*, depending on the degree of homology with *v*-Ha-*ras*, *v*-Ki-*ras* oncogenes, or the human cell line of initial description (i.e., neuroblastoma), respectively. All of the known *c-ras* oncogenes have normal or proto-oncogene forms that have only a single amino acid change from the corresponding activated oncogene forms. In general, the proteins encoded by the *ras*-gene family are approximately 21,000 daltons and are associated with the inner-surface plasma membrane. The only known biochemical property common to all forms of the *ras* proteins is the ability to bind guanine nucleotides. In the particular case of the *v*-Ha-*ras* protein, apparent autophosphorylation on threonine occurs (both in vivo and in vitro). The guanine-nucleotide-binding property is most likely an important property of the *ras* proteins, since in a temperature-sensitive viral mutant of the *v*-Ki-*ras* gene, the virus is temperature sensitive for transformation and the *v*-Ki-*ras* protein is temperature sensitive for the guanine-nucleotide-binding activity. Of the many alterations in the cellular phenotype associated with transformation by the *ras* oncogenes, the one relevant to the present work is the apparent loss of epidermal growth factor (EGF)-binding sites on the surfaces of the transformed cells. This has been postulated to occur as a result of either down-regulation of the receptors or occupancy of the receptors by the alpha-type transforming growth factor (TGFα), which is produced by the transformed cells. Because of the similar cellular location of the *ras* oncogene proteins and the growth factor receptors, and because several other hormone systems utilize guanine-nucleotide-binding proteins as regulatory elements (such as the *G* protein of the adenylate cyclase system), we investigated the possible relationship of *ras* oncogene proteins to the EGF receptors. We have found that the normally low EGF-binding activity of *ras*-transformed cell membranes is apparently stimulated by the addition of guanine nucleotides. By immunoadsorption of the *ras* proteins from the membranes, we found that this effect appears dependent on the presence of the *ras* protein. In addition, we have found that the phosphorylation of the *v*-Ha-*ras* protein and the guanine-nucleotide-binding activity of the *v*- or *c*-Ha-*ras* proteins in membranes isolated from transformed cells are stimulated by the addition of EGF and $MgGTP^{--}$. Taken together, these results suggest a role for the *ras* oncogene protein in the EGF receptor system, perhaps acting in a manner similar to the *G* protein.

TUMOR VIRUSES

PAPILLOMA VIRUSES

Human Papilloma Virus Expression Vectors

L.T. Chow, A.J. Pelletier, S. Cheng, R.L. Galli, T.R. Broker

Human papilloma viruses (HPVs) cannot be propagated in cell cultures. Nor do they generally express mRNA in warts, in organ cultures, or in transformed, transfected, or infected cell cultures in amounts sufficient for recovery and molecular analysis. Accordingly, to determine the structures of the mRNAs, we have adapted expression vectors that variously include transcriptional enhancer sequences, surrogate promoters, the SV40 replication origin (to amplify the DNA and increase gene dosage), or selectable drug resistances for isolation of transformed cells. Determination of the mRNA sequences will be necessary to ascertain how the open reading frames might be used alone or in combination after RNA splicing to encode proteins necessary for cellular transformation, DNA replication, and virion morphogenesis.

Our studies during the past year have focused largely on three types of HPVs. HPV-1 causes plantar warts and exhibits relatively prolific replication and gene expression in highly keratinized tissues. HPV-6 and HPV-11 are (along with HPV-16 and HPV-18) the leading causes of viral venereal diseases (condylomas, dysplasias, and, possibly, some carcinomas), resulting now in more than 1 million medical visits per year in the United States alone. HPV-11 also causes laryngeal papillomatosis.

PAPILLOMA VIRUS EXPRESSION VECTOR

We have assembled a vector that consists of a modified pML2 (pBR322 deleted for the *tet* resistance gene and for the "poison" sequences that inhibit replication in eukaryotes), the SV40 origin of replication and enhancer sequences, and the promoter for the *Drosophila* gene encoding the heat-shock protein (received from H. Pelham, Medical Research Council, Cambridge, England). Into this expression vector, the complete HPV-1 and HPV-6b DNA sequences have been cloned after addition of new restriction sites in the noncoding region upstream of the E region and downstream from the L region. In addition, small deletions have been made (using BAL-31 exonuclease) in the noncoding region of HPV-1 so that the surrogate heat-shock promoter is at different distances from the E-region open reading frames. Part of our anticipation is that possible regulatory sequences in this region that may result in the normally very low levels of HPV transcription might be eliminated. Forty hours after transfection into COS A2 cells and amplification of the plasmid DNAs, the cells were shocked at 43°C. The recovered cytoplasmic mRNAs were analyzed by electron microscopic heteroduplex methods. One of the clones gave about 100-fold more HPV-1-specific mRNA than had been observed previously. Most of the many mRNA species were indeed derived from the *Drosophila* heat-shock promoter. All contained one or more splices. The most abundant species spans the overlapping open translation frames E4 and the carboxyterminal half of E2 in the transformation region (Fig. 1); less-abundant species extend across the L1 region coding for the major capsid protein. Minor species have long leader sequences that span the E6 and E7 regions or long main bodies that extend through the E1 and L2 regions. Other clones were constructed to take advantage of the SV40 early promoter and enhancer. Transfection of COS cells with such clones produced less HPV-specific RNA than clones with the *Drosophila* heat-shock promoter.

CELLULAR TRANSFORMATION

Transformation of mouse C127 cells with clones driven by the Drosophila *heat-shock promoter.* Mouse C127 cells can be morphologically transformed with cloned bovine and HPV DNAs. The transformants contain the input DNA in an episomal state. We have attempted to transform C127 cells with a variety of cloned HPV-1 and HPV-6 DNAs, including those containing the *Drosophila* heat-shock promoter upstream of the E-coding region. Transfected cells were generally kept at 37°C but were heat-shocked at 42–43°C for 1–5 hours

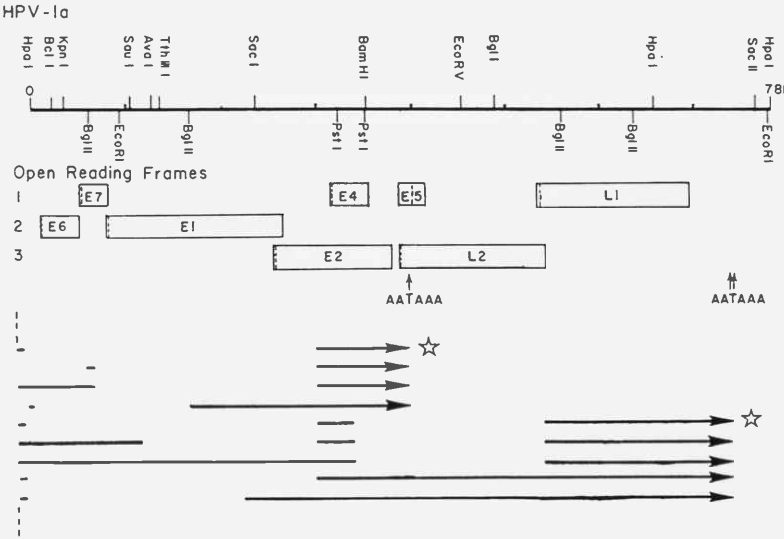


Figure 1

Electron microscopic mapping of RNA transcripts derived from COS cells transfected with a shuttle-expression vector containing the *D. melanogaster* heat-shock promoter, the SV40 replication origin and enhancer region, and a bacterial replication origin and the ampicillin resistance gene. The HPV-1a restriction site and open reading frames are based on the revised DNA sequence of Danos et al. (*EMBO J. 1:* 23 [1982]; see also Schwarz et al., *EMBO J. 2:* 2341 [1982]). The HPV-1 cloning site near nucleotide 7630 is indicated by the dashed vertical line adjacent to the RNA 5′ ends. Gaps in the RNA structures shown are intervening sequences deleted from the messages, the two most common of which are indicated by stars. The 3′ ends are just beyond AATAAA poly(A) signals evident in the sequence. E1 is required from episomal DNA replication, E2/E4 for transformation, and L2 and L1 for virion morphogenesis.

every other day. Morphological transformants were picked after 3 weeks, and these are being grown up for further characterization as to the state of the input DNA.

Transformation of Rat 4 cells. HPV-6b DNA sequences received from L. Gissman (German Cancer Research Center, Heidelberg) were originally represented in two separate *Bam*HI-*Eco*RI fragments. These were reassembled at the *Eco*RI site, and the entire HPV-6b was recloned in pKO-*neo* (constructed by D. Hanahan, Mammalian Cells Section) from which we deleted the "poison" sequences in the pBR322 portion. This recombinant DNA was then transfected into Rat 4 cells, and colonies resistant to the neomycin analog G418 were obtained. DNA was recovered from clonal isolates of the transformed cells and characterized. All clones experienced rearrangements, and the DNA is integrated. This result is different from what was observed with BPV-1 cloned into similar vectors (Matthais et al., *EMBO J. 2:* 1487 [1983]).

HPV-11, a virus related to HPV-6b, is associated with laryngeal papillomatosis. Cloned HPV-11 sequences, also received from Gissman, were removed from the original vector, recloned in a "poison"-minus pKO vector, and then used to transform C127 cells. The DNAs from morphological transformants were characterized by Southern blotting, and the input HPV-11 clone was found to be integrated, similar to the result we obtained with HPV-6b.

PHYSICAL CHARACTERIZATION OF HPV DNAs

Heteroduplexes were prepared between HPV-6b and HPV-11 DNAs, and the samples were analyzed under moderate stringency (30% formamide, 0.1 M salt, 23°C). The DNAs were paired over 90% of their lengths, indicating that the homology is at least 46% in the paired regions. Extensive pairing persists at higher stringencies (60% formamide), indicative of more than 73% homology in those regions. On the basis of these electron microscopic studies, the physical and genetic maps of HPV-6b and HPV-11 have been aligned.

Genomic Organization of HPV-5

S. Watts, L.T. Chow

Patients with epidermodysplasia verruciformis (EV) exhibit a rare familial disease characterized by multiple persistent flat warts. Many distinct types of HPVs have been isolated from these lesions, but thus far only HPV types 5 and 8 have been associated with the progression of sun-exposed lesions to primary and metastatic squamous cell carcinomas. Multiple episomal copies of HPV DNA are present both in the benign warts and in the primary and metastatic carcinomas, although carcinomas do not produce detectable viral particles. We have demonstrated that molecularly cloned HPV-5 DNA is able to induce morphological transformation of cultured mouse C127 cells and to persist as an episome in these transformed cells (S. Watts et al., in prep.). Accordingly, we chose to investigate the functional organization of the HPV-5 genome.

The nucleic acid sequence of HPV-5 DNA has not yet been determined. Therefore, to infer the approximate locations of the functional regions of the HPV-5 genome, we annealed HPV-5 DNA to HPV-1a DNA, for which the complete DNA sequence and the organization of open translation frames are known (Danos et al., *EMBO J. 1:* 231 [1982]), and we examined the heteroduplexes by electron microscopy. Under low-stringency hybridization conditions, partial hybrids were formed between HPV-5 and HPV-1 DNAs in two regions, the major one representing portions of the E1 and E2 open reading frames and another, less homologous region, representing a portion of the L2 open reading frame of the HPV-1 genome. Partial homologies in these particular regions have been seen consistently between many pairs of papilloma virus genomes annealed under conditions of reduced stringency. Therefore, alignment of the HPV-5 DNA homology to HPV-1 DNA allowed us to approximate the positions of the corresponding genetic regions of HPV-5 DNA (Fig. 2A,C).

In addition to wild-type genomes, several sub-

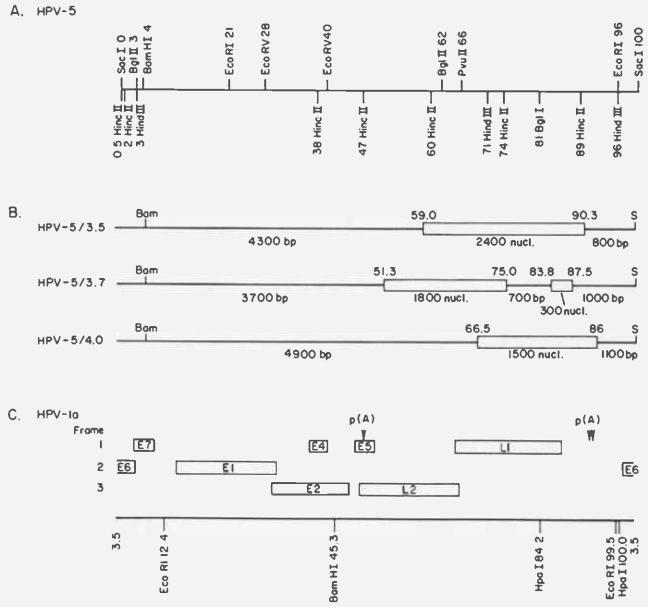


Figure 2

Diagrams of the genomic organizations of HPV-5 DNA and deletion mutants. (*A*) Restriction endonuclease map of HPV-5 DNA. Map positions indicate the percentage of the genome from the unique *Sac*I site. (*B*) Map positions of deletions in the 3.5-, 3.7-, and 4.0-MD subgenomic species of HPV-5 DNA. Map positions of the deletions as determined by electron microscopic heteroduplex formation are indicated by the open boxes. (*C*) Alignment of HPV-1 DNA relative to HPV-5 DNA by electron microscopic heteroduplex mapping. The positions of the HPV-1 open translation frames are based on the revised DNA sequence.

genomic species of episomal HPV-5 DNA have been detected in carcinomas of EV patients by Southern blot hybridization (Ostrow et al., *Proc. Natl. Acad. Sci. 79:* 1634 [1982]), and some have been molecularly cloned in bacteriophage λ. Restriction endonuclease mapping indicated that the sequences deleted in these mutants overlapped a single region of the wild-type genome. As depicted in Figure 2B, we mapped three of these subgenomic DNAs relative to wild-type DNA by electron microscopy of heteroduplexes to show that these DNA species lacked portions of the genomic region associated with virion morphogenesis.

All of the mutants appeared to retain the ability to transform C127 cells in culture. Preliminary results of Southern blots of transformed cells indicate that virus-specific DNA was present in the transformed cells in an episomal state. We have also assayed purified fragments of the HPV-5 genome for their ability to transform C127 cells. A 3.3-MD-fragment *Bam*HI (03)–*Hin*dIII (71) and a 2.9-MD-fragment *Bgl*II (03)–*Bgl*II (62) were able to induce transformation.

Thus, studies with HPV-5 subgenomic species and genomic fragments have delineated the genomic region necessary for in vitro transformation and episomal replication and are consistent with the organization of the genome that we propose as a result of heteroduplex mapping relative to HPV-1 DNA. Through analyses of transcripts and proteins in cells transfected with vectors containing HPV-5 DNA, we intend to help elucidate the requirements for HPV-5 expression and transformation.

ADENOVIRUSES/SV40

Adenovirus Early Gene Regulation

M. Rossini, R. Guilfoyle, W. Osheroff, L.T. Chow, T.R. Broker [Electron Microscopy Section]

During the past year we have continued to study the genome of human adenovirus type 2 (Ad2) as a model for gene regulation. We have concentrated our effort on the regulatory interactions between early regions 1A (E1A) and 2A (E2A). E2A encodes the DNA-binding protein (DBP) necessary for replication. An interesting and unusual property of E2A is that its transcription occurs from different promoters: At early times after infection, RNA synthesis initiates from a promoter located at map coordinate 75 (Berk and Sharp, *Cell 12:* 45 [1977]; Evans et al., *Cell 12:* 733 [1977]; Kitchingman et al., *Proc. Natl. Acad. Sci. 74:* 4392 [1977]) and at late times from a promoter at map coordinate 72 (Chow et al., *J. Mol. Biol. 134:* 265 [1979]). The expression of DBP is under the control of E1A (Berk et al., *Cell 17:* 935 [1979]; Jones and Shenk, *Proc. Natl. Acad. Sci. 76:* 3665 [1979]; Nevins, *Cell 26:* 213 [1981]).

E2A plasmids containing the DBP gene associated with its early or late promoter were microinjected into hamster cell nuclei in the presence or absence of plasmids expressing some or all of the E1A functions, and DBP production was monitored by indirect immunofluorescence. E1A stimulates the production of DBP from the early promoter and represses the synthesis of DBP from its late promoter (Rossini, *Virology 131:* 49 [1983]).

From these findings, we have analyzed which of the E1A products is involved in the stimulatory or inhibitory effects on E2A expression. This investigation has taken advantage of two preexisting and characterized adenovirus E1A mutants: *hr*1, in which a single-base mutation introduces a protein termination codon into the 13S mRNA while leaving the 12S mRNA functional (Ricciardi et al., *Proc. Natl. Acad. Sci. 78:* 6121 [1981]), and pEK*pm*975 (obtained from A. Berk, University of California, Los Angeles), in which a single-base transversion at the donor splice site for the 12S mRNA eliminates its production while the 13S message and its protein product are still made (Montell et al., *Nature 295:* 380 [1982]).

Last year, we reported as preliminary results that the E1A plasmid (pEK*pm*975) generating only the 13S mRNA maintained, in the microinjection system, the ability to stimulate the production of DBP from the early promoter clone but lost the ability to inhibit its expression from the late promoter plasmid. Conversely, the E1A plasmid with an intact 12S mRNA (*hr*1) lost the ability to stimulate the DBP early promoter but maintained the inhibitory effect on the late promoter clone (M. Rossini et al., in prep.). These results have been confirmed by many replicate experiments, and the same results have also been achieved using cDNA clones (constructed by B. Zerler and E. Moran, this section) of the 12S and 13S mRNAs.

To test the assumption that the effect of E1A on E2A synthesis occurs at the transcriptional level, we have constructed deletions at the E2A early and late promoter regions by BAL-31 exonucleolytic digestion to define the DNA sequences responsive to E1A regulation. Microinjection analyses of these deletion mutants have indicated that the sequences between nucleotides 87 and 51 upstream of

the E2A late transcription RNA cap site contain the E1A regulatory interaction site. Surprisingly, deletions in the early promoter region up to +5 (within the normal transcript) fail to abolish the ability of E1A to stimulate DBP production, suggesting a less-specific mechanism of activation of E2A early promoter by E1A.

Recognizing certain limitations of our experimental system based on the immunofluorescent staining of the final product (DBP) of a multistep process, we have initiated experiments leading to a more precise characterization of the regulation. These consist of (1) immunoprecipitation of DBP from microinjected cells for a better quantitation of the stimulation or the inhibition of its production, (2) analysis of RNA from cells transfected with wild types and deletion mutants in the presence or absence of E1A DNA, and (3) analysis of chloramphenicol acetyltransferase (CAT) activity in cells transfected with plasmids containing the E2A early or late promoter upstream of the bacterial *cat* gene, in the presence or absence of wild-type or mutant E1A plasmids.

Analysis of the sequences in the E2A early promoter has revealed the presence of a few stretches of alternating purines and pyrimidines 7 or 8 nucleotides long, apparently sufficient for the DNA conformation to switch to the Z-helix structure. This observation has been the basis to attempt in vivo studies using the microinjection technique to investigate the effect of antibodies against the Z-DNA structure (obtained from A. Rich, Massachusetts Institute of Technology) on the expression of DBP. Preliminary results have indicated that coinjection of anti-Z-DNA antibodies with the DBP plasmid containing both its early and late promoters significantly increases the percentage of cells fluorescent in response to DBP.

We have also examined human cells infected with Ad5*dl*312, a mutant virus in which the E1A region has been completely deleted (Jones and Shenk, *Cell 17:* 683 [1979]) and, accordingly, from which E2A is not expressed. As expected, the cells did not show any fluorescence for DBP. When an E1A plasmid was injected into Ad5*dl*312-infected cells, complementation occurred and the cells showed fluorescence for DBP. Cells infected with Ad5*dl*312 and then microinjected with anti-Z-DNA antibodies also produced DBP.

Although these results are preliminary, several possibilities can be envisioned to explain the mechanism of action of anti-Z-DNA antibodies: (1) A tract of alternating purines and pyrimidines observed in the early promoter for DBP is recognized by the antibodies, which act as Z-DNA-binding proteins. A change from B conformation to Z conformation or, conversely, stabilization of a transitory Z-DNA structure might occur by this interaction, with a subsequent activation of the gene. (2) The binding of antibodies to the early promoter region blocks entry of RNA polymerase II at this site and transcription initiates from the late promoter. (3) The action of anti-Z DNA antibodies could be indirect, through binding to a cellular gene coding for the factor with a repressor function on early gene transcription, as proposed by Nevins (*Cell 26:* 213 [1981]). In any case, the finding that an antibody directed to a specific structure of DNA has an effect on gene expression and can simulate the action of a known protein is of great interest, and additional investigation of the subject should shed more light on the complex problem of gene regulation.

Identification of Adenovirus DNA Replication Proteins

B. Stillman, T. Grodzicker, E. White

The replication of adenovirus DNA in cell-free extracts remains the only such system available in higher cells to study the detailed enzymology of DNA synthesis and its control. Studies from a number of laboratories over the past 5 years have identified and purified five proteins that, when combined, will initiate and complete one round of adenovirus DNA synthesis. Three of these proteins, encoded by the viral genome, are the single-stranded DNA-binding protein (DBP), the precursor terminal protein (pTP), and the adenovirus DNA polymerase (Adpol). The latter two are required for initiation of DNA synthesis at the replication origin (see below), and probably all three are required for elongation of DNA synthesis. Two proteins, encoded by the host cell, are also required to replicate adenovirus DNA in vitro and have been named factor I and factor II. Factor I is a site-specific DBP required for initiation of DNA synthesis and binds to sequences within the adenovirus origin of DNA replication (see below), whereas factor II is required for elongation of DNA synthesis and has been shown to be a type I topoisomerase.

Biochemical characterization in vitro of adenovirus mutants that fail to replicate their DNA in infected cells has proved an invaluable method to determine the role of virus-coded proteins in the replication process. Using such an approach, we had previously identified and characterized a novel DNA polymerase that is encoded by the virus and is defective in the mutant Ad5*ts*149. Another temperature-sensitive mutant Ad2*ts*111, isolated by Martin et al. (*J. Gen. Virol. 41:* 303 [1978]), has an unusual and interesting phenotype in infected HeLa cells; it is unable to replicate its DNA at the nonpermissive temperature, but it also induces degradation of the host cell's chromosomal DNA (D'Halluin et al., *J. Virol. 32:* 61 [1979]).

Replication extracts prepared from the nuclei of cells infected with Ad2*ts*111 were unable to repli-

cate adenovirus DNA at 37°C, but they could replicate DNA at 30°C. Thus, the virus contained a mutation in a gene that encoded a thermolabile protein. The replication defect was shown to be at the stage of elongation of DNA synthesis, since these extracts could form a functional initiation complex at 37°C.

A protein was purified from Ad2 wild-type-infected HeLa cells that could completely complement the Ad2*ts*111 replication defect in vitro and was shown to be the adenovirus DBP. This was an unexpected finding, since a previous report suggested that the mutation in Ad2*ts*111 did not map in the gene encoding DBP (D'Halluin et al., *J. Virol. 41:* 265 [1982]). However, this proved to be incorrect, and we have demonstrated that Ad2*ts*111 contains two mutations, one in the gene encoding DBP and the other in an E1B gene.

The mutation in the DBP gene produces a defective protein that can be distinguished by several criteria from the defective DBP produced in another temperature-sensitive mutant Ad5*ts*125. Unlike the Ad2*ts*111 DBP, which is stable and normally phosphorylated at the nonpermissive temperature, the Ad5*ts*125 DBP is rapidly dephosphorylated and degraded by proteases at the restrictive temperature. Second, the Ad5*ts*125 DBP, produced at the permissive temperature, is very difficult to heat-inactivate in vitro, whereas the Ad2*ts*111 DBP is readily inactivated in vitro. It is most likely that the mutation in the gene encoding the Ad2*ts*111 DBP occurs at an active site in the protein, and we are currently sequencing this gene to determine the change in primary protein structure.

A second mutation found in Ad2*ts*111 is responsible for the degradation of cellular DNA that occurs after infection with this virus. This defect occurs at all temperatures in vivo and has been localized by DNA sequence analysis and marker rescue techniques to the E1B 19K tumor antigen, a protein that is required for adenovirus-induced transformation of cells to tumor equivalents. Biochemical characterization of this protein and the mutant phenotype is described below.

Mutations in a Nuclear-envelope-associated Adenovirus Tumor Antigen Induce Degradation of Cellular DNA

E. White, T. Grodzicker, B. Stillman

As indicated above, we have shown that an adenovirus mutant, Ad2*ts*111, contains two apparently independent mutations that affect viral DNA synthesis and cellular DNA integrity. Upon infection with this mutant, cellular DNA is degraded into low-molecular-weight fragments (~40,000 to 300 bp) that can be visualized by electrophoresis of extrachromosomal DNA through agarose. Wild-type virus does not induce this DNA degradation. The amount of degraded DNA increases with time post-infection and with the multiplicity of infection, and it is not a temperature-sensitive phenotype, unlike the DNA replication defect (see above). The amount of degraded DNA is also increased by preventing viral and cellular DNA synthesis in infected cells, which suggests that cellular DNA synthesis is not required for the degradative process and that an early viral gene is responsible, at least in part, for the defect. This is consistent with our finding that this mutation is localized in the E1B 19K tumor antigen. A recombinant virus that contains the E1B mutation from Ad2*ts*111 and the wild-type DBP gene from Ad5 was obtained. This recombinant, named Ad2*cyt*106, retains the ability to induce cellular DNA degradation and, like its parent Ad2*ts*111, causes enhanced cytopathic effect in the host cell. These phenotypes are similar to those previously observed with some Ad12 mutants (Takemori et al., *Virology 36:* 575 [1968]; Ezoe et al., *J. Virol. 40:* 20 [1981]).

A number of mutants in the adenovirus E1B 19K tumor antigen have been reported, and we have studied three additional mutants, Ad2*lp*3, Ad2*lp*5 (Chinnandurai, *Cell 33:* 759 [1983]), and Ad5*dl*337 (T. Shenk, pers. comm.). All of these mutants cause enhanced cytopathic effect in infected HeLa cells, but only *lp*5 and *dl*337 induce degradation of cellular DNA. Thus, *lp*3 is an example of a cytopathic-effect mutant (*cyt*) that fails to induce degradation of cellular DNA and therefore eliminates the possibility that the DNA degradation is a result of general cell destruction by the virus.

The intracellular localization of the E1B 19K tumor antigen was investigated using antibodies that recognize synthetic peptides contained within either the carboxyterminal or aminoterminal region of the protein (Green et al., *J. Virol. 48:* 604 [1983]). Both antisera stain, by indirect immunofluorescence, the E1B 19K antigen in wild-type-infected HeLa cells. The protein is localized in both a cytoplasmic membrane structure (endoplasmic reticulum) and the nuclear envelope at early to intermediate times postinfection, but late in infection it localizes solely within the nuclear envelope (see Fig. 1). The nuclear-envelope staining was similar to that observed in cells stained with a human autoantibody (McKeon et al., *Proc. Natl. Acad. Sci. 80:* 4374 [1983]) that recognizes lamins A and C, two proteins that form part of the nuclear lamina at the internal surface of the nuclear envelope (see Fig. 1). Upon biochemical fractionation of infected cells, the E1B 19K protein copurifies with the lamin proteins, with 50% of the protein very tightly bound to the lamina and 50% eluting with high-salt treatment.

By indirect immunofluorescence, the E1B 19K protein produced in *lp*3-infected cells was indistinguishable from wild-type protein; however, in the

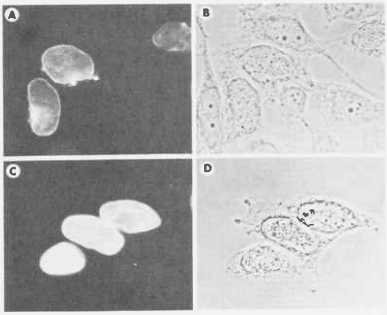


Figure 1
Indirect immunofluorescence (*A* and *C*) of HeLa cells infected with wild-type adenovirus and fixed at 40 hr postinfection. Cells were labeled with antisera against the E1B 19K protein (*A*) or the host-cell lamin protein (*C*). The corresponding phase-contrast photographs are shown in *B* and *D*.

mutants that induce degradation of cellular DNA, the E1B 19K protein had an altered intracellular location (*ts*111, *cyt*106, *lp*5; the protein is not detected in *dl*337-infected cells). These studies show that the degradation of cellular DNA induced by these mutants correlates with the failure of the E1B 19K tumor antigen to localize with the nuclear envelope. However, in mutants that fail to produce the E1B 19K protein (Ad5*dl*337 and Ad5*dl*313), the degradation is still observed. This suggests that it is not the E1B 19K protein that degrades cellular DNA, but that degradation is caused by either a cellular protein or another early adenovirus protein that is activated in the absence of the E1B 19K protein. We are currently searching for this activity in mutant-infected cells.

Adenovirus Mutants That Delay the Onset of DNA Synthesis In Vivo

B. Stillman

In addition to the three virus-coded proteins that are directly required for replication of viral DNA, a number of "early" viral proteins are indirectly required. These include the E1A proteins, which enhance transcription of the early region genes that encode replication proteins, and an E1B gene product. The replication of adenovirus DNA in HeLa cells infected with E1B host-range mutants was examined as part of a study to define the function of E1B gene products. These studies employed two host-range mutants, Ad5*hr*6 and Ad5*hr*7, which were originally isolated by Harrison et al. (*Virology 77:* 319 [1977]) and are capable of growth in 293 cells (a human embryo kidney cell that contains and expresses E1A and E1B gene products) but fail to grow in HeLa cells (which do not contain adenovirus gene products). When HeLa cells were infected at low multiplicities of infection with either mutant, the amount of accumulated intracellular viral DNA at late times in infection was low compared with the amount for similar infections with wild-type virus. At higher multiplicities of infection, the difference between the amount of mutant and wild-type DNAs was not as apparent.

Upon closer examination of the time course of DNA synthesis using one of the host-range mutants, Ad5*hr*7, it was found that the onset of viral DNA synthesis was delayed in infected HeLa cells by approximately 10 hours, relative to the onset of DNA synthesis observed in wild-type-infected HeLa cells. The rate of accumulation of viral DNA was also reduced in Ad5*hr*7-infected HeLa cells. But Ad5*hr*7 synthesizes normal amounts of early viral proteins with no apparent delay in their rate of accumulation (Ross et al., *Virology 103:* 475 [1980]), except for the E1B 57K tumor antigen, which cannot be detected in mutant-infected cells. This might suggest that the E1B 57K protein enhances the efficiency of viral DNA synthesis in an indirect manner. We have produced a number of hybridoma cells that make monoclonal antibodies against the E1B 57K tumor antigen, and these will aid in future biochemical characterization of the role that this protein plays in controlling DNA synthesis.

One intriguing feature of the E1B 57K host-range mutants is that they appear to grow normally in nontransformed, primary human embryo kidney cells (Harrison et al., *Virology 77:* 319 [1977]), which raises the possibility that some human embryonic cells may contain a protein with a function similar to that of the E1B 57K tumor antigen. A number of human cell lines that do not contain adenovirus sequences are currently being examined for their ability to complement the DNA replication defect in Ad5*hr*7.

Characterization of DNA Sequences Required for Adenovirus Replication Origin Function

B. Stillman, F. Tamanoi

We had previously cloned into bacterial plasmid vectors adenovirus DNA sequences that are required for the initiation and elongation of DNA synthesis, using partially purified enzymes prepared from adenovirus-infected HeLa cells. These studies demonstrated that specific sequences were required for the initiation of DNA synthesis in vitro, and recent work has concentrated on defining the precise boundaries of these sequences and their function, particularly their interaction with repli-

cation proteins. Recently, we have also collaborated with R. Guggenheimer and J. Hurwitz (Albert Einstein College of Medicine) and have examined the ability of purified replication proteins to interact with origin sequences and their mutant derivatives, and this has led to the functional origin being divided into two independent domains.

We introduced mutations into the origin sequences by creating deletions that remove sequences from either end of the cloned inverted terminal repeat of adenovirus DNA, a structure that is located at both ends of the linear adenovirus chromosome and contains within its boundaries the replication origin. In addition, site-specific mutations in this sequence were created using synthetic oligonucleotides. The individual mutant DNAs were tested for their ability to act as a template for initiation and elongation of DNA synthesis using both partially purified and purified replication proteins. Furthermore, the mutant DNAs were also examined for their ability to bind to purified factor I, a HeLa cell protein that is a site-specific DNA-binding protein required for efficient initiation of DNA synthesis in vitro. All plasmid DNAs that contained a deletion of sequences that overlap with the factor-I-binding site were not capable of binding to that protein and also were unable to support DNA synthesis. Thus, in the presence of HeLa factor I, the factor-I-binding site is required for efficient DNA synthesis, and we have called this region domain II (see Fig. 2). However, under conditions where factor I is present in suboptimal concentrations, as well as high DNA template concentrations, the factor-I-binding site was no longer necessary for DNA synthesis, but a sequence of only 20 bp (domain I, see Fig. 2) could support initiation in vitro. Domain I contains a 10-bp core sequence (sequence A in Fig. 2) that we had previously shown to be perfectly conserved in all human adenoviruses. The B region in Figure 2 is thought to be a spacer sequence between the core sequence and the 5′-terminal dC residue where initiation takes place.

The two domains of the adenovirus origin are both required for efficient initiation of DNA synthesis. The function of the factor I protein and its specific binding site in adenovirus DNA may shed light on the role that this protein plays in uninfected cells.

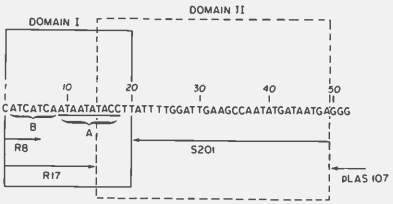


Figure 2
DNA sequences required for the adenovirus origin of DNA replication. The origin has been functionally divided into two domains and a cell protein binds to domain II. (Reprinted, with permission, from Guggenheimer et al., *Proc. Natl. Acad. Sci.* [1984, in press].)

Transcriptional Activation by the SV40 Enhancer: Possible Role for Z-DNA

W. Herr, Y. Gluzman

Enhancer elements were originally discovered as novel elements contained in the SV40 early promoter. They have received considerable attention because of their ability to increase the transcriptional activity in *cis* of a variety of heterologous promoters as well as the bona fide SV40 promoter. Enhancers are unique controlling elements because they are capable of stimulating transcription irrespective of their orientation or position in relation to the affected promoter. The mechanism of transcriptional activation is not yet known.

In a recent study, A. Nordheim and A. Rich (Massachusetts Institute of Technology) identified sequences within the SV40 genome that have the potential to form the Z configuration of DNA (Z-DNA). Z-DNA is a left-handed double helix that occurs in nucleotide sequences of alternating purine and pyrimidine residues and is stabilized in negatively supercoiled, circular DNA molecules, because the B−Z form transition relieves stress created by such supercoils. Using antibodies that bind specifically to Z-DNA, Nordheim and Rich identified two 8-nucleotide-long sequences of alternating purines and pyrimidines that can form Z-DNA in negatively supercoiled SV40 genomic DNA. Because these sequences lie within the enhancer region, Nordheim and Rich suggested that the potential to form Z-DNA may be important for enhancer activity. In support of their hypothesis, they found short stretches of alternating purines and pyrimidines in many other enhancer elements.

This model can be tested by mutating these short sequences within the SV40 enhancer so that the ability to form Z-DNA is either destroyed or maintained and then to determine whether the enhancer activity correlates with the ability to form Z-DNA. Transitions (i.e., pyrimidine to pyrimidine) should not markedly affect the ability to form Z-DNA, whereas transversions (i.e., pyrimidine to purine), which eliminate the alternating pattern of purines and pyrimidines, should both destroy the potential to form Z-DNA and, if the model is correct, diminish enhancer activity. We have tested the model in this fashion by point mutagenesis of the SV40 enhancer region.

We constructed two sets of mutated enhancers by oligonucleotide-directed mutagenesis in which two of the eight nucleotides of the alternating purine and pyrimidine stretches were each altered by either transition or transversion mutations. The potential of the mutated enhancers to activate transcription was measured by analyzing the relative levels of human β-globin transcription in a HeLa cell transient expression assay. We have found that, consistent with the model that Z-DNA is important for enhancer function, the mutants containing transversions exhibit a sevenfold reduction in the ability to activate the β-globin promoter, whereas the transition mutants show a less than twofold effect.

To examine the effects of these mutations on SV40 replication, we reinserted the altered enhancer elements into the SV40 genome and assayed the efficiency of plaque formation after transfection of the DNAs into CV-1 cells. As in the β-globin transcription assay, the wild-type SV40 and transition mutants replicated with similar efficiencies, whereas the mutants containing transversions formed plaques at least 100-fold less frequently.

By passage of the SV40 stock containing the transversion mutations, we have obtained 18 revertants that have an increased ability to grow in CV-1 cells, albeit less efficiently than wild-type SV40. The revertants are the result of tandem duplication of the mutated enhancer region that varies in size from 45 to 135 nucleotides. Although the exact boundaries of the duplicated sequences differ greatly, there is a 15-nucleotide-long sequence in common to all of the duplications, suggesting that this region may also be important for enhancer function. Curiously, this 15-nucleotide stretch contains the sequence GTGGAAAG that G. Khoury and P. Gruss (National Cancer Institute) have suggested is functionally important because the sequence is conserved in a variety of enhancers. We plan to mutate this latter sequence to verify its functional significance.

SV40 Mutants That Differentiate the Lytic and Transforming Functions of Large T Antigen

M. Manos, Y. Gluzman

The large T antigen of SV40 is a multifunctional phosphoprotein. It is essential both in the lytic cycle of the virus and in the process of oncogenic transformation of cells by SV40. T antigen is expressed early in the lytic pathway and functions in numerous processes throughout the infectious cycle. It is required for the initiation of viral DNA replication; it autoregulates early viral RNA synthesis, stimulates late viral transcription, and induces host-cell DNA synthesis and rDNA transcription. T antigen also possesses a "helper function" that enables adenovirus to grow in simian cells. Recent evidence also suggests that the T antigen is involved in the assembly of viral particles. In nonpermissive cells, T antigen is implicated in both the initiation and maintenance of the transformed phenotype. This 96-kD protein specifically binds to the SV40 origin of replication, has an ATPase activity, and is tightly bound to a 53-kD cellular protein in vivo.

Several of these functions can be mapped to specific regions of the SV40 *A* gene encoding large T antigen. This suggests that the T-antigen protein may contain distinct domains that are able to function somewhat independently. Mutants deficient in only a subset of the biochemical and physiological activities of T antigen have been invaluable in mapping activities to different regions of the protein. To investigate the role of the numerous activities of T antigen in cellular transformation and the SV40 lytic cycle, we have utilized early mutants that are deficient in viral DNA replication, but retain transforming activity.

Mutant SV40 DNA insertions have been rescued from a variety of permissive cells transformed by UV-irradiated SV40 (simian cell lines C2, C11, C8, T22, and BSC-SV1). Viral DNA was rescued by fusion of these lines with COS-1 cells, which provide a functional T antigen in *trans* that allows mutant insertions to excise and replicate in the heterokaryons. The rescued SV40 inserts were cloned into the plasmid vector pK1, restoring a complete SV40 genome containing the early region (*Bgl*I-*Bam*HI) from the mutants and a wild-type late region. These DNAs were analyzed by marker rescue and DNA sequencing. The mutants contain lesions in different parts of T antigen that render the protein defective for viral DNA replication but efficient in transforming cells in vitro.

Three mutants were sequenced and found to have mutations predicting amino acid changes at position 516, Lys→Arg (pC2); position 522, Pro→Ser, and position 549, Pro→Arg (pC11); and position 203, His→Gln (pT22). Two mutations of the double mutant (pC11) were separated by subcloning pC11 restriction fragments. Two new mutant DNAs (pC11-A and pC11-B) that contain only one of the two mutations present in pC11 were constructed. pC11-A (position 522) retains the replication-negative phenotype, whereas pC11-B (position 549) replicates at levels lower than those of wild type and forms pinpoint plaques. T antigen isolated from mouse cells transformed by pC11-A is deficient in ATPase activity (no activity was detected in our assays). C2 and C11-B T antigens were positive in ATPase assays.

The tumorigenicity of pC2 and pC11 was investigated by subcutaneous injection of the plasmids into newborn hamsters. Both pC2 and the double-mutant pC11 caused tumors. pC2 appears to have an enhanced tumorigenicity compared with plasmid pK1 containing the wild-type SV40 genome.

Characterization of the Biological Properties of cDNA Encoding SV40 Large T Antigen

Y. Gluzman

Last year, we reported cloning a complete cDNA copy of the SV40 large-T-antigen mRNA and placing it under the control of the bona fide SV40 regulatory region. This early region was reconstructed with the SV40 late region into the complete SV40 genome with the exception that the early region has the cDNA for large T antigen. This DNA could replicate on CV-1 cells with greatly reduced efficiency and produce very small plaques 15–20 days postinfection. Marker-rescue experiments located the deficiency in the cDNA construct to a region that spans only the splicing region. Complementation analysis with temperature-sensitive mutants demonstrated that cDNA SV40 DNA could be complemented by *tsB*, but not *tsA*, mutants. These data suggest that the cDNA construct has a deficiency in the early region, but not in the coding region per se. Preliminary data comparing the amounts of mRNA produced from the cDNA construct and the bona fide early region indicate that there is 3–5 times less mRNA made from the cDNA template.

The SV40 cDNA construct transforms primary baby rat kidney cells and mouse embryo fibroblast cells, although with lower efficiency than wild-type SV40. This reduced efficiency may be linked to the deficiency of producing normal quantities of mRNA or to the lack of small T antigen.

Heterologous Promoter Expression of SV40 T Antigen

R.D. Gerard, Y. Gluzman

COS cells produce a relatively limited amount of SV40 T antigen. For experimental purposes, it would be advantageous to derive a COS-like cell line that produces a higher level of T antigen. It would also be useful to be able to regulate the level of T antigen in these cells. Expression of the mouse metallothionein gene has been shown to be inducible by heavy metals such as cadmium and zinc. We have used the metallothionein promoter to express SV40 T antigen inducibly and to a high level.

A BAL-31 deletion series in the 5′-untranslated sequences of the SV40 early gene has been constructed. These deletions in the leader sequences have been fused to the metallothionein promoter to produce a series of chimeric genes lacking SV40 *ori* sequences and T-antigen autoregulatory sequences.

In transient assays on cells transfected with these constructs, immunofluorescent staining shows that the basal level of expression of T antigen is barely detectable. Induction by heavy metals gives efficient expression of T antigen from all of the hybrid constructs.

These hybrid genes have been used to transform both mouse embryo fibroblasts and monkey kidney cells. Transformants were obtained in the absence of any induction of T-antigen expression by heavy metals. The basal level of expression in these cell lines is variable, as judged from both immunofluorescent staining and immunoprecipitation of T antigen. Most lines increase the level of expression two- to threefold upon heavy-metal induction. Replication of SV40 *ori* sequences in at least some of the transformed monkey cell lines is much greater in the presence of heavy metals.

We have also used a bovine papilloma virus vector containing the metallothionein promoter/T antigen chimeric gene to transform C127 mouse cells and have characterized the subsequent expression of T antigen by these cells. In this system, the metallothionein promoter directs the synthesis of levels of T antigen equal to or greater than that in COS cells and two- to threefold higher upon heavy-metal induction. The T antigen produced by these cells is capable of rescuing SV40 *ori* sequences upon fusion with C6 monkey cells; however, no replication of SV40 *ori* sequences can be detected in the transformed mouse cells when transfected with *ori*-containing plasmids or infected with adenovirus-SV40 hybrids.

Transformation and Viral Tumor Induction

W.C. Topp, M. Dermody-Weisbrod, K. Lionetti, E. Rubin

Over the past several years, our group has been studying the phenomenon of rat breast fibroadenoma induction by certain members of group D of the human adenoviruses. We reported last year that susceptibility to tumor induction appeared to segregate as a single recessive locus linked to the MHC haplotype of the animal. The F_1 cross between a susceptible and a resistant strain gave 100% resistance. We have now completed the N_2 backcross to the susceptible parent and have recovered susceptibility in 17 of 100 animals inoculated at birth with human Ad9. To date, we have determined the MHC haplotype of six tumor-bearing and six nontumor-bearing animals. All six tumor-bearing animals expressed the MHC haplotype of the susceptible parent, whereas four of the six resistant animals were of the haplotype of the F_1 parent. Although this strongly suggests that susceptibility to viral tumor induction is linked to the MHC, we have good evidence that this is not the sole determining factor. A second inbred strain (Albany) of the same MHC haplotype as a known susceptible rat (Buffalo) has

proved resistant to tumor induction. We now plan genetic crosses between the Buffalo and Albany strains to determine the nature of this resistance.

Although they are all very similar to one another physically, only 2 of the more than 20 group-D viruses are tumorigenic. To date, we have tested ten. It is interesting that of these ten, only two are associated with a known disease state in man, keratoconjunctivitis, and the same two viruses are oncogenic. We reported earlier (in collaboration with L. Chow and T. Broker, this section) that a specific gene bank, early gene region 3 (E3), is substantially overexpressed in human Ad9-infected cells as compared with other adenovirus infections. We have now found that in cells infected by two nononcogenic group-D viruses (13 and 26), E3 is not abundantly expressed. This is particularly interesting in light of the fact that E3 encodes at least one cell-surface glycoprotein known to interact with the MHC. Furthermore, keratoconjunctivitis is thought to result from the formation of an antibody/antigen precipitate over the cornea of the eye, and it is intriguing that E3 may encode this precipitating antigen. Preliminary results, however, show that Ad10, also oncogenic, does not noticeably overproduce E3, but we are continuing to evaluate this hypothesis and are repeating a number of these experiments.

Monoclonal Antibodies to SV40 Nucleoprotein Complexes

R.D. Gerard, E. Harlow

During the lytic cycle of SV40 infection, the viral DNA can be recovered from the nucleus as a nucleoprotein complex. These complexes are heterogeneous in nature and include DNA that is being actively replicated and transcribed. Consequently, these complexes should contain a variety of eukaryotic proteins that are involved in replication and transcription. We have begun an analysis of the immunogenic proteins in these complexes.

SV40 nucleoprotein complexes (NPCs) were isolated from virus-infected CV-1 monkey cells and were purified by sucrose gradient centrifugation. After the complexes were fixed by formaldehyde treatment, they were used to immunize a BALB/c mouse. Hybridomas were constructed, and tissue-culture supernatants were screened for antibodies specific for NPCs by indirect immunofluorescence on SV40-infected CV-1 monolayers. Of the 384 cultures examined, 36 hybridomas produced antibodies recognizing antigens present in SV40-infected cells. Four distinct staining patterns were observed: cell surface, diffuse cytoplasmic, cytoplasmic network, and nuclear. Two of these hybridomas produce antibodies specific for the SV40-coded large T antigen, one produces antibodies against the viral VP1 protein, and four others secrete antibodies that recognize intermediate filaments. An eighth hybridoma from this series produces antibodies that precipitate NPCs that have been pulse-labeled with [^{3}H]thymidine. Initial experiments have indicated that this protein is not virus-coded, and we are currently attempting to identify this antigen.

Analysis of the Cellular Tumor Antigen p53

E. Harlow, N. Williamson, T. Adams

Animals bearing tumors induced by a wide variety of agents often produce antibodies specific for a normal cellular protein known as p53. This protein has been implicated in an obligatory role in one of the steps between mitogen stimulation and the initiation of cellular DNA synthesis. Several studies have demonstrated that the regulation of p53 synthesis, phosphorylation, and/or turnover is altered in a number of transformed cells. In SV40- and adenovirus-transformed mouse cells, p53 is found in a stable complex with one of the virus-coded early proteins. In collaboration with S. Benchimol and J. Jenkins (Imperial Cancer Research Fund Laboratories, London), we have isolated a cDNA copy of a portion of the mouse mRNA for p53. Using this cDNA as a hybridization probe, we have determined the levels of p53 mRNA in various tissues and during development. Analysis of mRNA isolated from different stages of embryonic development of the mouse has revealed that the expression of p53 mRNA is drastically reduced during the final stages of embryogenesis. Preliminary evidence suggests that the expression of p53 is also differentially controlled in tissues of the adult mouse. We have used the mouse cDNA clone to identify a human cDNA clone that appears to be a full-length copy of the human p53 mRNA. This cDNA is currently being cloned into several of the well-characterized eukaryotic expression vectors. Introduction of these vectors into the appropriate mammalian cells should allow the production of sufficient quantities of p53 to begin purification and biochemical analyses. Because of the small quantities of p53 found in normal cells and tissues, these experiments have not been possible until now.

Monoclonal Antibodies to the Adenovirus E1A Proteins

E. Harlow, C. Schley

Following infection of mammalian cells with adenovirus, the first virus-coded proteins synthesized

are the products of the E1A region. RNA from this region is differentially spliced to produce two early mRNAs of 12S and 13S. Translation of these RNAs yields two proteins with identical amino and carboxyl termini, but the 13S product has 46 additional amino acids in the central region of this polypeptide. These proteins are phosphorylated and are found primarily in the nucleus. Although their functions are poorly understood at present, these proteins have been implicated in the activation of transcription from other adenovirus early promoters as well as in the production of established cell lines. Very little is known about the biochemical or immunochemical properties of these proteins. To help in the studies of the E1A proteins, we have begun the construction of a library of monoclonal antibodies specific for these polypeptides. A bacterial expression plasmid (kindly supplied by K. Spindler and A. Berk, University of California, Los Angeles) that will direct the synthesis of a fusion protein between the bacterial tryptophan E gene product and a cDNA copy of the 13S mRNA was used as a source to purify an E1A-related protein. This polypeptide was purified by SDS-polyacrylamide electrophoresis, electroeluted, and used to immunize BALB/c mice. Hybridomas were prepared, and tissue-culture supernatants were screened for the production of antibodies that would bind to the fusion protein in a solid substrate radioimmunoassay. These antibodies were detected by their ability to bind to ^{125}I-labeled protein A. Seventy-seven hybridomas secreting antibodies specific for the fusion protein have been isolated and single-cell-cloned. Several of these hybridomas secrete antibodies that will bind to the authentic E1A proteins synthesized in 293 cells, an adenovirus-transformed human cell line. We are currently using these antibodies to immunoaffinity-purify the E1A proteins from infected cells.

Monoclonal Antibodies to the Rabbit Translation Release Factor

E. Harlow

The final step of the synthesis of a polypeptide is the release of the nascent polypeptide from the ribosome. The mechanism of termination and release of the nascent polypeptide is a poorly understood phenomenon, especially in eukaryotic cells. This is primarily because of the lack of drugs that specifically inhibit steps in the termination procedure. It has been demonstrated that termination is controlled by a protein known as the release factor (RF), and this process requires ATP. Using a partially purified preparation of RF obtained from T. Caskey (Baylor College of Medicine), we have produced monoclonal antibodies specific for RF. These antibodies will be used for the study of the termination of translation and also may be useful in the construction of an in vitro system for the production of large polysome complexes. A number of laboratories have demonstrated that rare mRNAs may be simply and efficiently cloned using antibodies to immunoselect mRNAs from a polysome-mRNA-nascent polypeptide complex. We are currently investigating the properties of rabbit reticulocyte lysates depleted in RF with the hope that these systems will allow the generation of large polysomes in vitro. These lysates could then be used as a convenient source of polysomes for mRNA isolation.

Monoclonal Antibodies to SV40 Virions

E. Harlow, M. Wylen

BALB/c mice were immunized with CsCl-purified virions of SV40, and hybridomas were prepared by standard procedures. Tissue-culture supernatants were screened for the production of antibodies specific for the external surface of viral particles. Fifty positive cultures were identified, and five of these were selected for further characterization. These five hybridomas were single-cell-cloned, and both tissue-culture supernatant and ascitic fluid were prepared. Analysis of these antibodies showed that all five were capable of precipitating both the native virions and the precursors of viral 250S particles. Western blotting and immunoprecipitations using proteins prepared from denatured virions have shown that two of the five monoclonal antibodies are specific for the VP1 protein. The specificities of the remaining antibodies have not been determined, but these antibodies are also likely to recognize VP1 but probably will bind to epitopes that are destroyed by denaturation. We are currently testing whether these antibodies can be used to purify SV40 virions on immunoaffinity columns. This would circumvent the tedious process of CsCl centrifugation and may serve as a test system for developing similar technologies for other viruses.

In Vitro Splicing

G.A. Freyer, K. Tohill

Our strategy for examining the mechanism of mRNA splicing in vitro has been to construct a recombinant plasmid that could be used to produce large amounts of a suitable precursor mRNA and then to use that RNA as a substrate in various cell extracts. The construction that was made positioned the λ leftward promoter (λp_L) next to the Ad2 major late transcript in such a way that transcription initiated at the exact nucleotide used in

vivo. Characterization of the in-vitro-synthesized transcript revealed that it does indeed initiate at the correct position. A key feature of this method is that a completely unmodified transcript is produced, and thus any requirements for posttranscriptional modification can be studied. To this end, a second clone, containing a poly(A) stretch adjacent to a unique restriction site, was made to allow a study of the effect of polyadenylation. In either case, the initial transcript can be modified in vitro by the addition of a 5′ cap structure, allowing its significance to be examined.

To date, we have been unsuccessful in demonstrating splicing with the in vitro transcript, irrespective of whether it is unmodified or modified, i.e., capped, polyadenylated, or both capped and polyadenylated. The extracts used have included the one described by Hernandez and Keller (*Cell 35:* 89 [1983]), which has been shown to splice successfully the Ad2 major late transcript prepared in vitro in HeLa cell extracts according to the protocol of Manley (*Proc. Natl. Acad. Sci. 77:* 3855 [1980]). These results suggest that our in vitro transcript is lacking some crucial modification. We are attempting to identify this modification by examining the fate of our transcript during incubation in whole-cell extracts. In addition, we are constructing two new clones that will produce a much simpler transcript (~200 bases) containing a large deletion in the intron.

Finally, experiments are under way to examine the possibility that the introns are removed by a reciprocal mechanism in which the intron sequences themselves become joined to generate circular molecules.

Early Region IV of Adenovirus 2

G.A. Freyer, Y. Katoh

A project aimed at mapping the splice junctions of the early region IV (E4) mRNAs of Ad2 is now complete. This has been accomplished by sequencing cDNA clones corresponding to early mRNA isolated from Ad2-infected HeLa cells. The cDNA libraries were constructed both by conventional cDNA cloning methods and by a novel method developed in this laboratory. The new procedure involved synthesizing a 20-base-long oligomer, whose sequence corresponded to a segment of the leader sequence present on all E4 mRNAs. This oligomer was ligated onto pBR322 to produce a single-strand 3′ extension, which was then used as a primer for second-strand synthesis of the cDNA. This strategy proved to be extremely useful in producing large numbers of E4-specific recombinants, essentially all of which contained the 5′ leader sequence. This new method was devised because of the low number (<5%) of cDNA clones found to contain the 5′ leader sequence, in cDNA libraries made by the conventional procedure.

From the sequence analysis of these cDNA clones, the exact splice sites of all but one of the previously characterized mRNAs was obtained. In addition, five new mRNAs were found that had not been detected by either electron microscopy or S1-nuclease analysis. A large number of cDNAs, containing the large common intron between coordinates 92.4% and 94.4%, were obtained and sequenced. All but one of these clones contained the same donor and acceptor splice sites, with the exception differing in the location of the acceptor splice site. From in vitro translation studies, it had been predicted that a large open reading frame present in this large intron was used to make a variety of E4 proteins. Our data failed to reveal any mRNAs from which this reading frame might be accessed directly. We could, however, tentatively assign predicted polypeptides to the various E4 mRNAs, which should help in future studies on the function of this region.

Adenovirus-2 Genomic Sequence

R.J. Roberts, K. O'Neill

The Ad2 DNA sequence is finally complete as a result of work in this laboratory and in others, most notably the groups of F. Galibert (Hôpital Saint-Louis, Paris), U. Pettersson (University of Uppsala, Sweden), and J. Sussenbach (University of Utrecht, Holland). A contiguous sequence of 35,937 nucleotides can be constructed, although because of sequence heterogeneity both within individual viral stocks and between stocks in different laboratories, the actual length varies ±9 nucleotides. Within this sequence, the coding regions for 25 well-characterized Ad2 proteins and 2 small virus-associated (VA) RNAs can be identified. An additional 30 open reading frames, which might encode polypeptides with molecular weights of 10,000 or greater, are present in the sequence, and it is likely that many of these encode polypeptides that are synthesized during the viral life cycle. One of the most striking features of this sequence is its extraordinarily high information content. There are numerous examples of overlapping genes, both on the same strand and on complementary strands. In general, distances between adjacent coding regions are extremely short, and signals for mRNA initiation, termination, and splicing are frequently found to overlap the coding regions. Thus far, 47 splice sites have been defined within the sequence, reflecting the extreme complexity of RNA processing that takes place during the expression of the viral genome. Current work is focused on discovering the fate of the intron sequences, which in many cases contain open reading frames within them.

Autoantibodies and Ribonucleoprotein Particles

M.B. Mathews, M.R. Sadaie, C. Herrmann

In autoimmune diseases, the organism's tolerance to "self" breaks down and antibodies arise to normal body components. Among the targets of this inappropriate immune response are molecules of intense biological interest, such as DNA, ribonucleoproteins (RNPs), and proteins. In collaboration with R. Bernstein of the Royal Postgraduate Medical School, London, we have screened a large number of patients' sera and measured the disease frequency of more than 20 antibody systems. At the same time, we have examined the molecular nature of the corresponding cellular antigens and have characterized a number of these in considerable detail.

The La antigen, described in previous annual reports, is a 46,000-dalton phosphoprotein that binds many RNA species transcribed by RNA polymerase III, including the adenovirus VA RNAs. Antibodies to this cellular protein are found in rheumatic diseases, such as systemic lupus erythematosus and Sjögrens syndrome, and have proved to be invaluable aids to studying the protein. We have now shown that the protein recognizes the 3′ tail of VA RNA, comprising a run of up to four uridylate residues. When there are three or four residues at the 3′ end, the RNA molecule is efficiently bound by the antigen; molecules with two residues are partially bound, but those with only one terminal uridylate residue fail to bind. We have demonstrated directly that the binding site is the 3′ terminus by cross-linking the RNA to the protein by UV-irradiation and then digesting it with ribonuclease T1. The only portion of the RNA molecule that is not released from the protein is its 3′ end. Thus, the La antigen recognizes and binds to the 3′ uridylate-rich terminus of these molecules. Current data suggest that the antigen is not involved in RNA synthesis or the release of new transcripts from the template, and there is also evidence against involvement in the translational role of VA RNA. We are therefore led by elimination to postulate that La antigen functions in the poorly understood transport or processing of these RNA species.

More definitive assignations have been obtained with autoantibodies found in polymyositis and dermatomyositis. Over one quarter of the patients suffering from these muscle-wasting diseases have antibodies of the Jo-1 type. Again, the antigen is an RNA-binding protein, but in this case, the RNA is a transfer RNA. By assaying for inhibition of amino-acid-charging activity, we have demonstrated that the antigen is the enzyme histidyl-tRNA synthetase, responsible for coupling histidine to its cognate tRNA.

As in the case of the La antigen that can bind the adenovirus VA RNAs, an indirect argument links the Jo-1 antigen with viral RNA. We believe that the viral connection is more than coincidental and may point to an origin for at least some autoimmune diseases in viral infection.

A third antigen recognized by autoantibodies is the proliferating cell nuclear antigen (PCNA). This antigen is characteristically seen in dividing cells, especially tumor cells, but not in resting cells. It is also known to alter its localization within the nucleus during the cell cycle, shuttling between the nucleolus and nucleoplasm. Although this antigen does not appear to be associated directly with RNA, it can be solubilized from the nucleus by DNase digestion, suggesting a possible association with chromatin. We have shown that the antigen is an acidic protein with a molecular weight of 35,000. In collaboration with J. Garrels and R. Franza of this laboratory, we have characterized the protein as a spot on a two-dimensional gel of human proteins and demonstrated its identity with the protein known as "cyclin." The regulation of cyclin has been described in the literature, and its properties mirror those of PCNA as expected. We are currently attempting to exploit the PCNA autoantibodies to isolate the gene for this interesting and potentially important nuclear protein.

Translational Role of VA RNA

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

In addition to some 40 mRNA species, adenovirus encodes two small RNAs known as the VA RNAs. These RNAs, about 160 nucleotides in length, differ from other viral transcripts in being synthesized by RNA polymerase III and in their inability to code for proteins. Previous work from this and other laboratories led to a description of the structure of the VA RNAs, their binding to other cellular components, and the organization of their genes on the chromosome, but their significance remained an enigma until Thimmappaya and his co-workers (*Cell 31:* 543 [1982]) constructed a mutant virus lacking the major species, VA RNA_I. This mutant fails to translate mRNA late in infection, suggesting that VA RNA might interact with elements of the protein synthetic apparatus of the cell. To explore this possibility, we have prepared cell-free systems from infected and uninfected HeLa cells. These cell-free systems duplicate the properties of the infected cells in that extracts of mutant-infected cells are unable to translate the viral mRNAs that they contain. The deficiency extends to mRNAs from other sources which, when added exogenously, are also not translated by the cell-free system from mutant-infected cells. This finding and results obtained with specific inhibitors of transla-

tion support the idea that the block is at the level of initiation of polypeptide chains.

We have tried to restore translational activity to the extract by supplementing it with purified VA RNA, but to no avail. Nor is the block overcome by the addition of a competent cell-free extract from uninfected cells or cells infected with wild-type virus. We conclude that the block is not due simply to the lack of VA RNA, but must result from a more complicated set of interactions involving VA RNA in an indirect way. Reciprocal mixing experiments indicated that the mutant-infected cell extract seems to contain an inhibitor capable of interfering with translation in either of the other systems. Likewise, some inhibition is seen when the mutant-infected cell extract is added to an unrelated translation system, prepared from rabbit reticulocyte lysate; but strikingly, the reticulocyte system makes use of the adenovirus mRNAs, showing that they are not irretrievably blocked. Fractionation of the reticulocyte system shows that the component chiefly responsible for rescue of the protein synthetic ability of the mutant system is not associated with ribosomes. We are currently engaged in purifying this factor and in comparing its properties with those of known initiation factors from reticulocytes.

Once the nature of this factor is understood, we should be able to work back to the reactions in which VA RNA takes direct part. As a complementary approach, we have also begun to study the interaction of VA RNA with adenoviral mRNAs in vivo. Plasmids containing a VA RNA gene and a gene encoding a viral mRNA are introduced into tissue-culture cells by transfection, and synthesis of the corresponding protein is examined. By systematically altering regions of the two genes, we hope to discover the sequences in each that are involved in this novel kind of translational control.

Functional Analysis of the Adenovirus E1A Gene

E. Moran, M.B. Mathews, R.J. Roberts, B. Zerler

Adenovirus E1A is required for adenovirus-induced cell transformation and is involved in the regulation of expression of other adenovirus genes. At least three different mRNAs are produced from the E1A region, and our aim is to distinguish the roles of the separate products of this gene. We have therefore isolated cDNA clones corresponding to the two largest known mRNA species, the 13S and 12S mRNAs. These cDNAs have been subcloned into plasmids so that they are expressed either from the adenovirus E1A promoter or from the inducible mouse metallothionein gene promoter. They have also been rebuilt into the entire viral genome in place of the wild-type E1A region. Ribonuclease protection analyses using SP6 probes have shown that only 13S E1A RNA is detectable from the 13S virus or plasmids, and only 12S RNA is detectable from the 12S constructs. In collaboration with E. Ruley (this section), it has been shown that transfection with either the 12S or 13S plasmids can establish primary baby rat kidney cells in culture in a manner similar to that for wild-type E1A plasmids. Both the 12S and 13S plasmids can also, like E1A, cooperate with plasmids carrying the *ras* genes to transform baby rat kidney cells. We have also studied these plasmids using a transient expression assay developed by Weeks and Jones (*Mol. Cell. Biol. 3:* 1222 [1983]) in which expression of the bacterial chloramphenicol acetyl transferase (*cat*) gene is under the control of the adenovirus E3 promoter and dependent on cotransfection with the E1A gene. The 13S plasmids stimulate E3-*cat* expression when substituted for the E1A gene, but the 12S plasmids do not. These assays will be extended by constructing plasmids in which the *cat* gene is placed under the control of various other adenovirus promoters.

In collaboration with T. Grodzicker (this section), we have studied the roles of the 12S and 13S products in the lytic and transforming functions of reconstructed adenovirus. Viruses containing the 13S cDNA region in place of E1A are competent for lytic functions in HeLa, whereas the 12S viruses are defective. Immunofluorescence studies of virus-infected HeLa cells indicate that the adenovirus DNA-binding protein, a product previously shown to be dependent on E1A, is made during infection of HeLa cells with the 13S virus but is not detectable during infection with the 12S virus. Regulation of gene expression from various other regions of adenovirus during infection by wild-type and the 13S or 12S viruses is currently under investigation. Although defective for lytic functions in human cells, the 12S virus transforms baby rat kidney cells in culture at very high efficiency. Infection of these cells with the 13S virus, as with wild-type E1A, results mainly in cell death.

We have recently constructed a 9S cDNA using synthetic oligomers that span the splice junction. The 9S cDNA has been placed under the control of the mouse metallothionein promoter, and recombinants with the E1A promoter in plasmids and in whole virus are under construction. The role of the 9S product is being studied using the transformation and transient expression assays. In addition to the "9S only" virus described above, a "9S minus" virus is being constructed using oligonucleotide-directed in vitro mutagenesis to alter the 9S splice donor site in E1A. Current experiments are directed toward the identification and purification of the protein synthesized from the 9S mRNA.

To investigate the functions of the E1A products in more detail, we are currently engaged in a mutational analysis of their active sites. Using oligo-

nucleotide-directed and random mutagenesis in bacteriophage M13, we have obtained various point mutations in the E1A gene or in the specific cDNA clones. These mutations will be subcloned into plasmids and viruses and assayed for their effect on lytic, transforming, and gene-regulating functions.

Two-step Transformation of Primary Cells

H.E. Ruley, J. Moomaw, K. Maruyama

During the past year, we have investigated the genetic requirements for the oncogenic transformation of cultured primary cells. Work in other laboratories has suggested that transformation of primary cells by polyoma virus and by human adenoviruses requires the expression of at least two viral functions. The first, an establishment function, is able to extend the life span of primary cells in vitro, whereas the second, a transformation function, is required for full expression of an oncogenic phenotype. Thus, establishment functions expressed by adenovirus E1A or portions of the polyoma virus large T antigen lead to the ability of primary cells to grow indefinitely in culture. Additional functions expressed by adenovirus E1B or the polyoma virus middle T antigen result in phenotypic changes characteristic of oncogenic transformation, such as anchorage-independent growth and the ability to form tumors when cells are transplanted into syngeneic animals. In contrast, transformation of established cell lines can require fewer viral functions. Thus, expression of the polyoma virus middle T antigen alone is sufficient to transform a variety of established cell lines. Apparently, such cells constitutively express establishment functions that can substitute for those of the virus. The interaction between establishment and transformation functions is poorly understood, as is the mechanism by which they combine to elicit the transformed phenotype.

Research in this area has addressed two questions. The first concerns the possibility of transforming cultured primary cells by mixing and matching viral establishment and transformation functions. For example, would it be possible to transform primary cells with a combination of the adenovirus E1A and polyoma virus middle T antigen genes? The second question concerns the ability of cellular oncogenes, isolated by virtue of their ability to transform NIH-3T3 cells, to transform cultured primary cells. Given the requirement for at least two functions in the case of viral transformation, it seemed quite likely that cellular oncogenes would also require two or more functions to transform primary cells.

To address these questions, cloned viral and cellular genes were introduced into primary cultures of baby rat kidney cells by coprecipitation with calcium phosphate. Plasmid DNAs were introduced both individually and in combinations to examine potential interactions leading to oncogenic transformation. The principal results from these studies are summarized below:

1. The polyoma middle T antigen and the T24 Ha-*ras*-1 genes are individually unable to transform primary baby rat kidney cells. This result is particularly interesting given that these genes are able to transform oncogenically a variety of established cell lines.
2. The adenovirus E1A is able to provide functions that enable these genes to transform primary cells, indicating (a) that viral establishment and transformation functions of polyoma virus and adenovirus can be interchanged and (b) that cellular *ras* oncogenes require additional functions in order to transform primary cells.
3. A nonactivated *c*-Ha-*ras*-1 gene is unable to transform even following cotransfer of E1A, indicating that oncogene activation is necessary but not sufficient for oncogenic transformation of cultured primary cells.

Transforming Functions of Adenovirus E1A Mutants

H.E. Ruley, K. Maruyama, J. Moomaw, T. Grodzicker [in collaboration with E. Moran, B. Zerler, M. Mathews, and R. Roberts]

During lytic infection, E1A transcripts are differentially spliced, generating coding sequences for three related proteins. Two of these are synthesized in adenovirus-transformed cells: Proteins containing 289 and 243 amino acids are synthesized from 13S and 12S mRNAs, respectively. The metabolic functions of the E1A proteins remain largely obscure. The larger of these proteins expresses E1A functions that are required for the transcriptional activation of the other adenovirus early-region genes, whereas no specific function has been attributed to the smaller protein. Activities attributed to the E1A region include (1) an establishment function that leads to the ability of primary cells to grow indefinitely in culture; (2) activities that produce cell-cycle effects, such as the induction of mitosis in quiescent cells and the induction of chromosomal aberrations; and (3) effects on or stimulation of the transcription of a variety of cellular genes. Whether any of these activities of E1A are related to one another is presently unknown.

To probe the function of E1A in transformation, plasmids containing mutations in E1A were transfected into primary baby rat kidney (BRK) cells alone and together with the polyoma middle T antigen and T24 Ha-*ras*-1 genes. Sequences con-

taining two E1A mutations, from Ad5*hr*1 and Ad5*hr*440 viruses, were cloned by D. Solnick (Institute of Cell and Tumor Biology, Heidelberg). *hr*1 contains a deletion within the intron of the 12S mRNA, resulting in the synthesis of a truncated form of the 289-amino-acid protein while having no effect on the 243-amino-acid peptide. *hr*1 is defective in E1A functions required to activate the transcription of the other adenovirus early-region genes. *hr*440 contains two base changes that abolish processing of 12S mRNA; consequently, the 243-amino-acid protein is not made. This lesion also introduces a termination codon in the reading frame of the 13S mRNA, resulting in the synthesis of a truncated form of the 289-amino-acid protein.

Both *hr*1 and *hr*440 plasmids express E1A establishment functions. Transfection of either plasmid alone into primary BRK cells gives rise to cells with extended growth potential in vitro, from which established cell lines can be isolated. *hr*1 is able to provide functions required by both the polyoma middle T antigen and T24 Ha-*ras*-1 genes to transform primary BRK cells; however, the T24 Ha-*ras*/*hr*1 cotransformants are not tumorigenic in syngeneic rats. *hr*440 is able to provide the functions required by the T24 Ha-*ras* gene for transformation but is unable to assist the polyoma middle T antigen gene in transformation. Despite this limitation, T24 Ha-*ras*/*hr*440 cotransformants are tumorigenic in syngeneic rats.

Plasmids separately expressing the 12S and 13S mRNAs have been constructed. Both plasmids express E1A establishment functions and both enable the polyoma middle T antigen and T24 Ha-*ras* genes to transform primary BRK cells. These results suggest the following: (1) The activity of E1A required for transformation of primary cells by viral and cellular transforming genes is linked to establishment functions and is not linked to E1A functions required for transcriptional activation of the other adenovirus early-region genes. (2) The establishment functions of E1A are located in the aminoterminal portion of the E1A proteins common to both the 12S and 13S products. (3) Although it is possible to mix and match establishment and transformation functions to a broad extent, certain combinations do not work. The reasons for this are not understood.

Establishment Functions of the *v-myc* Oncogene of MC29 Virus

H.E. Ruley, J. Moomaw, K. Maruyama

Since E1A is a viral gene, the question arises as to whether there are cellular genes that express activities similar to those of E1A that are important in malignant transformation. A candidate for such a gene is *myc*, which is activated in a variety of tumors, and yet *myc* is unable to transform NIH-3T3 cells. Consequently, the *v-myc* gene of MC29 virus was tested for establishment functions either by leading to the ability of primary baby rat kidney cells to grow indefinitely in culture or by providing the transforming functions required by the T24 Ha-*ras*-1 gene. The *v-myc* gene was found to express both activities, providing further evidence that where primary cells are concerned, the requirement for separate establishment and transformation functions reflects a basic mechanism of oncogenic transformation. The fact that proteins made early during the lytic infection by oncogenic DNA viruses express activities similar to those expressed by oncogenes activated in certain human tumor cells supports the conviction that the study of the oncogenic DNA viruses will provide basic knowledge concerning the mechanisms of oncogenic transformation.

Regulation of Viral Gene Expression: Anti-death

T. Grodzicker, H.E. Ruley

We have also investigated the ability of mutant adenoviruses to transform primary BRK cells. Ad5*hr*1 is defective in E1A functions required for the transcriptional activation of the other viral early-region genes and, consequently, is much less cytotoxic than wild-type virus on primary BRK cells. Ad5*hr*1 also expresses E1A establishment functions as evidenced by the high frequency with which Ad5*hr*1 induces colonies of cells with extended growth potential in vitro. Ad5*hr*440 also contains a mutant E1A region; however, the viral early regions are expressed to a much greater extent than in Ad5*hr*1. As a result, Ad5*hr*440 is cytotoxic to an extent that obscures detecting the establishment functions of he virus. We investigated the ability of Ad5*hr*1 and Ad5*hr*A to complement for transformation of primary BRK cells, reasoning that the establishment activity of Ad5*hr*1 might complement the activation of E1B by Ad5*hr*440. We were surprised to find that the establishment activity of Ad5*hr*1 was dominant over the cytotoxicity of Ad5*hr*440 (we have called this activity of Ad5*hr*1 anti-death). *dl*312 (a mutant lacking E1A) failed to suppress Ad5*hr*440, indicating that the interference by Ad5*hr*1 was not due to viral interference. In coinfection experiments in HeLa cells, Ad5*hr*1 was able to suppress the induction of the 72K DNA-binding protein by both *hr*440 and wild-type viruses, as judged from immunofluorescence. These results suggest that an activity associated with Ad5*hr*1 is able to repress the expression of adenovirus early-region genes. Mutant adenoviruses expressing only the 12S E1A

RNA behave in a manner similar to that of Ad5*hr*1, suggesting that the anti-death activity results from expression of the 12S E1A RNA and not from the truncated protein product of the 13S E1A RNA synthesized by Ad5*hr*1. The nature of the interference by the 12S E1A product is being investigated.

VIRAL VECTORS

Work on the development and use of eukaryotic viral vectors has continued this year. The major systems used are adenovirus and retrovirus vectors. A variety of genes have been inserted into these vectors, and the resulting viruses have been used for several purposes, including overproduction of proteins in mammalian cells; investigation of the biological role of inserted gene products and their controlling elements; study of the controlling elements, signals for RNA processing, and translation of the viral vectors themselves; and integration of vectors into mammalian cells to establish cell lines producing gene products of interest.

Expression of Foreign Eukaryotic Genes from Adenovirus Promoters and Controlling Elements

M. Yamada, M. Merle, T. Grodzicker

We reported last year studies of the SV40 *A* gene inserted into adenovirus as a model system for studying adenovirus late gene transcriptional and translational regulation. We also wanted to construct viruses that would produce large quantities of SV40 T antigen that could be easily purified and used in biochemical studies (Thummel et al., *Cell 23:* 825 [1981]; Thummel et al., *J. Mol. Appl. Genet. 1:* 435 [1982]; Thummel et al., *Cell 33:* 455 [1983]). These studies showed that the SV40 *A* gene lacking its own promoter is transcribed very efficiently when the gene is positioned in adenovirus so that it is controlled by the strong adenovirus major late promoter. Furthermore, the T-antigen protein is expressed at a high level when the *A* gene is located in the third segment of the late tripartite leader; in this case, the T-antigen mRNA that was efficiently translated in vivo carried almost the entire tripartite leader and the wild-type SV40 initiation codon.

The following features are essential for constructing a hybrid virus in our vector system: (1) Expression of the SV40 T-antigen helper function for growth of adenovirus in monkey cells. Adenovirus grows well in human cells but very poorly in monkey cells. This block can be overcome if SV40 T antigen is expressed in adenovirus-infected monkey cells. Thus, we can select and amplify hybrid adenoviruses that express T antigen. Furthermore, if the SV40 *A* gene has been previously linked to another foreign gene, the hybrid virus containing the foreign gene can be selected by the same mechanism. (2) Use of in vivo as well as in vitro recombination for construction of hybrid viruses. There are no convenient unique restriction sites that can be used to insert an exogenous gene in the third segment of the tripartite leader downstream from the adenovirus major late promoter. The starting material is a plasmid containing adenovirus DNA linked to the foreign gene that is lacking its own promoter. The joint between the adenovirus DNA and the foreign gene is at a position on the adenovirus genome (the third leader) where we want the foreign gene to be located in the hybrid virus to be constructed. Downstream from the gene (if it is a gene other than the SV40 *A* gene) are sequences that code for SV40 T antigen (including the SV40 early promoter). The entire insert is ligated to *Bam*-digested adenovirus DNA. The resulting molecule, which has normal adenovirus ends, contains a duplication of adenovirus DNA (in the left arm of the vector and the insertion). Such molecules, once introduced by transfection into 293 cells along with wild-type helper DNA, may recombine, leading to excision of the intervening DNA and placement of the foreign gene at the desired and predetermined location.

Integration and expression of the herpes simplex virus type I thymidine kinase gene and the human α-chorionic gonadotropin gene in adenovirus vectors. The thymidine kinase (*tk*) gene of herpes simplex virus type I (HSV) and the human α-chorionic gonadotropin (α-HCG) gene were chosen as representative exogenous genes. The *tk* gene contains no introns, whereas the coding sequences for α-HCG contain the two introns (Fiddes and Goodman, *J. Mol. Appl Genet. 1:* 3 [1981]). The coding sequences of these genes were separated from their promoters, ligated at their 5′ ends with a segment derived from adenovirus for proper insertion into the virus, and also ligated at their 3′ ends with the SV40 *A* gene for production of helper function. These manipulations were carried out in plasmids in *Escherichia coli*. The fragment carrying a piece of the adenovirus–exogenous gene–SV40 *A* gene was ligated with adenovirus DNA, mixed with

helper DNA, and transfected into 293 cells. The hybrid viruses were grown in monkey cells to select viruses that express T antigen. After trying many arrangements of the gene order and junction point, we have so far obtained four hybrid viruses containing either the *tk* gene (one) or the α-HCG gene (three) that produced Tk or α-HCG, respectively. In all cases, the exogenous gene was positioned in the third leader sequence, and the SV40 T-antigen gene had its own promoter. There were no other ATG codons in the 5′ end of the presumed mRNA before the authentic starting codon. The length of the exogenous DNA was 2.4 kb for the *tk* gene, 4.5 kb for full-length genomic α-HCG, 3.3 kb for the α-HCG gene with a deletion of its poly(A) site, and 1.5 kb for the α-HCG gene with both a deletion of the poly(A) site and a deletion of part of an intron. Each of the DNAs, along with a 3.1-kb insert of SV40 T-antigen DNA, replaced 11.9 kb of adenovirus DNA from 26.5 to 59.5 map units, as expected. There may be little preference in the length of exogenous DNA to be inserted. On the other hand, the presence of a poly(A)-addition signal in the α-HCG gene affected the propagation of hybrid virus. The proportion of the hybrid virus with a poly(A) site was estimated as 1–5% of the population, as judged from plaque formation on CV-1 monkey cells and HeLa cells. In contrast, the hybrid virus that did not contain a poly(A) site in the α-HCG gene comprised 10–30% of the viral population.

The following results concerning foreign gene expression have been obtained. (1) Hybrid viruses containing the *tk* gene (Ad-SVR-TK): Ad-SVR-TK produced, in the late stage of infection, a 1.55-kb mRNA that hybridized with a *tk* probe. This size corresponds to an mRNA containing the first, second, and part of the third tripartite leader sequences and the coding sequence of *tk*. It suggests that the *tk* gene was controlled by the major late promoter of adenovirus, and its mRNA was processed in the same way as normal adenovirus late mRNAs. The virus produced 2–5 times more *tk* activity in CV-1 monkey cells at late times after infection than is found in cells infected by HSV. A polypeptide with an apparent molecular weight of 47,000, which is indistinguishable from that produced by HSV, could be precipitated with anti-*tk* antiserum from infected cells. (2) Hybrid viruses containing the α-HCG gene (Ad-SVR-HCG): Three hybrid viruses isolated showed the same properties with respect to the expression of the protein. At late stages of infection of CV-1 cells, polypeptides with apparent molecular weights of 21,000, 16,000, 15,000, and 12,000 could be detected in the cytoplasm by immunoprecipitation experiments using anti-α-HCG antiserum. Only extremely low levels of polypeptide could be detected in the culture supernatant by immunoprecipitation. Uninfected CV-1 cells did not produce α-HCG. HeLa cells synthesize α-HCG endogenously; the molecular weights of the polypeptides are 20,000 and 16,000 in the cytoplasm, and a 22,000-molecular-weight form is secreted into culture medium. The amount of these products increased severalfold in late stages of infection with the hybrid virus, but the ratio among the three species was constant. This fact indicated that the virus-coded α-HCG was expressed and gave the same products as the endogenous ones. The different molecular-weight species probably represent differently glycosylated forms of α-HCG. These experiments indicated that there was a cell-line specificity for secretion of α-HCG.

These results indicate that our adenovirus vector system works well to express the exogenous genes and produce their protein products. Furthermore, we found several interesting phenomena in the course of these experiments. For example, the level of expression of the endogenous *tk* and α-HCG was affected by adenovirus infection. Also, as mentioned above, there is cell-line specificity for glycosylation and secretion of α-HCG. We are continuing to analyze these phenomena in various ways.

Expression of polyoma T antigens in adenovirus. In collaboration with R. Tjian and S. Mansour (University of California, Berkeley), we are studying the expression and production of polyoma T antigens expressed from the adenovirus major late promoter. These studies are being conducted in order to produce large quantities of protein in infected cells so that the T antigens can be analyzed and purified.

Using the methods described above, a segment of the polyoma early region that codes for large, middle, and small T antigens has been removed from its promoter and joined to a segment of adenovirus DNA so that they are joined at a position in the middle of the third segment of the tripartite leader. The SV40 *A* gene containing its promoter is located at the 3′ end of the polyoma DNA, and the entire insert is cloned in a pBR-derived plasmid and grown in *E. coli*. The insert is excised and inserted into adenovirus as described above. Hybrid viruses are grown in CV-1 cells.

Hybrid viruses have been isolated that contain polyoma DNA in the desired position. CV-1 cells infected with the virus synthesize polyoma large T antigen, as determined by indirect immunofluorescence using anti-polyoma T antisera. Immunoprecipitation of infected cell extracts with these antisera shows that the hybrid virus synthesizes middle and small T antigens as well as large T antigen. The proportion of these antigens relative to one another is different from that found in polyoma-infected mouse cells. Regardless of whether HeLa or CV-1 cells are infected with the hybrid virus, there is a large amount of middle and small T antigens and small amounts of large T antigen produced. We wish to investigate whether this is due

to preferential splicing of polyoma-containing RNAs in adenovirus-infected cells. We previously found that in our adenovirus vectors containing the SV40 *A* gene, much more large-T-antigen mRNA was produced than small-T-antigen mRNA and that the proportion of these mRNAs relative to one another differed from that found in SV40-infected cells.

We have also constructed viruses made with polyoma DNAs that code for only large T antigens (from R. Kamen, Genetics Institute). These viruses produce large, but not middle or small, T antigen, as determined by immunoprecipitation of infected cell extracts. They clearly produce more large T antigen than is found in polyoma-infected mouse cells.

Retroviral Vectors

S. Hughes, E. Kosik, J. Sorge

When compared with other vector systems that can be used either in vivo or in cultured cells from the higher eukaryotes, retroviruses offer several advantages. Since retroviruses are the only naturally occurring vectors in higher eukaryotes, it is reasonable to expect that they can be readily adapted as vectors capable of accepting a wide variety of inserts. Retroviral genomes are small, making it relatively easy to manipulate a cloned DNA copy of the genome. The viruses are efficient; in culture, essentially all of the cells can be infected. Retroviruses are nonlytic; infection has little or no effect on cultured cells. Certain strains of virus have little or no effect on the intact animal. Since the viral genome integrates into the host genome, the progeny of a single infected cell are all infected, and the provirus is in the same place in the genome of each of the progeny cells. Infection is self-limiting; each infected cell usually acquires 1–5 copies of the viral genome. Intervening sequences can be removed from genomic inserts cloned into a retroviral vector upon passage of the recombinant virus in cultured cells. Retroviral vectors have considerable potential for in vivo use, both for short-term infections and for insertion into the germ line.

Unfortunately, there are also some disadvantages. Retroviruses, and the vectors derived from them, are relatively unstable. When a helper virus is present, there is extensive recombination, and even in the absence of a helper, internal rearrangements are frequently seen. The total size of the vector, including both the viral and nonviral sequences, is limited to about 10–11 kb.

For several reasons, we have chosen, at least initially, to work with helper-independent retroviral vectors. Helper-independent vectors offer certain advantages. There is no requirement for a helper (cell or virus), and, in general, this gives a significant increase in stability. There is no requirement for the vector to carry a selectable marker. As long as the helper-independent construction is itself stable, any sequence inserted into the vector will be passively carried to all infected cells as a part of the viral genome. There are also disadvantages. The size of insert that can be introduced into the vector is further reduced. Our vectors can accept cDNAs about 2 kb in length. At least initially, helper-independent vectors are confined to the avian system. The avian system, however, has two advantages: Culturing a variety of different types of primary cells is trivial, and birds, both chickens and related species, are available that completely lack endogenous viruses homologous to the avian sarcoma-leukosis viruses.

Vector construction. With the above considerations in mind, we began to construct a family of helper-independent retroviruses a few years ago. All of the constructions are derived from the Schmidt-Ruppin A (SRA) strain of Rous sarcoma virus (RSV), and all the helper-independent constructions are based on the replacement of the cellular oncogene, *src*, carried by RSV, with other genes or sequences. In experiments initiated by J. Sorge (who was a postdoctoral fellow in our laboratory and is now at Scripps), a cloned copy of the unintegrated circular DNA from the SRA strain of RSV was manipulated in vitro to remove *src*. *Cla*I linkers were inserted at the end points of the deletions. Such vectors can both carry and express DNA sequences inserted in place of *src*; however, there is a problem with these constructions. In all known RSV strains, the *src* gene is flanked by direct repeats about 110 bp in length. Homologous recombination (presumably during reverse transcription) results in the frequent loss of *src* in wild-type RSV. It is hardly surprising that the vector constructions lose their inserts by the same route. We chose to try to eliminate one of two 110-bp direct repeats. This ultimately led to a series of experiments that tested the function in RSV of the regions lying between *env* and *src* and between *src* and the long terminal repeat (LTR) (Sorge and Hughes, *J. Virol. 43:* 482 [1982]; Hughes and Kosik, *Virology* [1984, in press]; J. Sorge and S. Hughes, unpubl.). The conclusions of this work are summarized below.

One copy of the direct repeat sequences must be retained for the virus to replicate; however, it is possible to eliminate the region of homology flanking *src*, either by eliminating the upstream copy of the direct repeat or by removing a small portion of the upstream copy and most of the downstream copy. To express *src*, or another gene inserted in place of *src*, there must be an appropriate splice acceptor. A partially synthetic splice acceptor can be substituted for the naturally occurring splice acceptor. Most of the segment of unknown origin (which we call E.T.) that lies just upstream of *src*

in the SRA strain of RSV can be deleted without interfering with viral replication or with *src* expression.

RNA splicing. The available constructions can be used for several purposes. Splicing has been studied with genomic inserts derived from both a homologous host (chick αD globin; Fischer et al., *Proc. Natl. Acad. Sci.* [1984, in press]) and a non-homologous host (human α-HCG; Sorge and Hughes, *J. Mol. Appl. Genet. 1:* 547 [1982]). Introns are removed from the genomic inserts relatively slowly. The two introns present in the chick αD globin are processed at different rates; viruses can be found that have one of the two introns removed (Fisher et al., *Proc. Natl. Acad. Sci.* [1984, in press]). It may be possible to use these constructions to examine splicing intermediates.

Since the virus replicates normally whether or not the *src* gene is expressed, the *src* splice acceptor can be modified and the effects on *src* expression analyzed. We have successfully substituted a new splice acceptor for the splice acceptor originally present in RSV. Three closely related constructions, which vary in the region ten bases upstream of the new splice acceptor, fail to express *src*. Such constructions not only are potentially useful vector constructions, but also provide information about splicing.

AUG choice in initiation. The vectors can also be used to study how ATG codons are selected during initiation. The four RSV messages *gag*, *gag-pol*, *env*, and *src* are all derived from a full-length transcript. All four messages contain the same 5′-leader segment. Three of the messages, *gag*, *gag-pol*, and *env*, use the same ATG to initiate translation. The *src* ATG initiation codon lies 3′ to the *gag* ATG in the spliced *src* mRNA. For the *src* ATG to be used efficiently in initiation there must be, in the completed message, a termination codon in frame with the *gag* ATG. A virus (1057 CGA) has been derived by oligonucleotide-directed mutagenesis that has this particular termination codon (TGA) altered to CGA. The 1057 CGA virus initiates *src* translation at the *gag* ATG. Cells infected by the mutant show a spindle-shape morphology distinct from the rounded morphology of cells infected with the wild-type SRA strain RSV (S.H. Hughes et al., unpubl.). The 1057 CGA mutant specifies a *src* protein of 63,000 daltons, which is currently being studied in J. Brugge's laboratory (SUNY, Stony Brook).

Expression of foreign genes. The vectors nonselectively express genes whose coding region is less than 2 kb (*v-src* and Tn*5 neo* have been tested; presumably, any other would also work). The vector derivatives are much more convenient for site-directed mutagenesis of *src* than is wild-type RSV, since in the vectors, *src* can be moved in and out of the virus via *Cla*I sites. (These experiments are being done in J. Brugge's laboratory.) In theory, the vectors can be used to study mutants of any gene whose coding region is less than 2 kb.

New vectors. In addition to the vector constructions already tested, we are currently working on a construction that will supply an initiation ATG to inserts that lack an ATG appropriate for translation initiation. Several different experiments would benefit from such a construction. For example, with such a vector, most or all of the retroviral portion of viral oncogene fusions could be removed; in addition, we have large *Escherichia coli* expression libraries that contain cDNAs inserted into the *lac* expression plasmids pUC8 and pUC9 (see Stuctural Studies in the Mammalian Cells Section). The majority of the plasmids we have isolated and characterized as expressing a portion of a particular protein in *E. coli* contain cDNA inserts that lack the 5′ end of the coding region. In some cases, there are reasons to express these incomplete genes in chick cells as well as in *E. coli*. For these experiments, the initiating ATG must be supplied by the retroviral vector.

We could make constructions that would initiate either at the *gag* ATG or at the *src* ATG. There are several reasons to think that the *src* ATG constructions will be better, and they will be tried first. The advantages and uses of such constructions are discussed below.

There is a simple route by which the reading frame of the retroviral vector constructions can be made to match the reading frame of pUC8 and pUC9. In such a system, a cDNA that is in frame in the *E. coli* vector will, perforce, be in frame upon insertion into the retroviral vector. Since the *src* ATG is a part of an *Nco*I site (CCATGG), the vector can be designed to add only 2–3 amino acids onto the amino terminus of the protein.

Although there are obvious limitations, such a system can be used to assay functional domains in proteins made from the same DNA fragment both in *E. coli* and in chick cells. Mutants can be made with the insert in the pUC plasmid and a preliminary screen done with the protein that is synthesized in *E. coli*. Potentially interesting mutants can be transferred directly into the retroviral vector for a more complete analysis in chick cells.

In vivo retroviral vectors. Although the vectors that have already been described lack an oncogene, these vectors are still oncogenic and can activate the endogenous oncogenes *c-myc* and *c-erbB*. Although such constructions are suitable for a variety of experiments in cultured cells and possibly even for some experiments in vivo, they may not be well suited for long-term in vivo experiments or for insertion into the germ line. The related endogenous virus RAV-0 is not oncogenic. We have replaced the

avian leukosis virus (ALV) LTR of the vectors with the RAV-0 LTR and, in collaboration with L. Crittenden (USDA Poultry Laboratory, Michigan), have shown that these recombinant viruses have little or no tumorigenic potential in vivo. Because only the LTR is replaced, essentially all of the ALV vector derivatives can be readily converted to nononcogenicity.

In addition to providing prototype in vivo vectors, these constructions locate the sequences responsible for the difference in the oncogenicity of the two viruses. Work from other laboratories (P.A. Luciw, H.E. Varmus, J.M. Bishop, and M.R. Capecchi, unpubl.) suggests that the RAV-0 LTR lacks an enhancer. We have subcloned the murine leukemia virus (MLV) enhancer to test its effect on replication, expression, and oncogenicity of the ALV/RAV-0 recombinants. If the RAV-0/ALV recombinants lack a functional enhancer, they may also be useful for cell-type-specific promoter assays (see below).

Since the ALV/RAV-0 recombinants appear to have a weaker "promoter" (lack of an enhancer?) than the ALV vectors, the ALV/RAV-0 recombinants should express inserts at lower levels. This is being tested with *v-src* and Tn*5 neo* inserts. In many cases, viruses that express a particular gene product at various levels would aid in the analysis of the gene product. For example, such a system could be used to ask questions about threshold levels of oncogene products required for transformation.

Crittenden's laboratory is trying to define conditions for efficient germ-line infection in chickens. We have agreed to supply nononcogenic recombinants for these experiments. If techniques can be developed that permit the ready introduction of retroviruses into the germ line of chickens, the nononcogenic vectors will be most useful; however, the retroviral vectors can be used to introduce genes into the intact animal without germ-line integration.

Foreign promoters. In addition to analyzing the proteins, it is possible that retroviral vectors can be used as vehicles for testing whether particular DNA segments are capable of initiating tissue-specific RNA expression. There are numerous ways in which such experiments can fail; however, some modest test is warranted. We may be able to avoid one potential problem simply by using constructions that have the ALV LTR (which contains an enhancer) replaced by the RAV-0 LTR (which appears to lack an enhancer). Initially, we plan to compare two promoters, the chicken β-actin promoter, which we have isolated and characterized (Kost et al., *Nucleic Acids Res. 11:* 8287 [1983]), and the chicken α-actin promoter, isolated and provided on a collaborative basis by C. Ordahl (University of California, San Francisco). Such promoters will be inserted into the retroviral vectors linked to a segment whose expression can be readily monitored at the protein or the nucleic acid level. The retroviral vectors can be introduced into a variety of cell types in culture and the cells checked for tissue-specific expression of the test segment that was attached to the cell-type-specific promoter. It may be useful to insert the promoter and the test sequence in the orientation opposite to the direction of transcription of the retrovirus, to avoid confusion from transcripts arising from the retrovirus.

Helper-free Adenovirus-5 Vectors

K. VanDoren, D. Hanahan, Y. Gluzman

Last year we reported the construction of helper-independent adenovirus-5 (Ad5) vectors and the cloning of several different genes in recombinant helper-independent viruses. These genes included (1) an SV40 T-antigen-coding region under control of the Ad2 major late promoter, (2) a wild-type or replication-defective SV40 early region with its own promoter, or (3) a neomycin resistance gene from Tn*5* under control of the SV40 promoter. Since then we have constructed recombinant viruses carrying (4) an SV40 T-antigen-coding region under control of the Ad2 major late promoter, including most of the tripartite leader that normally precedes all late adenovirus messages, (5) cDNA of SV40 large T antigen under control of the Ad2 major late promoter, and (6) cDNA of SV40 large T antigen under contol of the SV40 early transcription unit.

The biological properties of these genes were evaluated in a variety of assays. During lytic infection of 293 cells with recombinant viruses carrying the cDNA encoding SV40 large T antigen under control of either the Ad2 major late or SV40 early promoter, mRNAs were made and translated into protein. Both mRNA and protein levels were three- to fivefold lower than those of their wild-type counterparts. Nevertheless, this result indicates that this vector can be used to express certain cDNA constructs.

Infection of 293 cells with the recombinant virus carrying SV40 T-antigen-coding sequences under control of the Ad2 major late promoter, including most of the tripartite leader, results in production of large quantities of SV40 T antigen. Comparison with a similar construct of T antigen under control of the Ad2 major late promoter, but containing only half of the first leader (of the late tripartite leader), reveals that the first construct is more efficient than the latter one. These data are in agreement with the work of others (Thummel et al., *Cell 33:* 455 [1983]) and indicate that the presence of the tripartite leader in front of mRNA in adenovirus-infected cells improves the translatability of the messages.

Helper-independent adenovirus vectors carrying either a selectable marker for neomycin resistance (under control of the SV40 early promoter) or the early region of SV40 (origin positive or negative) integrate viral DNA into host-cell chromosomes in the absence of adenovirus early region 1. This indicates that viral functions encoded in early region 1 are not essential for the integration of viral DNA.

We have analyzed DNAs from CV-1 and Rat 2 cells transformed to neomycin resistance and from MEF (mouse embryo fibroblasts), HS74BM (human fibroblasts), and REF52 (rat fibroblasts) transformed by SV40 recombinant adenoviruses. Neomycin-resistant CV-1 cell lines contained usually 1 but sometimes 2 copies of the viral genome integrated colinearly with the infecting viral DNA. These cells contained between 60% and 100% of the infecting viral DNA integrated into host-cell chromosomes. Rat 2 cell lines transformed to neomycin resistance also incorporated full-length viral genomes, colinear with the infecting viral DNA.

One to several copies of the recombinant viral DNA molecules were integrated. Three of the five lines analyzed contained the left and right ends of the viral DNA linked together and viral genomes arranged in a tandem array. Tandem arrays were not observed in transformed CV-1 cell lines, suggesting the involvement of a cellular factor in either promoting or preventing interaction between viral DNA molecules. HS74BM cell lines established from transformation using origin-defective SV40 recombinant viruses contained 1–2 copies of viral DNA integrated in the host genome. The integrated copies in these cell lines appear to be full length and colinear with the infecting viral DNA, as was observed with the neomycin-transformed cells. SV40-transformed MEF cell lines contained 1–2 full-length copies of viral DNA as described for HS74BM transformants. The integration patterns in these cell lines differed from those of the human fibroblast lines in that some sequences had become amplified in MEF cell lines and two of the eight lines analyzed may contain the left and right ends of the viral genome linked together, as observed with the neomycin-resistant Rat 2 cell lines. Further analysis of these cell lines is in progress.

We have recently begun to analyze cell lines established from transformations of REF52 cells by the SV40 recombinant viruses. Preliminary results indicate that the integration patterns are different in cell lines transformed by origin-positive SV40 recombinant viruses and those transformed by origin-defective viruses. Transformed cell lines of origin-defective viruses, in general, contain fewer copies of integrated DNA than cells transformed with viruses carrying a functional SV40 origin of replication. These data are the first we have obtained to suggest that the SV40 origin of replication may affect transformation in some nonpermissive cell types. In addition, we have isolated cell lines of baby rat kidney (BRK) cells transformed with SV40 recombinant viruses alone or in conjunction with neomycin recombinant viruses. It will be interesting to analyze the integration patterns in the cells to see what effect, if any, the origin of replication has had in these cells. It will also be of interest to determine whether both the neomycin recombinant viruses and the SV40 recombinant viruses are treated the same in cells transformed by both viruses. We also wish to examine whether the presence of early region 1 will change the integration pattern during nonlytic infections with these recombinant viruses.

MAMMALIAN GENETICS

Mouse *t* Haplotypes: A Model System for Understanding the Organization, Expression, and Evolution of the Mammalian Genome

L.M. Silver, V. Bautch, L. Cisek, H. Fox, C. Jackson, M. Krangel, D. Lukralle, P. Mains, N. Sarvetnick

Mouse *t* haplotypes are naturally occurring, highly variant forms of a region of mouse chromosome 17 that has multiple and interacting loci with profound effects on embryogenesis and sperm differentiation. The *t* haplotypes maintain themselves as discrete genomic entities through recombination suppression, and these chromosomal units are propagated through wild populations by a male-specific transmission ratio distortion in their favor. Our understanding of *t* haplotypes has undergone radical changes over the last 5 years, and it is now possible to focus research efforts in attempts to answer specific questions through the combined use of molecular and genetic techniques.

t haplotypes represent a common polymorphism (often present at a level of 20%) in house mouse populations from all over the world and have been found in three separate commensal species, *Mus domesticus, Mus musculus,* and *Mus molossinus*, that evolved apart between 1 and 2 million years ago. A *complete t* haplotype is operationally defined as one that suppresses recombination in $+/t$ heterozygotes, along the entire 12-cM region of chromosome 17 encompassing *T* and the *H-2* complex. In general, *t* haplotypes recovered from wild mouse populations are complete. However, suppression of recombination is not absolute—rare recombinants have been isolated in the laboratory and are referred to as partial *t* haplotypes. These partial *t* haplotypes are portions of a complete *t* haplotype that continue to suppress recombination along their length and express only a subset of the *t*-specific properties.

Wild *t* haplotypes now appear to be genetically and evolutionarily isolated from normal mouse chromosomes, even as they coexist within the same genome. These isolated genetic units are propagating themselves through mouse populations as selfish chromosomes without any obvious benefit to their hosts. The very existence of *t* haplotypes in the mouse, and highly analogous genetic systems in other species (e.g., SD in *Drosophila*, spore killer in *Neurospora*, and pollen killer in tobacco), poses important questions concerning genomic evolution and the dynamics of chromosome polymorphism. Knowledge of the structure and origin of *t* haplotypes would certainly contribute to our understanding of these broader biological questions. Furthermore, an understanding of the "natural" mechanisms by which *t* haplotypes have thwarted the machinery of sperm differentiation to their advantage would yield insight into the differentiation machinery itself. Finally, we hope that studies of embryonic lethal genes will lead to a better understanding of normal embryogenesis.

Many of the molecular tools necessary for a concerted analysis of an isolated region of the mammalian genome are only now becoming available. Although we have chosen to concentrate our efforts on the *t* complex for the reasons just given, as an added benefit, we expect many of our molecular results to be indicative of general principles of mammalian genome structure and function.

Isolation and Mapping of Random DNA Clones of the *t* Complex

H. Fox, P. Mains

The pleiotropic effects of the *t* haplotypes indicate that they have diverged from the corresponding wild-type region of chromosome 17. As a result, it is possible that the entire region, and not just the relatively few genes in it so far identified, has diverged significantly from the wild type in arrangement and DNA sequence. Indeed, M. Lyon (Medical Research Council, England) suggested that a change in interstitial heterochromatin blocks recombination, and it has been proposed that *t* haplotypes were introduced into the house mouse as an introgression from another species.

To investigate these possibilities, as well as to gain a molecular entrance into the *t* region, we are collaborating with H. Lehrach's group (including D. Rohme, B. Herrmann, A.-M. Frischauf, and J.-E. Edstroem) at the European Molecular Biology

Laboratory in Heidelberg. They have obtained random DNA clones of the region by microdissecting the proximal half of chromosome 17 from metaphase chromosome spreads (chromosome 17 was marked by a Robertsonian translocation). This DNA was cloned into bacteriophage λ in nanoliter volumes and then subcloned into plasmids. These are referred to as "microclones." To show that these microclones were indeed from chromosome 17, we have performed Southern blots using DNA from a hamster-mouse hybrid cell line containing only chromosome 17 of the mouse. Of 18 clones tested, 17 mapped to chromosome 17. Nine of these clones were further mapped to the *t* region using 129.*t* congenic mice. If a clone shows a restriction-fragment-length polymorphism (RFLP) between 129 and 129.*t* mice, the clone must map to the only region that differs between the two lines—the proximal portion of chromosome 17. Similar analysis revealed no RFLPs between any of the different *t* haplotypes examined with 22 microclones, using an average of three different restriction enzymes each. However, there were no sequences present in wild type that were absent in *t*, and the level of polymorphism between *t* and inbred strains is roughly comparable to that between different inbred lines. Thus, the *t* haplotypes appear to have a common origin and have not radically diverged from other mice at the nucleotide sequence level.

The microclones have been used to confirm that the *t* complex is an extended region of the chromosome rather than a single point. Rare recombinants occur between *t* and wild-type chromosomes at a 100-fold reduced frequency. Lyon used these recombinants in an elegant genetic analysis of the *t* complex. Different recombinant chromosomes, termed partial *t* haplotypes, display different subsets of the *t* phenotypes. By examining the *cis* and *trans* interactions between different partial haplotypes, she proposed that the *T* interaction factor and the lethal factors mapped to single regions, sterility resulted from the interaction of two separable regions, and segregation distortion was controlled by three regions. She derived a genetic map and defined the crossover points for the partial haplotypes. We have used RFLPs between *t* and wild type to order the physical end points of the partial *t* haplotypes. This physical map agrees with Lyon's genetic map.

An α-Globin Pseudogene Maps within the *t* Complex

H. Fox

The mouse α-globin gene family consists of three "real" genes and two pseudogenes. With the use of somatic-cell hybrids, it was found that one pseudogene *Hba-ps4* was located on chromosome 17. To determine whether *Hba-ps4* is located in the *t* complex, we compared genomic DNA restriction patterns from mice carrying a series of complete or partial *t* haplotypes on the 129 background. We also analyzed 11 inbred strains of mice and other wild-derived (+) forms of chromosome 17. Restriction-fragment-length polymorphisms were observed when genomic DNA was digested with *Taq*I. All wild-type forms of chromosome 17 carry one of two alleles—either a single 3.4-kb fragment or a pair of fragments of 3.8 kb and 1.4 kb. In contrast, all complete *t* haplotypes carry an identical third allele of *Hba-ps4* represented by a single 5.2-kb *Taq*I restriction fragment. Analysis of partial *t* haplotypes allowed the localization of *Hba-ps4* to the distal portion of the *t* complex.

Among the *t* haplotypes examined was t^{h20}. This *t* haplotype arose from t^6 and was first identified by the appearance of a tufted phenotype in a tf/t^{h20} mouse. For a number of reasons, Lyon suggested that t^{h20} carries a deletion within the *t* chromatin, encompassing tufted as well as several other genes. We have found that the t^{h20} chromosome carries no α-globin pseudogene sequences; however, this chromosome is not missing any other distally located *t*-specific markers. Hence, it would appear that t^{h20} has deleted a small region of *t* chromatin located near the distal edge of the *t* complex and including the coat marker *tf*, the DNA marker *Hba-ps4*, and an embryonic lethal gene called *Kinky*. These three loci map close to one another in the central *t* complex region of wild-type chromosome 17 (P. D'Eustachio [New York University], personal communication for *Hba-ps4* mapping).

Molecular Cloning of a *t* Complex Structural Gene

N. Sarvetnick, L. Cisek, L.M. Silver

The *t* complex has been dissected biochemically during the past several years in our laboratory. Two-dimensional gel analysis has identified eight testicular proteins (TCP-1 to TCP-8) that are specified by this region of the mouse genome. Since *t* haplotypes have profound effects on spermatogenesis, it is likely that one or more of these proteins play a crucial role. To investigate this possibility further and obtain probes in the proximal region of the *t* complex, we set out to clone TCP-1.

Using methods developed by J. Fiddes (California Biotechnology, San Jose), we constructed a cDNA library starting with testis mRNA. The library was screened with several different radioactively labeled probes. The probes were obtained by sequential size fractionations of liver and testis mRNAs. In vitro translations identified enriched and nonenriched pools of TCP-1 message, and these pools were reverse-transcribed and used as differential probes for the library. Utilizing what we knew about TCP-1 message abundance and tissue

distribution, we made qualitative assessments from the autoradiograms and selected 70 clones for further analysis.

Northern blot analysis with a group of these confirmed the message length, and differential expression was inferred from the screen. DNA blotting experiments with a hybrid hamster cell line containing mouse chromosome 17 showed that two clones of the first dozen checked indeed mapped on chromosome 17. Hybrid-selection experiments with one of these clones (testis RNA was hybridized to immobilized clone DNA, eluted, and translated in vitro) produced a band with a molecular weight between 61,000 and 64,000 on an SDS gel. The accumulated data strongly suggest that this clone codes for the TCP-1 protein.

Further DNA blotting experiments were carried out to identify restriction-fragment-length polymorphisms (RFLPs), which would then allow this clone to be mapped within the *t* complex. Although extensive, these experiments produced no RFLPs between congenic lines. All strains examined possess several cross-hybridizing fragments, two mapping on another chromosome. A second set of blots revealed a polymorphism between C3H, DBA, and B6 inbred lines. We were therefore able to map certain alleles with recombinant inbred mouse DNAs provided by the Jackson Laboratory. The B6/DBA,C3H polymorphism is not surprising as B6 is considered somewhat "speciated" from the other inbred lines. However, we found the lack of RFLPs between *t*/+ unexpected, as the suppression of recombination described by *t* geneticists over the years led us to assume that there would be extensive nonhomology between the chromosome-17 alleles. We now realize that this view is narrow, as gross rearrangements do not necessarily predict the detection of polymorphisms on a local level. Furthermore, it would appear that the coding-sequence regions identified by this clone are highly conserved. Cross-hybridizing genomic clones have recently been isolated from a library provided by H. Lehrach (European Molecular Biology Laboratory, Heidelberg). Noncoding DNA could diverge more readily, so hopefully a third series of blots might allow RFLPs to be identified.

Studies are in progress to characterize this clone further. Sequence analysis and expression in a prokaryotic vector system are the main focus. The latter efforts should produce an antibody that will tell us more about TCP-1 intercellular localization and its pattern of expression during spermatogenesis.

Recombinational Analysis of the *t* Complex

P. Mains

The process of recombination, or crossing-over, takes place during meiosis and results in the exchange of genetic material between homologous chromosomes. As a result, even though an individual inherited a certain combination of alleles on the chromosomes from each parent, the combination would be randomized in subsequent generations. One property of *t* haplotypes is an inhibition of crossing-over with wild-type chromosomes, which causes the diverse set of phenotypes associated with *t* to be locked together as a unit.

Recombination is dependent on homologous chromosomes having the same order of genes; i.e., their genetic maps must be identical. The particular order is for the most part unimportant; what matters is that they be the same. It has recently been found that the coat marker tufted, *tf*, and the major histocompatibility complex, *H-2* (which itself is a large gene family), are in opposite orders in *t* and wild-type chromosomes. A chromosomal rearrangement would explain why one *t* chromosome can cross over with another, and one wild type can cross over with another wild type, but *t* cannot cross over with wild type. We are attempting to define precisely the chromosomal rearrangement between *t* and wild type. These chromosomes may carry a simple inversion or the rearrangement could be complex, such as multiple overlapping inversions or transpositions. A fine-structure genetic map of both *t* and wild type will define the rearrangement.

Mice carrying chromosomes that show recombination between the markers *T* and *tf* (which map to opposite ends of the *t* complex) are selected among the progeny of females heterozygous for complementing *t* haplotypes. These are then scored for *H-2* alleles by Southern blot analysis and for the presence of lethal alleles by further breeding experiments. We have currently isolated 29 recombinant chromosomes. One of these crossovers occurred within the *H-2* complex and shows that genes within the *H-2* region are probably inverted with respect to wild type.

We plan to extend the mapping analysis to include the chromosome-17 microclones. Because we have not yet found any restriction-fragment-length polymorphism between the parental *t* chromosomes with the microclones, we plan to use a gel system developed by L. Lerman (SUNY, Albany) that separates DNA fragments independently of size by the use of a gradient of DNA denaturants. When a restriction fragment reaches a concentration of denaturants such that a portion "melts," it stops migrating. Single-base-pair changes anywhere within the melted region (usually 50–100 bp) can have a profound effect on mobility. This should be a much more efficient method of detecting polymorphisms than by restriction fragment size. Thus, we hope to increase the resolution of our map to detect complex rearrangements by saturating the *t* region with microclones as genetic markers. Currently, Lehrach is performing a similar recombinational analysis on wild-type chromosomes.

A long-term goal of this work will be the cloning of the *t* lethal mutations. If enough microclones can be mapped (~ 100), a mutation could be localized to a 200-kb region. It may then be feasible to clone this area and attempt to rescue mutant embryos by microinjection of oocytes. This approach may allow the cloning of a lethal allele without prior knowledge of the gene product.

Genetic Analysis of Segregation Distortion of the Mouse *t* Haplotypes

P. Mains

The *t* complex is one of the few exceptions to Mendel's law of segregation. Normally, an organism heterozygous for two alleles passes each to its offspring in equal frequencies. However, male mice can transmit their *t*-bearing chromosomes to more than 90% of their offspring. This phenomenon, termed segregation distortion or transmission ratio distortion, probably maintains the otherwise deleterious *t* chromosomes at high levels in wild populations.

M. Lyon proposed that segregation distortion is controlled by three loci, one acting in *cis* and two acting in *cis* or *trans*. Detailed analysis is hampered by the fact that all complete *t* chromosomes appear to carry identical (or at least very similar) alleles at these loci; i.e., there are no polymorphisms to allow further genetic dissection. However, wild-type chromosomes do show polymorphisms in their interaction with segregation distortion. Both linked and unlinked suppressors of segregation distortion are found in different inbred strains of mice (this polymorphism has complicated the analyis of segregation distortion, since most *t* chromosomes are maintained as outbred stocks that are genetically heterogeneous). The fact that an allele can suppress (or enhance) segregation distortion indicates that it must interact with the *t* factors responsible for the phenomenon. Therefore, analysis of these loci in inbred mice may further elucidate the mechanism of segregation distortion.

As a first step, we wish to map these suppressors. Congenic 129.t^{w5} males (which show >90% transmission of *t*) are being mated to females from a variety of inbred mouse strains. The sons will then be tested for the level of segregation distortion. The F_1 males of the strains that show suppression will then be backcrossed to 129, and those sons will be tested. During a backcross, there is an equal probability that the suppressor or the permissive 129 allele in the F_1 animal will be transmitted. Those sons that happen to receive the suppressor(s) will show no segregation distortion. The relative frequencies of sons that show or do not show suppression in the backcross generation will indicate the number of unlinked suppressor loci present in the inbred strain. These suppressors can then be mapped quickly by the use of the appropriate panel of recombinant inbred (RI) strains. These are inbred mice that are derived from two different inbred strains. The panel chosen will be such that one parental strain shows suppression while the other does not.

Linked suppressors can be mapped by taking advantage of the fact that the *H-2* complex maps within the *t* complex. There exist a large number of mouse strains derived from intra-*H-2* recombination events between different inbred strains. By using the appropriate recombinants derived from parental strains in which one strain shows suppression of segregation distortion while the other does not, the suppressors can be mapped to subregions of chromosome 17.

Genetic Analysis of Dosage Effects of the Proximal Region of Chromosome 17

N. Sarvetnick, P. Mains

Two deficiencies within the proximal region of chromosome 17 display peculiar breeding patterns. One such chromosome, T^{hp} shows a deletion of 3 cM. Offspring that acquire two copies of T^{hp} die in utero at the morula stage of embryogenesis. When the deficient chromosome is acquired only through the father, the resulting heterozygotes are normal except for the short-tailed phenotype seen in all *T* mutations. However, when the T^{hp} chromosome is acquired through the mother, the heterozygous embryos die 15 days after conception. Analysis of this "maternal" effect is important because it may tell us that the same chromosome acts differently when it is derived from the egg or sperm. This nonequivalence of genetic material has been observed in a few other instances and is reconciled most easily by asserting that something is missing in the oocyte that cannot be compensated for by the paternal gene after fertilization.

A second chromosome showing the same maternal effect as T^{hp} has recently been identified by us and H. Winking (Medical Institute at Lubeck, West Germany). Biochemical studies indicate that the t^{lub2} chromosome has lost the TCP-1 protein that maps to this region, implying that it also has a deletion. t^{lub2} arose as a recombinant between a complete *t* haplotype and a "wild-type" chromosome 17. It appears that a deletion was introduced into this new distal *t* haplotype chromosome as a result of unequal crossing over.

We have been interested in reversing the maternal effect by manipulating the genetic backround of the female harboring t^{lub2} so that extra doses of a proximal portion of chromosome 17 are provided. During the last several years, two-dimensional gel analysis has enabled us to identify several other

partial *t* haplotypes that result from unequal crossing-over and have duplications. Chromosomes that duplicate TCP-1 should provide at least part of the region missing from t^{lub2}. By utilizing these chromosomes, we hope to determine when the region is required and whether or not two doses in the egg or sperm can rescue the embryo. A cross that attempts to reverse the phenotype by providing two doses of the proximal region in the sperm is as follows:

$$t^{lub2}/Ttf \times t^{h45}tf/t^{h45}tf \quad (t^{h45} \text{ has duplication})$$
$$\text{(F)} \qquad \text{(M)}$$

Rescued t^{lub2} offspring will have normal tails and be nontufted. Similarly, another cross provides two doses through the egg:

$$T^{h45}tf/t^{lub2} \times +tf/+tf$$
$$\text{(F)} \qquad \text{(M)}$$

By looking for nontufted progeny, we can score for the rescue of the maternally derived t^{lub2} embryos.

During oogenesis, half the chromosomes are set aside in polar bodies that can no longer contribute genetic information to the egg. If this is the critical period for the maternal effect, then the genes in the polar body will not be available to rescue the egg. However, a proximal duplication can accompany a t^{lub2} chromosome if crossing-over occurs. We could monitor this by marking a chromosome with a Robertsonian translocation that could be visualized by cytogenetic techniques. Such a genetic configuration will allow us to score recombination anywhere between the centromere and the tufted marker. If the t^{lub2} offspring are observed and are all recombinants, this would imply that the region is required after the first meiotic division.

Two-dimensional Protein Patterns of Embryos with Lethal *t* Haplotypes

V.L. Bautch, M.S. Krangel

Mice that are homozygous for certain *t* haplotypes die during embryogenesis as a consequence of lethal genetic factors within the *t* complex DNA. Each complete *t* haplotype is associated with a specific lethal genetic factor, and each factor causes morphological abnormalities and death at a different stage in mouse development. The nature of these lethal genetic factors is not known. In an effort to identify the products of these factors and to understand their role in development, we are comparing the two-dimensional protein patterns of normal embryos with those containing one (heterozygous) or two (homozygous) doses of the lethal factors. This work utilizes the QUEST system directed by J. Garrels (see Structural Studies in the Mammalian Cells Section). Embryos with each of these genotypes are all found in litters resulting from mating a male and female that are both heterozygous for the same *t* haplotype and associated lethal factor. Embryos at different developmental stages are dissected from the uterus, labeled in vitro with [^{35}S]methionine, and analyzed by high-resolution two-dimensional gel electrophoresis. The genotype of each embryo is determined after electrophoresis by the presence or absence of allelic forms of a *t*-complex-specific protein (TCP-1 or p63/6.9) in the two-dimensional gel pattern. The embryos carrying *t* haplotypes are congenic on a standard inbred background. This means that the DNAs of normal and mutant embryos differ only in the portion of chromosome 17 that contains the *t* complex, and that a comparison of the two-dimensional gel patterns of these staged embryos should yield only those protein polymorphisms that result from the lethal factors or other *t*-specific genes. Different *t* haplotypes are remarkably similar in terms of DNA structure and protein polymorphisms; therefore, the differences in two-dimensional gel patterns of embryos carrying different *t* haplotypes should reflect the action of the different lethal factors. The types of differences we are following in patterns of mice carrying *t* haplotypes include (1) the presence of an aberrant protein spot, (2) the absence of a normal protein spot, and (3) the inappropriate temporal expression of a normal protein spot during development. We have obtained two-dimensional gel protein patterns of normal embryos at several different developmental stages, and we are compiling data on mutant embryos of three different *t* haplotypes. Preliminary results indicate that embryos homozygous for a particular *t* haplotype and its associated lethal genetic factor show two-dimensional gel protein patterns very similar to those of normal embryos. Those proteins whose structure and/or temporal expression is altered in the mutant embryos will be mapped genetically and analyzed for tissue distribution. It is hoped that these studies will result in the identification of products of the lethal factors associated with *t* haplotypes and help elucidate their role in mammalian development.

Gene Transfer into Mice

D. Hanahan

During the past year, an embryology laboratory has been established in the Harris Animal Facility. One purpose of this laboratory is to manipulate mouse embryos experimentally, in particular to transfer cloned genes into the mouse germ line, as a means of studying gene regulation in the context of the organism. This is accomplished by injecting a solution of DNA into fertilized one-cell mouse embryos and then placing the inoculated embryos into a foster mother, where a significant fraction develop to term; about 10–30% of the mice contain

the injected DNA integrated into one of their chromosomes. The past few years have seen demonstrations that this approach can be used to study the expression of cloned genes. The most notable are the studies by Palmiter and Brinster (Palmiter et al., *Nature 300:* 611 [1982]), which have shown that the transfer of a rat growth-hormone gene into mice will produce unusually large mice as a consequence.

The ongoing studies at Cold Spring Harbor Laboratory focus on examining the structure and expression of a series of hybrid genes following their transfer into mice. The hybrid genes contain regulatory information derived from the rat insulin gene or the chicken alpha-2 (I) collagen gene, each fused to control expression of structural information derived from three viral genes: SV40 T antigen, MC29 *gag-myc* protein, and RSV-*gag* protein. The purpose of using these genes is twofold: (1) to define *cis*-acting regulatory information associated with insulin or collagen genes that elicits their correct expression in the organism and (2) to examine the phenotypic consequences of the expression of oncogenes in the tissues specified by the cellular regulatory sequences.

Recent work from the laboratory of B. Mintz (Wagner et al., *Cell 35:* 647 [1983]) has demonstrated that integration of injected DNA can produce recessive lethal mutations at a remarkably high frequency (33%). This result motivates examination of the integrated structure of genes transferred into mice. At Cold Spring Harbor Laboratory, this question is being addressed by retrieving integrated plasmids out of their resident status in mouse chromosomes and establishing them as episomes in *Escherichia coli*. It is possible to retrieve sequences flanking the integrated transgenes by this approach (plasmid rescue) and then to examine the mouse DNA before and after the integration event. This will facilitate assessment of the extent of deletion, rearrangement, and/or translocation that occurred upon integration, which in turn pertains to alternative mechanisms of mutagenesis (which could occur either by relatively clean insertion or by consequent large deletion and rearrangement).

Molecular Analysis of Human Histocompatibility Antigen Mutants

M.S. Krangel

Human histocompatibility antigens (HLA-A and HLA-B) are highly polymorphic cell-surface molecules that play important roles in self-nonself discrimination by the immune system. Polymorphism at these loci represents the major barrier to transplantation between different individuals of the species. However, the primary physiological role of these molecules is in mediating immune recognition of virally infected cells by cytotoxic T lymphocytes.

I have been interested in employing minor structural variants of these molecules to aid in drawing detailed structure-function relationships. In collaboration with D. Pious (University of Washington, Seattle), mutants in one particular HLA allele, HLA-A2, have been obtained. Mutagenesis of a human B-cell line, followed by immunoselection with a monoclonal antibody plus complement, has allowed the selection of mutants that have lost cell-surface antigenic determinants. Biochemical studies of the resulting HLA-A2 mutants have allowed the identification of that region of the molecule that is recognized by the selecting antibody. Other mutants have been obtained in which the molecules do not mature appropriately and fail to reach the cell surface.

One mutant cell line displayed a particularly complex phenotype and is the subject of ongoing experiments. These cells synthesize two forms of HLA-A2, a low-abundance cell-surface form and a predominant form that is secreted. The latter form is somewhat smaller than wild-type HLA-A2, and this difference was localized to that region of the molecule that interacts with the plasma membrane. Further experiments suggested that the secreted form was not derived from a membrane form by a posttranslational mechanism. It was proposed that the two forms of HLA-A2 in the mutant resulted from alternative splicing of the same primary transcript, the secreted molecule deriving from transcripts spliced so as to delete the exon encoding that portion of the molecule that interacts with the membrane.

To test this hypothesis, cDNA libraries were constructed from both the parent and mutant cell lines. cDNA clones that encode HLA-A and HLA-B were isolated from the mutant library using a general HLA-specific probe, and restriction mapping was used to identify a clone encoding the secreted HLA-A2 molecule. A comparison of the DNA sequence of this clone with that of an HLA-A2 encoding cDNA derived from another cell line (D. Arnot, pers. comm.) has demonstrated unequivocally that the exon encoding the membrane-anchoring region of the polypeptide has been precisely deleted. Other minor differences between the sequences are most probably cell-line-specific; however, this issue awaits the isolation of the appropriate clone from the wild-type cDNA library. An analysis of HLA-A2 genomic clones from both parent and mutant will likely be necessary to define precisely the mutation leading to aberrant splicing in these cells.

Hormonal Control of Gene Expression

D.T. Kurtz, W.R. Addison, J.I. MacInnes, D. Danna, J.-Z. Li, E. Nozik

We have been studying the hormonal and developmental regulation of gene expression in higher eu-

karyotes. The model system we have been using is a rat protein called α_{2u} globulin. This protein is encoded by a multigene family (20–25 copies per haploid genome), and its production is under complex hormonal and developmental control in vivo. The synthesis of α_{2u} in rats is regulated by glucocorticoids, insulin, sex steroids, and growth hormone.

DNA sequences involved in hormonal induction of α_{2u}. The generally accepted model for the molecular mechanism of action of steroid hormones postulates that the hormone binds to a cytoplasmic protein, the receptor; this binding activates the receptor and renders it competent to induce (or repress) the synthesis of specific genes. The simplest literal interpretation of this model predicts that hormone-responsive genes have specific sequences in or around them that are responsible for hormonal modulation and that a hormone-inducible gene should respond in any cell in which a competent receptor exists. We have tested this model by reintroducing cloned α_{2u} genes into mouse L cells, which contain a functional glucocorticoid receptor. The transfected α_{2u} genes indeed do respond to glucocorticoids, and the level of correctly initiated α_{2u} mRNA is induced 10–20-fold in individual clones.

We are identifying the sequences responsible for the induction using the "linker-scanning" method first devised by S. McKnight (Hutchinson Cancer Research Center, Seattle). In this method, BAL-31 deletions are made in plasmids containing the α_{2u} promoter region. Independent deletions are made coming from both the 5′ and 3′ directions, and an *Xho* linker is added. Clones are selected in which the deletion end points are in the α_{2u} promoter region. BAL deletions from each direction with end points near each other are then joined through the *Xho* linker. This leads to a clustered point mutation, in which the bases in the *Xho* linker substitute for the wild-type sequence (if the BAL deletions from opposite directions were exactly 8 bases apart), or leads to small deletions or duplications (if the BAL deletions were greater or less than 8 bases apart). We have saturated the region from the α_{2u} cap site back to -200 with such mutations. These mutant genes have been reintroduced into L cells, and the effects on hormonal modulation are currently being assessed. Although this method is more time consuming than simple 5′ deletions, in the long run, the information obtained from these clustered point mutations will be more meaningful. As many investigators are discovering, when BAL deletions are made, and heterologous sequences are brought in next to the end point of the deletion, these sequences (even from pBR322) can have unpredictable and artifactual effects on transcription. Our method ensures that the mutation is localized and that the neighboring sequences are identical to wild type.

Another possible method to localize the DNA sequences involved in hormonal modulation is to compare the sequences of several α_{2u} genes. We have already found that at least two of eight genes tested do not respond to hormones when transfected into L cells. Interestingly, the rate of transcription from these variant genes is constitutively high. This is consistent with a model in which the DNA region responsible for hormonal control is a negative control element; i.e., these DNA sequences keep the α_{2u} gene off in the absence of hormones. The hormonal induction of α_{2u} globulin, then, is a derepression, rather than a positive induction.

Tissue distribution of α_{2u} *synthesis in vivo.* Until recently, α_{2u} was thought to be synthesized exclusively in the liver of adult male rats. Indeed, the liver is the major site of synthesis of this protein: In adult male rats, α_{2u} represents 1–2% of hepatic mRNA. In the liver, the level of α_{2u} mRNA is influenced by several hormones: Androgens, glucocorticoids, thyroid hormone, insulin, and growth homone all induce α_{2u} mRNA, and estrogens repress the level of α_{2u}. However, we have found that α_{2u} is synthesized in several other rat tissues: the salivary gland, the lachrymal gland, and the mammary glands of pregnant females. The hormonal modulation in each tissue is different, and different α_{2u} genes or gene sets are transcribed in the various tissues.

1. Salivary gland: α_{2u} protein and mRNA are found in the submaxillary glands of both male and female rats of any age. The synthesis seems to be constitutive, at a level $\sim 1/20$ of that in the liver. No hormonal modulation is evident.
2. Lachrymal gland: α_{2u} is also found in the extraorbital lachrymal glands of rats and is present in both males and females. The level seems to rise somewhat at puberty, and in adults, it reaches a level $\sim 1/5$ that found in the liver.
3. Mammary gland: α_{2u} mRNA and protein are found in day-17–19 pregnant rats. The level seems to peak sharply but never reaches a level greater than $\sim 1/50$ that found in the liver. The α_{2u} mRNA declines precipitously following day 19, and by parturition ($\sim$ day 22) it is essentially absent. The hormonal modulation of α_{2u} in the mammary gland is currently under investigation. The time course of its appearance and subsequent repression is consistent with a model in which it is being induced by progesterone and then shut off by prolactin.

The α_{2u} mRNA found in each of the tissues appears to be a single species of approximately 1 kb. 5′-end mapping indicates that the cap site is identical in all tissues. Analysis of the α_{2u} protein being made in the various tissues revealed that, on one-dimensional gels, the protein made in the liver, submaxillary gland, and the salivary gland is a homogeneous species with a molecular weight of

20,000, whereas the protein made in the mammary gland is slightly smaller (~19,000). Isofocusing gels (one dimensional), however, reveal clear differences in the α_{2u} proteins being made in the different tissues.

Liver α_{2u} is represented by four or five isoelectric species with pI values ranging from approximately 5.0 to 6.0. The α_{2u} produced by the submaxillary and lachrymal glands appears as three to four species with pI values of approximately 4.0–4.8. The mammary-gland pattern is more complex and appears to comprise most of the liver proteins and several more basic species. This finding is consistent with different α_{2u} gene sets being transcribed in different tissues. We are now in the process of identifying which α_{2u} genes code for which tissue-specific proteins. Eighteen independent α_{2u} genomic clones have now been isolated from a library of rat DNA cloned in Charon 4A. These genes have been transfected into L cells in tissue culture to assess their response to various hormones. These same cells can then be used essentially as a linked transcription-translation system to determine which α_{2u} isoelectric species each gene encodes. This is currently being performed using isofocusing followed by Western blotting.

Once specific cloned α_{2u} genes have been "assigned" to various tissues, it may be possible to determine the molecular basis for the tissue specificity of expression of the different genes by reintroducing these genes into primary cultures of rat cells or into immortalized or transformed analogs of these primary cultures.

Cell-cycle-modulated Gene Expression

J. Lewis, D. Matkovich

Since the early 1960s, evidence has accumulated demonstrating that the expression of various genes in mammalian cells cultured in vitro is strictly growth-phase-dependent. The enzymes thymidine kinase, thymidylate synthetase, and dihydrofolate reductase, for example, important to the biosynthesis of DNA nucleotide precursors, can be readily assayed in cell cultures growing in mid-log phase but are virtually undetectable in cultures resting in stationary phase. This growth-phase-dependent gene expression is presently understood to reflect the exclusive expression of these genes during the S phase of the mammalian cell-division cycle. Our laboratory is working to define the genetic determinants that govern this mode of differential gene expression, and as a first step toward the development of an experimental system, we have cloned the thymidine kinase (*tk*) gene from Chinese hamster ovary DNA in recombinant bacteriophage λ (Lewis et al., *Mol. Cell. Biol. 3:* 1815 [1983]). Our experimental plan is to search for such genetic determinants by variously modifying the hamster *tk* gene in vitro, transfecting such modified *tk* genes into mammalian Tk⁻ cells, and then studying the growth dependence of the modified *tk*-gene expression in Tk⁺-transformed cells. In this way, by defining the character and genetic location of the determinants of growth-dependent gene expression, we can begin to infer the physiological processes they govern.

As we have previously reported, restriction enzyme sensitivity experiments and Southern and Northern blot hybridizations indicate that the hamster *tk* gene extends over 10–12 kb, from the *Hpa*I site in the 3.2-kb *Eco*RI fragment of λHaTK-5 through the *Hin*dIII site in the 5.5-kb *Eco*RI fragment at the left-arm end of λHaTK-5 (Fig. 1). To define the structure of this gene more precisely, we have isolated a nearly full sequence (1250 nucleotides) of double-stranded cDNA to hamster *tk* mRNA from a cDNA library prepared from poly(A)⁺ RNA from the Chinese hamster A-29 cell line. On the basis of preliminary S1 analysis, this double-stranded cDNA lacks at its 5′ end approximately 30 nucleotides of the hamster *tk* mRNA. Various subclones of this cDNA have been prepared in M13 and have been sequenced using the Sanger-Coulson dideoxynucleotide method. The cDNA sequence information, in conjunction with sequencing data derived from M13 subclones of the hamster *tk* gene, have permitted us to define the structure of the hamster *tk* gene. In brief, the mature hamster *tk* mRNA sequences are organized into

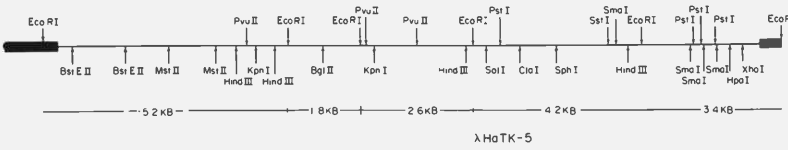


Figure 1

Restriction enzyme map of λHaTK-5. The Chinese hamster *tk* gene extends over 11 kb in the 17-kb recombinant insert in λHaTK-5, from the *Hpa*I site in the rightmost 3.4-kb *Eco*RI fragment through the leftmost *Hin*dIII site in the 5.2-kb *Eco*RI fragment.

at least seven exon blocks (distributed over 11 kb of genomic DNA) separated by intronic sequences that range in size from 100 to 5400 nucleotides. We are not yet certain whether the *tk* mRNA sequences that are not represented in our cDNA are contained in the genome as a continuous part of exon block (I). Nonetheless, the information we now have available makes it possible for us to undertake construction of the first modified hamster *tk* gene.

The genetic determinants of growth-dependent *tk*-gene expression might reside within 5′ or 3′ sequences flanking the gene, within its five introns, or conceivably even within *tk* coding sequences. Our experimental strategy, therefore, is to reduce the size and complexity of the hamster *tk* gene by first constructing a minimal hamster *tk* gene that depends on the hamster *tk*-gene promoter to direct transcription of the hamster *tk* cDNA and then testing the extent to which the expression of this minimal gene is growth-phase-dependent. We have constructed the prototype of a hamster minimal *tk* gene designated pHaTK·1 (Fig. 2) and have demonstrated that it is active in transforming a variety of Tk^- mammalian cell lines to the Tk^+ phenotype. This gene, however, still embodies some 1000 nucleotides to the 5′ side of the hamster *tk*-gene promoter, which we have tentatively mapped, on the basis of restriction enzyme sensitivity data and genomic nucleotide sequencing, to lie 200 nucleotides to the 3′ side of the *Hpa*I site. We are presently generating deletion derivatives of pHaTK·1, using the technique of Exo III/S_1 mutagenesis, to prepare a hamster *tk* gene of approximately 1400 nucleotides, of which 1250 nucleotides are derived from the double-stranded cDNA and 150 are derived from the promoter and its most proximal nucleotides. Should the expression of this gene be growth-phase-dependent, we will have quickly narrowed

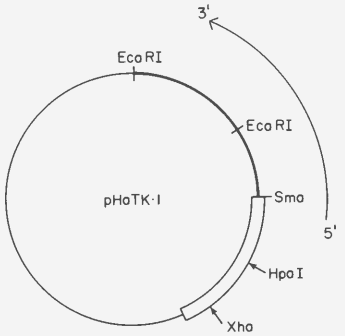


Figure 2
Structure of a minimal hamster *tk* gene. pHaTK·1 was constructed by fusing the hamster *tk*-gene promoter lying between the *Sma*I and *Xho*I sites (open box) to a 1180-bp *Eco*RI-*Sma*I fragment of the hamster *tk* cDNA (solid line). pHaTK·1 transforms a variety of mammalian Tk^- cells to the Tk^+ phenotype. Cells transformed with this construction are currently being studied for the growth dependence of their *tk* expression.

the search for the gene sequences that control growth-phase-dependent expression. Should the expression of this gene prove to be growth-phase-independent, as is true for the herpesvirus *tk* gene in Tk^+-transformed mammalian cells, we intend to modify our minimal *tk* gene further by adding to it either 5′ or 3′ flanking segments from the genomic *tk* clone or intron blocks that necessitate splicing of the primary *tk* RNA transcript. From a full analysis of this sort, we expect to provide insights into the mode of growth-phase-dependent *tk*-gene expression.

YEASTS

The year 1983 marked a new stage in the development of yeast genetics and molecular biology at Cold Spring Harbor Laboratory. The addition of David Beach and Mark Zoller and their research groups to the laboratory staff has broadened the scope of research to include regulation of the cell-division cycle and the application of site-directed mutagenesis to the study of DNA-protein interactions in yeast. In addition, the startling observation that proteins similar to mammalian cancer genes occur naturally in yeast and may play functionally similar roles in the control of the cell cycle (DeFeo-Jones et al., *Nature 306:* 707 [1983]; Powers et al., *Cell 36:* 607 [1984]; Lorincz and Reed, *Nature 307:* 183 [1984]) has led the group headed by M. Wigler (Oncogene Section) to focus new efforts on yeast as a model for cancer biology and has spawned the study of "yeast oncogenes."

These developments, along with the long-standing research program on cell-type switching in yeast and S. Bonitz' studies on mitochondrial RNA processing, have established yeast genetics as a major research area of the laboratory, with three research groups in the Delbrück Laboratory building and two groups in the Demerec building. This parallels the increased interest in yeast as an experimental organism world wide.

Mechanism of Cassette Transposition

J.N. Strathern, A.J.S. Klar, J.B. Hicks, M. Kelly, C. McGill, L. Miglio, R. Kostriken

Homothallic strains of the budding yeast *Saccharomyces cerevisiae* change from one mating type to the other by a mechanism that involves specific rearrangements of the genome. Copies of unexpressed regulatory genes are transposed from storage sites to an expression site. This is called the cassette mechanism of gene activation. The sequence present at the expression site, *MAT*, controls the mating type of the cell. Elsewhere in this report, experiments bearing on the mechanism by which the unexpressed copies are kept repressed are presented. Here, we describe recent experiments designed to determine the mechanism by which this site-specific transposition/substitution process occurs.

Several years ago, we cloned both alleles of the expression locus, *MAT***a** and *MAT*α, and both of the silent loci that serve as donors of the *MAT* cassettes, *HML* and *HMR*. These genes have been sequenced, and hence we have a complete end-product description of the cassette switching event (Fig. 1). The *MAT***a** and *MAT*α alleles differ by substitutions Y**a** and Yα, respectively. There are regions of homology (W, X, Z1, Z2) between the donor and recipient loci on each side of the Y sequences that one might expect have roles in the initiation and resolution of the switching intermediate. Several loci whose products might have catalytic roles in the switching process have been identified (*HO RAD52 SWI*). We are now in a position to examine a specific gene conversion event that is efficient, reproducible, and genetically regulated.

Over the past few years, we have shown that homothallic switching is initiated by a site-specific, double-strand cut in the recipient locus, *MAT*, in the Z region near the cell-type-specific Y sequences. This observation is in contrast to gene conversion models positing a nick in the donor locus. An enzyme that makes this cut (YZ endo) has been identified in homothallic cells. Variants of the *MAT* locus that have changes in this site are not able to switch. The Z1 region of homology between *MAT* and *HMR* is normally 230 bp. We made several deletions of the right side of Z1 but found that the rate of switching was not reduced when that homology was reduced to less than 100 bases. The results did suggest that aberrant events leading to fusion of *MAT* and the donor loci were elevated in these strains.

Several different approaches have been employed to determine whether sites essential to the switching reaction exist in the W and X regions of *MAT*. In one set of experiments, a variety of deletions throughout this region were tested for ability to switch. This approach involved having the recipient *MAT* locus on a plasmid. No essential sites were identified using this assay. Any homology between the donor loci and the recipient was sufficient to allow completion of the switching process. A second set of experiments involved making restriction-enzyme-site polymorphisms between the X region

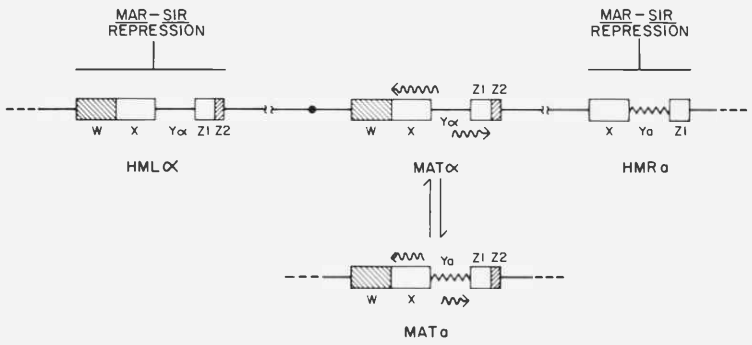


Figure 1
Diagram of the mating-type cassettes on chromosome III. W, X, Y, Z1, and Z2 represent regions defined by homology between *MAT* and the *HML* or *HMR* loci. The W region is 723 bases in length and is found at *MAT* and *HML*; the X region is 704 bases in length and is found at *MAT*, *HML*, and *HMR*; *MAT*, *HML*, and *HMR* can have either the **a**-specific sequence, Y**a** (642 bases), or the α-specific sequence, Yα (747 bases); Z1 is 239 bases in length and is found at *MAT*, *HML*, and *HMR*; finally, Z2 is 88 bases in length and is found only at *MAT* and *HML*. The *HML* and *HMR* copies of the mating-type genes are normally kept silent by the *MAR* and *SIR* genes. Interconversion of *MAT***a** and *MAT*α involves a unidirectional transposition-substitution event from *HML* or *HMR* to *MAT* and is regulated and/or catalyzed by the *HO*-gene product.

of the donor *HMR* and the X region of the *MAT* locus. These sequence differences could then be used to monitor the extent of the sequences transposed into *MAT*. In other words, when the Y region was switched, how much of the X region was also changed? We observed that the switched *MAT* loci sometimes had the X-region polymorphism of the original *MAT* and at other times had the sequence from the donor. These results suggested that the extent of the sequence transposed from *HMR* is variable. We are currently testing several polymorphisms throughout the X region to see if there is a gradient of switching that decreases with distance from the Y region.

This year, we have concentrated on the resolution step of recombination. *MAT* transposition is formally equivalent to the genetic process of gene conversion. Gene conversions studied at innumerable other loci are known to be associated with recombination of flanking markers in about 50% of the cases. This association is explained by the suggestion that the four-strand-stage Holliday structure freely isomerizes at the time of resolution. *MAT* transposition conversions, however, are not associated with crossing over. Quite interestingly, our recent results have shown that efficient recombination of flanking markers does occur, but during *MAT* to *MAT* interchromosomal gene transposition; i.e., crossing over is observed when transpositions occur between allelic sites (*MAT* to *MAT* between homologs) but not when transpositions occur from *HMR* to *MAT* (in both inter- and intrachromosomal transpositions). This constraint of lack of crossing over in the latter case is not regulated by the *MAR/SIR* control operative for keeping the *HM* loci unexpressed and unswitched. Either some other property of the system restricts resolution without the associated crossover or the extent of sequence homology between interacting segments may be limiting for isomerization.

Regulation of the *HML* and *HMR* Genes

J.B. Hicks, J.N. Strathern, A.J.S. Klar, J. Abraham, J. Ivy, S. Weisbrod, G. Livi, C. Stephens, J. Wood

The two major goals of this project group in 1983 were to characterize further the *MAR* and *SIR* genes and gene products and to study the mechanism of action of the *cis*-acting control sites at *HML* and *HMR*, known as E and I.

The four *MAR* and *SIR* genes were identified by mutations, unlinked to each other or any of the mating-type cassettes, which caused the silent cassettes at *HML* and *HMR* to be expressed. Formally, then, these genes are presumed to encode proteins that act in concert as repressors of *HML* and *HMR*. The site of *MAR/SIR* action is presumed to be the same at both *HML* and *HMR*, since all known *mar*$^-$ or *sir*$^-$ mutations cause coordinate expression of *both* *HML* and *HMR*.

The mechanism by which the *MAR/SIR* genes control the silent cassettes is especially intriguing because of the structure of the cassettes themselves. When expressed at the *MAT* locus, both **a** and α cassettes produce two transcripts divergently from a central promoter region. The DNA sequence surrounding the promoter is identical at all

three locations, yet transcription is prevented at *HMR* and *HML*. The sites of negative regulation must therefore be located outside the silent cassettes, at the 3′ termini of the transcripts, rather than at the 5′ end as in most regulated genes. During the past 2 years, we have located the negative control sites by in vitro mutagenesis and have developed the working hypothesis that the *MAR/SIR* genes act through these sites to "turn off" transcription between them (Fig. 2).

As a test of this hypothesis, we have placed a gene for a biosynthetic enzyme not normally under *MAR/SIR* control between the E and I control sites and reintroduced the hybrid constructions into the genome. Transcription assays have shown that the yeast *HIS3* gene, complete with its normal upstream regulatory sequences, can be effectively turned off by the *MAR/SIR* system, rendering cells lacking a normal *HIS3* allele auxotrophic for histidine in Mar$^+$ cells but His$^+$ when the *MAR/SIR* genes are missing. Thus, *MAR/SIR* control is not specific to the **a** or α promoters but represents a general mechanism for negative regulation of a "domain" on the chromosome as diagramed in Figure 2. Such a mechanism might be employed in more complex eukaryotic organisms to regulate "banks" of genes during development.

The individual *MAR* and *SIR* genes have been cloned by complementation in yeast, and the cloned segments have been used to test several possible roles for these genes in the control of *HMR* and *HML*. Because the E and I sites at the two storage loci share a maximum of 10 bp of sequence homology—less than would be required to bind four separate repressor proteins—it was possible that the four genes represented a sequentially regulated cascade culminating in the production of a single repressor. Transcription studies have shown, however, that each of the four genes is transcribed even in the absence of function of the other three. Conversely, we have shown that overexpression of the *SIR3* gene can complement several different mutations in the *SIR4* locus, indicating that a more complex protein-protein interaction may be involved. In additional studies, we have used the cloned *SIR1* and *SIR4* genes to map the positions of these previously unmapped genes to chromosomes XI and IX, respectively.

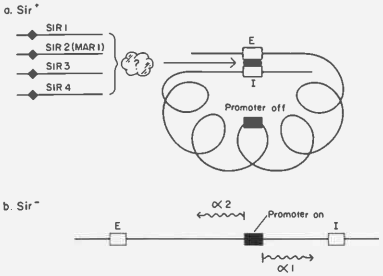


Figure 2
Schematic diagram of the control sites flanking the *HML* and *HMR* storage cassettes. The E site is essential for control. Deletion of this site causes full constitutive expression of the cassette. Deletion of the I (Important) site on the right side allows only partial expression. E and I are presumed to be sites of interaction with the *SIR* (*S*ilent *I*nformation *R*egulator) proteins (*SIR1*, *2*, *3*, *4*). Interaction of the *SIR*-gene products in an as yet unspecified way is believed to cause a change in chromatin structure in the domain between E and I, preventing access of RNA polymerase to the promoter.

Schizosaccharomyces pombe Mating-type Switching

A.J.S. Klar, D. Beach

The fission yeast, *Schizosaccharomyces pombe*, also switches efficiently between h^+ (Plus) and h^- (Minus) mating types. Last year, we reported that mating-type switching in this yeast, as in *Saccharomyces cerevisiae*, also involves transposition of "cassettes" from the silent *MAT2-P* (containing the Plus element) and *MAT3-M* (Minus element) loci into the expressed *MAT1* locus to generate alternate alleles of *MAT1-P* and *MAT1-M* in the homothallic h^{90} strains. These strains spontaneously generate quite stable *Plus* and *Minus* cultures. We have now shown that such "heterothallic" cells result from aberrant transposition events. h^{+N} results from transposition of *MAT2* and *MAT3* cassettes (along with the intervening sequence) into *MAT1*. The proximal cassette with the *P* element confers *Plus* mating type. This cassette switches infrequently to generate h^{-U} phenotype. At low frequencies, h^{+N} and h^{-U} can "revert" back to h^{90} by removing both cassettes from *MAT1* and thus restoring the original h^{90} configuration (Fig. 3).

As in *S. cerevisiae*, switching proceeds through formation of a double-strand cleavage at the *MAT1* locus (see Fig. 3). In collaboration with R. Egel (University of Copenhagen), we have conducted studies on mutants that are defective for switching. By genetic studies, over 50 mutants have been assigned to one of ten loci. One class of mutants consists of *cis*-acting mutations at *MAT1* that reduce the amount of the double-strand cut at *MAT1*. The remaining nine groups are mutations in genes that are not linked to *MAT*. Three genes are required for formation of the double-strand cut. Mutations of three other genes seem to be defective in proper resolution of switching intermediates. The remaining three have normal levels of cut, do not make errors of resolution, and are possibly required either for efficient utilization of the cut or for determining the directionality of switching.

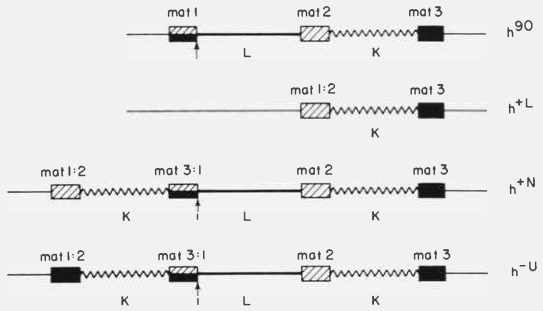


Figure 3
Diagram of the structure of the mating-type locus in different strains. Each 1.3-kb cassette is marked as a box, separated by the L and K regions, each approximately 15 kb in length. The arrow marks the cut site of the double-stranded DNA.

The mating-type cassettes have recently been sequenced in the laboratory of M. Smith (University of Vancouver). Future work in this system will consist of identification of the role of each of the mating-type gene products and also further characterization of the mechanisms of switching.

Cell-cycle Control in Fission Yeast

D. Beach, S. Silbiger [in collaboration with P. Nurse, University of Sussex]

The cell cycle of fission yeast is controlled at two points: one in G_1 referred to as "start" and the other in G_2. Genes in which mutations cause no defect in general cell metabolism but arrest cell-cycle progression are called *cdc* genes. *cdc2* is one such gene, and it has been found that its product is required both in G_1 at start and in G_2. The percentage of cells caught in the G_1 or G_2 block depends entirely on the age distribution of the cells at the time of shift up to the restrictive temperature for *cdc2*.

The *cdc2* gene was previously isolated, and by cross-complementation experiments it was shown that this gene of fission yeast and the *cdc28* gene of budding yeast are functionally equivalent. Sequencing of both genes reveals that they are evolutionary homologs, which are also related to the tyrosine kinase family of mammalian oncogenes. The *cdc2* gene contains at least four introns. Our present work consists of isolating a cDNA clone of the *cdc2* gene so that the gene product can be expressed in *Escherichia coli*. The protein expressed in *E. coli* will be used for raising antibodies against the protein and to establish whether it is a protein kinase.

During the isolation of *cdc2*, a second sequence was found that is capable, when present on an autonomously replicating plasmid, of rescuing certain alleles of *cdc2*. This sequence integrates at a locus unlinked to *cdc2*. The gene, *suc1*, has been subjected to "gene destruction" by insertion of a yeast selectable marker, *leu2*, into a transcribed region. When this construction is integrated into the chromosome in a manner that displaces the original copy of the gene, the cell is inviable. Spores carrying this mutation germinate and undergo between one and three cell divisions before dying. The dead cells do not have an obvious *cdc* phenotype. The relationship between *cdc2* and *suc1* will be the subject of further investigation.

Regulation of Yeast Mitochondrial Gene Expression

S. Bonitz, C. Moomaw

Gene expression in yeast mitochondria is regulated by proteins made from yeast nuclear genes. To determine which nuclear genes regulate mitochondrial gene expression, a set of nuclear mutants that affect mitochondrial functions have been isolated by A. Tzagoloff at Columbia University. Over 200 different nuclear genes regulate various mitochondrial functions, such as RNA splicing and mitochondrial protein synthesis. Many of these nuclear genes regulate the gene expression of one of the mitochondrial proteins, cytochrome oxidase.

Cytochrome oxidase is a protein required for the synthesis of adenosine triphosphate. It contains seven subunits, four made from nuclear DNA and three made from mitochondrial DNA. The three largest subunits, subunits 1, 2, and 3, are made from mitochondrial DNA. The gene encoding the subunit-1 protein is a complex gene coding for a protein with a molecular weight of 56,000. DNA sequence analysis has revealed that the subunit-1 gene is a split gene containing 10,000 nucleotides. The

subunit-1 coding sequence consists of six to eight exons separated by five large introns. The entire gene is transcribed, and the introns are removed in several steps to generate the subunit-1 mRNA. The mRNA is then translated to give the subunit-1 protein. Therefore, the possibility exists that the expression of the subunit-1 gene can be regulated at either of three different levels: transcription, splicing, or translation.

To determine how the expression of the subunit-1 gene is regulated, we are studying the set of nuclear mutants that control the synthesis of subunit 1 of cytochrome oxidase. Of the 200 different nuclear genes affecting mitochondrial functions, 10–15 different nuclear genes specifically affect the subunit-1 gene. These mutants lack only the subunit-1 protein of cytochrome oxidase and contain all other mitochondrial proteins. To characterize these mutants at a more detailed level, we have been studying the mitochondrial RNA from these mutants. Mitochondria were isolated and lysed to release their RNA. This RNA was separated by size on an agarose gel and then transferred to DBM paper. Using a radioactively labeled exon fragment, the RNA was probed to determine whether any subunit-1 mRNA was present. RNA prepared from wild-type mitochondria contained the subunit-1 mRNA. However, several nuclear mutants contained no mRNA but instead had some of the higher-molecular-weight, subunit-1 RNA precursors. These mutants may be defective in RNA splicing. Other mutants have the subunit-1 mRNA but no subunit-1 protein. Therefore, it appears that there may be several different nuclear genes that control expression of the subunit-1 gene of cytochrome oxidase. Studies with the nuclear mutants indicate that regulation by these nuclear genes may occur at the level of mitochondrial splicing or translation. Further characterization at the mitochondrial RNA level is continuing to determine exactly how the nuclear gene products are affecting subunit-1 gene expression.

Point Mutations in *MAT*a That Affect the Function of the *HO* Endonuclease

M. Zoller, R. Kostriken, K. Johnson

This is a new laboratory that began operation in November, 1983. We are studying the interaction between the *HO* endonuclease and *MAT* DNA in the yeast *Saccharomyces cerevisiae*. Our aim is to understand the role this enzyme plays in the transposition of mating-type DNA. In addition, we are interested in the general problem of the means by which a protein recognizes a specific sequence of DNA. The *HO* endonuclease is required for efficient transposition of mating-type DNA from an unexpressed locus to *MAT*, the expressed locus. The mechanism by which this occurs is not fully understood. It is believed that the *HO* endonuclease initiates the event by creating a double-strand break in the chromosome at *MAT*. R. Kostriken, who recently joined the laboratory as a postdoctoral fellow, has demonstrated endonuclease activity in yeast extracts from *HO* strains and has determined the specific nucleotides that are cleaved in *MAT* DNA. This work was done with F. Heffron in collaboration with the Yeast group at Cold Spring Harbor. Kostriken is currently purifying the *HO* endonuclease from a strain of *Escherichia coli* that has been engineered to express the *HO*-gene product. This construction demonstrated that the *HO* gene encodes the endonuclease.

The current effort in the laboratory stemmed from the characterization of nonswitching strains of yeast (*in*convertible mutations). DNA sequence analysis indicated that these strains contained point changes within the *HO* recognition sequence at *MAT*. To understand the specific determinants by which the *HO* endonuclease recognizes the cut site, we are analyzing the effects of point changes at other positions within this region.

Seventeen single-point changes have been constructed in a clone of *MAT*a using oligonucleotide-directed mutagenesis. Wild type and each mutant substrate were analyzed by an in vitro cutting assay using extracts containing the *HO* endonuclease. The results indicate that the major determinant of the *HO* recognition sequence is a 13-bp region that spans the YZ junction (see Fig. 4). *MAT* DNAs with alterations at positions Z1, Z2, Z3, or Z6 are completely resistant to cleavage by the *HO* endonuclease. Substrates with base-pair changes at other positions within this region are cleaved at slower rates compared with wild type. By using *HO* endonuclease purified by Kostriken, we hope to identify which mutations affect binding and which affect catalysis. In addition, we plan to conduct footprinting experiments in order to identify major- and minor-groove interactions.

The second phase of these experiments will be to analyze the effects of these mutations on in vivo switching. Each mutant will be integrated into the yeast genome and assessed for the ability to serve

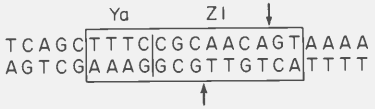


Figure 4
Sequence of the *HO* endonuclease cut site at the Y-Z junction of the mating-type cassette (see Fig. 1). The enclosed base pairs represent the extent of the recognition sequence as determined by site-directed mutagenesis. Arrows denote the actual cleavage sites, generating a 4-bp 3′ overhang.

as either a donor or a recipient of a switch. In strains that carry the mutations at *MAT*, we predict that the frequency of switching will be correlated with the efficiency of cutting. Furthermore, we predict that the mutations will have no effect on the ability of the cassette to serve as a switch donor. Mutants that are unable to switch will be used to obtain revertants. Some may be in the cut site and others may be in the *HO* endonuclease gene, thus encoding an enzyme that recognizes and cuts the altered recognition sequence. The latter mutations will shed light on the amino acids that function in specific protein/DNA interactions. These experiments are currently in progress.

PLANTS

Maize Transposable Elements

S.L. Dellaporta, P.S. Chomet, J.B. Hicks [in collaboration with J.P. Mottinger, University of Rhode Island]

Genetic studies in maize have shown that progeny from plants that are systemically infected with virus exhibit genetic instabilities, such as chromosomal breakage and high rates of spontaneous mutation, even in the absence of the inciting virus for many generations postinfection. We have been investigating the molecular basis for this aberrant genetic behavior in spontaneous mutations at the *Shrunken* (*Sh1*) locus originally isolated by J. Mottinger from maize lines exposed to systemic viral infection. In work reported last year, we determined that several of these mutations resulted from genomic rearrangements, probably insertions of transposable elements. Genomic blotting data establishing the positions of the rearrangements are shown in Figure 1.

Recently, our efforts have been focused on the molecular characterization of the *sh-5586* and *sh-5584* mutations for several reasons: The *sh-5586* mutation mapped at a position near the 3′ end of the *shrunken* gene, and in F_3 and subsequent populations, numerous phenotypic reversion events have been isolated. The *sh-5584* mutant has the most interesting genetic behavior; it appears to exhibit a dosage effect due to reduced levels of the *shrunken*-gene product sucrose synthase (J.P. Mottinger, pers. comm.).

We have cloned the *sh-5586* allele and its Sh^B progenitor using standard genomic library construction in bacteriophage vectors. Restriction mapping data demonstrated that the alleles are identical, with the exception of a 3.6-kb insertion in *sh-5586*. This insertion maps to the wild-type 0.7-kb *Hin*dIII fragment located in the 3′ region of the *shrunken* gene. This region was sequenced from both Sh^B and *sh-5586*, indicating that the *sh-5586* element, which we refer to as Tz86, is integrated

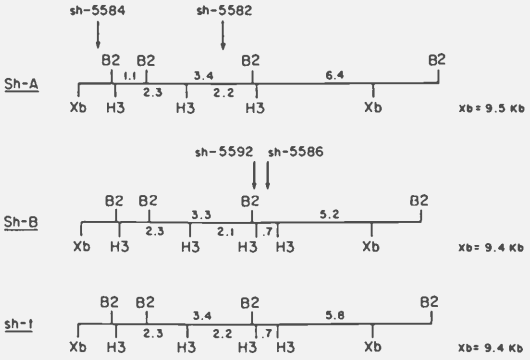


Figure 1

Restriction mapping data obtained from genomic blot analysis of the dominant *Sh* isoalleles (Sh^A and Sh^B and the *sh* tester allele (*sh′*) used to generate the *sh* mutants described in this study. Positions of four of the seven *sh* mutants are indicated by arrows in the appropriate progenitor allele. The *Sh* transcriptional unit begins close to the position of the *sh-5584* insertion (>3.5 kb) and ends within the 700-bp *Hin*dIII fragment of the Sh^B allele. The *sh-5586* insertion (3.6 kb) is 5′ to the end of *Sh* transcription (see Fig. 2). *sh-5582* represents a 2.1-kb insertion within the Sh^A gene.

within a *Sh* exon (Fig. 2). Like mobile elements of other organisms including maize, the insertion in the *sh-5586* mutation has caused a duplication of host DNA at the site of insertion. In this case, a 10-bp duplication of the sequence GAGGCTGATG is present as a direct repeat at the termini of Tz86 and only once in the wild-type gene. However, unlike most transposable elements, this transposon does not contain simple inverted or direct repeats immediately adjacent to the insertion site. Instead, a complex series of perfect and imperfect inverted and direct repeats comprise the terminal regions. This structural feature has also been identified recently in the maize controlling elements *Ac*, *Ds* (J. Messing, pers. comm.), and the *Antirrhinum* transposon Tam1 (H. Saedler, unpubl.). Complex stem-and-loop secondary structures developed from these regions may be important in controlling element function.

We have also observed that the transposition ability of the *sh-5586* element remains active for the three generations we have studied this mutant. In genetic studies of advanced progeny of the *sh-5586* mutant, we have isolated phenotypic revertants (Sh^R) to the wild-type *Sh* phenotype. Genomic blot analysis of the Sh^R DNAs indicated that in one revertant the element has excised and in another phenotypic revertant a secondary rearrangement has occurred without loss of the element at *shrunken*.

Gel-blot analysis of maize genomic DNA has shown that the Tz86 element represents a repetitive family of heterogeneously related sequences with different regions of the insertion represented at different levels ranging from about 5 to 50 copies per genome. It is important to note that the element is *not* homologous to the genome of barley stripe mosaic virus, the virus used to induce the original mutations. It is thus possible that increased transposition activity of endogenous maize elements is a response to the stress of viral infection. Tests of this hypothesis will be the subject of work in the coming year.

Cell and Developmental Genetics of Tobacco

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

We are interested in studying the regulation of the polyamine synthesis pathway in tobacco cells. The polyamines—putrescine, spermidine, and spermine—are small molecules with a high positive charge that play a variety of roles in the cell and in the whole organism. They have been implicated in processes as diverse as the cell cycle, hormone response, stress response, nucleic acid structure, and protein synthesis. Our hope is that, by collecting mutations altered in their synthesis, we will learn more about the role of polyamines in the cell and plant, in addition to studying the molecular genetic regulation of the pathway. A simplified diagram of the pathway is given in Figure 3.

One feature of this pathway that makes it suitable for biochemical and genetic analyses is the existence of specific inhibitors that block the action of the enzymes in the pathway, as shown in Figure 3. When applied to cell cultures of tobacco, these inhibitors cause the levels of the polyamines to fall; the cells become starved for the polyamines, stop dividing, and eventually die. This suggests a simple strategy for selection of mutants with altered polyamine synthesis: Start with wild-type tobacco cells, mutagenize, and then select for resistance to the inhibitor. These mutants would then be analyzed to determine the nature of the genetic change.

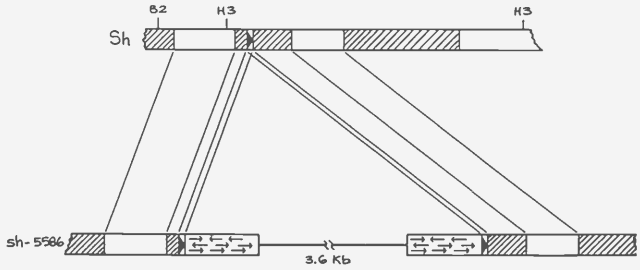


Figure 2

Insertion site of the *sh-5586* element (Tz86). The *sh-5586* insertion element was determined to be within a 700-bp *Hin*dIII fragment near the 3′ end of the Sh^R gene. Sequence analysis of the corresponding wild-type and mutant insertion regions indicated that the position of Tz86 was within *Sh* exon DNA (hatched rectangles). Integration of Tz86 caused a duplication of 10 bp of *Sh* DNA (solid arrowheads), which is found as a direct repeat at the termini of the element. A 200-bp region at each end of Tz86 is rich in small direct and inverted repeats (arrows), which have the potential for cruciform structures.

Figure 3
Polyamine synthesis pathway. (OrnDC) Ornithine decarboxylase; (SamDC) *S*-adenosylmethionine decarboxylase; (ArgDC) arginine decarboxylase; (DFMO) difluoromethylornithine; (DFMA) difluoromethylarginine; (MGBG) methylglyoxal *bis*(guanylhydrazone). OrnDC, ArgDC, and SamDC are enzymes in the pathway; DFMO, DFMA, and MGBG are specific inhibitors that block the action of the enzymes indicated.

The power of working with tobacco cell cultures is that the mutants can then be regenerated into whole plants. This allows us to perform conventional genetic crosses and also to examine the effects of a lesion in polyamine synthesis on the whole plant and its development.

We have isolated a variety of mutants that are either deficient in polyamine synthesis or resistant to the specific inhibitors. When these mutants are regenerated back into whole plants, we found the surprising result that many of the mutants had abnormal flowers. All were male sterile, some were female sterile, and most had altered morphologies, i.e., anthers apparently turned into petals, ovules turned into stamens, sepals turned into petals, and/or anthers turned into pistils in individual mutants.This suggests that polyamines are important in the normal development of the flower; however, a direct cause-and-effect relationship has not yet been demonstrated. Genetic tests to determine causality are currently in progress.

Our mutant collection has provided a molecular entry into the pathway. One of our mutants makes abnormally large amounts of the second enzyme in the pathway, *S*-adenosylmethionine decarboxylase. Also, we can induce wild-type cells to make an excess of the enzyme by treating them with methylglyoxal *bis*(guanylhydrazone) (MGBG). Recently, we have shown that in wild-type cells, MGBG causes an increase in abundance of the enzyme by protecting it from degradation, rather than by increasing its rate of synthesis. Our goal for future research is to use our mutant collection further to explore the regulation of polyamine synthesis and to clarify the effects of polyamines on flower development.

PROKARYOTES

Cloned Genes for the *Msp*I Restriction-Modification System

P.-M. Lin, R.J. Roberts

The *Msp*I restriction-modification system recognizes the sequence 5′-CCGG-3′ and is found in an organism originally characterized as a *Moraxella* species, although there is now reason to believe that this identification was incorrect. DNA from this organism has been cloned in a random fashion into the plasmid vector pUC9, and a clone has been isolated that expresses both the methylase and restriction endonuclease activities of the *Msp*I restriction system. This clone contains a 15-kb insert. Both plasmid DNA and host DNA from the clone have been shown to be resistant to *Msp*I cleavage in vitro, and the endonuclease has been partially purified from cell-free extracts of the clone.

A series of subclones of the original 15-kb insert have been prepared, and one of these, containing a 1.55-kb insert, still retains methylase activity. This insert has been sequenced and contains a single open reading frame that could code for a polypeptide of 418 amino acids (M_r = 47,664). This is the only large open reading frame present in the sequence and must therefore encode the *Msp* methylase. A series of specific deletions of this clone have been prepared. Removal of the aminoterminal sequences of the open reading frame destroys the ability of the clone to produce active methylase. One deletion, which removes 14 amino acids from the carboxyl terminus of the reading frame, still retains methylase activity. Immediately upstream of the first AUG in this reading frame is a sequence that closely resembles the Pribnow box found in *Escherichia coli* promoters. This is probably the natural promoter, since clones containing the 1.55-kb insert express the methylase gene irrespective of its orientation in pACYC184. Another subclone carries an insert of 3 kb and, like the parent clone, carries both the methylase and the restriction enzyme genes. The latter is currently being sequenced.

One interesting feature of plasmids carrying the *Msp*I methylase gene is that they show considerable variation in their ability to transform different *E. coli* strains. The original clones were isolated using the host *E. coli* RR1, in which plasmid propagation is normal. However, attempts to transfer the plasmid into the *E. coli* strains MM294, JM107, or DHI lead to extremely low transformation efficiencies. This suggests that the expression of this gene in an inappropriate genetic background can severely disrupt normal cell metabolism. A similar effect has been observed with the *Bsu*RI methylase gene (see A. Kiss, this section)

*Eco*RII Restriction-Modification Genes and *dcm* Gene

A. Bhagwat

N3, a 60-kb natural plasmid of *Escherichia coli*, codes for the *Eco*RII restriction-modification enzymes. The enzymes recognize the sequence 5′-CC(A/T)GG-3′; the endonuclease breaks the DNA before the first C residue unless the methylase has methylated the second C residue. The genes that code for these enzymes have been cloned from N3 into the plasmids pBR322 and pACYC184. A 5.8-kb fragment of N3 contains all the information required to express these proteins.

Random insertions of the transposon Tn*3* into the pACYC clone and a series of deletions constructed in these plasmids have helped narrow the coding region to about 2.8 kb. Of this, no more than 350 bp is likely to contain the noncoding space between the two genes. A *Stu*I site within this region may lie within the methylase promoter. Tn*3* insertions, 350 bp upstream of this site, do not significantly affect the methylase, but the insertion of an 8-bp *Pst*I linker at the *Stu*I site gives only partial methylase expression. This and other data suggest that the two genes are transcribed from the central noncoding region in divergent directions.

The product of the endonuclease gene does not appear to be required for the methylase expression, and vice versa. Tn*3* insertions in the putative endonuclease gene have the phenotype R^-M^+. A set of eight clones containing Tn*3* insertions spanning a 1.3-kb region can survive only when a functional *Eco*RII methylase gene exists on another plasmid in the same cell. This suggests that these insertions lie within the methylase gene and that the continued

synthesis of the endonuclease in these cells threatens their survival unless a functional methylase is provided by a complementing plasmid. A further conclusion from these data is that the functional *dcm* gene within the chromosome of these cells is unable to complement the Tn*3* insertions in the *Eco*RII methylase gene. The product of *dcm* has been reported to have the same specificity as the *Eco*RII methylase (Schlagman et al., *J. Bacteriol. 126:* 990 [1976]). This may mean that either (1) the amount or the efficiency of the *dcm* product in the cell is much less than that of the *Eco*RII methylase or (2) the methylation specificities of the two enzymes are not identical.

The *Bacillus subtilis* R Restriction-Modification System

A. Kiss

The enzymes of the restriction-modification system of *Bacillus subtilis* R recognize the sequence 5′-GGCC-3′. The endonuclease cleaves between the G and C residues to produce blunt-ended fragments, whereas the modification enzyme methylates the internal C residue. I began studies of this system while at the Biological Research Center of the Hungarian Academy of Sciences, and these studies resulted in the cloning of the *Bsu*RI methylase gene. *Escherichia coli* clones carrying this gene expressed the *Bsu*RI methylase but did not express the *Bsu*RI endonuclease (Kiss and Baldauf, *Gene 21:* 111 [1983]). The methylase gene was located close to one end of the cloned segment, and one possibility was that the endonuclease gene was wholly, or partially, on an adjacent fragment, since genetic data from T. Trautner's laboratory (Max Planck Institute) indicated that the two genes were linked.

The cloned methylase gene was used as a hybridization probe, in Southern experiments, to identify fragments of *B. subtilis* R DNA that would carry the methylase gene and would extend into the neighboring sequences. One such fragment was a 9.5-kb *Sal*I-*Sph*I fragment. This fragment was cloned into pBR322, using *E. coli* RR1 as the host, by in vitro selection for the presence of the methylase gene. Both the recombinant plasmid and the chromosomal DNA from the clone were resistant to *Hae*III digestion (*Hae*III is an isoschizomer of *Bsu*RI), indicating the presence and expression of the *Bsu*RI methylase gene. Cell-free extracts from the clone were prepared and partially purified by Bio-Gel A0.5m chromatography. Fractions were tested for the presence of the *Bsu*RI endonuclease and were found to be positive. Preliminary estimates suggest that the amounts of endonuclease are comparable with those found in the parent strain. In vivo restriction-modification by the clone was demonstrated using bacteriophage λ. The degree of restriction was between 10^{-3} and 10^{-4}. The endonuclease and the methylase genes were further localized to a 4.3-kb *Hpa*I-*Sal*I fragment, and a detailed restriction map of the DNA region coding for the *Bsu*RI enzymes was established. The two genes are less than 0.5 kb apart.

The attraction of the *Bsu*RI enzymes as a model system for the study of sequence-specific DNA-protein interaction is that genes of three other modification methylases, recognizing the sequence GGCC, have also been cloned. These are M.*Bsp*RI, M.SPR, and M.*Hae*III. Comparison of the primary sequence of these enzymes is likely to give information about the structural determinants of this type of specific DNA recognition.

During these studies we found that the related *E. coli* strains, RR1 and HB101, can be transformed easily with the plasmid carrying the *Bsu*RI genes. In contrast, several other *E. coli* strains (DH1, GM272, GM161) cannot be transformed, even with plasmids carrying only the methylase gene. Plasmids coding for other cloned GGCC-specific methylases (M.*Bsp*RI, M.SPR, and M.*Hae*III) showed a similar pattern of transformation. We have shown that this phenomenon is related to the methylase function and its cause is being examined.

Transposase Recognition Sites

A.I. Bukhari

The temperate bacteriophage Mu is a very efficient transposon and yet the sequences at its ends are not the typical inverted repeats of most prokaryotic transposons. To gain more insight into the function of these terminal sequences, we have determined the nucleotide sequences at the left end of bacteriophages D108 and Mu. The rationale for this experiment is that D108 and Mu are essentially identical viruses, both in sequence and in mechanism of transposition. However, as shown by Toussaint et al. (*Mol. Gen. Genet. 190:* 70 [1983]), the *A*-gene product (the transposase) of Mu specifically acts at its own left end and does not recognize the D108 left end. Similarly, the D108 transposase is specific to its own left end. This difference in specificity must reflect a difference in the nucleotide sequences at the left ends of Mu and D108 and must signify at least one site that is recognized by the respective transposases. The left-end 1-kb *Hin*dIII fragment of Mu and the left-end 1.5-kb *Eco*RI fragment of D108 were purified and cloned into M13 vectors for dideoxy sequencing. The fragments were positioned so that extension of the primer yielded the sequence of the host segment attached to the left end, followed by the specific phage sequence. Sequencing of several independent clones

has shown the following: (1) The first 53 bp in Mu and D108 are identical. At that point the 8-bp pair sequence GATCTGAT in Mu is replaced by TATTTGGC in D108. (2) The sequences between nucleotides 61 and 200 are substantially the same with only scattered base changes. (3) The host sequences show some common features. For example, a sequence TTTGGCGG is found in several host segments. This sequence is also present in D108 and overlaps the 8-bp change between Mu and D108. A part of this sequence is present in Mu. (4) The first five nucleotides of the host sequence preceding the host-phage junction are generally, but not always, GC-rich.

These results suggest that the transposase recognizes a sequence at least 53 bp from the end of Mu and that it probably makes a large complex that covers the Mu-host junction during transposition.

Studies on the Regulation of the Bacteriophage Mu Transposition Functions

T. Patterson, K. Martin, R. Weiss, J. Gould, A.I. Bukhari

The regulation of the transposition functions of a transposable element is crucial to its persistence, since unchecked transposition would certainly result in the death of the host. Since transposition is an integral part of the Mu lytic cycle, the mode of regulation employed by Mu should share features in common with nontransposing bacteriophage as well as with nonviral transposable elements. Indeed, what has been learned thus far about Mu regulation is consistent with this notion.

The current picture of Mu regulation has been developed from a number of different experimental systems, many of which have involved the use of high-copy-number plasmids carrying cloned fragments of the Mu genome. We feel that the use of these plasmids may have precluded the formulation of a unified model of Mu regulation, consistent with most of the available evidence. For example, the involvement of host functions in Mu regulation could be swamped out by the increased gene-dosage effect when using high-copy-number plasmids.

We are taking a different, systematic approach to the study of Mu regulation that involves the use of fusions between the Mu genome and a *lacZ* gene located on a F′ *prolac* episome. Starting with an insertion of a temperature-inducible Mu*amp* prophage in the *lacI* region of an episome carrying fused *lacI* and *lacZ* genes, we have isolated 100 independent Lac$^+$ revertants that carry defective Mu prophage. We have been able to divide these revertants into four categories: (I) those in which *lacZ* expression is tightly regulated in *cis* by the temperature-sensitive Mu repressor, (II) those in which *lacZ* expression is loosely regulated in *cis* by the Mu repressor, (III) those in which *lacZ* expression is unaffected by temperature but is affected by host-strain mutations, and (IV) those in which *lacZ* expression is not influenced by the Mu repressor or any host mutations tested so far.

Since the fusions were isolated on an F′ episome, we can easily transfer them into strains carrying different alleles of host genes that may affect regulation, strains carrying different genetically marked Mu prophage, or strains carrying various plasmids. Thus far, we have transferred the Mu-*lacZ* fusion episomes into strains with mutations in the *dam* (deoxyadenine methylase) gene and the *himA* gene (encoding the α subunit of the integration host factor protein), into a strain carrying a wild-type Mu prophage, and into a strain carrying a low-copy-number mini-Mu plasmid. The resulting strains were tested for *lacZ* activity at high and low temperatures, and the following observations were made: (1) *lacZ* activity from category I fusions becomes noninducible by temperature in the presence of a wild-type Mu prophage, indicating that the fusions are subject to regulation in *trans*. These fusions are apparently unaffected by *himA* or *dam* mutations, but interestingly, some of them lose temperature regulation in the presence of the mini-Mu plasmid. (2) *lacZ* activity from category II fusions becomes noninducible in the presence of a wild-type Mu prophage and becomes tightly regulated in the presence of the mini-Mu plasmid. This suggests that the fusions retain the target sites necessary for regulation but do not synthesize sufficient or necessary regulatory molecules. These fusions appear to become more tightly regulated at low temperature in the presence of a *himA* mutation and are unaffected by *dam* mutations. (3) *lacZ* activity from category III fusions is unaffected by the presence of a wild-type Mu prophage or by the mini-Mu plasmid. However, these fusions become subject to temperature regulation in the presence of a *dam* mutation and exhibit increased but unregulated *lacZ* expression in the presence of a *himA* mutation. These studies are being extended by transferring the episomes to strains carrying the host mutations, prophage, or mini-Mu plasmids in various combinations.

In conjunction with the work described above, we have been approximating the extent of Mu DNA in the fusions by hybridization of DNA from the episome-bearing strains to plasmid DNA carrying defined restriction fragments of Mu. We have also been successful in moving some of the fusions onto a multicopy plasmid by in vivo recombination. This will allow us to define more precisely the fusion junctions through restriction mapping and/or DNA sequence analysis and to test the effects of gene dosage. Correlation of the location of the fusion end points with their phenotypic properties will pro-

vide valuable insight into the regulatory elements of Mu.

As a corollary to the studies employing the fusions isolated in vivo, we are creating fusions between Mu and *lacZ* in vitro on multicopy plasmids and are developing a method for moving the fusions into a chromosomal or episomal location. Using information gained from our experiments with the F′ Mu-*lacZ* fusions, we will be able to target specific regulatory regions of Mu for detailed study employing techniques of in vitro mutagenesis.

Synthetic Transposons

T. Patterson, A.I. Bukhari

The basic requirements for the movement of bacterial transposable elements are the ends of the elements, element-encoded transposition proteins that recognize the ends and catalyze transposition, and various functions provided by the host. With the goal of performing a detailed study of the role of the terminal sequences in the transposition process, we are constructing synthetic transposons in collaboration with S. Narang's group at the National Research Council, Canada. The synthetic transposons consist of chemically synthesized right- and left-end terminal sequences of a naturally occurring transposable element cloned into a plasmid. The ends are synthesized with a restriction enzyme recognition site in between to allow the in vitro insertion of a selectable marker, such as a drug resistance determinant. The presence of a selectable marker will allow us to monitor transposition of the synthetic transposon. Our initial efforts have been with the ends of two elements, the insertion element IS*5* and bacteriophage Mu.

The 16-bp inverted terminal repeats of IS*5* (with 1 bp difference) have been synthesized with a *Bgl*II restriction site in between. This oligonucleotide was inserted into the *Eco*RI site of pBR328, and the resulting plasmid was designated pSN162. We inserted a 1-kb kanamycin resistance determinant into the *Bgl*II restriction site in pSN162 but have been unable to detect transposition of kanamycin resistance mediated by the synthetic IS*5* ends. Since we are relying on IS*5*-specific transposition proteins synthesized from the 10–12 copies of IS*5* present in the *E. coli* chromosome to act efficiently in *trans*, it may be necessary to insert IS*5* (without its ends) into the plasmid carrying the synthetic transposon to provide the transposition proteins in *cis*. Alternatively, it may be necessary to increase the length of the synthetic sequences to include regions located outside the inverted repeats that may be required for transposition. Both of these approaches are being tried.

Although we have been unable to detect transposition of the IS*5* transposon, we have observed another activity of the synthetic IS*5* ends. Plasmid pBR328 carries an inverted duplication of a 482-bp segment that lies at the end of the tetracycline resistance gene. We have observed the low-frequency *recA*-independent appearance of a dimeric pBR328 molecule that has a structure consistent with having been formed by intermolecular recombination within the region of the inverted repeats. The plasmid carrying the synthetic IS*5* ends (pSN162) also generates this dimeric form. However, the proportion of pSN162 in the dimeric form after 30 generations of growth is almost tenfold higher than with pBR328. Our preliminary results indicate that this increase in the amount of dimeric pSN162 does not occur in a *recA*$^-$ strain of the closely related species, *Salmonella typhimurium*. Since *S. typhimurium* does not carry IS*5* in its chromosome, this suggests that the increase in dimer formation in *E. coli* is due to an IS*5*-encoded function acting on the synthetic ends present in pSN162. Experiments to test this hypothesis are in progress.

The construction of a plasmid analogous to pSN162, which will consist of the chemically synthesized right- and left-end sequences of bacteriophage Mu cloned into pUC9, is almost complete. A kanamycin resistance determinant will be inserted between the ends, and the transposition of the resulting mini-Mu will be tested. An advantage of the Mu system is that the production of Mu-specific transposition proteins can be triggered at will using a temperature-inducible prophage and that these functions will act efficiently in *trans*. Once transposition of the synthetic transposons is successful, we will perform site-specific mutagenesis on the ends to determine which nucleotides are important in the transposition process.

Visualization of the Mu Lytic Cycle

A.I. Bukhari, K. Martin

The structure of the bacterial chromosome throughout the lytic cycle of bacteriophage Mu is being studied using a technique that allows the rapid isolation of genetically active nucleic acid–protein complexes for analysis in the electron microscope. During the time required for one doubling of the *Escherichia coli* chromosome, the phage is able to increase from 1 copy to 100 copies. The replication of Mu is accompanied by its transposition within the chromosome. Analyses of structures associated with the chromosome's replication forks are being carried out in an attempt to observe directly the process of Mu DNA transposition. Preliminary experiments show that when cells are opened and prepared for microscopy immediately prior to lysis, viral particles are observed extruding from the cell. At this time point, the bulk of the chromosomal DNA appears to be degraded, but there are many

visible polysomes. The DNA that is observed appears to be significantly more supercoiled than the DNA from cells harvested less than 15 minutes after induction of the Mu lytic cycle. The significance, if any, of this finding awaits further analysis. A more surprising preliminary finding is the presence of significant numbers of circular molecules in cells harvested approximately 10 minutes prior to the time at which mature viruses are observed. These molecules are heterogeneous in size (range, 4–50 kb), and one putative circle displays a polysome gradient, suggesting the presence of an active transcription unit. One hypothesis is that these molecules arise as a result of the removal of Mu DNA from the bacterial genome during phage maturation. The circles would then be the result of a recircularization of the bacterial DNA present between two copies of the Mu DNA within the chromosome. This hypothesis is being tested by spreading cultures at earlier time points after the induction of Mu to determine whether larger circles that still contain Mu are observable prior to the appearance of the small, circular DNA molecules. Electron microscopic analysis of the structure of the chromosomal DNA–protein complex during the proliferation of Mu DNA should contribute to a better understanding of the mechanism of transposition and the action of the bacteriophage Mu proteins in the cell.

Shifty Ratchets: Molecular Mechanics of Protein Synthesis Revisited

R. Weiss

The physical mechanism of ribosome translocation has remained shrouded in mystery, inaccessible to experimental tests. Fourteen years ago, a potential solution to the problem of a plausible physical basis for the mechanics of translocation was proposed by C.R. Woese (University of Illinois). His model replaced the notion of phantom movements of the peptidyl-tRNA across the ribosome's surface with a simple, precise, and defined conformational flip of the codon:anticodon helix. This conformational flip, termed ratcheting, serves to move the mRNA by precise triplet intervals and provides a mechanism for the strict maintenance of reading frame. One of the more important aspects of the ratchet model is the implication for the evolution of ribosomes and genetic code, which has been pursued in detail by F.H.C. Crick (Salk Institute), S. Brenner (MRC, England), A. Klug (MRC, England), and G. Piecznik (*Origins Life 7:* 389 [1976]). However, Woese's model has not gained general acceptance, and most investigators still labor under an insufficient paradigm: the A-site–P-site model.

Recently, I discovered a hidden prediction in Woese's original proposal; the prediction involves how abnormal translocation (2- or 4-base mRNA movement) should occur. The prediction is very precise (Weiss, *Proc. Natl. Acad. Sci.* [1984, in press]). When all observations concerning abnormal translocation, from frameshifting by normal tRNA to frameshift suppressor tRNAs, are examined under this prediction, a unified explanation of the mechanics of normal and abnormal translocation emerges. This new view of the ratchet model provides specific testable predictions concerning the mechanism, and these are now under investigation.

Homology-dependent Repair of Double-strand Breaks and Chromosomal Transformation in Exonuclease-deficient *Escherichia coli* Strains

A.I. Bukhari, R. Weiss

Recent evidence from *Saccharomyces cerevisiae* suggests that double-strand DNA breaks are recombinogenic. The free duplex end is thought to invade a homologous segment, thus establishing a Holliday junction. The repair of double-strand breaks and gaps within regions of homology can be easily demonstrated by the rescue of linearized plasmid DNA during transformation. Such experiments have not been attempted in *Escherichia coli* because of high levels of exonuclease activity in wild-type strains. Use of *recB*$^-$ *recC*$^-$ *sbcB*$^-$ strains as recipients overcomes the exonuclease problem by removing two major nucleases (*Exo*I and *Exo*V) but leaving the cell "recombination$^+$." The *recB recC sbcB* strain is known to be competent for high-level chromosomal transformation.

Initial experiments with plasmids linearized or gapped by restriction endonucleases and transformed into *recB recC sbcB* strains have revealed frequencies of high-level, homology-dependent rescue of linear plasmid DNA comparable to the frequency of double-strand break repair in *S. cerevisiae*; i.e., the double-strand break reduces the transformation frequency by only 3–5-fold in a *recB recC sbcB* strain versus 1000-fold in wild-type *E. coli*. The majority of transformants, however, suffer deletions encompassing the site of the break or gap. This is in sharp contrast to the high fidelity of double-strand break and gap repair in yeast. The implication is that *E. coli* is unable to prime the repair DNA synthesis needed to establish the postulated double-Holliday-junction intermediate necessary to repair a gapped plasmid. Since resolution of the postulated double Holliday junction should yield an equal ratio of *integrated repaired plasmid* versus *extrachromosomal repaired plasmid*, the ability to detect integration events is important. The *recB recC sbcB* strains are unable to maintain ColE1-derived replicons in a stable form, and this

may prove useful for detecting integration of cut circular molecules. The homology requirement for mutagenic repair of cut plasmid can be dissected by introduction of various *rec*⁻ alleles (such as *recA* and *recF*) into the *recB recC sbcB* strain. Involvement of heteroduplex regions of DNA, indicative of Holliday junction formation, can be probed by scoring chromosomal markers flanking the area of the double-strand break.

The *recB recC sbcB* strain also provides a unique tool for disrupting *E. coli* chromosomal genes by direct chromosomal transformation with cloned segments altered in vitro. The coding sequences of the cloned release factor genes (Weiss et al., *J. Bacteriol.* [1984, in press]) are being disrupted with antibiotic resistance genes, and these markers will be used to select for disruption of the chromosomal genes during transformation. A genetic analysis can then be applied to the unique codon-recognition properties of the release factors. This should illuminate the mysterious "tRNA-like" properties of these protein molecules.

Nucleotide Sequences Encoding Mu Transposition Proteins

A.I. Bukhari, P. Koka

The transposition of bacteriophage Mu into the host genome occurs by replication. The product of the *A* gene, transposase, is essential for transposition. The function encoded by the *B* gene, which also affects transposition by enhancing the transposition frequency, is implicated in the replication. Thus, both the *A*- and *B*-gene products play a role in Mu transposition. However, the relative amounts of the 70,000-dalton protein of the *A* gene and the 33,000-dalton protein of the *B* gene present in the cells vary substantially, with the *B*-gene protein much in excess over the *A*-gene protein. This differential expression of these genes could be due to the nature and presence of the DNA sequences that control the expression of the *A* and *B* genes. One line of investigation to establish the reasons for this varying content of *A*- and *B*- gene proteins is to determine the DNA sequence of the *A* and *B* genes and the sequences preceding these genes. Knowledge of the DNA sequence will also provide information on the nature of the proteins. Both orientations of the left-end *Bal*I-*Hpa*I fragment of Mu DNA consisting of 3.6 kb have been cloned into the *Sma*I site of M13mp8. This fragment consists of the *A* gene carrying an amber mutation and the majority of the *B* gene. The remaining portion (74 bp) of the *B*-gene sequence and the sequence preceding the *A* gene and of its first 260 bp are available. Using in-vitro-synthesized primers (M. Zoller, Yeast Section), the 3.6-kb fragment is being sequenced from both the *Bal*I and the *Hpa*I sites. This will provide information on the DNA and protein sequences responsible for Mu transposition. The amber mutant sequence will allow us to identify unambiguously the coding sequence of the *A* gene.

Photosynthetic Reaction Center and Light-harvesting Genes

D. Youvan, S. Ismail

Purple nonsulfur bacteria such as *Rhodopseudomonas capsulata* have a very diverse metabolism that includes the ability to grow photosynthetically under anoxygenic conditions. At least seven polypeptides (three reaction-center and four light-harvesting polypeptides) are induced, along with the biosynthetic enzymes for carotenoid and bacteriochlorophyll biosynthesis during the differentiation of the respiratory membrane into the invaginated photosynthetic membrane. These polypeptides are necessary for the proper binding of the carotenoid and bacteriochlorophyll pigments, quinones, and other cofactors that absorb light, mediate primary charge separation, and generate reductant. An adjacent oxidoreductase complex utilizes reduced quinones to generate a transmembrane proton gradient. Many of the bacterial photosynthetic processes are analogous or identical to processes that occur in the thylakoid membranes of higher plant chloroplasts. Because it is easier to study and manipulate photosynthetic bacteria, we have chosen *R. capsulata* as a model organism for the study of the photosynthetic membrane.

Several genetic techniques have been employed in the past 10 years in the characterization of photosynthetic mutants and in the localization of the genes involved in the differentiation of the photosynthetic apparatus. Initially, the gene-transfer agent (GTA) was isolated from *R. capsulata* and was used to show that mutations in carotenoid and bacteriochlorophyll biosynthesis were linked. Cotransduction of these markers was used to generate a genetic map, and mutations were correlated with intermediates in bacteriochlorophyll and carotenoid biosynthesis. Later, these genes were mobilized for conjugation using a broad host-range conjugative plasmid. R-prime plasmids were obtained carrying the photosynthetic gene cluster. The 46-kb photosynthetic gene insert on the R-prime plasmid pRPS404 was shown to complement all known mutations in carotenoid and bacteriochlorophyll biosynthesis.

The complete nucleotide sequence (8867 bp) and the predicted polypeptide sequence have now been determined for 11 proteins from the photosynthetic gene cluster of *R. capsulata* (46 kb), including the photosynthetic reaction center (RC) L, M, and H subunits and the light-harvesting I (LHI) β and α polypeptides. Hydropathy plots indicate that the L

and M subunits are transmembrane proteins that may cross the membrane five times with highly hydrophobic stretches of amino acids. The H subunit has only one hydrophobic section near the amino terminus that may be transmembrane. The L and M subunits are homologous over their entire length and have a high degree of homology with the 32-kD quinone-binding (Qβ) protein from photosystem II of higher plants (Youvan et al., *Cell* [1984, in press]).

We have recently identified the light-harvesting II (LHII; infrared maxima: B800 + 850 complex) genes from *R. capsulata* by probing a λ library of chromosomal *Eco*RI fragments with oligonucleotide probes. Two oligonucleotide probes with a degeneracy of 32 were synthesized based on the amino acid sequence of the β and α subunits of LHII. Both probes hybridize specifically to a 6-kb *Eco*RI restriction fragment in genomic Southern hybridizations (hybridization to total bacterial DNA digested with *Eco*RI). Several λ phages that carry the 6-kb *Eco*RI fragment were identified by filter hybridization to a library of *R. capsulata* chromosomal *Eco*RI fragments (courtesy of P. Scolnick, Cold Spring Harbor Laboratory). The 6-kb *Eco*RI fragment has been recloned in pBR322, and both probes hybridize to an internal 1250-bp *Sma*I fragment. This indicates that the structural genes for β and α are adjacent. Southern hybridization of the β and α probes to the 46-kb photosynthetic gene cluster carried by pRPS404 indicates that the LHII genes are outside of this cluster. The structural genes for β, α, and the upstream regulatory elements (light-intensity-modulated) will be sequenced.

When the sequencing of LHII is completed, *R. capsulata* will be the only organism in which all of the structural genes involved in the primary light reactions of photosynthesis have been mapped and sequenced. DNA mutagenized in *Escherichia coli* by transposons, or in the future by oligonucleotides, may be returned to *R. capsulata* using genetic techniques specifically developed for this organism. Structure-function problems involving specific residues in the RC and LH polypeptides and their role in the light reactions of photosynthesis may be first addressed in *R. capsulata*.

5′ Control Region of *Escherichia coli pfkB* Gene

F. Daldal, J. Applebaum

The *pfkB* gene encodes the *Escherichia coli* minor phosphofructokinase Pfk-2. In the past, two interesting mutants affecting this gene have been isolated. One of them has a mutation that increases the expression of the wild-type gene about 20- to 40-fold. The other one, apparently a double mutant, not only has increased Pfk-2 activity, but also produces a cold labile enzyme that is less sensitive to its inhibitor, fructose-1,6-bisphosphate.

The wild-type gene and its mutant derivatives have been cloned and sequenced. The 5′ control region has been defined by construction of operon fusions between the 5′-proximal part of *pfkB* and the coding part of the galactokinase genes, and by in vitro transcription. Comparison of the overproducing mutant with the wild-type gene revealed that overproduction is mediated by increased transcription due to a C→T single-base-pair change in the −10 region of the *pfkB* promoter. The nature of the second mutation affecting the structure of the enzyme has yet to be defined at the nucleotide sequence level.

The 5′-proximal region controlling the expression of the *pfkB* gene is complex. In addition to the in vitro functional promoter, proximal to the coding part of the gene, there is another potential promoter, about 100 bp upstream of the first one. An operatorlike sequence and a ρ-dependent terminatorlike hairpin structure are both located within this second promoter region. Furthermore, adjacent to the −35 region of the first promoter, a CRP-binding consensus sequence is present. The functions, if any, of these features are not yet known. We have shown that a fragment carrying this CRP sequence is able to bind in vitro, although weakly, the CRP protein in the presence of cAMP. However, since the *pfkB* gene is thought to be expressed constitutively, the meaning of this interaction is not clear.

To analyze the role of these features, we took advantage of the galactose-sensitive phenotype of *pfkB* up promoter–*galK* fusions. *galK* strains carrying such fusions on multicopy plasmids are inhibited by galactose, presumably due to the accumulation of galactose-1-phosphate. Several mutants resistant to galactose and still presenting a functional galactokinase were selected. By retransformation back into the *galK* strain, it was found that most of the mutations were localized on the plasmids. Analysis of the total cell proteins by SDS-PAGE indicated the presence of two major classes of mutants. In the first class, the galactokinase protein is present at a much lower amount than the parent, and in the second class, it is barely detectable. Since, in the fusion strains, galactokinase production is under *pfkB* control, apparently these mutations affect the *pfkB* promoter. Their molecular nature is now being determined by DNA sequence analysis. It is hoped that some of these mutations will help us to understand the functions of the features found in the 5′ control region of the *pfkB* gene.

Clostridium pasteurianum Galactokinase

F. Daldal, J. Applebaum

In the past, no gene from a bacterium like *Clostridium pasteurianum*, which has a very high A + T

content (72%), has been isolated. To study the structure of such a gene, and also to use it as a marker in genetic studies, we cloned the *galK* gene of *C. pasteurianum* by complementation of an *Escherichia coli* galactokinase-defective mutant.

Initial screening of a *C. pasteurianum* chromosomal library in an *E. coli galK* mutant revealed no plasmid conferring complementation. However, after longer incubation, several galactose-utilizing papillae were detected. A recombinant plasmid isolated from one of them provided a galactokinase activity detectable by enzyme assay. The amount of the activity is lower than would be expected from a gene carried by a multicopy plasmid. Restriction analysis, subcloning, and Tn*5* mutagenesis indicated that the *galK* gene is located on a 2-kb *Eco*RI-*Bam*HI fragment. Use of "maxicells" and in vitro transcription-translation-coupled *E. coli* extracts showed that this plasmid encodes a protein with a relative molecular weight of approximately 40,000. Strong hybridization detected by Southern analysis between this gene and *C. pasteurianum*, but not *E. coli*, chromosomal DNA indicated that the *galK* gene originated from *C. pasteurianum*. It seems that even though the subunit molecular weights of both gene products are similar, there is little homology at the DNA level.

In the absence of selection, this Gal^+ phenotype is unstable in *E. coli*. Even when the maintenance of plasmid is forced by the use of antibiotic selection, about 0.1% of the cells are still Gal^-. Analysis of these derivatives showed that about 25% of them had insertions. In two distinct cases, we have detected the presence of IS*1* and IS*5* within the gene. Therefore, it appears that in *E. coli*, the *C. pasteurianum galK* gene presents a hot spot for IS insertions. Using this gene as a probe, by colony hybridization, the original clone from the starting library has also been isolated and was found to be unable to grow on galactose. However, analysis of the total cell proteins by SDS-PAGE revealed that galactokinase was overproduced in this clone. Thus, as is also the case for the *E. coli* enzyme, overproduction of the *C. pasteurianum* galactokinase is deleterious to *E. coli* in the presence of galactose. Papillae growing upon longer incubation are presumably mutants expressing the kinase at a lower, but tolerable, amount.

Comparison of these plasmids, producing low or high levels of galactokinase, indicated that the low-level producer carries a deletion of approximately 300 bp located in the 5′ region of the gene. This region is now being characterized by DNA sequencing. Assuming that in *E. coli*, the *C. pasteurianum* galactokinase is expressed from its authentic control region, comparison of these sequences will define the molecular structures governing the expression of a clostridial gene. However, the reasons for the instability in *E. coli* of the active *C. pasteurianum galK* gene, even when expressed at a low level, are not yet understood.

Isolation of Mutants from *Clostridium pasteurianum*

F. Daldal, J. Applebaum

The genetics of strict anaerobes belonging to the genus of Clostridiae is not well studied. We have chosen *Clostridium pasteurianum* as a prototype for such studies because it is a true prototroph, it has no reported pathogenicity, and it has been the anaerobic microorganism of choice for several biochemical studies.

To explore possible genetic exchange between cells, we first isolated and characterized a collection of spontaneous or mutagen-induced mutants of *C. pasteurianum* ATCC 6013 either by direct selection or by screening after enrichment. To date, about 25 independent mutants, including several auxotrophs, several antibiotic and UV resistants, and several others defective in sporulation, have been obtained. A procedure for the formation of osmotically sensitive cells, "protoplasts," by lysozyme treatment and for the regeneration of such cells on protective media has also been developed. However, we have not yet been able to detect prototrophs by fusing the auxotrophic mutant protoplasts with polyethylene glycol. It is hoped that emerging techniques, such as Zimmerman dielectric field fusion, will be useful in the future to overcome such difficulties.

Molybdenum Metabolism in *Clostridium pasteurianum*

S. Hinton

For several years I have been studying molybdenum (Mo) metabolism in *Clostridium pasteurianum*, a nitrogen-fixing anaerobe. To date, it is not known how the nutrient, molybdate, is biologically processed or chemically transformed into a biological catalyst (molybdenum cofactors). My efforts have been directed toward identifying and characterizing molybdoproteins that may play a role in the synthesis of the Mo-containing cofactors.

An anaerobic native gel-electrophoresis procedure was developed to identify all (oxygen-sensitive) molybdoproteins in clostridial crude extracts. Six distinct molybdoproteins were identified by anaerobic electrophoresis, and four of the molybdoproteins have characteristics that suggest they might play a role in the synthesis of the Mo-containing cofactor(s). One of the molybdoproteins identified as a "suspected" intermediate in the synthesis of the

Mo cofactor was purified and biochemically characterized. The purified molybdoprotein, Mop, has an unusually low molecular weight (~6000), and UV, visible, and fluorescence spectroscopy suggest that a pterin derivative is associated with it. J. Johnson (University of North Carolina) has proposed a model that all molybdoenzymes (except the MoFe protein of nitrogenase) have a common Mo cofactor (Mo-co) in which the Mo atom is bound to a pterin derivative via sulfur ligands. X-ray absorbance spectroscopy (XAS) has shown that all molybdoproteins studied to date have at least one molybdenum-sulfur ligand. In collaboration with S. Cramer (Exxon Research Co.), the structure of the Mo environment in Mop was determined by XAS. The mononuclear Mo site in Mop is unusual in that it has three terminal oxo groups and three oxygen or nitrogen ligands (no sulfur ligands). Recently, formate dehydrogenase found in *C. pasteurianum* has been shown to have a Mo site identical to Mop. Evidence suggests that Mop binds a precursor of the Mo-co that eventually is inserted into formate dehydrogenase, the end product of the Mo processing pathway. From biochemical analysis, it is apparent that Mop might be useful in studying the structure of the Mo-co and how the Mo-co is bound to the molybdoproteins.

There is a parallel effort using recombinant DNA technology to complement the biochemical studies of Mop. In collaboration with G. Freyer and M. So (Tumor Virus Section), a genomic library of *C. pasteurianum* was constructed in a pBR322 vector, and an expression clone of the *mop* gene was identified using immunoscreening. The coding region of the *mop* gene was identified by aligning the sequence of the first 26 amino acids of the amino terminus with the DNA sequence. The DNA sequence predicts that the protein is hydrophobic and is enriched with lysine and glutamic acid residues that are potential ligands to the Mo atom. The DNA sequence upstream of the *mop* gene shows complementarity to *Escherichia coli* promoter sequences, which may explain why Mop is expressed in *E. coli*. Since *Clostridium* as yet has no gene-transfer system, further characterization of the cloned gene will have to be performed in *E. coli*. If *mop* is functional (binds Mo-co) in *E. coli*, site-directed mutagenesis will be used to attempt to determine the Mo-co binding site.

Isolation of Nitrogenase Genes from *Clostridium pasteurianum*

D.J. Lundell

Certain prokaryotic organisms can satisfy their nitrogen requirement by reduction of atmospheric dinitrogen to ammonia. Dinitrogen is quite unreactive under ambient conditions and therefore understanding the mechanism of its reduction is of some interest. The reaction is carried out by two proteins, collectively called nitrogenase—an $\alpha_2\beta_2$ molybdenum-iron-sulfur protein (MoFe protein; coded for by the *nifD* and *nifK* genes in *Klebsiella pneumoniae*; nif, nitrogen fixation) of 250,000 daltons that appears to contain the active site for dinitrogen reduction and an α_2 iron-sulfur protein (Fe protein; *nifH*-gene product) that acts as the unique reductant of the MoFe protein. My aim is to use in vitro mutagenesis to understand the structure and mechanism of nitrogenase. To this end I have started cloning the Fe and MoFe protein genes from *Clostridium pasteurianum*. This organism is being used because an X-ray crystal structure is being determined for its MoFe protein.

Prior to my arrival, various genomic libraries of *C. pasteurianum* DNA were constructed in the expression vectors, pUC8 and pUC9, by F. Daldal and S. Hinton (this section). Two MoFe protein clones were identified by immunoscreening. The smaller clone (MF4), containing an insert of ~550 bp, was shown by Southern hybridization to be completely represented within the larger clone (MF21), which contained a 2700-bp *Pst*I-*Bam*HI fragment. Western blot analysis showed that MF21 directed synthesis of a ~60,000-dalton polypeptide corresponding in molecular weight to the *nifK*-gene product and a ~35,000-dalton polypeptide, probably a fragment of the *nifD*-gene product. DNA sequence analysis of MF4 showed it to match a portion of the *nifD* polypeptide sequence recently published. Knowing the position of MF4 within the larger clone, MF21 can be shown to contain the carboxyterminal portion (~330 amino acids out of 529) of the *nifD*-gene product immediately followed by the entire *nifK* gene. This gene order, *nifDK*, is the same as is seen in *K. pneumoniae*.

The *Pae* Restriction-Modification System

T.R. Gingeras, J.E. Brooks, D. O'Loane

Restriction and modification systems are present in many bacteria as a means of preventing foreign DNA from entering the host cell. Such systems consist of a restriction endonuclease that recognizes and cleaves a specific short nucleotide sequence and a DNA methylase that modifies the same recognition sequence, thus protecting the host from self-destruction.

Because the activity of the endonuclease produces a double-strand break in the chromosomal DNA, expression of this gene without the protection of the companion methylase gene has been considered to be lethal. Consequently, expression

of both of these genes is thought to be highly controlled and coordinated. Such regulation would be needed most in those instances where restriction-modification systems are located on mobilizable plasmids.

The *Pae*R7 restriction-modification system from an R-type plasmid (pMG7) found in *Pseudomonas aeruginosa* has been cloned and sequenced in collaboration with G. Theirault (Lavelle University, Quebec) over the course of the last year. The organization of the two genes in this system is in the form of an operon with the methylase gene preceding the endonuclease gene on the same strand of DNA. This operon covers approximately 2300 nucleotides and is regulated by a control region whose sequence has been determined. Deletions of varying segments of this control region by BAL-31 nuclease give rise to a collection of clones that displays a spectrum of restriction-modification phenotypes. The DNA sequence of these clones indicated deletions concentrated within an 80-bp region at the 5′ end of the operon. Such deletions affect the expression of both genes. Analysis of the DNA sequence of this region showed a sequence used as a Pribnow box by the *araC* gene of *Escherichia coli*. Deletions within and proximal to this putative Pribnow box region of the operon resulted in a reduction in the levels of phage restriction.

The most striking feature first noted about this *Pae* system was the ability to construct subclones that express only the endonuclease gene. Such clones were viable but incapable of restricting infecting phage. How such a phenotype was possible was somewhat puzzling. However, measurement of the levels of expression by these unusual clones indicated that they, like the deletion mutants involving the control region, made 100-fold less endonuclease than the clones carrying the intact system. This was due to the deletion of most of the 5′ end of this system and, presumably, the activation of a secondary promoter. A lower level of endonuclease appears tolerable in vivo when no methylase is present. Since dimers are the active form of the endonuclease, it may be that at such low levels of endonuclease expression, formation of dimers is retarded.

Deletion mutants involving both the regulatory and structural segments of this operon produced restriction phenotypes. Thus, it appears that the levels of endonuclease within bacteria are important in order for the restriction phenotype to be observed.

NEUROBIOLOGY

Our strategy for probing the molecular basis of neuronal connectivity is to isolate monoclonal antibodies (mAbs) that stain single types of neurons or sets of different neurons. Now, after 4 years, we have reached a vantage point from which we can begin to formulate categories for the various patterns stained by our monoclonal antibodies. We recognize three broad categories of staining patterns that are informative with respect to the molecular heterogeneity of the leech nervous system, one of them already pointing promisingly to combinatorial markers that may specify neuronal connectivity.

The first category of antibodies that we are generating binds to 130K glycoproteins on Western blots. In CNS tissue, some of these antibodies are specific probes for select cell bodies and axons that run in tight bundles; other antibodies bind to 130K antigens that are shared by select axons and glial cells. Possibly, these 130K surface antigens are nCam-like molecules subserving specific adhesion between axons or between axons and glial cells, with implications for such phenomena as axonal guidance.

The second type of monoclonal antibody that we are generating gives staining patterns similar to those created by polyclonal antisera against peptides. These antibodies stain neuronal processes with varicosities that resemble typical leech nerve terminals. Usually, these antibodies only stain a few neurons per ganglion, or sometimes even only a few neurons found in specialized ganglia. None of these monoclonal antibodies react with proteins on Western blots, but they might very well be directed against low-molecular-weight peptides.

The third category consists of those antibodies that are currently the most promising in terms of pointing to molecular markers important for identifying synaptic connectivities in leech CNS. Interestingly, antibodies in this category were generated not only against leech CNS, but also against the *Drosophila* CNS by S. Benzer's laboratory (California Institute of Technology, Pasadena). Both leech and *Drosophila* monoclonal antibodies stain different intersecting sets of neurons and thereby reveal an assortment of combinatorial markers. For example, a class of primary mechanosensory neurons, the pressure-sensitive P cells, are stained by four different monoclonal antibodies as part of different-sized neuronal sets. Thus, the four monoclonal antibodies point to four different combinatorial markers in these identified neurons. Another example is the α cell for which one leech and three *Drosophila* monoclonal antibodies have also discovered four combinatorial markers (Fig. 1).

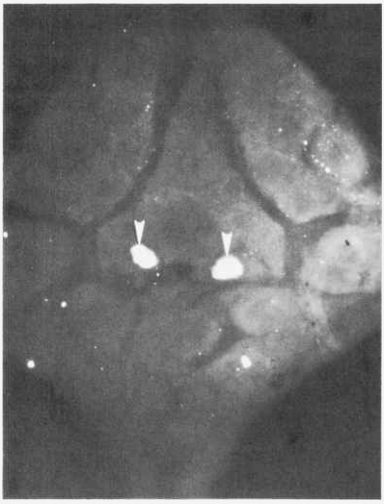


Figure 1
Drosophila monoclonal antibody 2G4B specifically stains the α cell (arrowheads) and is one of our four α-cell-specific markers.

The Macroglial Cells of the Leech Are Molecularly Heterogeneous

M.S. Flaster, B. Zipser

Using monoclonal antibodies obtained from either whole nerve cords or CNS extract run on polyacrylamide gels of the leech *Haemopis marmorata*, we have cataloged the distribution of several monoclonal antibodies directed against the macroglia of the nerve cord and have partially characterized some of these glial antigens biochemically. The identifiable macroglia of the leech, those in the lateral connectives, the ganglionic neuropil, the ganglionic packets, and the ganglionic roots, are distinguishable anatomically by position. Here, we report several monoclonal antibodies that prominently stain some, but not all, macroglia, differentiating these cells at the molecular level. One monoclonal antibody stains the processes of the macroglial cells of the lateral connectives, the ganglionic neuropil, and the ganglionic roots but does not stain the packet glia appreciably. Immunoblots of SDS gels indicate that this monoclonal antibody binds a single protein antigen with an apparent molecular weight of 130,000. The antigen is a glycoprotein. A second monoclonal antibody strongly stains throughout the interior of the connective and root macroglia but does not appear to stain the other macroglial cells, whereas a third monoclonal antibody stains throughout the interior of only the connective macroglial cells. This monoclonal antibody binds a single protein antigen with an apparent molecular weight of 77,000, and this antigen is not a glycoprotein, as judged from multiple lectin column chromatography. The underlying significance of the differences in these identified glia to the development or adult function of the leech nervous system remains to be thoroughly explored, although in developmental studies, the time of appearance of one of these monoclonal antibodies has already been established.

Combinatorial Leech Antigens

B. Zipser, C. Schley, T. Flanagan, J. Perez

A panel of monoclonal antibodies generated against either leech or *Drosophila* CNS divide the leech ganglion into different sets of antigenetically homologous neurons. Some of the smaller sets are entirely contained within larger sets; other sets overlap only partially. An electrophysiological analysis of the set of Laz2-1-stained neurons has identified sensory and motor neurons and interneurons, which are already shown to be in synaptic contact. Laz2-1, together with leech mAbs Lan3-5, and *Drosophila* mAbs, 3A4 and 8G1 (Fujita et al., *Proc. Natl. Acad. Sci. 79:* 7929 [1982]), identify an assortment of combinatorial markers. For sensory neurons (the pressure cells), three different markers have been identified using three leech monoclonal antibodies. For a ventral medial pair of neurons, one leech and two *Drosophila* monoclonal antibodies have also identified three markers. Neurons are hypothesized to use an assortment of combinatorial factors to form precise synaptic connections. These hypotheses predict the type of overlapping chemically labeled sets our monoclonal antibodies have discovered.

Analysis of a Neuron-specific Leech Antigen

B. Zipser, T. Flanagan, M. Flaster, C. Schley [in collaboration with E. Macagno and R. Stewart, Columbia University]

Is there a common denominator to leech neurons carrying the Laz2-1 antigen? In the CNS, the monoclonal antibody labels about 40-odd neurons, or 20 bilateral pairs of different neurons. Five of these neurons have been identified through intracellular dye injections and all were observed to project their primary axons into roots, enabling them to innervate the periphery as sensory or motor neurons. Among these neurons are both pairs of the extensively studied primary mechanosensory neurons responding to pressure. The function of the other three cells has not yet been determined. Of particular interest is the one pair next to the Retzius cells that is the most likely candidate to be the first neuron expressing the Laz2-1 antigen during CNS development. The expression of the Laz2-1 antigen has been extensively characterized both embryonically and postembryonically and is seen to appear in a precise spatial and temporal pattern both within the CNS and in the periphery.

In the periphery, Laz2-1 stains putative sensory neurons in each annulus of the body wall. It also stains neurons within the esophagus and cells that appear to be part of the testicular primordia. A possible mechano- or chemosensory function of the peripheral neurons remains to be explored. In our attempt to find a common functional denominator for Laz2-1-labeled neurons, we are very interested in identifying the functions of the other Laz2-1-stained CNS neurons to determine whether they, like the pressure cells, are associated with first- or higher-order sensory function.

Our other interest in the Laz2-1 pattern relates to the temporal pattern of antigen expression in the CNS. Measured immunocytochemically, some of the Laz2-1-labeled neurons only express their antigen postembryonically. This offers the opportunity

to analyze a given neuron before and after antigen expression has begun. Since some of the neurons within the staining patterns are known to be synaptically connected, the possibility arises that neurons accumulate the Laz2-1 antigen as they become sequentially inserted in a functional network.

A Monoclonal Antibody Specific to the Leech Pressure-sensitive Mechanosensory Neurons

C. Schley, B. Zipser [in collaboration with C. Loer and W. Kristan, University of California, San Diego]

Two pairs of pressure-sensitive mechanosensory neurons (P cells) are found in the standard midbody ganglion of the glossophoniid leech, *Haementeria*. We have produced a monoclonal antibody specific to the somata and neurites of these cells and to a small number of unidentified fibers. The antibodies were prepared by immunizing BALB/c mice with lightly fixed, homogenized *Haementeria* nerve cords. Their spleen cells were fused with myeloma cells to produce hybridomas. After a standard HAT selection, the clones were screened on paraformaldehyde-fixed ganglia of adult *Haementeria* using an HRP-conjugated secondary antibody and staining with DAB and H_2O_2. Four large cell bodies stain in the standard adult ganglion, along with their neurites in the neuropil, peripheral nerves, and connectives. A few as yet unidentified fibers are found more medially in the neuropil and connectives; these also sometimes appear to be sending branches out the nerves to the periphery. Some specialized ganglia stain differently. The two sex ganglia have only one pair of cell bodies staining, and the last two unfused ganglia have one pair of large cell bodies and one pair of small cell bodies that stain. The tail brain, composed of seven fused ganglia, has cells staining that appear to be the segmental homologs of the P cells; the head brain, composed of four fused ganglia and a supraesophogeal ganglion, segmental homologs are again apparent, as well as a few additional cell bodies.

We have found as well that the antibody cross-reacts with another glossophoniid leech, *Helobdella triserialis*, in which experimental manipulations of P-cell progenitors are possible. We are now using the antibody to examine the consequences of ablations and other experimental manipulations of P-cell progenitors. We are investigating the possible function of the antigen by a variety of means. First, by light microscopy, the antigen appears to be located both on the surface and inside the cell; these observations will be confirmed by other methods. Second, the molecular weight of the antigen will be ascertained. Finally, the antibody will be applied to live ganglia while recording from the P cells and their postsynaptic targets.

Varicosity Patterns and Rare Neuron Types in the Leech Central Nervous System

T. Flanagan, B. Zipser

Our studies indicate that the neuropile of leech ganglia is compartmentalized with reference to the distributions of several types of immunoreactive varicosities. The leech neuropile is also compartmentalized with reference to the characteristic distribution of neurites from identified cell types. We suspect that such compartments may correspond to regions restricted to specific types of cellular communication. We report here the results of our immunocytological studies of 11 varicosity patterns and an in depth morphological and electrophysiological description of one set of these immunoreactive cell types.

Monoclonal antibodies raised against leech nerve cords react with specific neuronal antigens. Of the 1200 hybridoma lines screened, 57% were immunoreactive, 3% stain restricted neuronal sets, and 1% stain varicosities. These immunoreactive varicosities resemble leech varicosities stained with commercial anti-Leu-enkephalin antisera in terms of varicosity size, approximate number, and ganglionic distribution pattern. Varicosities extend throughout the leech nervous system and are particularly conspicuous within the cephalic neurohemal sites. Antisera that stain varicosities also stain a restricted number of immunoreactive soma that either lie clustered within specialized ganglia or are regularly distributed in sparse cell sets in segmental ganglia. The mAb Lan3-11, for example, stains soma within the subesophageal and the second segmental ganglia. These cells are well suited for electrophysiological and morphological analyses and allow us to demonstrate that immunoreactive varicosities arise from immunoreactive soma. Using double-labeling methods, we have established that these cells are interganglionic interneurons projecting to ganglionic sites that contain immunoreactive varicosities and that their neurites display swellings comparable to these varicosities. We recognize three Lan3-11 subtypes, and thus we are presently studying the extent to which Lan3-11 projection fields overlap and varicosity distribution patterns correspond. Perhaps distinct neuropile target sites will suggest separate follower cell types and shed light on their individual physiological functions.

Identifying Cell Types in Adult and Developing Mammalian CNS

S. Hockfield, E. Walvogel, C. Bautista

The mammalian CNS is composed of many different neuronal types. Much of neurobiology over the last 100 years has been concerned with the description of neuronal diversity on the basis of anatomical, physiological, and pharmacological criteria. These studies have shown that the morphology and physiology of identified neuronal types are consistent among animals of the same species and often among animals of different mammalian species. Many hypotheses for the generation and expression of neuronal diversity have postulated that molecular differences among neurons could direct cellular differentiation and the establishment and maintenance of specific connections. In our laboratory we are using hybridoma and recombinant DNA technologies to explore the molecular basis of neuronal diversity. During the past year, we have used these techniques to examine further the cellular organization of the adult mammalian brain and to study the development of the CNS by identifying early cell types and the sequence of events that give rise to the adult structure.

Monoclonal antibodies identify subsets of neurons in the adult CNS. Monoclonal antibody Cat-301 recognizes subsets of neurons in many areas of the cat and monkey CNS. In the dorsal lateral geniculate nucleus (LGN) of both cat and monkey, the subset of Cat-301-positive neurons appears to correlate with a physiological class of neurons. In the visual cortex of monkeys, Cat-301 recognizes patches of neurons in both normal and monocularly enucleated animals. These groups of neurons lie in register with ocular dominance columns and may define a functionally distinct subset of cells. These studies show that the neurons in the LGN and visual cortex are molecularly heterogeneous and that the heterogeneity correlates with features of the organization of each of these areas. We have further identified antibody-positive neurons by showing that they project to specific sites within the CNS using retrograde transport techniques. In addition, in collaboration with M. Sur (Yale University), we are electrophysiologically identifying individual LGN neurons and marking them using intracellular electrodes. These neurons are then stained with Cat-301 to determine precisely which physiological classes of neurons carry the Cat-301 antigen.

Defining cell types in the developing mammalian CNS. In our initial efforts to study developmental events, we used the markers we have developed that recognize antigens in the adult CNS. All of these markers are not expressed until relatively late in development. For example, Cat-301, which recognizes a surface antigen, is not expressed on the surface of most spinal cord neurons until after the second postnatal week. At this time, synapse elimination, one of the late events in neuronal maturation, is at its peak. This suggests a role for Cat-301 in the establishment or maintenance of adult synaptic patterns.

We have now raised monoclonal antibodies to rat embryonic day-15 spinal cord. At this age the nervous system contains both the rapidly proliferating neuroepithelial cells and the recently differentiated postmitotic neurons, some of which have begun to extend their axons. We have obtained reagents that identify (1) postmitotic neurons; (2) early develop-

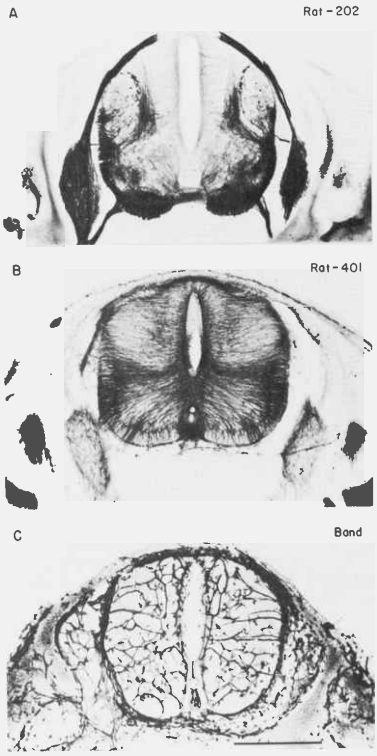


Figure 2
Markers that differentiate among prevalent cell types in developing CNS. Transverse sections of embryonic day-15 rats. (*A*) Monoclonal antibody Rat-202 stains axons in the developing spinal cord and periphery. (*B*) Monoclonal antibody Rat-401 stains radial glia in the CNS, nonneuronal cells in nerve routes, and developing muscle cells. (*C*) Lectins from *Bandeiria simplicofolia* stain developing blood vessels in embryos but not in adults. Bar, 1 mm.

ing axons and their growth cones; (3) a class of proliferating cells, the radial glia; and (4) the embryonic vascular system (Fig. 2). We have used these monoclonal antibodies to define major cell types and to study the developmental events that give rise to the diverse cell types in the adult nervous system. One example of this approach is given by our studies using antibody Rat-401.

The antigen recognized by antibody Rat-401 (a 200-kD species on immunoblots of SDS-polyacrylamide gels) first appears in the developing rat CNS after neural tube closure, on embryonic day 11 (E11). As neural development proceeds, antibody-positive cells come to resemble the radial glial cells described by Ramon y Cajal. Electron microscopic immunocytochemistry has shown that the antibody recognizes mitotic cells.

Rat-401 recognizes radial cells in all areas of the developing rat brain but not in the adult. In each area we have examined, antibody staining begins to decrease at the end of the period of neuronal migration (Fig. 3). This has important implications as the radial glial cells are thought to guide neuronal migration. We have shown that a radial cell carrying the Rat-401 antigen is transiently present during the period of neuronal migration—a cell that is spatially and temporally suited to serve as a structural guide for migrating neurons.

We have used other monoclonal antibodies to follow the development of axons. One antibody, Rat-202, recognizes axons at very early stages and recognizes the entire axon, including the growth cone and its filopodia. As these early axons travel into the periphery, they appear to be in close contact

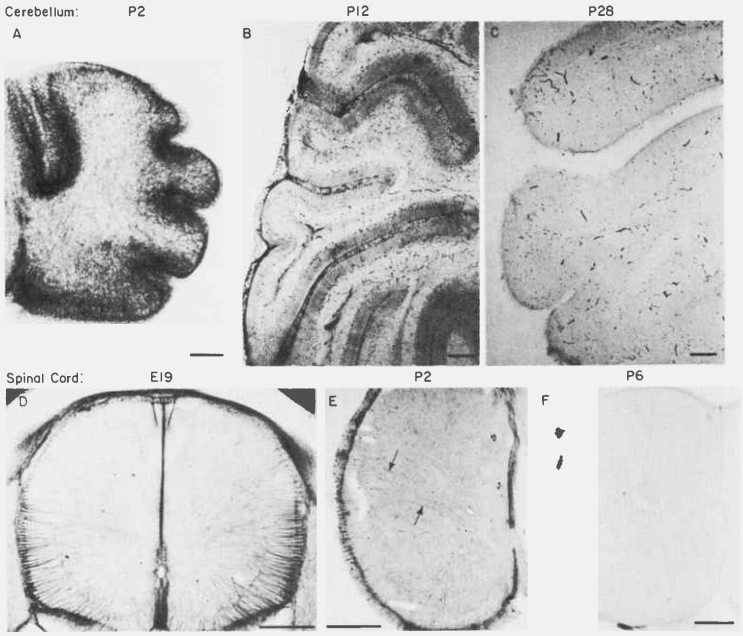


Figure 3

Antigens recognized by Rat-401 are lost during development in cerebellum (*A–C*) and spinal cord (*D–F*). (*A*) Postnatal day-2 (P2) cerebellum contains numerous Rat-401-positive profiles. (*B*) By P12 the intensity of Rat-401 staining begins to decrease. (*C*) At P28 the cerebellum contains no Rat-401-positive profiles. The loss of Rat-401 staining is achieved by P21. In the developing cerebellum, the majority of neuronal proliferation and migration is complete by P21. (*D*) In the spinal cord, Rat-401 staining begins to decrease at embryonic day 19 (compare with E15 in Fig. 2). (*E*) At P2 very few antibody-positive profiles remain in the spinal cord (arrows). (*F*) By P6 the spinal cord contains no positive profiles. This section of P12 spinal cord illustrates the lack of Rat-401 staining at later stages. In the spinal cord, neuronal proliferation and migration are complete by E19. Bar, 1 mm.

with a peripheral Rat-401-positive cell. This cell is present at the location of axon routes before axons have grown out. This suggests that these nonneuronal cells may pioneer axon pathways and that the first axons may use these cells as a guide from CNS to PNS.

Other axonal antigens are expressed later in axonal maturation than Rat-202. We have used antibodies against these antigens to follow different time courses in axon development. Together, these studies on antigen expression in the developing and adult mammalian CNS show that the amount of diversity among the cells in the CNS is large. They also indicate that an elaborate, but tractable, pattern of antigenic expression occurs during neuronal development.

Molecular Studies of Neural Organization

R. McKay, S. Hockfield, K. Frederiksen, O. Sundin

Recently, the tools of molecular biology have become sufficiently simple to allow the experimental investigation of patterns of gene expression in complex tissues. We have used hybridoma technology to study both the molecular complexity and the cellular organization of the nervous system. More recently, we have applied recombinant DNA techniques, particularly cDNA cloning, plus-minus screening, and in situ hybridization to these same problems.

Using hybridoma technology, we showed that the nervous systems of the leech and of the cat were composed of antigenically diverse cell types. With both invertebrate and vertebrate systems, it became clear that specific monoclonal antibodies allowed a high-resolution analysis of in vivo cellular organization (Hockfield and McKay, *J. Neurosci. 3:* 369 [1983]; Hockfield and McKay, *Proc. Natl. Acad. Sci. 80:* 5758 [1983]; Hockfield et al., *Cold Spring Harbor Symp. Quant. Biol. 48:* 877 [1983]; Hendry et al., *Nature 307:* 267 [1984]).

Axon Organization

R. McKay, K. Frederiksen, S. Hockfield

The large numbers of antibodies that stained different subsets of neurons in the leech suggested that we were very far from saturating the pool of subset-specific antigens. To combat this complexity, we concentrated our efforts on the specific problems raised by a set of antibodies that recognized a particular subset of central neurons, the nociceptive or N cells. These cells give the leech information about noxious stimulation of the skin. We mapped these N cells in different ganglia physiologically and showed that all the segmental N cells carry specific antigens (Johansen et al., *J. Comp. Neurol.* [1984, in press]). These specific antigens are found on axons as well as cell bodies, and we showed that these axons occupy specific locations in the CNS. The specific location of leech central axons was confirmed by electron microscopy of labeled axons after electrophysiological identification of cells (Johansen et al., *J. Comp. Neurol.* [1984, in press]). The regulation of axonal morphology has been recognized as a central question of neural development. Although there are many properties of neurons that may contribute to axonal morphology, it has often been postulated that different surface molecules might play a role in axon pathfinding as the growth cone establishes axonal morphology. We then showed that the specific antigens which were present on stereotypically organized axons were present on the axon surface (McKay et al., *Science 222:* 788 [1983]) and that these protease-sensitive, glycosylated antigens are found on axons and filopodia during the earliest stages of axon outgrowth (McKay et al., *Cold Spring Harbor Symp. Quant. Biol. 48:* 599 [1983]). This work established that growing axons can carry distinct surface molecules. Using a cell culture of identified invertebrate neurons that send out neurites, we plan to explore further the biochemistry of axon pathfinding.

Genetic Complexity of the Rat Brain

O. Sundin, R. McKay, S. Hockfield

We are now using recombinant DNA techniques to obtain systematic data on the molecular complexity of the vertebrate brain. cDNA libraries in various plasmid vectors have been made with oligo(dt)-selected sequences from the embryonic neural tube and different regions of the adult brain of the rat. We are now using plus-minus screening and in situ hybridization to obtain probes that mark cell types during particular stages of differentiation. Our first results with these cDNA libraries show that there is a high degree of genetic complexity in the vertebrate brain. We now plan to use these techniques to obtain a more complete description of the number and organization of different cell types in the vertebrate brain.

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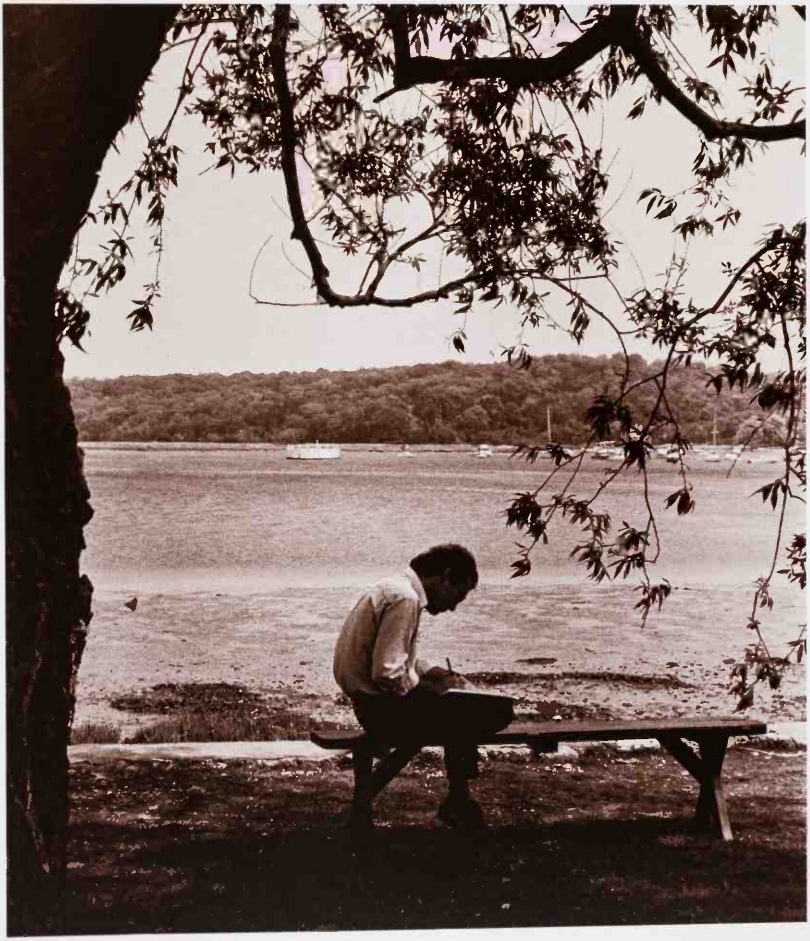
AUTHOR INDEX

COLD SPRING HARBOR MEETINGS



48TH COLD SPRING HARBOR SYMPOSIUM ON QUANTITATIVE BIOLOGY

MOLECULAR NEUROBIOLOGY

June 1–June 8

223 participants

Ever since the genetic code was solved in 1966, the brain and the phenomena it oversees—perception, memory, and thinking—have stood out like Himalayan peaks to the world of molecular biologists. Until very recently, however, most of us have believed that we have little unique to offer those long professionally involved in neurobiological phenomena and that the brain in particular was far beyond our capabilities. Those few molecular biologists who did move into neurobiology we have in the past regarded as frightfully brave or recklessly silly.

Apparently particularly hopeless was the approach that should be the most natural to us, that of focusing first on the structure and functioning of the molecules out of which the nerve cells are made. Although such research of itself could become fun to do, for many years the chances seemed nil that it would be through molecular biology that the intimate secrets of the brain would fall out. So an audacious few of us had moved into the fields of psychophysics, hoping that bouts of hard thinking could do the job. But here again the odds that fundamental revelations would quickly emerge seemed painfully low.

Today, however, we sense a new mood among those molecular biologists who gaze upwards toward the brain. By using recombinant DNA, monoclonal antibodies, and the facts of receptor biochemistry to the fullest, we should have a fighting chance to understand the uniqueness of nerve cells and the ways they come together to form functional networks. To mark this new mood, we decided to hold the 48th Symposium on Molecular Neurobiology.

In putting together this meeting, we received excellent advice from many colleagues, especially Eric Kandel and Charles Stevens, both long associated with the Cold Spring Harbor summer courses in neurobiology. The final result was a Symposium that will be long remembered as a decisive turning point in the development of neurobiology.

Funding for this meeting was provided by the National Institutes of Health, National Science Foundation, and the Department of Energy.

C. ELEGANS

May 4–May 8

Arranged by
Robert H. Waterston, *Washington University, St. Louis*
David Hirsh, *University of Colorado*
Jonathan Hodgkin, *MRC Laboratory of Molecular Biology*
Sam Ward, *Carnegie Institution of Washington, Baltimore*

178 participants

The fourth international *C. elegans* meeting at Cold Spring Harbor attracted more than 170 scientists from 40 laboratories studying diverse aspects of this small nematode. The results presented at the meeting included a description of the complete neuroanatomy; genetic and molecular studies of the specification of neural development and neuron-specific polypeptides; a genetic pathway of sex determination and the identification of genes specifying sex-specific proteins; a description of mutants affecting developmental lineages, including some with homeotic effects; the localization of gene products within cells, from the asymmetric distribution of proteins during cell division both in the embryonic cleavages and in spermatogenesis to the specific positions occupied by myosin isoforms in the thick filament; the molecular and genetic analysis of proteins of the cuticle and muscle, especially of the myosin heavy-chain gene; the search for transposable elements by genetic and molecular approaches; the conclusive identification of a tRNA amber nonsense suppressor; a detailed deletion map of a region of the second chromosome; and the first successful DNA transformation of *C. elegans*. The gathering of workers from all fields to share results produced a wealth of ideas and collaborations, demonstrating the value of the meeting.

This meeting was supported in part by funds from the National Institute on Aging, National Institutes of Health.

MICROBIAL DEVELOPMENT

May 11–May 15

Arranged by
Richard Losick, *The Biological Laboratories, Harvard University*
Lucy Shapiro, *Albert Einstein College of Medicine*
Amar Klar, *Cold Spring Harbor Laboratory*

143 participants

Relatively simple microorganisms are exceptionally attractive systems in which to study development because of the facility with which they can be manipulated by genetic and molecular techniques. The meeting on Microbial Development was unusual in bringing together workers in a wide variety of prokaryotic and eukaryotic microbial systems in which general problems of cellular differentiation, cell-cell interaction, and morphogenesis are being addressed. A special feature of the meeting was the comparison of similar biological problems posed by very different experimental systems, such as cell division and differential gene expression in *E. coli*, *Caulobacter*, and yeast; spore formation in *Bacillus*, *Streptomyces*, yeast, and *Aspergillus*; mating interactions in *Streptococcus*, *Streptomyces*, *E. coli*, *Chlamydomonas*, and yeast; and parallel patterns of social behavior in *Myxobacteria* and *Dictyostelium*. Despite the diversity of systems, a coherent set of recurring issues shaped the discussions: the role of nutritional signals in triggering development, the way in which sets of genes are activated in ordered sequences during differentiation, polarity, and the role of positional information, cell communication, and the transduction of sensory information. An important success of the meeting was that workers in diverse systems found themselves on common ground with respect to the questions being addressed and the experimental approaches being employed. The meeting conveyed a strong sense of excitement, since it was clear that many microbial developmental systems were yielding rapidly to genetic and molecular approaches, with many molecular details and, in some instances, important general principles already emerging.

This meeting was supported in part by Abbott Laboratories; American Cyanamid Co.; Biogen S.A.; Eli Lilly Co.; Miles Laboratories, Inc.; Monsanto; Monsanto Agricultural Products Co.; Upjohn Co.; the National Science Foundation; and Searle Research and Development.

RNA PROCESSING

May 18–May 22

Arranged by
John Abelson, *University of California, San Diego*
James Dahlberg, *University of Wisconsin*
Michael Mathews, *Cold Spring Harbor Laboratory*

224 participants

Modification of primary transcripts plays an essential role in the mobilization and expression of genetic information. The alterations can be of several kinds, from changes in single nucleotides to the ad-

dition or deletion of lengthy sequences, and they can modulate the stability and function of RNAs of all types. It is not surprising, then, that the topics discussed at this year's meeting on RNA Processing ranged widely, including nuclear structure and function, ribonucleoprotein particles, and RNA structure, as well as mRNA, tRNA, and rRNA processing. In some areas, definitive answers have begun to emerge—the detailed chemistry and biochemistry of tRNA cleavage and ligation reactions are a notable example. In others, such as mRNA splicing and polyadenylation, it was clear that sufficiently active cell-free systems are now in hand and rapid advances are anticipated. Yet other areas are moving through a more descriptive phase: The structural analysis of ribonucleoprotein particles is well advanced, for instance, while their functions offer food for thought and subjects for debate.

This meeting followed a successful predecessor in 1982, and plans for a further meeting in 1984 are now in hand. We are fortunate that the granting agencies, recognizing the significance of RNA processing, approved funding for two consecutive years—a rare act. Support comes from grants from the National Science Foundation, the National Institutes of Health, the National Institute of General Medical Sciences, and the Fogarty International Center.

RNA TUMOR VIRUSES

May 25–May 29

Arranged by

Edward Scolnick, *Merk Sharp & Dohme Research Laboratories*

Nancy Hopkins, *Massachusetts Institute of Technology*

441 participants

The annual RNA Tumor Virus meeting once again achieved a record number of applicants and abstracts, the abstract booklet being almost one inch thick. The continuing intense interest in this field is, of course, in large part due to viral oncogenes, those cellular sequences captured by retroviruses and thereby turned into cancer-inducing genes. At least 50% of the abstracts dealt directly or indirectly with viral oncogenes and their cellular homologs, and it was clear that the existence of mutant *v-onc* genes will continue to provide an important probe into *c-onc* function. Central issues concerned the various mechanisms of oncogene activation and the structure of *c-onc* genes in species as diverse as *Drosophila*, the frog *X. laevis*, man, and most novel and striking, yeast, where one group reported finding *ras*-gene homologs.

Retroviruses as vectors was a subject of increasing interest. Also, there were numerous abstracts dealing with human retroviruses, both endogenous sequences and the exogenous human T-cell leukemia viruses. The mechanism of HTLV-induced leukemogenesis remains obscure and clearly of great interest. There is no reason to think that the RNA Tumor Viruses meeting will be any smaller next year.

This meeting was supported in part by funds from the Cancer Center Grant to Cold Spring Harbor Laboratory from the National Cancer Institute, National Institutes of Health.

THE MOLECULAR BIOLOGY OF YEAST

August 16–August 21

Arranged by

James B. Hicks, Amar Klar, and Jeffrey N. Strathern, *Cold Spring Harbor Laboratory*

473 participants

Well over 600 scientists applied to attend the 1983 Yeast meeting. This reflects both the proliferation of the laboratories and the rapid progress in application of molecular approaches to a variety of biological problems in yeast. As a result of this oversubscription, it was both more difficult to organize the sessions and more rewarding to absorb their content. One take-home lesson obvious from the meeting is that the recombinant DNA and transformation technologies have progressed to the point where the cloning of most genes is routine. Nearly a quarter of the mapped genes have been cloned. Several molecular approaches to simplify mapping techniques have been developed that are expanding our understanding of the organization of the genome. Finally, enriched messages, genes from heterologous systems, immunological screening, and synthetic probes are allowing the cloning of genes for which there are no mutations. All of these techniques provided the raw material for several elegant molecular studies on the regulation and expression of genes that were reported at this meeting. Talks and posters addressed such topics as the control and expression of cell-cycle-specific genes; the coordinated expression of banks of genes such as cell-type-specific, ribosomal, and sporulation; and the induction of metabolic genes. This past year has seen the development of techniques for returning in-vitro-generated mutant sequences to their proper place in the chromosome. Thus, the analysis of the function and regulation of genes is progressing to the point of making single base-pair changes. Cloned sequences have allowed the physical characterization of genetic rearrangements such as recombination between reiterated sequences, hops by

the transposon Ty1, amplification of genes, and mating-type switching. These same technologies provide the raw material for the characterization of yeast extracts exhibiting replication or recombination of yeast plasmids. Furthermore, this meeting provided a forum for exchanging observations on the use of yeast to express and properly localize or secrete proteins for use in biochemical or commercial enterprises. In summary, this meeting provided an invaluable opportunity to bring major yeast laboratories together to compare notes on their progress toward utilizing the various molecular approaches to understand the biology of yeast.

This meeting was supported by financial contributions from Biogen Research Corp.; Hoffman-LaRoche, Inc.; Pfizer, Inc.; Smith Kline & French Laboratories; Biogen N.V.; Genentech, Inc.; Zymos Corp.; and LaBatt Brewing Company.

PHAGE AND BACTERIAL REGULATORY MECHANISMS

August 23–August 28

Arranged by

Ahmad Bukhari, *Cold Spring Harbor Laboratory*
Sankar Adhya, *National Cancer Institute*
Harrison Echols, *University of California, Berkeley*

228 participants

This meeting formally joined the phage and its host for the first time at the level of their scientific admirers. Presentation of recent work on the genetics, physiology, and biochemistry of prokaryotic organisms attested to the value of this union because of the similarity of molecular mechanisms and of techniques for elucidating them. One highlight of the meeting was the opportunity to note the enormous diversity of regulatory mechanisms, an aspect of prokaryotic control once overshadowed by the early understanding of the *lac* system of negative regulation. Transcription is regulated positively and negatively at the initiation and termination stage; posttranscriptional regulatory mechanisms abound, including initiation and completion of protein synthesis and protein stability. Another highlight was the chance to view the integration of diverse individual mechanisms into a unified regulatory response, as in the lysis-lysogeny decision by bacteriophage λ and the SOS and heat-shock responses of bacteria.

MODERN APPROACHES TO VACCINES

August 31–September 4

Arranged by

Robert Chanock, *National Institutes of Health*
Richard Lerner, *Research Institute of Scripps Clinic*

241 participants

This meeting concentrated on the latest contributions of a worldwide selection of leading laboratories in the field of vaccines. Topics included viral structure and function as they relate to the development of vaccines; chemistry of virus neutralization; new experimental approaches to the construction of viral proteins, including cloning and the expression of viral genes in prokaryotic and eukaryotic cell systems; attenuation of virulence; and enhancement of antigenicity and immunogenicity.

The broad scope of this meeting was of interest to individuals doing research on infectious disease, public health officials concerned with new strategies in the study and control of infection, members of the pharmaceutical industry involved in the development of vaccines, regulatory authorities who set safety and efficacy standards for vaccines, and immunologists.

Funding was provided in part by Merieux Institute, Inc.; Merck and Co., Inc.; and Wellcome Biotechnology, Ltd.

THE CANCER CELL

September 8–September 13

Arranged by

Arnold Levine, *State University of New York, Stony Brook*
William Topp, *Cold Spring Harbor Laboratory*
George Vande Woude, *NCI, National Institutes of Health*
James D. Watson, *Cold Spring Harbor Laboratory*

262 participants

The Cancer Cell meeting begins a new series of meetings on this topic that will replace the Cell Proliferation and Cancer Conferences. The meeting format sought to bring together scientists from diverse disciplines and approaches to focus on the molecular and cellular aspects of the cancer cell. Both RNA and DNA tumor virologists discussed the gene structure, gene products, and functions of oncogenes and virogenes involved in transforma-

tion and tumorigenesis. Cell biologists contributed sessions on growth factors, the keratins and cytoskeletal elements, chromosomal rearrangements, tumor promoters, and cancer cell surface antigens. The meeting succeeded in providing some unifying principles for the mechanisms of action of cell-derived oncogenes and DNA-virus-derived virogenes in the transformation process. The bridge from virology to cell biology has begun with new relationships between oncogene products and growth factors, chromosome rearrangements and oncogenes, and altered cytoskeletal genes and gene products.

The first clues to these unifying principles of oncogenic mechanisms and their apparent relationships to the problem of human cancer provided an appropriate level of excitement for this meeting dedicated to Mrs. Albert D. Lasker. Her contributions to the funding of this research over four decades were celebrated by the high caliber of science and the rapid rate of progress now evident in this field.

This meeting was supported by grants from the American Cancer Society, Becton-Dickinson and Company, the Fogarty International Center, and the National Cancer Institute.

HUMAN T-CELL LEUKEMIA VIRUSES

September 14–September 15

Arranged by

Myron Essex, *Harvard School of Public Health*
Robert Gallo, *National Cancer Institute*

113 participants

This was the first international meeting on human T-cell leukemia viruses (HTLV). HTLV is the first retrovirus that has been characterized that is clearly associated with human leukemia/lymphoma. The initial isolation in 1979 by Gallo and co-workers was followed by additional isolations in Japan and elsewhere. To date, there are approximately 35 isolates of HTLV worldwide. All of the HTLV isolates except two belong to the HTLV-I subgroup. The other two isolates belong to a related but distinctly different group designated HTLV-II, originally isolated from a patient with hairy-cell leukemia. The clustering of adult T-cell leukemia/lymphoma (ATLL) in the southern islands of Japan led to the suspicion that ATLL might be caused by an infectious agent. Further epidemiologic analysis worldwide has exposed other areas of HTLV-associated disease. HTLV is endemic in the southern islands of Japan, regions of Africa, southeastern United States, the Caribbean, and regions of Central and South America. Another disease of current worldwide interest is the acquired immune deficiency syndrome (AIDS). HTLV has been isolated from several AIDS patients. Although there is no clear etiology, HTLV has been linked to AIDS by virus isolation, molecular biology, and seroepidemiology.

This meeting was supported in part by grants from the National Cancer Institute and Fogarty International Center, Cancer Research Institute, and N.Y.S. Department of Health.

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 which 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.

THE CELLULAR AND MOLECULAR BIOLOGY OF BEHAVIOR

June 10–June 23

INSTRUCTORS

Kandel, Eric, M.D., *Columbia University, New York, New York*
Koester, John, Ph.D., *Columbia University, New York, New York*
Nottebohm, Fernando, Ph.D., *Rockefeller University, New York, New York*
Pearson, Keir, Ph.D., *University of Alberta, Canada*

This lecture course was designed to introduce students to cellular and molecular biological approaches to the study of behavior and learning. Rather than being exhaustive, the lectures provided an intensive coverage of four main areas: (1) general principles of behavior and of cellular neurobiology, including regulation of gene expression; (2) simple forms of behavior, learning, and motivation, and the role of biogenic amines, hormones, and peptides in their modulation; (3) initiation and maintenance of complex locomotor sequences, including voluntary movement and motor learning; and (4) communication. To illustrate general principles, suitable systems for study were selected from both invertebrate and vertebrate behavior. Emphasis was placed on the application of recombinant DNA technology in the analysis of behavior. To put the cellular work into perspective, selected examples were also taken from human behavior and its abnormalities. Guest lecturers included: R. Axel, A. Fuchs, P. Getting, C. Ghez, I. Kupfermann, G. Ojemann, R. Scheller, N. Suga, T. Thach, J. Truman, J. Wine, R. Wurtz, and R. Zigmond. This course was designed for advanced graduate students and research workers. A prior course in neurobiology was required.

MOLECULAR BIOLOGY OF PLANTS

June 10–June 30

INSTRUCTORS

Bedbrook, John, Ph.D., *Advanced Genetic Sciences, Berkeley, California*
Malmberg, Russell, Ph.D., *Cold Spring Harbor Laboratory, New York*
Sussex, Ian, Ph.D., *Yale University, New Haven, Connecticut*

ASSISTANTS

Waldren, John, *CSIRO, Canberra, Australia*
Brown, Susan, *Massachusetts General Hospital, Boston*
Dunn, Barbara, *Harvard University, Cambridge, Massachusetts*
McIndoo, Jean, *Cold Spring Harbor Laboratory, New York*

LABORATORY INSTRUCTORS

Dunsmuir, Pamela, Ph.D., *CSIRO, Canberra, Australia*
Jones, Jonathan, Ph.D., *Advanced Genetic Sciences, Berkeley, California*
Dellaporta, Stephen, Ph.D., *Cold Spring Harbor Laboratory, New York*
Miles, Don, Ph.D., *University of Missouri, Columbia*
Steinbeck, Kit, Ph.D., *Advanced Genetic Sciences, Berkeley, California*
Ho, David, Ph.D., *University of Illinois, Chicago*

This course provided an introduction to current techniques for the manipulation of plant material as applied to experiments in molecular biology and genetics. It was designed primarily for scientists working in other areas who wish to pursue research in plants. It was assumed that applicants had a general working knowledge of molecular biology, but no previous experience with plants was required. Experiments were designed to emphasize aspects unique to plant systems, including mutagenesis and analysis of mutants, tissue culture techniques, protoplast isolation and culture, regeneration of plants from culture, DNA and RNA isolation and cloning procedures, crown gall tumorigenesis, chloroplast genetics, and nitrogen fixation. Guest lecturers provided a background in plant morphogenesis, physiology, pathology, genetics, and cytology. Different plant species were used to illustrate particular experimental techniques, but the course emphasized tobacco and maize as model systems.

NERVOUS SYSTEM OF THE LEECH

June 10–June 30

INSTRUCTORS

Nicholls, John, Ph.D., M.D., *Stanford University Medical School, California*

Muller, Ken, Ph.D., *Carnegie Institution of Washington, Baltimore, Maryland*
Thompson, Wesley, Ph.D., *University of Texas, Austin*

In this laboratory course students used intracellular and extracellular recording techniques to recognize individual motor and sensory nerve cells, to map the fields they innervate in the periphery, to measure their membrane properties, and to trace their synaptic connections.

Other techniques used included: staining cells with horseradish peroxidase and Lucifer yellow, making chronic lesions in the CNS, and recording from cells in swimming preparations. In addition, the technique involved in staining leech cells with monoclonal antibodies was demonstrated. Last year, seminars and demonstrations were given by B. Zipser, W. Kristan, G. Stent, D. Weisblat, O. Friesen, R. McKay, and S. Hockfield.

Our aim was to accept students who want to go on and work with the leech or a similar preparation. On the other hand, students that wished to use this course as an advanced techniques course were accepted if their interest was great enough. Some prior knowledge of neurobiology and experience in electrical recording were required for this course.

MOLECULAR CLONING OF EUKARYOTIC GENES

June 10–June 30

INSTRUCTORS

Fritsch, Edward, Ph.D., *Genetics Institute, Boston, Massachusetts*
Davis, Mark, Ph.D., *Stanford University, California*
Mullins, James, Ph.D., *Harvard School of Public Health, Boston, Massachusetts*

ASSISTANTS

Kim, Stu, B.S., *California Institute of Technology, Pasadena*
Boyer, Paul, B.S., *Michigan State University, East Lansing*
Federspiel, Mark, B.S., *Michigan State University, East Lansing*

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 cellular structural genes were emphasized. Among the topics covered were the construction of cDNA libraries in plasmid or bacteriophage λ vectors, construction of bacteriophage λ and cosmid libraries of high-molecular-weight eukaryotic DNA, screening DNA libraries with gene-specific hybridization probes 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 lecturers discussed the application of molecular cloning procedures to the study of specific eukaryotic gene systems.

GENETICS AND MOLECULAR BIOLOGY OF *CHLAMYDOMONAS*

June 26–June 30

CHAIRMAN

Gillham, Nicholas W., Ph.D., *Duke University, Durham, North Carolina*

ORGANIZING COMMITTEE

Boynton, John E., Ph.D., *Duke University, Durham, North Carolina*
Harris, E.H., Ph.D., *Duke University, Durham, North Carolina*
Luck, David J.L., M.D., Ph.D., *Rockefeller University, New York, New York*
Goodenough, Ursala, Ph.D., *Washington University, St. Louis, Missouri*

The green alga *Chlamydomonas* is recognized as a particularly favorable model system for the study of a variety of basic biological problems. Students in this 4-day combined course and conference participated in workshops with invited scientists working on the molecular biology, physiology, and genetics of this organism. Through review lectures, panel discussions, and demonstrations, specialists working on *Chlamydomonas* explored the use of this organism in investigations of photosynthesis, flagellar structure and function, the cell cycle, porphyrin biosynthesis, chloroplast genetics, phototaxis, mating, and other topics. In addition, techniques such as genetic analysis, transformation, and gene cloning in *Chlamydomonas* were covered. This conference/course was designed to bring together established workers in the field with people interested in using *Chlamydomonas* as a research tool and was conducted in an informal setting where maximum interaction is possible between the participants.

MOLECULAR EMBRYOLOGY OF THE MOUSE

July 3–July 16

INSTRUCTORS

Hogan, Brigid, Ph.D., *Imperial Cancer Research Fund, London, England*

Costantini, Frank, Ph.D., *Columbia University, New York, New York*

Lacey, Liz, Ph.D., *Memorial Sloan-Kettering Cancer Institute, New York, New York*

ASSISTANTS

Barlow, Denise, Ph.D., *Imperial Cancer Research Fund, London, England*

Raphael, Katherine, *Columbia University, New York, New York*

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 into 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 germ layers, 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.

SINGLE-CHANNEL RECORDING

July 3–July 23

INSTRUCTORS

Aldrich, Richard W., Ph.D., *Yale University, New Haven, Connecticut*

Cahalan, Michael D., Ph.D., *University of California, Irvine*

Corey, David P., Ph.D., *Yale University, New Haven, Connecticut*

Dionne, Vincent E., Ph.D., *University of California, San Diego*

Hume, Richard I., *Washington University, St. Louis, Missouri*

Lewis, Richard, Ph.D., *California Institute of Technology, Pasadena*

Stevens, Charles F., M.D., Ph.D., *Yale University, New Haven, Connecticut*

LECTURERS

Breakfield, Xandra O., Ph.D., *Yale University, New Haven, Connecticut*

Yellen, Gary, Ph.D., *Yale University, New Haven, Connecticut*

ASSISTANT

Stevens, Meg, *Harvard University, Boston, Massachusetts*

Single-channel recording and other applications of the patch-clamp technique were taught in this intensive lab/lecture course.

The laboratory work, in which two participants work with each instructor, began with all groups working on a common preparation. Participants learned to make electrodes; establish giga-seals; form inside-out, outside-out, and cell-attached patches; voltage clamp whole cells; and to recognize and specifically activate different channel species. The third week was spent on individual projects with preparations of particular interest to the participants. Lectures covered electrode fabrication; properties of glasses and formation of a seal; design of the patch-clamp circuit and sources of electronic noise; primary cell culture and cell lines; ion permeation and single-channel conductance; procedures for whole-cell clamp, separation of currents; rate theory and macroscopic kinetics; stochastic processes and single-channel statistics; single-channel behavior and noise spectra; stimulation and data acquisition with computers; and algorithms for single-channel analysis.

ADVANCED NEUROANATOMICAL METHODS

July 3–July 23

INSTRUCTORS

Jones, Edward G., Ph.D., *Washington University, St. Louis, Missouri*

Hand, Peter J., Ph.D., *University of Pennsylvania, Philadelphia*

Pickel, Virginia M., Ph.D., *Cornell Medical School, New York, New York*

Wise, Steven P., Ph.D., *National Institute of Mental Health, Bethesda, Maryland*

Hendry, Stewart, Ph.D., *Washington University, St. Louis, Missouri*

ASSISTANTS

Donoghue, John, *National Institute of Mental Health, Bethesda, Maryland*

McClure, Bertha, *Washington University, St. Louis, Missouri*

Hand, Carol, *University of Pennsylvania, Philadelphia*

This is an intensive laboratory course in basic and advanced neuroanatomical methodology given by six or more faculty, three assistants, and six visiting speakers. Students, individually or in pairs, carried out an anatomical analysis of one of several parts of the mammalian CNS. Methods used were anterograde and retrograde axonal tracing by autoradiography, enzyme histochemistry, and fluorescent dyes; neurotransmitter uptake and receptor binding techniques;

immunocytochemistry using conventional and monoclonal antibodies; and metabolic mapping by 2-deoxy-D-glucose uptake and certain oxidative enzymes.

Students received training in conventional light and electron microscopy methods, fluorescence microscopy, animal surgery, microinjection methods under electrophysiological control, photomicrography, autoradiography, and immunocytochemistry, as well as practice in the use of most of the more common laboratory instruments.

Lectures by the faculty and by a series of visiting speakers covered the methodologies used, dealt with selected research on the parts of the CNS being investigated, and provided insights into newly developed techniques.

ADVANCED BACTERIAL GENETICS

July 3–July 23

INSTRUCTORS

Berman, Michael L., Ph.D., *NCI, Frederick Cancer Research Facility, Frederick, Maryland*

Enquist, Lynn W., Ph.D., *Molecular Genetics, Inc., Minnetonka, Minnesota*

Silhavy, Thomas J., Ph.D., *NCI, Frederick Cancer Research Facility, Frederick, Maryland*

ASSISTANTS

Bear, Susan, Ph.D., *NCI, National Institutes of Health, Bethesda, Maryland*

Garrett, Stephen, B.A., *NCI, Frederick Cancer Research Facility, Frederick, Maryland*

Bremer, Erhard, Ph.D., *NCI, Frederick Cancer Research Facility, Frederick, Maryland*

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 regulon; however, the experimental techniques presented were sufficiently general to be applicable to any gene in *E. coli* for which there exists a mutation conferring a recognizable phenotype.

MOLECULAR AND CELLULAR NEUROBIOLOGY

July 3–July 23

INSTRUCTORS

Rahamimoff, Rami, M.D., *Hebrew University, Jerusalem, Israel*

McMahan, U. Jack, Ph.D., *Stanford University, California*

PART-TIME INSTRUCTORS

Herbert, Edward, Ph.D., *University of Oregon, Eugene*

Stevens, Charles, M.D., Ph.D., *Yale Medical School, New Haven, Connecticut*

Yoshikami, Doju, Ph.D., *University of Utah, Salt Lake City*

The aim of this intensive course was twofold: (1) to examine in detail the principles of nervous system function, structure, and development at the cellular and molecular level, and (2) to define specific problems in neurosciences that can be studied by recently developed techniques in molecular and cellular biology. One week was devoted to the application of molecular biological methods to nervous system problems. Specific topics included general principles of molecular biology; the use of recombinant DNA methodology to study the regulation of neuropeptides and acetylcholine receptors; use of hybridoma technology for identification and characterization of specific molecules in the nervous system; and examination of the genetic basis of specific patterns of behavior. The cellular approach focused on the synapse. Topics covered included synaptic excitation and inhibition; structure of presynaptic and postsynaptic elements; release, storage, and inactivation of neurotransmitters; noise analysis and single-channel recording; structure, distribution, and metabolism of receptor molecules; synaptic immunology; electrical synapses and gap junctions; slow synaptic potentials; synaptic development; degeneration and regeneration; role of peptides in neural function; and neurobiology of certain diseases.

NEUROBIOLOGY OF HUMAN DISEASE

July 24–July 30

INSTRUCTORS

Black, Ira B., M.D., *Cornell University Medical College, New York, New York*

Breakefield, X.O., Ph.D., *Yale University School of Medicine, New Haven, Connecticut*

This intensive seminar course explored the cellular and molecular basis of abnormal neural function, delineating the pathogenesis of neuropsychiatric disorders. Experimental models and human disease counterparts were examined in detail. Topics included: (1) molecular basis of neurotransmitter derangement; (2) mechanisms of consciousness and the genesis of coma; (3) neurophysiology of lateralization of brain function; (4) cellular basis of regeneration of brain systems; (5) mechanisms of dementia, including Alzheimer's disease; (6) experimental models of degenerative disorders, including Huntington's disease; (7) genesis of movement disorders, including dystonia; (8) mechanisms underlying epilepsy; (9) biochemistry of the lipidoses; (10) slow virus infections of the nervous system; (11) molecular basis of pain syndromes; (12) cellular biology of peripheral neuropathy; (13) pathogenesis of myasthenia gravis; and (14) diseases of DNA repair.

ADVANCED TECHNIQUES IN MOLECULAR CLONING OF EUKARYOTIC GENES

July 26–August 15

INSTRUCTORS

Smith, Michael, Ph.D., *University of British Columbia, Vancouver, Canada*
Zoller, Mark, Ph.D., *University of British Columbia, Vancouver, Canada*
Atkinson, Tom, B.S., *University of British Columbia, Vancouver, Canada*
Fiddes, John, Ph.D., *California Biotechnology, Inc., Mountain View*
Shortle, David, Ph.D., *State University of New York, Stony Brook*

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.

ELECTROPHYSIOLOGICAL METHODS USED IN ANALYZING THE MODE OF ACTION OF TRANSMITTERS

July 26–August 15

INSTRUCTORS

Schuetze, Stephen, Ph.D., *Columbia University, New York, New York*
Siegelbaum, Steven, Ph.D., *Columbia University, New York, New York*
Ascher, Philippe, D.Sc., *Ecole Normale Supérieure, Paris, France*

In this experimental course, students were introduced to the various electrophysiological techniques that are currently applied to the study of transmitter action on ion channels in skeletal muscle and invertebrate neurons. They used both basic voltage-clamp techniques (including a single microelectrode voltage clamp) as well as the patch-clamp technique for single-channel recording. Aspects of the theory of the techniques as well as some basic biochemical and biophysical principles of transmitter actions were also covered.

YEAST GENETICS

July 26–August 15

INSTRUCTORS

Sherman, Fred, Ph.D., *University of Rochester, New York*
Fink, Gerald, Ph.D., *Massachusetts Institute of Technology, Cambridge*
Hicks, James, Ph.D., *Cold Spring Harbor Laboratory, New York*

ASSISTANT

Kim, Jinimi, *Massachusetts Institute of Technology, Cambridge*

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.

IMMUNOGLOBULINS: MOLECULAR PROBES OF THE NERVOUS SYSTEM

July 26—August 15

INSTRUCTORS

Brockes, Jeremy, Ph.D., *Kings College, London, England*

McKay, Ronald, Ph.D., *Cold Spring Harbor Laboratory, New York*

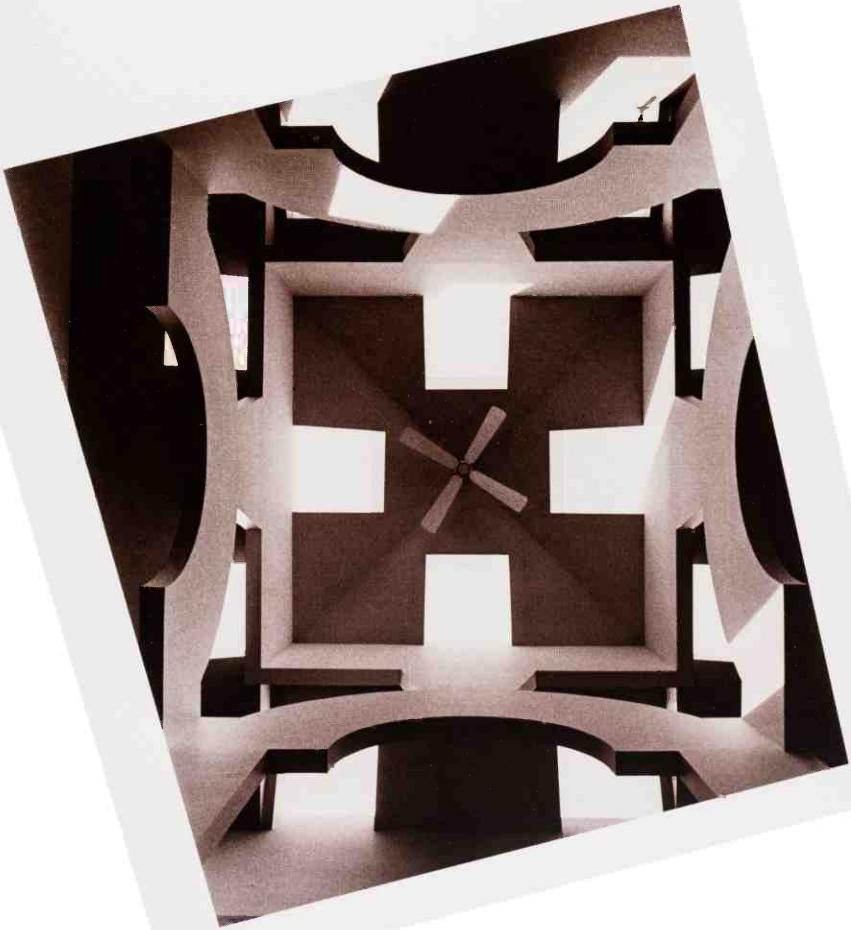
ASSISTANT

Kitner, Chris, Ph.D., *Kings College, London, England*

Through laboratory work and lectures, this course was designed to provide an advanced understanding of the power and limitations of immunoglobulins as a means of identifying and characterizing molecules of interest to neuroscientists. It was intended for research scientists at all levels. A series of lectures by invited speakers covered the following topics: structure and function of immunoglobulins; thermodynamics of antigen-antibody interaction; molecular genetics of antibody diversity; cellular regulation of the immune response; hybridoma technology; c-DNA expression vectors; synthetic peptides; cell recognition and differentiation antigens; immunological characterization of the acetylcholine receptor, the neuromuscular junction, and synaptic components of the central nervous system; and immunological characterization of cellular diversity in the invertebrate and vertebrate nervous system.

The laboratory work focused around a practical introduction to generating monoclonal antibodies to simple and complex antigens. In addition, some of the growing number of associated immunological techniques were taught, e.g., antigen preparation, complement-mediated lysis, solid-phase immunoassays, immunoprecipitation, Western blotting, purification and conjugation of immunoglobulins, double diffusion, affinity purification, and immunocytochemical techniques.

BANBURY CENTER



Recto: *Interior view of Sammis Hall, Banbury Center's guest house, designed by Moore Grover Harper, P.C., 1981.*

REPORT OF THE DIRECTOR

Scientific meetings have been recognized as being important to the progress of science at least since the 1660s, when the newly established Royal Society assumed the regular holding of such meetings at Gresham College in London as one of its main functions. The establishment, in 1977, of an intimate and informal biological sciences conference center within the magnificent estate atop Banbury Lane was entered upon very much within this tradition. The small, highly interactive conferences of the Banbury Center have proven of particular value not just in the promotion of rapid and efficient communication of new data and concepts, but also in their further refinement and development during the spontaneous discussions and analyses that are an intrinsic part of the meeting dynamic itself. This is especially important in areas where previously divergent approaches may be coming together for the first time as well as in facilitating progress in existing fields either through identification of critical areas to be addressed or by placing possible impediments to progress within broader disciplinary perspectives.

This, the sixth year of such activities at the Banbury Center of Cold Spring Harbor Laboratory, saw a continuation and development of the Center's three broad areas of identified interest: human health risk assessment; pivotal areas accruing from newer approaches such as recombinant DNA methodologies and related molecular genetic manipulations; and the bringing of such perspectives to the attention of major public health concerns, especially in the area of biological aspects of, and possible interventions in, aging.

The first 1983 meeting occurred early in February and addressed the increasingly pressing public health problem presented by acquired immunodeficiency syndrome (AIDS). This meeting, by bringing together immunologists, epidemiologists, pathologists, virologists, and public health officials in a small, informal yet intensive environment, proved particularly useful for setting priorities and developing a greater coherence of effort in approaches to this growing health concern. It was fairly clear, by the meeting's conclusion, that a consensus was developing for a concerted search for a blood-borne viral agent, the properties of which could at least begin to be loosely defined. Subsequent events are tending to bear out the soundness of what was then still a tentative and perhaps somewhat controversial proposal.

If the AIDS conference was meant to help facilitate the coalescing of divergent approaches addressing the same central problem, the next two Banbury meetings were perhaps more useful in delineating and better defining critical areas to be addressed in already recognized important research areas. The first of these meetings, Plant Viruses and Viroids, considered an area of great potential importance not only in molecular biology, but also with regard to practical application. These often strange infectious agents, apparently unique to plants, present possible new mechanisms by which genetic material may be replicated, perpetuated, and transmitted from cell to cell or from plant to plant. The second meeting, held in the early spring, considered an equally important topic from both research and practical application perspectives—the ways in which the activities of genes may be regulated. This conference, Enhancers and Controlling Elements, focused on eukaryotic DNA sequences that, when placed anywhere in the vicinity of a given gene, have great effects upon the expression of that gene within the cell. Identification of such sequences will have great significance for fundamental questions of biology, such as molecular mechanisms of development, as well as for the manipulation of gene products in practical application. *Plant Infectious Agents* and *Enhancers and Eukaryotic Gene Expression* have both been published in extended abstract form as part of the Cold Spring Harbor Laboratory series Current Communications in Molecular Biology.

The Banbury Center publishing program also remained quite active in 1983 with the appearance of three new titles, two in the ongoing Banbury Report series and one as the first Banbury publication directed to a wider, nonscientific audience. Banbury Report 14, *Recombinant DNA Applications to Human Disease*, grew out of a fall 1982 conference considering new approaches that combine DNA cloning and hybridization techniques with more traditional genetic methodologies for identification and diagnosis of genetic defects at the level of the defective gene itself. The second 1983 book emanating from the 1982 conference program was Banbury Report 15, *Biological Aspects of Alzheimer's Disease*. Reflecting the impetus of the original conference in bringing together clinicians and basic researchers for an exploration of the origins and pathological bases of this devastating major health concern, this volume has proved a useful compendium both

of the current state of knowledge and of possible leads on how this might further be developed in the future. The final publication of 1983 also grew out of a 1982 Banbury conference, but it was the first Banbury publication outside of the Banbury Report series. *Gene Therapy: Fact and Fiction,* published as a "Banbury Public Information Report," combined the edited transcribed proceedings of the original conference with additional interpretive and descriptive material in an attempt to make known to the public at large the import of this high-level scientific meeting on a topic that continues to be of significant public interest and concern.

Although *Gene Therapy: Fact and Fiction* was a departure in publishing for the Banbury Center, the function of the Center in broadly disseminating information about developments in key areas of the biological sciences has been ongoing almost since Banbury's inception. Under continuing support from the Sloan Foundation, Banbury Center has carried out a series of informational workshops specifically designed either for media representatives or for congressional aides and staff members. Two such Sloan Informational Workshops were held in 1983. The first of these, New Concepts in Mutation, was a summer workshop for journalists. For many years, mutational mechanisms could be studied productively mainly in microorganisms. The advent of current DNA methodologies, however, has presented opportunities for the highly effective study of such processes in higher organisms as well. The cumulative body of microorganismal data, together with the rapidly emerging new concepts derived from approaches in higher organisms, is leading to new perspectives on the origins and roles of mutation and on the mutational dynamic underlying biological evolution. These topics were considered in depth at the Sloan Journalists' Workshop, as well as at the subsequent full Banbury scientific conference, Mechanisms of Mutagenesis.

The second Sloan workshop, Biological Imaging and Nuclear Magnetic Resonance, was held early in December for congressional staff members. This rapidly developing area of medical technology is achieving spectacular results in imaging both structure and function in the living body while employing minimally invasive procedures. However, the costs can also be spectacular, and during this relatively early stage of development, it is imperative for congressional decision makers to have an understanding of what these technologies are likely to achieve and of the relative advantages and disadvantages of competing approaches. They must also have an overall feel for the benefits, risks, and costs that these technologies will potentially bring to the U.S. health care capability at large. The December workshop, which considered everything from the theoretical foundations of this technology to the practical installation of the apparatuses themselves, together with multiple examples of just what such approaches are currently achieving and are likely to achieve in the near future, helped to clarify considerably the complexities of this new area of medical technology.

Finally, two full Banbury Conferences, each with an ensuing Banbury Report, were held in the fall of 1983. The first of these, held in early October, considered the genetic predisposition of the individual in response to chemical exposure. Many of the bodily systems concerned with maintenance and integrity of function, or with metabolic activation or detoxification of chemicals, have such a large degree of underlying genetic variability that each individual almost certainly presents a unique profile of strengths and susceptibilities. Individualized genetic susceptibility profiles could clearly have a place in preventive medicine or even as personalized guides toward maximizing health and safety. There have already been controversial nascent attempts to apply such concepts to workers employed in hazardous workplace environments. The October conference, considering in depth the scientific data base underlying such possible approaches, is to be published as Banbury Report 16, *Genetic Variability in Responses to Chemical Exposure.*

The second full Banbury Conference, held at the end of October and into early November, examined the health consequences of coffee consumption. Due to the great popularity and prevalence of this beverage, even possible minor deleterious effects have potentially far-reaching impact. The Coffee and Health conference assembled researchers from both private and public sectors representing both epidemiological and experimental approaches to this question. These proceedings, reviewing major ongoing studies together with assessment of where such research thrusts may be leading, is to be published as Banbury Report 17 in the spring of 1984.

Michael Shodell

1983 SUPPORT

Together with the donation to Cold Spring Harbor Laboratory of his estate on Banbury Lane, Charles S. Robertson also generously supplied an endowment for upkeep of the grounds and of Robertson House proper. However, maintenance of Sammis Hall and the conference center, as well as support for Banbury Center conferences and publications, remains strictly dependent upon government and foundation grants and corporate contributions. In 1983, Banbury Center activities were greatly facilitated by unrestricted core support accruing from the generous donations of nine corporations: The Bristol-Myers Fund, Inc., Conoco, Inc., The Dow Chemical Company, E.I. du Pont de Nemours & Company, Exxon Corporation, Getty Oil Company, International Business Machines Corporation, Eli Lilly & Company, and Texaco Philanthropic Foundation, Inc. In addition, the Center was able to proceed with specific conference programs as a result of the following grants and contributions: The Role of Genetic Predisposition in Responses to Chemical Exposure was supported by the National Cancer Institute, the National Institute of Environmental Health Sciences, and the Fogarty International Center, together with essential additional support from the American Occupational Medical Association, the Aluminum Company of America, E.I. du Pont de Nemours & Company, Johnson & Johnson, the Occupational Health Institute, Inc., and United States Steel. Support for Coffee and Health was obtained from Cold Spring Harbor Laboratory, with major additional funding from the National Coffee Association of the U.S.A., Inc., and the International Coffee Organization; contributions toward publication of these proceedings were received from the Folger Coffee Company, the Nestle Company, Inc., and Tetley, Inc. The conference on Plant Viruses and Viroids received corporate contributions from Monsanto Company, ARCO Plant Cell Research Institute, Pfizer Central Research, Agrigenetics Corporation, and Calgene Corporation. The Enhancers and Controlling Elements conference was made possible by donations provided by Abbott Laboratories, Applied Molecular Genetics, Inc., Biogen N.V., the Cetus Corporation, Collaborative Research, Inc., E.I. du Pont de Nemours & Company, Genentech, Inc., Lilly Research Laboratories, and the Monsanto Company. The conference on Acquired Immunodeficiency Syndrome was supported by a grant from the Cancer Research Institute and the National Cancer Institute (National Institutes of Health). The meeting on Mechanisms of Mutagenesis was supported in part by a grant from the March of Dimes Birth Defects Foundation. These generous grants and donations in support of the Banbury Center program are gratefully acknowledged. Collectively, these grants and contributions, the ongoing Sloan Foundation sponsorship of informational workshops for journalists and congressional staff, and contributions received from the Kaiser Family Foundation financing the publication of *Gene Therapy: Fact and Fiction* and the original conference on this topic have formed the financial base that has permitted continuation of the Banbury Center program through its sixth year of operation.

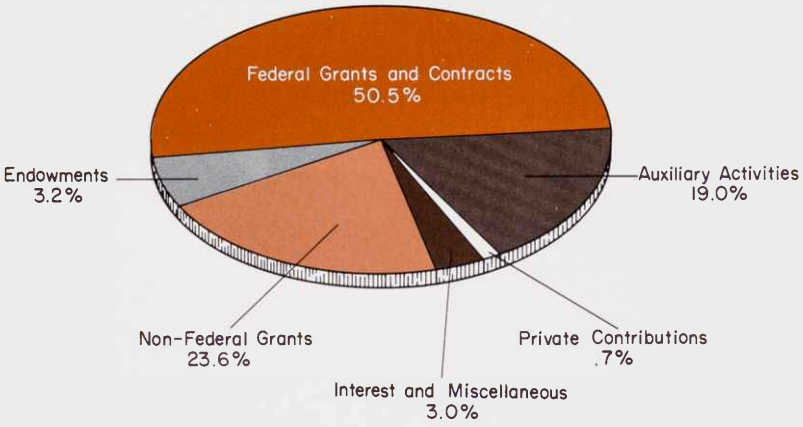
Alfred Pfeiffer

1919 – 1984

When the Banbury estate was donated to Cold Spring Harbor Laboratory, the Laboratory was especially fortunate in being able to retain the services of the estate's head groundskeeper, Fred Pfeiffer. Fred not only continued to give his special type of care to the estate's grounds, but also brought to the Banbury community a special sense of culture and thoughtfulness. Fred's sudden death in March, 1984, just months before he was due to retire, was a great loss. He will continue to be missed by the staff and visitors to the Banbury Center.

FINANCIAL REPORT

COLD SPRING HARBOR LABORATORY
SOURCES OF REVENUE
YEAR END DECEMBER 31, 1983



FINANCIAL SUPPORT OF THE LABORATORY

Cold Spring Harbor Laboratory is a non-profit 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 Information Services, Cold Spring Harbor Laboratory, Box 100, Cold Spring Harbor, N.Y. 11724, or call 516-367-8397.

FINANCIAL STATEMENT

BALANCE SHEET

year ended December 31, 1983
with comparative figures for year ended December 31, 1982

ASSETS

	1983	1982
CURRENT FUNDS		
Unrestricted		
Cash and Short-term investments	$ 4,119,413	$ 2,658,863
Accounts Receivable	375,687	464,261
Prepaid expenses and other assets	289,940	258,587
Inventory of books	194,111	203,899
Due from restricted fund	–	286,290
Due from Banbury Center	115,810	284,630
Total unrestricted	5,094,961	4,156,530
Restricted		
Grants and contracts receivable	3,779,375	4,615,780
Due from unrestricted fund	660,906	–
Total restricted	4,440,281	4,615,780
Total current funds	$ 9,535,242	$ 8,772,310
ENDOWMENT FUNDS		
Robertson Research Fund		
Cash	3,536,782	508,041
Marketable securities (quoted market 1983 – $13,466,772; 1982 – 13,987,572)	11,446,963	11,585,922
Total Robertson Research Fund	14,983,745	12,093,963
Olney Memorial Fund		
Cash	4,623	1,420
Marketable Securities (quoted market 1983 – $23,252; 1982 – $23,284)	27,538	27,538
Total Olney Memorial Fund	32,161	28,958
Total endowment funds	$15,015,906	$12,122,921
PLANT FUNDS		
Investments	454,212	461,239
Due from unrestricted fund	983,773	961,628
Land and improvements	1,129,875	1,111,213
Buildings	9,621,165	7,307,583
Furniture, fixtures and equipment	2,485,774	1,647,248
Books and periodicals	365,630	365,630
Construction in progress	960,709	727,245
	16,001,138	12,581,786
Less allowance for depreciation and amortization	4,013,770	3,371,273
Total plant funds	$11,987,368	$ 9,210,513

LIABILITIES AND FUND BALANCES

	1983	1982
CURRENT FUNDS		
Unrestricted		
Accounts payable	$ 835,166	$ 171,326
Deferred income	140,000	–
Mortgage payable	–	33,300
Due to plant fund	983,773	961,628
Due to restricted fund	660,906	–
Fund balance	2,475,116	2,990,276
Total unrestricted	5,094,961	4,156,530
Restricted		
Due to unrestricted fund	–	286,290
Fund balance	4,440,281	4,329,490
Total restricted	4,440,281	4,615,780
Total current funds	$ 9,535,242	$ 8,772,310
ENDOWMENT FUNDS		
Fund balance	$15,015,906	$12,122,921
PLANT FUNDS		
Fund balance	$11,987,368	$ 9,210,513

ASSETS (*continued*)

	1983	1982
BANBURY CENTER		
Current funds		
Unrestricted		
Cash	700	700
Prepaid and deferred expenses	15,025	70,001
Inventory of books	36,857	25,777
Due from Banbury restricted fund	95,505	35,999
Total unrestricted	148,087	132,477
Restricted		
Grants and contracts receivable	142,988	91,046
Total restricted	142,988	91,046
Total current funds	291,075	223,523
Endowment Funds		
Robertson Maintenance Fund		
Cash	494,054	89,768
Marketable securities (quoted market 1983 – $2,314,259; 1982 – $2,385,039)	1,978,853	1,971,628
Total endowment funds	2,472,907	2,061,396
Plant funds		
Land	772,500	772,500
Buildings	845,967	846,028
Furniture, fixtures and equipment	176,318	176,284
Construction in progress	1,386	1,386
	1,796,171	1,796,198
Less allowance for depreciation	320,450	275,311
Total plant funds	1,475,721	1,520,887
Total Banbury Center	$ 4,239,703	$ 3,805,806
Total – All funds	$40,778,219	$33,911,550

LIABILITIES AND FUND BALANCES (*continued*)

	1983	1982
BANBURY CENTER		
Current funds		
Unrestricted		
Accounts payable	32,277	11,376
Due to CSHL unrestricted fund	115,810	284,630
Fund balance	–	(163,529)
Total unrestricted	148,087	132,477
Restricted		
Due to Banbury unrestricted	95,505	35,999
Fund balance	47,483	55,047
Total restricted	142,988	91,046
Total current funds	291,075	223,523
Endowment funds		
Fund balance	2,472,907	2,061,396
Plant funds		
Fund balance	1,475,721	1,520,887
Total Banbury Center	$ 4,239,703	$ 3,805,806
Total–All funds	$40,778,219	$33,911,550

CURRENT REVENUES, EXPENSES AND TRANSFERS
year ended December 31, 1983
with comparative figures for year ended December 31, 1982

COLD SPRING HARBOR LABORATORY

	1983	1982
REVENUES		
Grants and contracts	$11,650,901	12,175,270
Indirect cost allowances on grants and contracts	3,626,022	3,155,513
Contributions		
Unrestricted	27,800	30,682
Restricted and capital	868,528	225,000
Long Island Biological Association	33,300	46,500
Robertson Research Fund Distribution	453,000	453,000
Summer programs	491,049	433,188
Laboratory rental	20,732	20,732
Marina rental	56,170	53,192
Investment income	481,992	376,587
Publications sales	1,655,691	1,650,263
Dining Hall	490,678	469,196
Rooms and apartments	216,179	269,061
Other sources	26,371	12,347
Total revenues	20,098,413	19,370,531
EXPENSES		
Research*	10,098,170	8,855,612
Summer programs*	993,338	775,528
Publications sales*	1,306,539	1,132,856
Dining hall*	671,602	535,399
Research support	256,676	203,631
Library	223,117	191,344
Operation and maintenance of plant	1,921,985	1,719,729
General and Administrative	1,632,045	1,192,215
Depreciation	643,097	505,252
Total expenses	17,746,569	15,111,566
TRANSFERS		
Capital building projects	1,738,298	2,003,518
Banbury Center to (from)	(80,420)	212,235
Total transfers—net	1,657,878	2,215,753
Total expenses and transfers	19,404,447	17,327,319
Excess of revenues over expenses and transfers	$ 693,966	$ 2,043,212

**Reported exclusive of an allocation for research support, operation and maintenance of plant, general and administrative, library, and depreciation expenses.*

BANBURY CENTER

	1983	1982
REVENUES		
Endowment income	$100,000	$ 99,000
Grants & contributions	261,436	288,226
Indirect cost allowances on grants and contracts	13,576	19,880
Rooms and apartments	76,522	46,353
Publications	191,993	136,391
Conference fees	54,096	20,375
Dining Hall	4,708	6,484
Transfer from (to) Cold Spring Harbor Laboratory	(80,420)	212,235
Total revenues	621,911	828,944
EXPENSES		
Conferences	206,659	151,631
Publications	126,618	168,011
Operation and maintenance of plant	93,548	109,474
Program administration	202,651	163,312
Depreciation	45,107	53,954
Capital plant	–	53,271
Total expenses	674,583	699,653
Excess (deficit) of revenues over expenses	$(52,672)	$129,291

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.

GRANTS
January 1, 1983–December 31, 1983

COLD SPRING HARBOR LABORATORY

Grantor	*Principal Investigator and Program*	*Total Award*	*Duration of Grant*
National Institutes of Health			
Program Project Grants	Dr. Bukhari–Genetics	$ 2,375,371	06/01/80–11/30/83
	Dr. Roberts–Cancer Research Center	18,604,426	01/01/77–12/31/86
	Dr. Sambrook–Gene Organization	5,626,997	04/01/81–03/31/86
Research Grants	Dr. Blose	449,929	12/01/78–11/30/84
	Dr. Feramisco	686,968	07/01/80–07/31/86
	Dr. Gething	976,587	*02/01/83–01/31/87
	Drs. Gingeras & Roberts	408,085	03/01/80–02/28/83
	Dr. Hicks	1,710,283	07/01/81–06/30/86
	Dr. Hockfield	282,400	02/01/82–01/31/85
	Dr. Klar	1,572,978	07/01/81–06/30/86
	Dr. Klar	286,070	*12/01/83–11/30/85
	Dr. Kurtz	606,389	04/01/80–03/31/86
	Dr. Lin	290,636	09/24/82–08/31/85
	Dr. Mathews	729,283	04/01/80–03/31/86
	Dr. Matsumura	385,649	*07/01/83–06/30/86
	Dr. McKay	288,546	07/01/81–06/30/84
	Dr. Silver	625,803	02/01/82–01/31/85
	Dr. Stillman	677,384	*07/01/83–06/30/86
	Dr. Topp	279,134	03/01/82–02/28/84
	Dr. Topp	303,452	07/01/80–06/30/83
	Dr. B. Zipser	299,219	12/01/81–11/30/84
	Dr. B. Zipser	358,081	*12/01/83–11/30/86
Biomedical Research Support Grants	Dr. Watson	104,822	04/01/82–03/31/83
	Dr. Watson	111,124	*04/01/83–03/31/84
Fellowship Training	Dr. Grodzicker (Institutional)	852,578	07/01/78–11/30/83
	Dr. Bautch	57,244	*09/01/83–08/31/86
	Dr. Flanagan	36,204	*09/16/83–09/15/85
	Dr. Flaster	34,776	09/15/82–09/14/84
	Dr. Ivy	38,776	01/27/82–01/26/84
	Dr. Krangel	54,584	10/01/82–09/30/85
	Dr. LeMaster	34,681	06/01/81–02/15/83
	Dr. Livi	57,244	*10/16/83–10/15/86
	Dr. Roth	36,776	*09/01/83–08/31/85
	Dr. Taparowsky	54,584	10/16/82–10/15/85
	Dr. Welch	56,584	*09/01/83–08/31/85
Course Support	Dr. Hockfield–Neurobiology	131,277	05/01/82–04/30/85
	Dr. Hockfield–Neurobiology	646,143	06/01/79–03/31/86
	Dr. Grodzicker–Cancer Center	657,174	06/01/82–12/31/86
	Dr. Hicks–Advanced Bacterial Genetics	302,523	05/01/80–04/30/88

*New grants awarded in 1983.

Grantor	*Principal Investigator and Program*	*Total Award*	*Duration of Grant*
National Institutes of Health (*continued*)			
Meeting Support	Dr. Mathews – RNA Processing	18,000	04/15/82–04/30/84
	Dr. Watson – Human T-Cell Leukemia Viruses	27,436	*09/13/83–08/31/84
	Dr. Watson – Symposium: Molecular Neurobiology	41,977	*04/01/83–03/31/84
	Dr. Watson – Genetics of *Chlamydomonas*	5,000	*06/26/83–06/25/84
	Dr. Watson – *C. elegans*	29,290	*04/01/83–03/31/84
	Dr. Watson – Cell Proliferation Conference: The Cancer Cell	61,350	08/15/81–07/31/84
Construction	Mr. Udry – Cancer Research Facility	1,411,011	09/15/77–12/31/83
National Science Foundation			
Research Grants	Dr. Bukhari	60,000	07/82–12/83
	Dr. Garrels	140,000	09/80–02/83
	Dr. Harshey	78,001	01/82–05/83
	Dr. Heffron	167,000	02/81–07/83
	Drs. Hicks, Klar, Stratherm & Malmberg	330,134	07/82–12/84
	Dr. Lewis	140,000	*11/83–12/85
	Dr. Roberts	210,000	12/79–05/83
	Dr. Roberts	270,000	08/80–07/83
	Dr. Roberts	28,442	*01/83–08/86
	Dr. Roberts	200,000	*01/83–12/86
	Dr. Roberts	252,000	*07/83–12/86
	Dr. So	32,194	07/82–12/83
	Dr. Tamanoi	88,000	07/82–12/84
	Dr. D. Zipser	100,000	01/81–06/83
Course Support	Dr. Hicks – Plant Molecular Biology Workshop	99,600	05/81–10/84
Meeting Support	Dr. Bukhari – 4th Bacteriophage Mu Workshop (Pakistan)	32,000	*11/83–10/84
	Dr. Klar – Microbial Development	8,940	*04/83–03/84
	Dr. Mathews – RNA Processing	5,000	*05/83–04/84
	Dr. Watson – Symposium: Molecular Neurobiology	6,000	*07/83–06/84
	Dr. Watson – Genetics of *Chlamydomonas*	8,000	*06/83–11/84
Construction	Dr. Watson – Plant Genetics Laboratory	406,350	*12/83–05/85

Grantor	*Principal Investigator and Program*	*Total Award*	*Duration of Grant*
Research Grants			
A.B.C. Foundation	Dr. Wigler	600,000	05/01/82–04/30/87
Rita Allen Foundation	Dr. Stillman	150,000	*01/01/83–12/31/87
American Cancer Society	Dr. Chow	150,000	01/01/82–12/31/84
	Dr. Rossini	110,000	01/01/82–12/31/84
	Dr. Stillman (Institutional)	50,000	07/01/82–06/30/84
Cancer Research Institute	Dr. Garrels	36,000	*07/01/83–06/30/84
Cell Biology Corporation	Dr. Sambrook	627,746	01/01/82–12/31/83
Central General Hospital	Research	2,000	*01/01/83–12/31/83
Exxon	Research	7,500,000	01/01/82–12/31/86
McKnight Foundation	Dr. Malmberg	105,000	*03/01/83–02/28/86
Muscular Dystrophy Assn.	Dr. Garrels	80,000	01/01/80–12/31/83
	Dr. Lin	95,350	01/01/81–12/31/83
	Dr. Mathews	25,000	*07/01/83–06/30/84
	Dr. Matsumura	15,228	*01/01/83–12/31/83
New England Bio-Labs	Dr. Roberts	14,000	
Marie Robertson Memorial Fund	Dr. Sambrook – Neurobiology Support	75,000	*01/01/83–12/31/83
Stauffer Chemical	Dr. Hicks	15,000	
Strasser Company	Dr. Hicks	20,000	
State University of New York, Stony Brook	Dr. Topp (Subcontract)	7,267	08/01/82–07/31/83
Yamasa Shoyo Co., Ltd.	Dr. Katoh	94,480	10/01/81–09/30/83
Fellowships			
Jane Coffin Childs	Dr. Sundin	32,000	07/01/82–06/30/84
Helen Hay Whitney	Dr. Herr	39,250	*01/01/83–06/14/85
Leukemia Society of America, Inc.	Dr. Kost	22,375	07/01/82–09/15/83
	Dr. Powers	34,000	*12/31/83–12/30/85
Muscular Dystrophy Assn.	Dr. Helfman	17,000	*07/01/83–06/30/84
Robert P. Olney Memorial Cancer Fund	Dr. Dudley, Jr.	2,800	*1983
Damon Runyon–Walter Winchell Cancer Fund	Dr. Abraham	35,000	08/01/81–07/31/83
	Dr. Bhagwat	35,000	07/01/82–06/30/84
	Dr. Gerard	35,000	*05/01/83–03/31/85
	Dr. Mains	35,000	*04/01/83–03/31/85
	Dr. Roth	17,000	09/01/82–08/31/83
	Dr. White	35,000	*11/01/83–10/31/84
Training			
Bayer Aspirin (Glen Brook Labs)	Undergraduate Research Program	3,500	*1983
Burroughs Wellcome Fund	Undergraduate Research Program	7,000	*1983
J.M. Foundation	Undergraduate Research Program	25,300	*1983
Grass Foundation	Neurobiology Scholarships	45,190	1980–1983
Volkswagen Foundation	Neurobiology Scholarships	64,049	1980–1983

Grantor	*Principal Investigator and Program*	*Total Award*	*Duration of Grant*
Construction			
Pew Memorial Trust	Matching Grant Support	400,000	1981–1983
Equipment			
Fannie E. Rippel Foundation	Matching Grant Support	100,000	*1983–1984
Course Support			
National Foundation for Jewish Genetic Diseases, Inc.	Neurobiology of Human Diseases Course	2,000	*1983
Hereditary Diseases Foundation	Neurobiology of Human Diseases Course	2,000	*1983
The Esther A. & Joseph Klingenstein Fund	Neurobiology Support	150,000	1982–1983
Meeting Support			
Abbott Laboratories	Microbial Development	500	*1983
American Cancer Society	The Cancer Cell	34,000	*1983
American Cyanamid Company	Microbial Development	500	*1983
Becton-Dickinson	The Cancer Cell	5,000	*1983
Biogen	Microbial Development	1,000	*1983
	The Molecular Biology of Yeast	1,500	*1983
Cancer Research Institute	Human T-Cell Leukemia Viruses	4,060	*1983
Department of Energy	Genetics of *Chlamydomonas*	5,000	*05/15/83–05/14/84
	Symposium: Molecular Neurobiology	9,000	*07/15/83–07/14/84
Genentech, Inc.	The Molecular Biology of Yeast	1,000	*1983
Hoffmann-La Roche, Inc.	The Molecular Biology of Yeast	2,000	*1983
Eli Lilly Research Labs	Microbial Development	750	*1983
LaBatt Brewing Company	The Molecular Biology of Yeast	250	*1983
Long Island Biological Association–Dorcas Cummings Memorial Fund	Community Conference on the Gypsy Moth	4,000	*1983
Martin Marietta Laboratories	Genetics of *Chlamydomonas*	3,192	*1983
Merck & Co., Inc.	Modern Approaches to Vaccines	5,000	*1983
Merieux Institute	Modern Approaches to Vaccines	15,000	*1983
Miles Laboratories	Microbial Development	750	*1983
Monsanto	Genetics of *Chlamydomonas*	3,000	*1983
	Microbial Development	6,000	*1983
N.Y.S. Department of Health	Human T-Cell Leukemia Viruses	4,060	*1983
Pfizer, Inc.	The Molecular Biology of Yeast	1,000	*1983
Searle Research & Development	Microbial Development	500	*1983
Standard Oil Co. of Ohio	Genetics of *Chlamydomonas*	2,000	*1983
Smith Kline & French	The Molecular Biology of Yeast	1,000	*1983
Upjohn Company	Microbial Development	2,000	*1983
Wellcome Biotechnology, Ltd.	Modern Approaches to Vaccines	6,000	*1983
Zoecon Corporation	Genetics of *Chlamydomonas*	1,500	*1983
Zymos Corporation	The Molecular Biology of Yeast	1,000	*1983

BANBURY CENTER

Grantor	*Principal Investigator and Program*	*Total Award*	*Duration of Grant*
Meeting Support			
National Institutes of Health	Dr. Topp–AIDS and Kaposi's Sarcoma	20,367	*01/07/83-12/31/83
	Dr. Watson–The Role of Genetic Predisposition in Responses to Chemical Exposure	31,575	*08/01/83-07/31/84
Abbott Laboratories	Enhancers and Controlling Elements	1,000	*1983
Agrigenetics Research Associates, Ltd.	Plant Viruses and Viroids	500	*1983
Alcoa Foundation	The Role of Genetic Predisposition in Responses to Chemical Exposure	5,000	*1983
American Occupational Medical Association	The Role of Genetic Predisposition in Responses to Chemical Exposure	5,000	*1983
Applied Molecular Genetics	Enhancers and Controlling Elements	2,000	*1983
Arco Solar, Inc.	Plant Viruses and Viroids	2,000	*1983
Biogen	Enhancers and Controlling Elements	3,000	*1983
Calgene, Inc.	Plant Viruses and Viroids	250	*1983
Cancer Research Institute	AIDS and Kaposi's Sarcoma	4,050	*1983
Cetus Corporation	Enhancers and Controlling Elements	3,000	*1983
Collaborative Research	Enhancers and Controlling Elements	3,000	*1983
E.I. du Pont de Nemours & Co., Inc.	Enhancers and Controlling Elements	3,000	*1983
	The Role of Genetic Predisposition in Responses to Chemical Exposure	5,000	*1983
Genentech, Inc.	Enhancers and Controlling Elements	1,000	*1983
International Coffee Organization	Coffee and Health	15,000	*1983
Johnson & Johnson	The Role of Genetic Predisposition in Responses to Chemical Exposure	3,000	*1983
Eli Lilly Research Laboratories	Enhancers and Controlling Elements	1,000	*1983
March of Dimes	Mechanisms of Mutagenesis	4,000	*1983
Massachusetts Institute of Technology	Marr Memorial Conference: Mechanisms of Perception	23,369	*1983
Monsanto	Enhancers and Controlling Elements	3,000	*1983
	Plant Viruses and Viroids	2,000	*1983
Pfizer, Inc.	Plant Viruses and Viroids	1,000	*1983
National Coffee Association of USA, Inc.	Coffee and Health	10,000	*1983
Occupational Health Institute, Inc.	The Role of Genetic Predisposition in Responses to Chemical Exposure	5,000	*1983
Sloan Foundation	Journalists' Workshop: New Concepts in Mutation	10,000	*1983
	Congressional Workshop: Biological Imaging and NMR	10,000	*1983
United States Steel	The Role of Genetic Predisposition in Responses to Chemical Exposure	5,000	*1983

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THE LONG ISLAND BIOLOGICAL ASSOCIATION

Ambassador John P. Humes with Mr. and Mrs. Norris Darrell, Jr., at a dinner party held at Airslie House to honor a combined 40 years of service to the Laboratory by Walter Page and Edward Pulling.

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 one of the first Fish Commissioners 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 1974, LIBA made possible the building of the James Laboratory Annex and the renovation of Blackford Hall; and in 1976 the rebuilding of Williams House.

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's administrative director, Mr. William R. Udry, at (516) 367-8300.

REPORT OF THE CHAIRMAN FOR 1983

The major effort of LIBA in 1983 was a continuation of our campaign launched in 1982 to raise at least $500,000 over a two-year period to help the Lab finance the construction of the urgently needed new auditorium.

The Cold Spring Harbor Laboratory has become the world center for the exchange and distribution of information about the latest discoveries in molecular biology. As the number of molecular biologists from all over the world who apply for participation in the June Symposium and other seminar meetings continues to increase, the need for an auditorium large enough to accommodate them has become increasingly urgent.

Of special interest to LIBA members and to all North Shore residents is the fact that the new auditorium has been planned to make it suitable for concerts, lectures, and other appropriate community functions during the off-season for Laboratory meetings.

I am pleased to report that as of February 1984 we have reached our original goal of $500,000 and are now striving substantially to increase our support of the auditorium project, which has turned out to be more expensive than was anticipated at the time we began our campaign.

As usual, LIBA members and their guests were invited to two lectures during the year. At the first, the annual Dorcas Cummings Memorial Lecture, our speakers were Amory and Hunter Lovins of the Rocky Mountain Institute at Snowmass, Colorado. They have become world famous for their study of the sources and proper use and conservation of energy, and gave us a fascinating account of their findings, followed by provocative suggestions for the solution of energy problems.

At the Annual Meeting in December, the lecturer was Dr. Arnold Levine, Chairman of the Department of Microbiology at Stony Brook. He gave his audience a remarkably clear and exciting over-view of recent significant research in molecular biology, with a detailed explanation of the way mutation can change a healthy gene into an oncogene.

The custom of giving dinner parties for visiting scientists during the June Symposium was successfully continued in 1983. It is a custom enjoyed equally by the scientists and by their hosts and hostesses. This year's parties were given by

Mrs. Gilbert A. Ball
Mr. & Mrs. F. Sedgwick Browne
Mr. & Mrs. John P. Campbell
Mr. & Mrs. David C. Clark
Mr. & Mrs. Miner D. Crary
Mr. & Mrs. Henry U. Harris, Jr.
Mr. & Mrs. George J. Hossfeld, Jr.
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Mr. & Mrs. Franz Schneider
Mr. & Mrs. Martin B. Travis
Mr. & Mrs. Bronson Trevor, Jr.
Mr. & Mrs. Robert P. Walton
Mr. & Mrs. R. Ray Weeks
Mr. & Mrs. William A. Woodcock

At the Annual Meeting regret was expressed that the terms of office as directors for Messrs. David Clark, Roderick Cushman, Norris Darrell, Walter Frank, and Clarence Galston had terminated after eight years of faithful service, and that Mrs. Shirley Ames and Mrs. Stephen Ulman had found it necessary to resign from the Board. To fill the vacant places, the following directors were elected to office: Mrs. Donald Arthur, Mr. Arthur M. Crocker, Mr. George W. Cutting, Mr. Lawrence L. Davis, Mr. Arthur C. Merrill, and Mr. Harvey E. Sampson.

At the subsequent meeting of the Board of Directors the previous officers and members of the Executive Committee were re-elected to office with the exception of Mrs. Ames, who had resigned. Mr. Callaway was elected to take her place in the Executive Committee.

Edward Pulling, *Chairman*
Long Island Biological Association
February 21, 1984

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Mr. & Mrs. Bronson Trevor, Jr.
Mr. & Mrs. Stanley Trotman
Mr. & Mrs. A.M. Trotter
Mr. & Mrs. E.T. Turner
Mr. & Mrs. William R. Udry
Mr. & Mrs. Stephen Van R. Ulman
Dr. & Mrs. Thornton Vandersall
Mr. & Mrs. Martin Victor
Mr. Eric Von Raits
Mr. & Mrs. Colton P. Wagner
Ms. Christine M. Walker
Mrs. George Walker
Mr. & Mrs. R.W. Walker
Mrs. Robert P. Walton
Dr. & Mrs. David E. Warden
Mrs. Ethelbert Warfield
Mr. & Mrs. Bradford A. Warner
Mr. & Mrs. Harold L. Warner, Jr.
Dr. & Mrs. James D. Watson
Edwin S. Webster Foundation
Mrs. Arthur D. Weekes, Jr.
Mr. & Mrs. Bradford G. Weekes
Mr. & Mrs. R. Ray Weeks
Mrs. F. Carrington Weems
Mr. John C. Weghorn
Mr. & Mrs. Richard J. Weghorn
Mr. & Mrs. Taggart Whipple
Mrs. Alexander M. White
Mr. & Mrs. Warren D. White
Mr. Theodore S. Wickersham
Mr. Malcolm D. Widenor
Mr. & Mrs. Douglas Williams
Mr. & Mrs. Duane N. Williams
Mr. Henry S. Williams
Mr. & Mrs. Ichabod Williams
Mr. & Mrs. Thorndike Williams
Mrs. John C. Wilmerding
Mrs. Henry S. Wingate
Mrs. Scudder Winslow
Mr. & Mrs. William A. Woodcock
Mr. & Mrs. James A. Woods
Mrs. Ford Wright
Miss Flavia Helen Wyeth
Mr. & Mrs. Woodhull Young
Mr. Robert Zakary

COLD SPRING HARBOR MEETINGS PROGRAMS

48TH COLD SPRING HARBOR SYMPOSIUM ON QUANTITATIVE BIOLOGY

Molecular Neurobiology

June 1–June 8

Opening Remarks: W.M. Cowan, Salk Institute, San Diego, California

SESSION 1 *Electrically Excitable Channels*

Chairperson: A. Karlin, Columbia University College of Physicians and Surgeons, New York, New York

Aldrich, R.A., Stevens, C.F., Dept. of Physiology, Yale University School of Medicine, New Haven, Connecticut: Rates of sodium channel inactivation depend on the channel's state.

Catterall, W.A., Talvenheimo, J.A., Hartshorne, P., Tamkun, M.M., Messner, D.J., Sharkey, R.M., Dept. of Pharmacology, University of Washington, Seattle: Structure and functional reconstitution of the sodium channel from rat brain.

Fritz, L.C.,[1] Moore, H.-P.H.,[2] Raftery, M.A.,[1] Brockes, J.P.,[3] [1]Division of Chemistry, California Institute of Technology, Pasadena; [2]Dept. of Biochemistry, University of California, San Francisco; [3]MRC, Biophysics Unit, London, England: Monoclonal antibodies as probes for the eel electroplax sodium channel.

Agnew, W.S.,[1] Miller, J.A.,[1] Ellisman, M.H.,[2] [1]Dept. of Physiology, Yale University School of Medicine, New Haven, Connecticut; [2]Dept. of Neurosciences, University of California, San Diego: The voltage-regulated sodium channel from electroplax of *Electrophorus electricus.*

SESSION 2 *The Biochemistry and Molecular Genetics of the Acetylcholine Receptor*

Chairperson: C.F. Stevens, Yale University, New Haven, Connecticut

Karlin, A.,[1] Cox, R.,[1] Dwork, A.,[1] Kaldany, R.-R.,[1] Kao, P.,[1] Lobel, P.,[1] Yodh, N.,[1] Holtzman, E.,[2] [1]Dept. of Biochemistry and Neurology, [2]Dept. of Biological Sciences, Columbia University, New York, New York: The arrangement and functions of the chains of the acetylcholine receptor of *Torpedo* electric tissue.

Raftery, M.A., Conti-Tronconi, B., Dunn, S.M.J., Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena: Signaling to and through nicotinic acetylcholine receptors.

Forrest, J.W., Ingraham, H.A., Ballivet, M., Patrick, J., Heinemann, S., Molecular Neurobiology Laboratory, Salk Institute, La Jolla, California: Isolation and analysis of a cDNA clone synthesized from mRNA isolated from the electric organ of *Torpedo californica.*

Stengelin, S., Ueno, S., Ballivet, M., Heinemann, S., Patrick, J., Molecular Neurobiology Laboratory, Salk Institute, La Jolla, California: Molecular cloning of acetylcholine receptor.

Noonan, D., Hershey, N.D., Mixter, K.S., Claudio, T., Davidson, N., Dept. of Chemistry, California Institute of Technology, Pasadena: Structure and function of *Torpedo* acetylcholine receptor genes.

Merlie, J.P.,[1] Sebbane, R.,[2] Gardner, S.,[2] Olson, E.N.,[3] [1]Dept. of Pharmacology, Washington University, St. Louis, Missouri; [2]Dept. of Biochemistry, Washington University, St. Louis, Missouri: Studies of the regulation of acetylcholine receptor synthesis.

Barnard, E.A.,[1] Richards, B.M.,[2] [1]Dept. of Biochemistry, Imperial College of Science and Technology, London, England; [2]Searle Research and Development Laboratories, G.D. Searle Co., High Wycombe, England: Structural requirements in nicotinic acetylcholine receptors, as revealed by translation of mRNA and cloning of cDNA.

Numa, S., Noda, M., Takahashi, H., Tanabe, T., Toyosato, M., Furutani, Y., Kikyotani, S., Dept. of Medical Chemistry, Kyoto University Faculty of Medicine, Japan: Molecular structure of *Torpedo californica* acetylcholine receptor.

Ballivet, M., Nef, P., Spierer, P., University of Geneva, Switzerland: Genomic sequences encoding the alpha subunit of the nicotinic acetylcholine receptor are highly conserved in evolution.

SESSION 3 *Genetic Analysis of the Nervous System*

Chairperson: N. Davidson, California Institute of Technology, Pasadena, California

Hahn, W., Chaudhari, N., Beck, L., Morrison, K., Dept. of Anatomy, University of Colorado Health Sciences, Denver: Genetic expression in postnatally developing brain.

Sutcliffe, J.G.,[1] Milner, R.J.,[2] Bloom, F.E.,[2] [1]Scripps Clinic and Research Foundation; [2]Salk Institute, La Jolla, California: Cellular localization of the proteins encoded by brain-specific mRNAs.

Axel, R., Buck, L., Stein, R., Palazzolo, M., Claudio, T., Gallay, B., Anderson, D., Jackson, J., Madaule, P., Schwartz, J., Institute of Cancer Research and Center for Neurobiology and Behavior, Columbia University, New York, New York: Isolating genes expressed in single neurons.

Kuenzle, C.C., Heizmann, C.W., Hübscher, U., Hobi, R., Winkler, G.C., Jaeger, A.W., Morgenegg, G., Dept. of Pharmacology and Biochemistry, School of Veterinary Medicine, University of Zürich, Switzerland: Chromatin changes accompanying neuronal differentiation.

Sulston, J.E., Laboratory of Molecular Biology, MRC, Cambridge, England: Neuronal cell lineages in the nematode *C. elegans*.

Horvitz, R., Ellis, H.M., Fixsen, W., Greenwald, I., Sternberg, P., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Mutations that affect neuronal development in the nematode *C. elegans*.

SESSION 4 *Studies on Neuronal Proteins with Recombinant DNA Techniques*

Chairperson: E. Barnard, Imperial College, London, England

Mallet, J., Biguet, N.F., Grima, B., Julian, J.F., Lamouroux, A., Institut de Microbiologie, Université Paris, France: A molecular genetics approach to the study of catecholamine biosynthesis.

Chikaraishi, D.M., Brilliant, M.H., Lewis, E.J., Tomasiewicz, H.G., Molecular and Cellular Biology, National Jewish Hospital/National Asthma Center, Denver, Colorado: Cloning and characterization of brain-specific transcripts—Rare, brain-specific transcripts and tyrosine hydroxylase.

O'Malley, K., Mauron, A., Kedes, L., Barchas, J., Stanford University School of Medicine, California: Structure, regulation, and brain localization of mRNA for rat dopamine-β-hydroxylase.

Joh, T.H., Baetge, E.E., Ross, M.E., Moon, H.M., Albert, V.R., Reis, D.J., Laboratory of Neurobiology, Cornell University Medical College, New York, New York: Evidence for the existence of a gene family coding for catecholamine biosynthetic enzymes.

DeGennaro, L.J.,[1] Wallace, W.C.,[2] Kanazir, S.,[2] Lewis, R.M.,[2] Greengard, P.,[2] [1]Max-Planck Institute for Psychiatry, Martinsried, Federal Republic of Germany; [2]Dept. of Pharmacology, Yale University School of Medicine, New Haven, Connecticut: Neuron-specific phosphoproteins as models for neuronal gene expression.

Lewis, R.M., Wallace, W.C., Kanazir, S., Greengard, P., Dept. of Pharmacology, Yale University School of Medicine, New Haven, Connecticut: Expression of cell-type-specific neuronal phosphoproteins.

Fambrough, D.M., Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: Regulation of the sodium pump.

SESSION 5 *Neuronal Surface Molecules*

Chairperson: Z. Hall, University of California, San Francisco, California

Rutishauser, U., Dept. of Anatomy, Case Western Reserve University School of Medicine, Cleveland, Ohio: Chemistry and biology of a neural cell adhesion molecule.

Goridis, C., Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, France: Neural surface antigens during nervous system development.

Schubert, D., LaCordbiere, M., Salk Institute, La Jolla, California: A role for adherons in the adhesion of nerve and muscle cells.

Edelman, G.M., Hoffman, S., Cunningham, B.A., Rockefeller University, New York, New York: Structure and modulation of neural cell adhesion molecules.

Zipser, B., Flanagan, T., Flaster, M., Macagno, E., Stewart, E., Cold Spring Harbor Laboratory, New York: Analysis of neuron-specific leech antigen.

Schachner, M., Faissner, A., Meier, D., Kruse, J., Lindner, J., Rathjen, F., Wernecke, H., Dept. of Neurobiology, University of Heidelberg, Federal Republic of Germany: Characterization of cell-surface components involved in cell adhesion.

Raff, M.C.,[1] Miller, R.H.,[1] Noble, M.,[2] [1]MRC Neuroimmunology Project, Dept. of Zoology, University College London, England; [2]Institute of Neurology, London, England: Glial cell lineages in the rat optic nerve.

SESSION 6 *Biochemical Analysis of Electrically Active Proteins*

Chairperson: G. Edelman, Rockefeller University, New York, New York

Lindstrom, J.,[1] Tzartos, S.,[1] Gullick, W.,[1] Hockschwender, S.,[1] Swanson, L.,[1] Montal, M.,[2] [1]Salk Institute for Biological Studies; [2]University of California, San Diego: Use of monoclonal antibodies to studv acetylcholine receptors from electric organs, muscle, and brain and the autoimmune response to receptor in myasthenia gravis.

Anderson, D.J.,[1] Blobel, G.,[2] [1]Institute for Cancer Research, Colum-

bia University College of Physicians and Surgeons, New York, New York; [2]Laboratory of Cell Biology, Rockefeller University, New York, New York: Biosynthesis of the acetylcholine receptor in vitro.

Changeux, J.P., Institut Pasteur, Paris, France: Allosteric properties of the acetylcholine receptor.

Kristofferson, D., Fairclough, R., Love, R., Moore, J., Young, E., Stroud, R., Dept. of Biochemistry and Biophysics, University of California, San Francisco: The structure of an acetylcholine receptor and what it tells us about function.

Papazian, D.,[1] Chan, S.,[2] Hess, E.J.,[2] Rahamimoff, H.,[3] Goldin, S.,[2] [1]Dept. of Biochemistry; [2]Dept. of Pharmacology, Harvard Medical School, Boston, Massachusetts; [3]Dept. of Biochemistry, Hadassah Medical School, Jerusalem, Israel: Purification and characterization of synaptosomal Ca pumps.

Triggle, D.J.,[1] Bolger, G.T.,[1] Gengo, P.J.,[1] Luchowski, E.M.,[1] Rampe, D.,[1] Janis, R.A.,[2] [1]Dept. of Biochemical Pharmacology, State University of New York, Buffalo; [2]Miles Institute for Preclinical Pharmacology, New Haven, Connecticut: Ca-channel antagonists—Radioligand binding in neuronal and nonneuronal tissue.

Birdsall, N.J.M.,[1] Hulme, E.C.H.,[1] Stockton, J.,[1] Wong, E.H.F.,[2] [1]Division of Molecular Pharmacology, National Institute for Medical Research, London, England; [2]Dept. of Biochemistry, University of California, Riverside: Muscarinic receptor subclasses—Allosteric interactions.

SESSION 7 *Behavior*

Chairperson: M.C. Raff, University College London, London, England

Adler, J., Dept. of Biochemistry and Genetics, University of Wisconsin, Madison: How *E. coli* does neurobiology .

Boyd, A.,[1] Kirkos, A.,[2] Mutoh, N.,[2] Simon, N.,[2] [1]Leicester Biocentre, University of Rhode, England; [2]Division of Biology, California Institute of Technology, Pasadena: The structure of proteins that act as receptors and sensory transducers in bacteria.

Koshland, D.E., Jr., Russo, A.F., Gutterson, N.I., Dept. of Biochemistry, University of California, Berkeley: Information processing in a sensory system.

Schwartz, J.H., Bernier, L., Castellucci, V.F., Eppler, C.M., Saitoh, T., Kandel, E.R., Center for Neurobiology and Behavior, Columbia University College of Physicians and Surgeons and New York State Psychiatric Institute, New York, New York: What enzymatic steps determine the time course of short-term memory in *Aplysia?*

Kandel, E.R., Abrams, T.W., Carew, T.J., Hawkins, R.D., Columbia University College of Physicians and Surgeons, New York, New York: Classical conditioning in *Aplysia*—Conditioning and sensitization seem to share aspects of the same molecular cascade.

Aceves-Pina, E.O., Brooker, R., Duerr, J.S., Livingston, M.S., Quinn, W.G., Smith, R.T., Sziber, P.P., Tempel, B.L., Tully, T., Dept. of Biology, Princeton University, New Jersey: Genetic studies of learning and memory in *Drosophila.*

SESSION 8 *The Regulation of Axon Organization*

Chairperson: U.J. McMahan, Stanford University, Stanford, California

Bentley, D., Caudy, M., Dept. of Zoology and Biophysics Group, University of California, Berkeley: Pathways and pathfinding by peripheral pioneer neurons in grasshoppers.

Goodman, C.S., Bastiani, M.J., Kotria, K.J., Dept. of Biological Sciences, Stanford University, California: Guidance of neuronal growth cones—Cell recognition and filopodial interactions during embryonic development.

McKay, R.D.G., Hockfield, S., Johansen, J., Cold Spring Harbor Laboratory, New York: The organization of molecularly distinct axons in the leech connective.

Reichardt, L.F., Calof, A.L., Greenspan, R.J., Greig, K.F., Lander, A.D., Matthew, W.D., Tomaselli, K.J., Winter, J., Depts. of Physiology and Biochemistry, University of California, San Francisco: Studies on factors that promote neurite outgrowth in vitro.

White, J.G., Southgate, E., Thomson, J.N., Brenner, S., Laboratory of Molecular Biology, MRC, Cambridge, England: Patterns of connectivity in the nervous system of *C. elegans.*

Wyman, R.J., Thomas, J.B., Dept. of Biology, Yale University, New Haven, Connecticut: Genetic approach to the molecular basis of neural connectivity.

SESSION 9 *Molecular Aspects of Neuropeptides I*

Chairperson: H. Reuter, University of Berne, Berne, Switzerland

Jan, Y.N., Bowers, C., Evans, L., Jan, L.Y., Dept. of Physiology, University of California, San Francisco: Roles of peptides in neuronal function—Studies using frog autonomic ganglia.

Gould, R.J., Murphy, K.M.M., Snyder, S.H., Dept. of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, Maryland: Studies of voltage-operated Ca channels using radioligands.

Evans, R.M.,[1] Swanson, L.,[1] Sawchenho, P.,[1] Vale, W.,[1] River, J.,[1] Amara, S.,[2] Rosenfeld, M.G.,[2] [1]Salk Institute for Biological Studies; [2]University of California School of Medicine, San Diego: Generation of

diversity in the nervous system—Exon invasion, splicing control, and complex genes.

Scheller, R.H., Dept. of Biological Sciences, Stanford University, California: The molecular basis of a simple behavior.

Shine, J., Mason, A., Evans, B., Richards, R., Centre for Recombinant DNA Research, Australian University, Canberra: Proteolytic processing and regulation of neuropeptide biosynthesis.

SESSION 10 *The Cellular Organization of Neurons*

Chairperson: H. Thoenen, Max-Planck Institut, Martinsried, Federal Republic of Germany

Weber, K., Shaw, G., Osborn, M., Debus, E., Geisler, N., Max-Planck Institute for Biophysical Chemistry, Göttingen, Federal Republic of Germany: Neurofilaments—Structural aspects and expression during development.

Lasek, R.J., Drake, P.F., Oblinger, M.M., Neurobiology Center, Dept. of Developmental Genetics and Anatomy, Case Western Reserve University, Cleveland, Ohio: Regional differences in the periphery of neuronal cytoskeletons may be specified in the cell body.

Steward, O., Dept. of Neurological Surgery, University of Virginia, Charlottesville: Polyribosomes at the base of dendritic spines of CNS neurons—Their possible role in synapse construction and modification.

Stallcup, W., Beasley, L., Levine, J., Molecular Neurobiology Laboratory, Salk Institute, La Jolla, California: Cell-surface molecules that characterize different stages in the development of cerebellar interneurons.

Matus, A., Friedrich Miescher Institute, Basel, Switzerland: Neuronal microdifferentiation.

Ginzburg, I., Scherson, T., Rybak, S., Kimhi, Y., Neuman, D., Schwartz, M., Littauer, U.Z., Dept. of Neurobiology, Weizmann Institute of Science, Rehovot, Israel: Expression of mRNA for microtubule proteins in the developing nervous system.

Baitinger, C.,[1] Cheney, R.,[1] Clements, D.,[1] Glicksman, M.,[1] Hirokawa, N.,[1] Levine, J.,[2] Meiri, K.,[1] Simon, C.,[1] Skene, J.H.P.,[3] Willard, M.,[1] [1]Dept. of Anatomy and Neurobiology, Washington University, St. Louis, Missouri; [2]Dept. of Neurobiology, Salk Institute, La Jolla, California; [3]Dept. of Neurobiology, Stanford University, California: Intriguing axonally transported proteins.

SESSION 11 *Synaptic Organization and Function*

Chairperson: D. Bentley, University of California, Berkeley, California

McMahan, U.J., Dept. of Neurobiology, Stanford University School of Medicine, California: Molecular components of synaptic basal lamina that cause aggregation of acetylcholine receptors on regenerating myofibers.

Sanes, J.R., Dept. of Physiology, Washington University School of Medicine, St. Louis, Missouri: Basal lamina in neuromuscular regeneration and development.

Thoenen, H., Korsching, S., Barde, Y.-A., Edgar, D., Dept. of Neurochemistry, Max-Planck Institute for Psychiatry, Martinsried, Federal Republic of Germany: Quantitation and purification of neurotropic factors from physiologically relevant sources.

Dodd, J., Hamilton, P., Heath, M., Jahr, C., Jessell, T., Dept. of Neurobiology, Harvard Medical School, Boston, Massachusetts: Neurotransmission and neuronal markers at sensory synapses in the spinal cord.

Kelly, R.B., Carlson, S., Burgess, T., Moore, H.-P., Buckley, K., Schweitzer, E., Hooper, J., Pfeffer, S., Gumbiner, B., Miljanich, G., University of California, San Francisco: Sorting in neurons and peptide-secreting cells.

Patterson, P.H., Dept. of Neurobiology, Harvard Medical School, Boston, Massachusetts: Surface membrane and secreted glycoproteins of developing neurons.

SESSION 12 *Aspects of Vision*

Chairperson: T. Wiesel, Rockefeller University, New York, New York

Stryer, L., Dept. of Structural Biology, Stanford University School of Medicine, California: Signal-amplifying proteins in the cyclic nucleotide cascade of vision.

Dunn, R.J., Hackett, N.R., Huang, K.-S., Jones, S.S., Khorana, H.G., Liao, M.-J., Lo, K.-M., Satterthwait, A., Seehra, J.S., Yatsunami, K., Depts. of Biology and Chemistry, Massachusetts Institute of Technology, Cambridge: Studies on the light-transducing pigments, bacteriorhodopsin and rhodospin.

Benzer, S., Zipursky, S.L., Venkatesh, T.R., Fujita, S.C., Biology Division, California Institute of Technology, Pasadena: A monoclonal antibody study of the development of the *Drosophila* retina.

Barnstable, C.J., Akagawa, K., Hofstein, R., Dept. of Neurobiology, Harvard Medical School, Boston, Massachusetts: Monoclonal antibodies that label discrete cell types in the mammalian nervous system.

Nirenberg, M., Krueger, K., Fukui, H., Rotter, A., Wilson, S., Higashida, H., NHLBI, National Institutes of Health, Bethesda, Maryland: Synapse formation by neuroblastoma hybrid cells.

Hockfield, S.,[1] McKay, R.D.G.,[1] Hendry, S.H.,[2] Jones, E.G.,[2] [1]Cold Spring Harbor Laboratory, New York; [2]Dept. of Neurology and Neurosurgery, Washington University School of Medicine, St. Louis, Missouri: Structural and organizational features of mammalian CNS demonstrated with monoclonal antibodies.

SESSION 13 *Electrophysical Analysis of Membrane Properties*

Chairperson: J. Schwartz, Columbia University College of Physicians and Surgeons, New York, New York

Reuter, H.,[1] Cachelin, A.B.,[1] de Peyer, J.E.,[1] Kokubun, S.,[1] Levitan, I.,[2] [1]Dept. of Pharmacology, University of Berne, Switzerland; [2]Dept. of Biochemistry, Brandeis University, Waltham, Massachusetts: Modulation by phosphorylation of Ca^{2+} channels and Ca^{2+}-activated channels.

Nowycky, M.C.,[1] Bean, B.P.,[2] Hess, P.,[2] Tsien, R.W.,[2] [1]Dept. of Neuroanatomy; [2]Dept. of Physiology, Yale University School of Medicine, New Haven, Connecticut: Properties of Ca channels in neurons and heart cells.

Salkoff, L., Dept. of Physiology, University of California School of Medicine, San Francisco: Genetic and voltage-clamp analysis of a K^+ channel in *Drosophila*.

Jan, L.Y., Barbel, S., Timpe, L., Laffer, C., Salkoff, L., O'Farrell, P., Jan, Y.N., Depts. of Physiology and Biochemistry, University of California, San Francisco: Mutating a gene for a potassium channel by hybrid dysgenesis—An approach to the cloning of the *shaker* locus in *Drosophila*.

Cull-Candy, S.G., Dept. of Pharmacology, University College London, England: Glutamate and GABA-receptor channels at the locust nerve muscle junction—Noise analysis and single-channel recording.

Barker, J.L., Owen, D.G., Segal, M., NINCDS, National Institutes of Health, Bethesda, Maryland: Regulation of excitable membrane properties in cultured CNS neurons.

Sakmann, B., Hamill, O.P., Max-Planck Institut für Biophysikalische Chemie, Göttingen, Federal Republic of Germany: The mode of action of excitatory and inhibitory transmitters.

Schramm, M., Dept. of Biological Chemistry, Hebrew University, Jerusalem, Israel: Mechanism of receptor action in the adenylate cyclase system.

SESSION 14 *Molecular Aspects of Neuropeptides II*

Chairperson: S. Numa, Kyoto University, Kyoto, Japan

Herbert, E., Dept. of Chemistry, University of Oregon, Eugene: Regulation of expression of opioid peptide genes in man, rat, and mouse.

Roberts, J.L., Dept. of Biochemistry and Center for Reproductive Sciences, Columbia University College of Physicians and Surgeons, New York, New York: Pro-opiomelanocortin—A model for differential regulation of neuropeptide hormone gene expression.

Darling, T.,[1] Petrides, P.,[1] Beguin, P.,[1] Frey, P.,[1] Shooter, E.M.,[1] Selby, M.,[2] Rutter, W.J.,[2] [1]Dept. of Neurobiology, Stanford University School of Medicine, California; [2]Dept. of Biochemistry and Biophysics, University of California, San Francisco: The biosynthesis and processing of proteins in the mouse 7S nerve growth factor complex.

Ullrich, A., Gray, A., Berman, C., Dull, T.J., Dept. of Molecular Biology, Genentech, Inc., South San Francisco, California: Characterization of the gene encoding the β subunit of nerve growth factor.

Rossier, J., Liston, D., Patey, G., Chaminade, M., Foutz, A., Vanderhaeghen, J.J., Laboratoire de Physiologie Nerveuse, CNRS, Gif-sur-Yvette, France: The enkephalinergic neuron—Implications of a polyenkephalin precursor.

Summary: E. Kandel, Columbia University College of Physicians and Surgeons, New York, New York.

C. ELEGANS

May 4–May 8

SESSION 1 *Neurobiology*

Chairperson: P. Anderson, University of Wisconsin, Madison, Wisconsin

Rand, J., Dept.of Biological Sciences, University of Pittsburgh, Pennsylvania: *cha-1*, *unc-17*, and choline acetyltransferase in *C. elegans*.

Perkins, L.,[1] Hedgecock, E.,[2] Culotti, J.,[1] Thomson, N.,[2] [1]Northwestern University, Evanston, Illinois; [2]MRC Laboratory of Molecular Biology, Cambridge, England: Behaviors of mutants defective in fluorescein-concentrating sensory neurons.

Chalfie, M., Dept. of Biological Sciences, Columbia University, New York, New York: Still more on touch sensitivity in *C. elegans*.

Johnson, C.D., Stretton, A.O.W., Dept. of Zoology, University of Wisconsin, Madison: Monoclonal antibodies to *Ascaris* neurons.

Angstadt, J.D.,[1] Stretton, A.O.W.,[2] [1]Neurosciences Training Program; [2]Dept. of Zoology, University of Wisconsin, Madison: Intracellular recordings and Lucifer yellow-fills of interneurons in the nematode *Ascaris*.

Kenyon, C.J., Hedgecock, E.M., MRC Laboratory of Molecular Biology, Cambridge, England: A cell lineage mutation that blocks neuroblast formation in the lateral hypodermis.

Trent, C., Desai, C., Tsung, N., Horvitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Egg-laying defective mutants of *C. elegans*.

SESSION 2 *Sex Determination and Gametogenesis*

Chairperson: D.L. Riddle, University of Missouri, St. Louis, Missouri

Meneely, P.M., Wood, W.B., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Autosomal genes affecting dosage compensation and sex determination.

Doniach, T., Hodgkin, J., MRC Laboratory of Molecular Biology, Cambridge, England: New alleles of *intersex-1 (isx-1)*.

Edgar, L.,[1] Kimble, J.,[2] Hirsh, D.,[3] [1]NIADDK, National Institutes of Health, Bethesda, Maryland; [2]Laboratory of Molecular Biology, University of Wisconsin, Madison; [3]Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: The role of *isx-2* in male sexual differentiation.

Fixsen, W., Horovitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: A *lin-22* mutation causes apparent spatial and sexual transformations in cell fates.

Goldstein, P., Dept. of Biology, University of North Carolina, Charlotte: Associated autosomal structures (nondisjunction regulator regions) may specifically influence X-chromosome nondisjunction.

Burke, D.J., Ward, S., Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: Identification of a large multigene family encoding the major sperm protein of *C. elegans*.

Klass, M.,[1] Kinsley, S.,[1] Lopez, L.C.,[2] [1]Biology Dept., University of Houston, Texas; [2]Biochemistry Dept., M.D. Anderson Hospital and Tumor Institute, Houston, Texas: Isolation and characterization of a developmentally regulated sperm-specific gene family in the nematode *C. elegans*.

Blumenthal, T., Donegan, M., Kirtland, S., Sharrock, W., Spieth, J., Dept. of Biology, Indiana University, Bloomington: The yolk protein gene family of *C. elegans*.

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Edgley, M.L., Swanson, M.M., Riddle, D.L., Division of Biological Sciences, University of Missouri, Columbia: *Caenorhabditis* genetics center.

SESSION 3 *Poster Session: Neurobiology, Sex, and Gametes*

White, J.G., Southgate, E., Thomson, J.N., Brenner, S., MRC Laboratory of Molecular Biology, Cambridge, England: The nervous system of *C. elegans*.

Rand, J., Bashor, O., Cavalier, L., Russell, R., Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: Anatomy and function of the juvenile motor nervous system in *C. elegans*.

Albert, P.S., Riddle, D.L., Division of Biological Sciences, University of Missouri, Columbia: Reversible developmental alterations in dauer larva sensory neuroanatomy.

Perkins, L.,[1] Hedgecock, E.,[2] Thomson, N.,[2] Culotti, J.,[1] [1]Northwestern University, Evanston, Illinois; [2]MRC Laboratory of Molecular Biology, Cambridge, England: Ultrastructural analysis of mutants of the fluorescein concentrating sensory neurons.

Chen, V.,[1] Hieb, W.,[2] [1]Dept. of Biophysics, [2]Dept. of Biological Sciences, State University of New York, Buffalo: Coordinated mass movement in *C. elegans* and *Tubatrix aceti*.

Hieb, W.F.,[1] Chen, V.K.-H.,[2] [1]Dept. of Biological Sciences, [2]Dept. of Biophysical Sciences, State University of New York, Buffalo: Culture morphology of *C. elegans* behavioral mutants.

Lewis, J.A., McLafferty, S., Dept. of Biological Sciences, University of Missouri, Columbia: The determent-solubilized levamisole receptor.

Kolson, D.L., Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: New alleles of *ace-2*.

Zuckerman, B.,[1] Damon, R.,[2] Kahane, I.,[3] [1]Dept. of Plant Pathology; [2]Dept. of Veterinary and Animal Science, University of Massachusetts, Amherst; [3]Dept. of Biomembranes, Hebrew University-Hadassah Medical School, Jerusalem, Israel: Regulation of chemotaxis of *C. elegans* by Con-A-binding sites.

Siddiqui, S., Culotti, J., Dept. of Biochemistry, Molecular Biology, and Cell Biology, Northwestern, University, Evanston, Illinois: Characterization of a 34 kD antigen present on the surface of neurons and their support cells in *C. elegans* using antibodies against horseradish peroxidase (HRP).

Culotti, J.,[1] Siddiqui, S.,[1] Gremke, L.,[1] Stretton, A.O.W.,[2] Johnson, C.D.,[2] [1]Northwestern University, Evanston, Illinois; [2]University of Wisconsin, Madison: Monoclonal antibodies to nerve-enriched tissue from *Ascaris* bind to *C. elegans*.

Okamoto, H., MRC Laboratory of Molecular Biology, Cambridge, England: Monoclonal antibodies to *C. elegans* nervous tissue.

Davis, R.E.,[1] Stretton, A.O.W.,[2] [1]Neurosciences Program, [2]Dept. of Zoology, University of Wisconsin, Madison: Motorneuron membrane constants and signaling properties in the nematode *Ascaris*.

Donmoyer, J.E., Desnoyers, P.A., Stretton, A.O.W., Dept. of Zoology, University of Wisconsin, Madison: Synaptic interactions of ventral cord interneurons in *Ascaris*.

Hedgecock, E., MRC Laboratory of Molecular Biology, Cambridge, England: Q neuroblast migration mutants.

Finney, M., Horovitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: The genetic analysis of *unc-86*.

Trent, C., Ellis, H.M., Horovitz,R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Weakly sexually transformed XX animals are hermaphrodites that lack HSN neurons.

Hodgkin, J., MRC Laboratory of Molecular Biology, Cambridge, England: Chauvinist dumpy genes.

Pavalko, F.M., Roberts, T.M., Dept. of Biological Science, Florida State University, Tallahassee: Monoclonal antibodies as probes for membrane movement on *C. elegans* sperm.

SESSION 4 *Cell Biology*

Chairperson: H.R. Horvitz, Massachusetts Institute of Technology, Cambridge, Massachusetts

Miller, D.M., III, Ortiz, I., Berliner, G., Epstein, H.F., Dept. of Neurology, Baylor College of Medicine, Houston, Texas: The location of two myosins within nematode thick filaments.

Francis, R., Waterston, R.H., Dept. of Genetics, Washington University School of Medicine, St. Louis, Missouri: Minor muscle proteins in *C. elegans*.

Brown, S.J., Riddle, D.L., Division of Biological Sciences, University of Missouri, Columbia: Mutations interfering with indirect suppression of muscle defects in *C. elegans*.

Edgar, L.G., McGhee, J.D., NIADDK, National Institutes of Health, Bethesda, Maryland: Development of digestive enzymes in the intestine of *C. elegans*.

Clokey, G., Jacobson, L.A., Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: Endocytosis by intestinal cells of *C. elegans*.

Ward, S., Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: Asymmetric distribution of specific proteins during spermatogenesis.

Roberts, T.M., Dept. of Biological Science, Florida State University, Tallahassee: Crawing *C. elegans* spermatozoa contain 2-nm filaments.

SESSION 5 *Postembryonic Development and Aging*

Chairperson: D. Hirsh, University of Colorado, Boulder, Colorado

Sternberg, P., Ferguson, E., Tsung, N., Greenwald, I., Horvitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Genetic control of *C. elegans* vulval cell lineages.

Ferguson, E., Horvitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Synthetic multivulva mutations.

Hedgecock, E., MRC Laboratory of Molecular Biology, Cambridge, England: Two genes affecting cell proliferation in many lineages.

Greenwald, I., Sternberg, P., Horvitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: The *lin-12* locus determines cell fates in *C. elegans*.

Ambros, V., Horvitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Genes affecting temporal patterns of development.

O'Riordan, V., Burnell, A.M., Dept. of Biology, St. Patrick's College, Maynooth, Ireland: Intermediary metabolism in dauer larvae of *C. elegans*.

Mendis, A.H.W., Keene, N.M., Rees, H.H., Goodwin, T.W., Dept. of Biochemistry, University of Liverpool, England: Identification, titers, and functions of molting hormones in *C. elegans*.

Johnson, T.E.,[1] Roberts, L.,[2] Cuccaro, P.M.,[1] [1]Dept. of Molecular Biology and Biochemistry, University of California, Irvine; [2]Institute for Behavioral Genetics, Boulder, Colorado: Genetics of long-lived variants of *C. elegans*.

SESSION 6 *Poster Session: Genetics and Development*

Rosenbluth, R., Cuddeford, C., Scott, G., Baillie, D.L., Dept. of Biological Science, Simon Fraser University, Burnaby, Canada: EMS and gamma-ray mutagenesis in *C. elegans*.

Pulak, R., Samoiloff, M.R. Madrid, A. Ager, D. Bogaert, T., Dept. of Zoology, University of Manitoba, Winnipeg, Canada: The 1983 genetic map of *Panagrellus redivivus*.

Denich, K., Samoiloff, M., Dept. of Zoology, University of Manitoba, Canada: Mutation rates induced by large doses of gamma, proton, and neutron irradiation of the X-chromosome of the nematode *Panagrellus redivivus*.

Hartman, P., Dept. of Biology, Texas Christian University, Fort Worth: Wild-type and *rad* mutant radiation sensitivities—Effects of parental genotype and developmental stage.

Emmons, S.W., Roberts, S., Katzenberg, D. Sanicola, M. Rubinfeld, B., Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Mutations in Bergerac induced by heat shock.

Park, J., Horvitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Dominant mutations of *C. elegans*.

Hosono, R., Kuno, S., Dept. of Biochemistry, Kanazawa University School of Medicine, Ishikawa, Japan: Isolation and partial characterization of temperature-sensitive paralytic mutants of *C. elegans*.

Ferguson, E., Sternberg, P., Greenwald, I., Horvitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Mutations in the locus *sup-17* reduce the level of *lin-12* gene activity.

Greenwald, I., Horvitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: *sup-10(n983)* confers a phenotype that is similar to the of *unc-93(e1500)*.

Rose, A.M.,[1] Baillie, D.L.,[2] Curran, J.,[2] [1]Dept. of Medical Genetics, University of British Columbia, Vancouver, Canada; [2]Dept. of Biological Sciences, Simon Fraser University, Burnaby, Canada: Overlapping duplications cover entire chromosome I.

Sigurdson, D.C., Spanier, G., Herman, R.K., Dept. of Genetics and Cell Biology, University of Minnesota, St. Paul: A deficiency map for a region of chromosome II.

Rogalski, T.M., Baillie, D.L., Dept. of Biological Sciences, Simon Fraser University, Burnaby, Canada: Genetic analysis of the *unc-22 IV* region of *C. elegans*.

Golden, J.W., Riddle, D.L., Division of Biological Sciences, University of Missouri, Columbia: Influence of environmental cues on development of the *C. elegans* dauer larva.

Klass, M., Biology Dept., University of Houston, Texas: Age-correlated changes in DNA in *C. elegans*.

Ishii, N., Suzuki, K., Dept. of Molecular Biology, Tokai University

School of Medicine, Japan: Effect of radiation on the life span of the nematode *Rhabditidae tokai*.

Yarbrough, P.O., Hecht, R.M., Dept. of Biochemical and Biophysical Sciences, University of Houston Central Campus, Texas: Developmental regulation of two glyceraldehyde-3-phosphate dehydrogenases in *C. elegans*.

Abdulkader, N., Otsuka, A., Dept. of Genetics, University of California, Berkeley: Mutations affecting the morphology of the tail of *C. elegans*.

Kemphues, K., Wolf, N., Hirsh, D., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Studies at the *zyg-11* locus.

Cowan, A., McIntosh, J.R., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Effect of repositioning the first cleavage plane on the development of *C. elegans* embryos.

Denich, K.,[1] Cassada, R.,[2] Isnenghi, E.,[2] Schierenberg, E.,[3] [1]Dept. of Zoology, University of Manitoba, Winnipeg, Canada; [2]Dept. of Molecular Biology, Max-Planck Institute for Experimental Medicine, Göttingen, Federal Republic of Germany; [3]Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Cell lineage and developmental defects of temperature-sensitive embryogenesis mutants in *C. elegans*.

Cassada, R., Denich, K., Isnenghi, E., Schierenberg, E., Smith, K., Dept. of Molecular Biology, Max-Planck Institute for Experimental Medicine, Göttingen, Federal Republic of Germany: Temperature-sensitive embryonic mutants—The Göttingen set revisited.

SESSION 7 *Transposons, Suppressors, and Transformation*

Chairperson: J. Hodgkin, MRC Laboratory of Molecular Biology, Cambridge, England

Eide, D., Anderson, P., Dept. of Genetics, University of Wisconsin, Madison: Spontaneous *unc-54* mutations.

Moerman, D.G., Waterson, R.H., Dept. of Genetics, Washington University School of Medicine, St. Louis, Missouri: Spontaneous unstable *unc-22 IV* mutations in *C. elegans* var. Bergerac.

Emmons, S.W., Yesner, L., Ruan, K., Katzenberg, D., Rubinfeld, B., Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Evidence for a transposon in *C. elegans*.

Liao, L.W., Rosenzweig, B., Hirsh, D., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Tc1, a transposable element in *C. elegans*.

Rose, A.M., Mawji, N., Harris, L., Donati, L., Dept. of Medical Genetics, University of British Columbia, Vancouver, Canada: Molecular and genetic analysis of the *dpy-14 unc-13* region of chromosome I.

Waterston, R.H., Bolten, S., Dept. of Genetics, Washington University, St. Louis, Missouri: Evidence that *sup-7* of *C. elegans* is a $tRNA^{Trp}$ gene.

Stinchcomb, D., Shaw, J., Wood, W., Hirsh, D., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: DNA transformation of *C. elegans*.

SESSION 8 *Genes and Molecular Biology*

Chairperson: R.K. Herman, University of Minnesota, Minneapolis, Minnesota

Sanford, T.R., Golomb, M., Riddle, D.L., Division of Biological Sciences, University of Missouri, Columbia: RNA polymerase II from wild-type and amanitin-resistant strains of *C. elegans*.

Hedgecock, E., Salvato, M., Thomson, J.N., MRC Laboratory of Molecular Biology, Cambridge, England: Nucleolar mutants.

Snutch, T.P., Baillie, D.L., Dept. of Biological Sciences, Simon Fraser University, Burnaby, Canada: Structure and molecular organization of genes coding for the major heat-shock peptide in *C. elegans*.

Russnak, R.H., Jones, D., Candido, E.P.M., Dept. of Biochemistry, University of British Columbia, Vancouver, Canada: The 16kD heat-shock protein gene family of *C. elegans*.

Kramer, J., Cox, J., Hirsh, D., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Organization and sequence analysis of the collagen multigene family of *C. elegans*.

Cox, J., Kramer, J., Hirsh, D., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Expression of collagen genes during *C. elegans* development.

Politz, J.C., Kusch, M., Politz, S.M., Edgar, R.S., University of California, Santa Cruz: The search for cuticle collagen primary gene products.

Wild, M., Landel, C., Krause, M., Begley, M., Hirsh, D., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: *C. elegans* actin genes.

Karn, J., MRC Laboratory of Molecular Biology, Cambridge, England: More myosins (not more of my sins.)

SESSION 9 *Poster Session: Proteins, DNA, and Polymorphisms*

Burke, D.J., Ward, S., Kirschner, B., Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: Identification of sperm-specific proteins and isolation of their genes.

Otsuka, A., Wang, D., Dept. of Genetics, University of California, Berkeley: A general method for the identification of proteins affected by null mutations.

Kolston, D.L., Russell, R.L., Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: Class C—A new class of acetylcholinesterase in *C. elegans*.

Carton, M.,Culotti, J., Northwestern University, Evanston, Illinois: Electrophoretic separation of acetylcholinesterase forms from *C. elegans*.

Jen-Jacobson, L., Jacobson, L.A., Shah, M.V., Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: Biochemical characterization of Cathepsin D.

Jacobson, L.A., Jen-Jacobson, L. Bolanowski, M., Shah, M.V., Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: Mutants deficient in lysosomal Cathepsin D activity.

Jameel, S., McFadden, B.A. Biochemistry/Biophysics Program, Washington State University, Pullman: Isocitrate lyase from *C. elegans*.

Starck, J., Dépt. de Biologie Générale et Appliquée, Universite Claude Bernard Lyon, France: Synthesis of the four specific proteins in *C. elegans* mutant strains, affected in their gametogenesis or in their embryogenesis.

Albertson, D.G., MRC Laboratory of Molecular Biology, Cambridge, England: Localization of genes on *C. elegans* chromosomes.

Benian, G.M., Waterston, R.H., Dept. of Genetics, Washington University School of Medicine, St. Louis, Missouri: Cloning of myosin heavy chain genes and adjacent regions.

Eide, D., Anderson, P., Dept. of Genetics, University of Wisconsin, Madison: A novel insertion mutation in *unc-54*.

Prasad, S., Baillie, D.L., Dept. of Biological Sciences, Simon Fraser University, Burnaby, Canada: Cloning and characterization of the gene coding for the muscle protein tropomyosin in *C. elegans*.

Nelson, D.W., Honda, B.M., Dept.of Biological Sciences, Simon Fraser University, Burnaby, Canada: 5S RNA genesin *C. elegans*.

Stinchcomb, D., Mello, C., Roberts, J., Hirsh, D., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: *C. elegans* DNA that directs segregation in *S. cerevisiae*.

Baillie, D.L., Beckenbach, K.A., Dept. of Biological Sciences, Simon Fraser University, Burnaby, Canada: Molecular analysis of the *unc-22* region of chromosome IV.

Felsenstein, K.M., Emmons, S.W., Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Short, interspersed repetitive sequences in *C. elegans*.

Emmons, S.W., Felsenstein, K., Katzenberg, D., Rubinfeld, B., Yesner, L., Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Identification of distinct *C. elegans* strains on the basis of restriction fragment polymorphisms.

Carr, S., Cox, J., Hirsh, D., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Polymorphism mapping.

Jefferson, R., Hirsh, D., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Plasmid expression vectors for the in vivo analysis of regulatory sequences.

Himmelhoch, S.,[1] Zuckerman, B.,[2] [1]Dept. of Biomembranes, Weizmann Institute, Rehovot, Israel; [2]Dept. of Plant Pathology, University of Massachusetts, Amherst: Characterization from ultrathin cryosections of negatively charged groups on the cuticle and intestine of *C. elegans*.

Emmons, S.W., Yesner, L., Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Optimal bacterial strains for cultivation of *C. elegans*.

SESSION 10 *Embryogenesis*

Chairperson: S. Ward, Carnegie Institution of Washington, Baltimore, Maryland

Priess, J., Hirsch, D., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Embryonic cuticle development.

Ellis, H.M., Tsung, N., Horvitz, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Activity of the gene *ced-3* is necessary for the initiation of programmed cell death.

Herman, R.K., Dept. of Genetics and Cell Biology, University of Minnesota, St. Paul: Genetic mosaics of *C. elegans*.

Schierenberg, E., Wood, W.B., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Experimental embryology.

Sulston, J.E., Thomson, J.N., MRC Laboratory of Molecular Biology, Cambridge, England: Ontogeny of the anterior sensilla.

Strome, S., Wood, W.D., Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Segregation of germline-specific antigens during embryogenesis in *C. elegans*.

Gossett, L.A., Hecht, R.M., Dept. of Biochemical and Biophysical Sciences, University of Houston Central Campus, Texas: *zyg-4* and its allele *emb-7* exhibit muscleless phenotypes during embryogenesis in *C. elegans*.

MICROBIAL DEVELOPMENT

May 11–May 15

SESSION 1

Chairperson: J. Adler, University of Wisconsin, Madison, Wisconsin

Chemotaxis

Krikos, A., Mutah, N., Boyd, A., Simon, M., Dept. of Biology, California Institute of Technology, Pasadena: Sensory transducers of *E. coli* are composed of discrete structural and functional domains.

Callahan, A.M., Sherris, D., Slocum, M.K., Parkinson, J.S., Dept. of Biology, University of Utah, Salt Lake

City: Genetics of a chemotactic transducer protein in *E. coli*.

Stock J., Dept. of Biochemical Sciences, Princeton University, New Jersey: The role of receptor methylation in chemotaxis as revealed by the behavior of *S. typhimurium* mutants lacking the methyltransferase (*cheR*) or methylesterase (*cheB*) enzymes.

Flagellar Structure

Huang, B., Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas: Genetic dissection of eukaryotic flagella.

Dutcher, S.K., Ramanis, Z., Luck, D.J.L., Rockefeller University, New York, New York: Genetic behavior of a new linkage group in *Chlamydomonas*.

SESSION 2

Chairperson: N. Agabian, University of Washington Medical School, Seattle, Washington

Cell Division in E. coli

Donachie, W., Dept. of Molecular Biology, University of Edinburgh, Scotland: Regulation of expression in a cluster of cell-division genes in *E. coli*.

Hirota, Y., Maruyama, I.N., Nakamura, M., Yamamoto, A., Nishimura, Y., National Institutes of Genetics, Mishima, Japan: Entire nucleotide sequence of *E. coli* structure gene coding for penicillin-binding protein 3 (pbp-3).

Regulation of Polarity and Morphogenesis during Cell Division in Caulobacter

Ely, B.,[1] Schoenlein, P.V.,[1] Winkler, M.E.,[1] [1]Dept. of Biology, University of South Carolina, Columbia; [2]Dept. of Molecular Biology, Northwestern University Medical School, Chicago, Illinois: Gene organization of *Caulobacter crescentus*.

Shapiro, L., Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Membrane biogenesis and the regulation of differentiation in *Caulobacter*.

Agabian, N., Gill, P.R., Milhausen, M., Dept. of Biochemistry, University of Washington, Seattle: The flagellin gene family of *Caulobacter crescentus*.

Ohta, N., Chen, L.-S., Newton, A., Dept. of Biology, Princeton University, New Jersey: Regulation of flagellum biosynthesis and assembly in the *Caulobacter crescentus* cell cycle.

Cell Division in Physarum

Burland, T.G.,[1] Dove, W.,[1] Gull, K.,[1,2] Schedl, T.,[1] [1]McArdle Laboratory, University of Wisconsin, Madison; [2]Biological Laboratories, University of Kent, Canterbury, England: Expression of the tubulin gene families in the cycles of *Physarum polycephalum*.

SESSION 3 *Poster Session*

Bellofatto, V., Amemiya, K. Shapiro, L., Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: An RNA processing enzyme from *Caulobacter crescentus*.

Curiel-Quesada, E., Fliss, E.R., Setlow, P., Dept. of Biochemistry, University of Connecticut Health Center, Farmington: Cloning and analysis of the gene for the C protein—A spore-specific protein degraded during *Bacillus megaterium* spore germination.

DeWitt, J.P., Abbott Laboratories, North Chicago, Illinois: Genetic instability involving regulation of secondary metabolism in *Streptomyces erythreus*.

Donnelly, C.E., Sonenshein, A.L., Dept. of Molecular Biology and Microbiology, Tufts University, Boston, Massachusetts: Mutations in the promoter region of a *Bacillus subtilis* gene.

Liu, C.-J.,[1] Pogell, B.M.,[1] Meade, H.,[2] [1]Dept. of Medicinal Chemistry/Pharmacognosy, School of Pharmacy, University of Maryland, Baltimore; [2]Biogen, Inc., Cambridge, Massachusetts: Complete deletion of the *argG* gene occurs in aerial mycelium-negative isolates of streptomycetes.

Manis, J.J., Olsen, M.K., Fermentation Research and Development, Upjohn Company, Kalamazoo, Michigan: Construction of an *E. coli-Streptomcyes* promoter-probe shuttle vector.

Nelson, D.R.,[1] Zusman, D.R.,[2] [1]Dept. of Microbiology, University of Rhode Island, Kingston; [2]Dept. of Microbiology and Immunology, University of California, Berkeley: Transport and localization of protein S, a spore coat protein, during fruiting body formation in *Myxococcus xanthus*

Piret, J.M., Chater, K.F., John Innes Institute, Norwich, England: Cloning of genes involved in differentiation of *Streptomyces coelicolor* A3(2).

Ruby, E.G., Dept. of Biological Sciences, University of Southern California, Los Angeles: Differentiation of intraperiplasmically growing *Bdellovibrio bacteriovorous*—The control of initiation of DNA replication.

Yee, T., Correia, F., Inouye, M., Dept. of Biochemistry, State University of New York, Stony Brook: Two-dimensional S1 nuclease heteroduplex mapping—Detection of rearrangements in bacterial genomes.

SESSION 4 *Spore Formation in* Bacillus

Chairperson: R. Losick, Harvard University, Cambridge, Massachusetts

Ferrari, F., Hoch, J., Research Institute of Scripps Clinic, La Jolla, California: Control of sporulation by *spoO* genes.

Smith, I., Dubnau, E., Ramakrishna, N., Weir, J., Dept. of Microbiology, Public Health Research Institute of the City of New York, Inc., New York: The *Bacillus spoOH* gene.

Zuber, P., Johnson, W.C., Losick, R., Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: Developmental regulation of a sporulation promoter in *Bacillus subtilis*.

Sonenshein, A.L., Rosenkrantz, M.S., Donnelly, C.E., Fisher, S.,

Emond, R., Dept. of Molecular Biology and Microbiology, Tufts University, Boston, Massachusetts: Regulation in vivo of *Bacillus subtilis* genes active during growth or sporulation.

Setlow, P., Dept. of Biochemistry, University of Connecticut Health Center, Farmington: Protein degradation during bacterial spore germination.

Youngman, P.J., Perkins, J.B., Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: Developmental genetics in *Bacillus* using the *Streptococcus* transposon Tn*917.*

SESSION 5

Chairperson: D. Hopwood, John Innes Institute, Norwich, England

RNA Polymerase Heterogeneity in Bacillus

Pero, J., Costanzo, M., Hannett, N., Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: Sigma factors, promoters, and the temporal control of gene expression.

Gilman, M.Z., Glenn J., Chamberlin, M.J., Dept. of Biochemistry, University of California, Berkeley: In vivo expression of *Bacillus subtilis* transcripts controlled by σ^{28} RNA polymerase.

Truitt, C.L., Ray, G.L., Trempy, J.E., Haldenwang, W.G., Dept. of Microbiology, University of Texas Health Science Center, San Antonio: Isolation of *Bacillus subtilis* mutants with altered expression of a gene transcribed in vitro by σ^{37}-containing RNA polymerase.

Price, C.W., Gitt, M.A., Wong, S.-L., Doi, R.H., Dept. of Biochemistry and Biophysics, University of California, Davis: Cloning and genetic mapping of the RNA polymerase σ^{55} gene of *Bacillus subtilis.*

Differentiation in Streptomyces

Chater, K.F., Bruton, C.J., Ikeda, H., King, A.A., Piret, J.M., Rodicio, M.R., John Innes Institute, Norwich, England: Genetics in *Streptomyces* differentiation.

Baltz, R.H., Seno, E.T., Stonesifer, J., Matsushima, P., Lilly Research Laboratories, Indianapolis, Indiana: Genetic control of tylosin biosynthesis in *Streptomyces fradiae* – A model to study regulation of antibiotic gene expression and DNA amplification.

Westpheling, J., Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: RNA polymerase heterogeneity in *Streptomyces coelicolor.*

Solenberg, P.J., Mooney, P.Q., Schaus, N.A., Lilly Research Laboratories, Indianapolis, Indiana: Development of a model system for the study of the regulation of gene expression in *Streptomyces.*

SESSION 6 *Sporulation and Bud Formation in Yeast and Other Fungi*

Chairperson: A. Klar, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Pringle, J.R., Coleman, K., Adams, A., Lillie, S., Oeller, P., Robinson, J., Kelley, M., Division of Biological Sciences, University of Michigan, Ann Arbor: Cellular morphogenesis in the *Saccharomyces* cell cycle.

Henry, S., Letts, V.A., Klig, L.S., Loewy, B.S., Dept. of Genetics, Albert Einstein College of Medicine, Bronx, New York: The role of phospholipid synthesis and regulation in membrane biogenesis in yeast.

Nurse, P., Dept. of Biological Sciences, University of Sussex Falmar, Brighton, England: Cell cycle controls in fission yeast.

Magee, P.T.,[1] Clancy, M.J.,[2] Primerano, D.,[1] [1]Dept. of Microbiology, Michigan State University, East Lansing; [2]Dept. of Microbiology, Notre Dame University, South Bend, Indiana: Transcriptional control and mutant phenotype of genes involved in sporulation in *Saccharomyces cerevisiae.*

Ninfa, E.G., Kaback, D.B., Dept. of Microbiology, UMDNJ, New Jersey Medical School, Newark: Isolation of DNA sequences complementary to sporulation-specific transcripts.

Timberlake, W.E., Gwynne, D.I., Miller, B.L., Miller, K., Zimmermann, C.R., Dept. of Plant Pathology, University of California, Davis: Organization and developmental regulation of the *spoC1* gene cluster of *Aspergillus nidulans.*

Champe, S.P., Yager, L.N., Butnick, N.J., Kurtz, M.B., Waksman Institute of Microbiology, Rutgers University, New Brunswick, New Jersey: Sporulation-defective mutants of *Aspergillus nidulans.*

SESSION 7 *Cell Communication and Morphogenesis in* Dictyostelium

Chairperson: L. Shapiro, Albert Einstein College of Medicine, Bronx, New York

Lodish, H.F., Barklis, E., Chisholm, R., Pontius, B., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Regulation of *Dictyostelium discoideum* mRNAs specific for prespore or prestalk cells.

Mehdy, M.C., Mann, S.K.O., Firtel, R.A., Dept. of Biology, University of California, San Diego, La Jolla: Induction and modulation of cell type-specific gene expression in *Dictyostelium.*

Gerisch, G., Max-Planck-Institut für Biochemie Martinsried, Federal Republic of Germany: Control of early cell differentiation in *Dictyostelium discoideum.*

MacWilliams, H., Dept. of Zoology, University of Munich, Federal Republic of Germany: Morphogenesis in cellular slime mold slugs.

David, C.N.,[1] Sternfeld, J.,[2] [1]Zoologisches Institut, Munich, Federal Republic of Germany; [2]Dept. of Biology, University of Southwestern Louisiana, Lafayette:

Formation of the prestalk prespore pattern in *Dictyostelium* occurs by cell sorting.

Kay, R., Brookman, J., Kopachik, W., Pogge, R., Dhokia. B., Jermyn, K., Peacey, M., Gross, J., Imperial Cancer Research Fund, London, England: The morphogens and intracellular responses controlling the choice between stalk- and spore-cell differentiation in *Dictyostelium*.

Williams, J.G., Garreau, H., Imperial Cancer Research Fund, Mill Hill Laboratories, London, England: Nuclear DNA-binding proteins of *Dictyostelium discoideum*.

Fontana, D.R., Devreotes, P.N., Dept. of Physiological Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland: Inhibition of the cAMP signal relay response in *Dictyostelium discoideum* amoebae by agents which cross-link surface components.

SESSION 8

Chairperson: D. Kaiser, Stanford University, Stanford, California

Cell Communication and Morphogenesis in Myxobacteria

Shimkets, L.,[1] Gill, R.,[2] Kaiser, D.,[3] [1]Dept. of Microbiology, University of Georgia, Athens; [2]Dept. of Microbiology, University of Colorado Health Center, Denver; [3]Dept. of Biochemistry, Stanford University, California: Cell interactions in *Myxococcus* development.

Nelson, D.R.,[1] Downard, J.S.,[2] Zusman, D.R.,[2] [1]Dept. of Microbiology, University of Rhode Island, Kingston; [2]Dept. of Microbiology and Immunology, University of California, Berkeley: Long-lived messenger RNA during fruiting body formation in *Myxococcus xanthus*.

Dworkin, M., Dept. of Microbiology, University of Minnesota, Minneapolis: Cell-surface interactions during development in *Myxococcus xanthus*.

Inouye, S., Teintze, M., Inouye, M., Dept.of Biochemistry, State University of New York, Stony Brook: Development-specific calmodulin-like protein of *Myxococcus xanthus*.

Bacteroid Formatin by Rhizobium *and Heterocyst Formation by* Anabaena

Ausubel, F., Buikema, W., Sundaresan, V., Szeto, W., Zimmerman, L., Dept. of Molecular Biology, Massachusetts General Hospital, Boston: Identification and characterization of *Rhizobium meliloti* symbiotic genes.

Pühler, A., Klipp, W., Priefer, U., Simon, R., Weber, G., Lehrstuhl für Genetik, Fakultat fur Biologie, Universität Bielefeld, Federal Republic of Germany: Analysis of symbiotic genes of *Rhizobium meliloti*.

Haselkorn, R., Curtis, S.E., Richaud, C., Robinson, S.J., Turner, N., Dept. of Biophysics, University of Chicago, Illinois: Organization and transcription of *Anabaena* genes regulated during heterocyst differentiation.

Pattern Formation in Pseudomonas

Shapiro, J.A., Dept. of Microbiology, University of Chicago, Illinois: Pattern formation in *Pseudomonas putida* colonies revealed by insertions of a Mud *lac* phage.

SESSION 9 *Mating Interactions*

Chairperson: I. Herskowitz, University of California, San Francisco, California

Hopwood, D.A., Bibb, M.J., Kieser, T., Lydiate, D.J., Wright, H.M., John Innes Institute, Norwich, England: Plasmid-mediated conjugation in *Streptomyces*.

Clewell, D., Ike, Y., White, B., Craig, R., Yagi, Y., Dept. of Oral Biology, University of Michigan, Ann Arbor: Sex pheromones in *Streptococcus faecalis*.

Silverman, P., Sambucetti, L., Albins, R., Cuozzo, M., Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Cellular contributions to plasmid-dependent conjugal donor activity of *E. coli* K-12.

VanWinkle-Swift, K., Aubert, B., Burrascano, C., Dept. of Biology, San Diego State University, California: Genetic anlaysis of sexual reproduction in the homothallic alga *Chlamydomonas monoica*.

Julius, D.,[1,2] Brake, A.,[1,3] Emr, S.,[2] Flessel, M.,[1] Thorner, J.,[1] [1]Dept. of Microbiology and Immunology; [2]Dept. of Biochemistry, University of California, Berkeley; [3]Chiron Corporation, Emeryville, California: Yeast peptide pheromones—Biosynthesis and cell-type-specific gene expression.

Herskowitz, I., Wilson, K., Jenson, R., Fields, S., Dept. of Biochemistry and Biophysics, University of California, San Francisco: Determination of yeast cell type by the mating-type locus.

Beach, D., Klar, A., Cold Spring Harbor Laboratory, New York: Developmental switches of the mating-type locus in *Schizosaccharomyces pombe*.

RNA PROCESSING
May 18–May 22

SESSION 1 *Nuclear Structure and Function*

Chairperson: G. Blobel, Rockefeller University, New York, New York

Berrios, M.,[1] Filson, A.,[1] Blobel, G.,[1] Fisher, P.,[2] [1]Laboratory of Cell Biology, Rockefeller University, New York; [2]Dept. of Pharmacological Sciences, State University of New York, Stony Brook: An ATPase/dATPase of the nuclear matrix-pore, complex-lamina fraction specifically identified by direct UV photoaffinity labeling.

Risau, W., Symmons, P., Saumweber, H., Frasch, M., Bonhoeffer, F., Ab-

teilung für Physikalische Biologie, Max-Planck-Institut für Virusforschung, Tübingen, Federal Republic of Germany: Nonpackaging and packaging proteins of *Drosophila* hnRNP.

Trendelenberg, M.F.,[1] Oudet, P.,[2] Franke, W.W.,[1] Gallwitz, D.,[3] [1]Institute of Cell and Tumor Biology and Experimental Pathology, German Cancer Research Center, Heidelberg, Federal Republic of Germany; [2]CNRS, Strasbourg, France; [3]University of Marburg, Federal Republic of Germany: Structural organization of defined pre-mRNP transcripts and patterns of posttranscriptional RNP modifications.

Leser, G.P., Monsma, S.A., Martin, T.E., Dept. of Biology, University of Chicago, Illinois: Immunological studies of nuclear hnRNP and snRNP complexes.

Scheer, U.,[1] Rose, K.M.,[2] [1]Institute of Cell and Tumor Biology, German Cancer Research Center, Heidelberg, Federal Republic of Germany; [2]Milton S. Hershey Medical Center, Pennsylvania State University, Hershey: Localization of transcribed rRNA genes in interphase cells and detection of RNA polymerase I bound to mitotic chromosomes by light and electron microscope immunocytochemistry.

Bringmann, P., Rinke, J., Reuter, R. Appel, B., Lührmann, R., Max-Planck-Institut für Molekulare Genetik, Berlin, Federal Republic of Germany: Structure-function relationships of U-snRNPs as studied with snRNA cap-specific antibodies.

Mattaj, I.W., Bürglin, T., De Robertis, E.M., Biocenter, University of Basel, Switzerland: 7S and 42S RNP particles are excluded from the nucleoplasm of *X. laevis* oocytes.

Forbes, D., Kirschner, M., Dept. of Biochemistry and Biophysics, University of California, San Francisco: New nuclear structures containing snRNP antigens in the developing *Xenopus* embryo.

Atkinson, N.S., Nolan, S.L., Dunst, R.W., Hopper, A.K., Dept. of Biological Chemistry, Milton S. Hershey Medical Center, Pennsylvania State University, Hershey: Characterization of a yeast gene affecting RNA processing.

SESSION 2 *RNPs and Processing*

Chairperson: T. Martin, University of Chicago, Chicago, Illinois

LeStourgeon, W.M., Arenstorf, H.P., Lothstein, L., Dept. of Molecular Biology, Vanderbilt University, Nashville, Tennessee: Observations on the arrangement of protein and RNA in 40S hnRNP particles.

Wilk, H.-E., Schäfer, K.P. Lehrstuhl Biochemie, Ruhr-Universität Bochum, Federal Republic of Germany: The in vitro reconstitution of hnRNP-like complexes.

Thomas, J.O., Glowacka, S.K., Szer, W., Dept. of Biochemistry, New York University School of Medicine, NewYork: Structure of an hnRNP protein-polynucleotide complex.

Kloetzel, P.M., Bautz, E.K.F., Dept. of Molecular Genetics, University of Heidelberg, Federal Republic of Germany: Organization of hnRNP complex in heat-shocked *Drosophila* cells.

Economidis, I.V., Pederson, T., Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Assembly of a pre-mRNP in vitro.

Wieben, E., Madore, S., Pederson, T., Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Maturation of U1 and U2 RNAs.

Dahlberg, J.E.,[1] Lund, E.,[1] Mitchen, J.L.,[1] Skuzeski, J.M.,[1] Burgess, R.R.,[2] Murphy, J.T.,[2] Steinberg, T.,[2] [1]Dept. of Physiological Chemistry, [2]Dept. of Oncology, University of Wisconsin, Madison: Synthesis and process of wild-type and mutant human U1 RNA transcripts.

Branlant, C.,[1] Krol, A.,[1] Ebel, J.P.,[1] Haendler,B.,[2] Lazar, E.,[2] Jacob, M.,[2] [1]University of Strasbourg; [2]CNRS, Strasbourg, France: A study of snRNAs within hnRNPs using DMS as a probe.

Sri-widada, J., Brunel, C., Liautard, J.P. Jeanteur, P., Laboratoire de Bochimie, Laboratoire de Biologie Molécular, Université des Sciences et Techniques du Languedoc, Montpellier, France: Interaction of snRNAs with rapidly sedimenting subnuclear structures (hnRNP).

Calvet, J.P., Myers, J.A., Dept. of Biochemistry, University of Kansas Medical Center, Kansas City: In vivo secondary structure and intermolecular base-pairing interactions involving the snRNAs analyzed by psoralen cross-linking.

SESSION 3 *Poster Session*

Aloni, Y., Abulafia, R., Hay, N., Ben-Ze'ev, A., Dept. of Genetics, Weizmann Institute of Science, Rehovot, Israel: The control of late SV40 transcription by the attenuation mechanism, and transcriptionally active ternary complexes are associated with the nuclear matrix.

Aloni, Y., Hay, N., Skolnik-David, H., Ben-Asher, E., Pruzan, R., Dept. of Genetics, Weizmann Institute of Science, Rehovot, Israel: Attentuation in the control of viral gene expression.

Alonso, A.,[1] Jorcano, J.L.,[1] Beck,E.,[2] Spiess, E.,[1] Kubli, E.,[3] [1]German Cancer Research Center, Heidelberg, Federal Republic of Germany; [2]Institute of Microbiology, University of Heidelberg, Federal Republic of Germany; [3]Institute of Zoology, University of Zürich, Switzerland: Cloning and characterization of U2 and U1 snRNA genes from *D. melanogaster.*

Beccari, E.,[1] Amaldi, F.,[1] Annesi, F.,[1] Bozzoni, I.,[1] Fragapane, P.,[1] Mileo, A.M.,[1] Pierandrei-Amaldi, P.,[2] [1]Centro Acidi Nucleici; [2]Instituto di Biologia Cellulare, CNR, Rome, Italy: *X. laevis* ribosomal protein genes—Processing of transcripts from genomic clones injected in *X. laevis* oocytes.

Benyajati, C.,Dray, J.F., Frederick Cancer Research Facility, Frederick, Maryland: Expression of the cloned *adh* gene in transfected *Drosophila* tissue culture cells.

Billings, P.B., Hoch, S.O., Division of Cellular Biology, Research Institute of Scripps Clinic, La Jolla, California: Isolation of U1 and U2, U4, and U5 plus U6 snRNP populations.

Brandt, C.R., Morrison, S.L., Milcarek, C., Dept. of Microbiology, Columbia University, New York, New York: Altered RNA splicing in

immunoglobulin in heavy-chain mutants.

Dreyfuss, G., Choi, Y.D., Adam, S.A., Dept. of Biochemistry, Molecular and Cell Biology, Northwestern University, Evanston, Illinois: Characterization of proteins in direct contact with mRNA and hnRNA in vivo.

Frayne, E., Yeung, C., Hook, A., Al-Ubaidi, M., Bobonis, C., Ingolia, D., Kellems, R., Dept. of Biochemistry, Baylor College of Medicine, Houston, Texas: ADA gene-amplification mutants—A model system for studying ADA mRNA metabolism.

Fritz, A., Zeller, R., Carrasco, A., Mattaj, I., De Robertis, E.M., Biocenter, University of Basel, Switzerland: snRNA genes and snRNA-binding proteins of *X. laevis*.

Hammarström, K., Monstein, H.-J., Westin, G., Bark, C., Zabielski, J., Philipson, L., Pettersson, U., Dept. of Medical Genetics and Microbiology, Biomedical Center, Uppsala, Sweden: Loci for human U1, U2, and U4 RNAs.

Jacobson, A.B., Dept. of Microbiology, State University of New York, Stony Brook: Studies on the folding of MS2 RNA.

Khandjian, E.W., Darlix, J.-L., Weil, R., Dept. of Molecular Biology, University of Geneva, Switzerland: Interaction of SV40 large T antigen with noncoding and repetitive sequences of primary transcripts.

Lagrimini, L.M., Donelson, J.E., Dept. of Biochemistry, University of Iowa, Iowa City: The use of an SV40 expression vehicle to study splicing of the rat prolactin primary transcript.

Leys, E.J.,[1] Hook, A.G.,[1] Crouse, G.F.,[2] Kellems, R.E.,[1] [1]Dept. of Biochemistry, Baylor College of Medicine, Houston, Texas; [2]Frederick Cancer Research Facility, Frederick, Maryland: Control of DHFR mRNA production.

Lund, E.,[1] Schenborn, E.T.,[1] Bostock, C.,[2] Mitchen, J.L.,[1] Dahlberg, J.E.,[1] [1]University of Wisconsin, Madison; [2]MRC-MGU Edinburgh, Scotland: Expression of human U1 snRNA genes in mouse cells containing either human chromosome 1 or a human gene cloned in BPV.

McKenzie, D., Aiken, J.M., Dixon, G.H., Dept. of Medical Biochemistry, University of Calgary, Alberta, Canada: mRNP particles in the developing testis of rainbow trout.

Morris, G.F.,[1] Brown, D.T.,[2] Marzluff, W.F.,[1] [1]Dept. of Chemistry, Florida State University, Tallahasse; [2]Dept. of Biochemistry, University of Mississippi Medical Center, Jackson: In vivo and in vitro synthesis of snRNA N1 in sea urchin embryos.

Patton, J.R., Ross, D.A., Chae, C.-B., Dept. of Biochemistry, University of North Carolina, Chapel Hill: β-Globin RNA-protein interaction in chicken reticulocyte nuclei.

Pawar, S., Ahmed, C.M.I., Watkins, R., Zain, S., Cancer Center, University of Rochester, New York: In vitro and in vivo processing of mRNA encoding the SV40 T-antigen-specific 30K protein in the nondefective adeno-SV40 hybrid virus (Ad2$^+$ ND1).

Schrier, W., Feinbaum, R., Okarma, T., Dept. of Medicine, Stanford-University School of Medicine, California: Characterization of snRNP particles isolated for L-cell nuclei.

Setyono, B.,[1] Pederson, T.,[2] [1]Institute of Biology, University of Stuttgart, Federal Republic of Germany; [2]Worchester Foundation for Experimental Biology, Cell Biology Group, Shrewsbury, Massachusetts: Interaction between snRNPs and hnRNPs as revealed by UV light and psoralen cross-linking in vivo.

Tollervey, D., Wise, J.A., Maloney, D., Dunn, E., Guthrie, C., Dept. of Biochemistry and Biophysics, University of California, San Francisco: Cloning and genetic analysis of yeast snRNAs.

Villarreal, L.,[1] White, R.,[2] [1]Dept. of Microbiology and Immunology, University of Colorado Health Sciences Center; [2]Dept. of Biochemistry, University of California, Berkeley: A splicing-junction deletion deficient in the transport of RNA does not polyadenylate nuclear RNA.

Wilk, H.-E., Kecskemethy, N., Schäfer, K.P., Lehrstuhl Biochemie, Ruhr-Universität Bochum, Federal Republic of Germany: An affinity matrix for capped snRNAs and mRNAs.

SESSION 4 *Messenger RNA Processing*

Chairperson: A. Shatkin, Roche Institute of Molecular Biology, Nutley, New Jersey

Padgett, R.A.,[1] Mount, S.M.,[2] Steitz, J.A.,[2] Sharp, P.A.,[1] [1]Massachusetts Institute of Technology, Cambridge; [2]Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Splicing of adenoviral RNA in a cell-free transcription system requires U1 RNP.

Hernandez, N., Frick, M., Keller, W., Institute of Cell and Tumor Biology, Germany Cancer Research Center, Heidelberg: In vitro transcription and splicing of adenoviral RNA in HeLa cell extracts.

DiMaria, P.R., Goldenberg, C.J., Dept. of Pathology, Washington University, St. Louis, Missouri: In vitro splicing of purified Ad2 precursor RNAs.

Fradin, A.,[1] Jove, R.,[1] Hemenway, C.,[1] Michaeli, T.,[1] Keiser, H.D.,[1] Manley, J.L.,[2] Prives, C.,[2] [1]Dept. of Medicine, Albert Einstein College of Medicine, Bronx, New York; [2]Dept. of Biological Sciences, Columbia University, New York: Antibodies to snRNPs inhibit SV40 RNA processing in *X. laevis* oocytes.

Green, M., Maniatis, T., Melton, D., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Eukaryotic mRNA processing in microinjected frog oocytes.

Wieringa, B., Weissmann, C., Institut für Molekularbiologie I, Universität Zürich, Switzerland: What are the minimal sequence requirements for splicing the large intron of the rabbit β-globin pre-mRNA?

Langford, C.J., Donath, C., Klinz, F.-J., Gallwitz, D., Institut für Physiologische Chemie I, Universität Marburg, Lahnberge, Federal Republic of Germany: The identification of an intron sequence necessary for the splicing of the yeast actin gene transcript.

Pikielny, C., Teem, J., Abovich, N., Rosbash, M., Dept. of Biology, Brandeis University, Waltham, Massachusetts: Yeast mRNA splicing.

Kaüfer, N.F., Warner, J.R., Albert Einstein College of Medicine, Bronx, New York: Intron deletion studies with the cycloheximide-resistance gene of *S. cerevisiae*.

Nelson, K.J.,[1] Haimovich,J.,[2] Perry, R.P.,[1] [1]Institute for Cancer Research, Philadelphia, Pennsylvania; [2]Tel Aviv University Medical School, Israel: Characterization of productive and sterile transcripts from the immunoglobulin heavy-chain locus—Processing of μ_m and μ_s mRNA.

Nishikura, K., Wister Institute, Philadelphia, Pennsylvania: Regulation of immunoglobulin heavy μ-chain gene expression in Cos cells.

Amara, S.G.,[1] Evans, R.M.,[2] Rosenfeld, M.G.,[1] [1]Dept. of Medicine, University of California, San Diego, La Jolla; [2]Molecular Biology and Virology Laboratory, Salk Institute, San Diego, California: Poly(A) site selection is a critical determinant in the alternative expression of mRNAs from the calcitonin gene.

Manley, J.L., Dept. of Biological Sciences, Columbia University, New York, New York: Accurate and specific polyadenylation of mRNA precursors in a soluble whole-cell lysate.

SESSION 5 *Ribosomal RNA Processing*

Chairperson: A. Dahlberg, Brown University, Providence, Rhode Island

Stark, J.R.,[1] Gourse, R.L.,[2] Dahlberg, A.E.,[1] [1]Section of Biochemistry, Brown University, Providence, Rhode Island; [2]Institute for Enzyme Research, University of Wisconsin, Madison: A mutation in the *rrnB* operon that affects the production of precursor 23S rRNA by RNase III.

King, T.C., Schlessinger, D., Division of Biology and Biological Sciences, Washington University School of Medicine, St. Louis, Missouri: rRNA processing in wild-type and processing-deficient *E. coli*.

Loughney, E., Lund, E., Dahlberg, J.E., Dept. of Physiological Chemistry, University of Wisconsin, Madison: Processing sites for precursor rRNAs of *B. subtilis*.

Szeberenyi, J., Roy, M.K., Apirion, D., Dept. of Microbiology, Washington University, St. Louis, Missouri: Precursor nucleotides at the 5′ end are not required for processing by RNase E at the 3′ end of 5S rRNA.

Grabowski, P.J., Cech, T.R., Dept. of Chemistry, University of Colorado, Boulder: Accurate exon ligation accompanies IVS excision during self-splicing of the rRNA precursor of *Tetrahymena*.

Wollenzien, P.,[1] Cantor, C.,[1] Jacobson, A.,[2] Garriga, G.,[3] Lambowitz, A.,[3] [1]Dept. of Human Genetics and Development, Columbia University College of Physicians and Surgeons, New York, New York; [2]Dept. of Microbiology, State University of New York, Stony Brook; [3]Dept. of Biochemistry, St. Louis University Medical School, Missouri: The intervening sequence in the *N. crassa* mitochondrial rRNA gene—Comparison of experimentally determined contacts and the predicted secondary structure.

Garriga, G.,[1] Bertrand, H.,[2] Lambowitz, A.M.,[1] [1]St. Louis University, Missouri; [2]University of Regina, Saskatchewan, Canada: RNA splicing in *Neurospora* mitochondria—A relationship between 3′-end synthesis and splicing of the large rRNA.

Strittmatter, G., Natt, E., Kössel, H., Institüt fur Biologie III, Universität Freiburg, Federal Republic of Germany: Processing sites of transcripts from the maize chloroplast rRNA operon and its tRNA genes.

Prince, D.L., Dubin, D.T., UMDNJ, Rutgers Medical School, Piscataway, New Jersey: Possible effects of the methylation inhibitor cycloleucine on the processing of mammalian mitochondrial RNA.

SESSION 6 *Poster Session*

Adeniyi-Jones, S., Romeo, P., Zasloff, M.A., Human Genetics Branch, NICHD, National Institutes of Health, Bethesda, Maryland: Human $tRNA^{Met}$ gene as a portable eukaryotic promoter.

AbuBakar, U.,[1] Schmidt, J.C.,[1,2] [1]Dept. of Chemistry and Biochemistry, [2]Medical Biochemistry, Southern Illinois University, Carbondale: Defective rRNA processing in a mutant strain of *N. crassa*.

Anziano, P.Q.,[1] Perlman, P.S.,[1] Sass, P.,[2] Lamb, M.R.,[2] Mahler, H.R.,[2] [1]Genetics Dept. and MCD Program, Ohio State University, Columbus; [2]Chemistry Dept., Indiana University, Bloomington: Isolation and characterization of nuclear supressor of splicing mutants in yeast mitochondria.

Boak, A.M.,[1] Agris, P.F.,[1] Chakraborty, D.,[2] Sarkar, S.,[2] [1]Dept. of Biological Sciences, University of Missouri, Columbia; [2]Dept. of Muscle Research, Boston Biomedical Research Institute, Massachusetts: Association of Sm with cytoplasmic mRNA-containing RNP particles and polysomes of chick embryonic muscle.

Bonen, L., Boer, P.H., Hensgens, L.A.M., Grivell, L.A., Section for Molecular Biology, University of Amsterdam, Kruislaan, The Netherlands: A dispersed, multigene family of intron maturases in yeast mitochondria.

Bonitz, S., Tzagoloff, A., Cold Spring Harbor Laboratory, NewYork: RNA processing in yeast mitochondria.

Cimino, G.D., Hearst, J.E., Dept. of Chemistry, University of California, Berkeley: Localization of secondary structure regions in *E. coli* 23S RNA by psoralen cross-linkage.

Cooley, L., Burke, D.J., Söll, D., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Posttranscriptional guanylate addition to the 5′ terminus of histidine tRNA.

Edlind, T., Cooley, T., Ihler, G., Texas A&M College of Medicine, College Station: Potential secondary structures in papovavirus late RNA—Implications to splicing.

Francoeur, A.M., Mathews, M.B., Cold Spring Harbor Laboratory, New York: Purification and characterization of the lupus antigen, La.

Frendewey, D., Willis, I., Hottinger, A., Hottinger, H., Schaack, J., Söll, D., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Processing of dimeric tRNA precursors from *S. pombe*.

Furdon, P., Altman, S., Dept. of Biology, Yale University, New Haven, Connecticut: Site-specific mutagenesis of the dihydrouridine loop of the $tRNA^{Tyr}$ su_3^+ gene from *E. coli*.

Gurevitz, M., Apirion, D., Dept. of Microbiology, Washington University, St. Louis, Missouri: Interplay among processing and degradative enzymes and a precursor RNA in the selective maturation of tRNA molecules.

Hickey, A., Dept. of Biology, University of Ottawa, Canada: Introns as genomic parasites.

Jacobson, A., Favreau, M., Manrow, R.E., Steel, L., Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester: Possible involvement of poly(A) in protein synthesis.

Kinlaw, C.S., Robberson, B.L., Brandt, C., Berget, S.M., Dept. of Biochemistry, Rice University, Houston, Texas: Characterization of the polypeptides of human snRNPs U1, U2, and U4.

Klein, B.K., Forman, P., Staden, A., Romero, J., Schlessinger, D., Division of Biology and Biomedical Sciences, Washington University School of Medicine, St. Louis, Missouri: Stabilizing factors for tRNA and ribosome secondary structure assessed by electron microscopy.

Kotin, R.M., Dubin, D.T., Dept. of Microbiology, UMDNJ, Rutgers Medical School, Piscataway, New Jersey: Further support for attenuation as a mechanism for preferential transcription of rRNA in hamster mitochondria.

Krause, M.O., Sohn, U., Division of Molecular and Microbiology, University of New Brunswick, Fredericton, Canada: A small RNA from SV40-transformed cells recognizes the viral early promoter.

Krol, A., Laboratoire de Biochimie, IBMC, Strasbourg, France: Isolation and sequence of the U-RNA family from plant nuclei.

Lee, M.-C., Knapp, G., Dept. of Microbiology, University of Alabama, Birmingham: Secondary and tertiary structures of unspliced yeast tRNA precursors.

Pape, L.K., Tzagoloff, A., Dept. of Biological Sciences, Columbia University, New York, New York: A nuclear gene required for processing the *S. cerevisiae* mitochondrial apocytochrome-B gene.

Roe, B.A., Wong, J.F.H., Ma, D.P., Wilson, R.K., Dept. of Chemistry, University of Oklahoma, Norman: Nucleotide sequence analysis of the *X. laevis* mitochondrial genome.

Schnare, M.N., Gray, M.W., Dept. of Biochemistry, Dalhousie University, Halifax, Nova Scotia, Canada: The 3′-terminal sequence of wheat mitochondrial large subunit (26S) rRNA.

Skuzeski, J.M., Jendrisak, J.J., Dept. of Botany, University of Minnesota, St. Paul: A family of wheat embryo U2 snRNAs.

Szeberenyi, J.,[1] Elford, R.M.,[2] Holmes, W.M.,[2] Apirion, D.,[1] [1]Dept. of Microbiology, Washington University Medical School, St. Louis, Missouri; [2]Dept. of Microbiology, Medical College of Virginia, Richmond: Initiation, processing, and termination of rRNA from a hybrid 5S rRNA gene in a plasmid.

Tanner, N.K.,[1] Cech, T.R.,[1] Tinoco, I., Jr.,[2] Weir, B.R.,[2] Zuker, M.,[3] Perlman, P.S.,[4] [1]Dept. of Chemistry, Univeristy of Colorado, Boulder; [2]Dept. of Chemistry and Laboratory of Chemical Biodynamics, University of California, Berkeley; [3]Division of Biological Sciences, National Research Council of Canada, Ottawa; [4]Dept. of Genetics, Ohio State University, Columbus: Determination of the secondary structure of the *Tetrahymena* rRNA intervening sequence and comparison with fungal mitochondrial intervening sequences.

Wallace, J.C., Edmonds, M., Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: Localization of branches within nuclear polyadenylated RNA molecules.

Watson, N., Gurevitz, M., Ford, J., Apirion, D., Dept. of Microbiology and Immunology, Washington University School of Medicine, St. Louis, Missouri: Possible self-cleavage of a precursor RNA of a tRNA-like molecule from bacteriophage T4.

Winicov, I., Dept. of Biochemistry, University of Nevada, Reno: tRNA processing in isolated mouse L-cell nuclei.

SESSION 7 *Transfer RNA Processing*

Chairperson: D. Söll, Yale University, New Haven, Connecticut

Sakamoto, H., Kimura, N., Shimura, Y., Dept. of Biophysics, Kyoto University, Japan: Structure and expression of the gene coding for the RNA component of RNase P from *E. coli*.

Dallmann, G.D., Gurevitz, M., Dallmann, K., Apirion, D., Dept. of Microbiology, Washington University Medical School, St. Louis, Missouri: Studies on the RNA moiety of the tRNA processing enzyme, RNase P, and the concept of an RNA processing complex.

Guerrier-Takada, C., Furdon, P., Baer, M., Reed, R., Altman, S., Dept. of Biology, Yale University, New Haven, Connecticut: Recognition of tRNA precursor substrates by RNase P from *E. coli*.

Marsh, T.L.,[1] Pace, N.R.,[2] [1]University of Colorado Medical Center, Denver; [2]Dept. of Molecular and Cellular Biology, National Jewish Hospital and Research Center: Precursor tRNA substrate binding by *B. subtilis* RNase P protein.

Deutscher, P., Asha, P.K., Blouin, T., Zaniewski, R., Dept. of Biochemistry, University of Connecticut Health Center, Farmington: RNase BN–Description of a new tRNA processing enzyme and comparison with other *E. coli* exoribonucleases.

Barkay, T., Goldfarb, A., Daniel, V., Weizmann Institute, Rehovot, Israel: RNase PC, an *E. coli* endonuclease responsible for processing of tRNA precursors.

Kaine, B.P., Gupta, R., Woese, C.R., Dept. of Genetics and Development, University of Illinois, Urbana: tRNA genes in archaebacteria.

Pearson, D., Willis, I., Chisholm, V., Söll, D., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Second-site mutations of the *sup3-e* locus of *S. pombe*–New insights to transcription and processing of eukaryotic tRNAs.

Tocchini-Valentini, G.P., Baldi, M.I., Mattoccia, E., Gandini-Atardi, D., Margarit, I., Institut of Cell Biology, CNR, Rome, Italy: Mutations that affect tRNA processing.

Castaño, J.G., Zasloff, M.A., NICHD, National Institutes of Health, Bethesda, Maryland: Human $tRNA^{Met}$ formation by nucleolytic cleavage and evidence of its nuclear transport.

SESSION 8 *Transcripts of Cytoplasmic Organelles*

Chairperson: N. Martin, University of Texas Health Science Center, Dallas, Texas

Greenberg, B.M.,[1] Gruissem, W.,[2] Prescott, D.M.,[2] Hallick, R.B.,[1] [1]Dept. of Chemistry, [2]Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: In vitro transcription and processing of chloroplast tRNAs in chloroplast soluble extracts.

Attardi, G., Doersen, C., Gaines, G., King, M., Montoya, J., Shuey, D., California Institute of Technology, Pasadena: In vivo and in vitro synthesis and processing of human mitochondrial RNA and their control by the cytoplasm.

Osinga, K.A., Van der Bliek, A.M., De Vries, E., Groot Koerkamp, M., Tabak, H.F., Laboratory of Biochemistry, University of Amsterdam, Kruislaan, The Netherlands: Identification of promoters on yeast mtDNA.

Edwards, J.C., Christianson, T., Rabinowitz, M., Dept. of Medicine, Biochemistry, and Biology, University of Chicago, Illinois: Transcriptional initiation in yeast mitochondria.

Hopper, A.K.,[1] Hurt, D.J.,[1] Morales, M.,[2] Martin, N.C.,[2] [1]Dept. of Biological Chemistry, Milton S. Hershey Medical Center, Pennsylvania; [2]Dept. of Biochemistry, University of Texas Health Science Center, Dallas: Modification of both cytoplasmic and mitochondrial tRNAs are affected by single nuclear mutations.

Simon, M., Faye, G., Institut Curie, Orsay, France: MSS51 – A yeast nuclear gene implicated in the mitochondrial processing of the cytochrome oxidase subunit I pre-mRNA.

Dieckmann, C.L., Homison, G., Koerner, T.J., Dept. of Biological Sciences, Columbia University, New York, New York: Characterization of *CBP1*, a nuclear gene involved in maturation and cytochrome-b mRNA.

McGraw, P., Tzagoloff, A., Dept. of Biological Sciences, Columbia University, New York, New York: The role of the *CBP2* gene in splicing cytochrome-*b* pre-mRNA.

Sass, P.,[1] Mecklenburg, K.,[2] Ralph, D.,[2] Robinson, S.,[2] Mahler, H.R.,[1] Perlman, P.S.,[2] [1]Dept. of Chemistry, Indiana University, Bloomington; [2]Genetics Dept. and MCD Program, Ohio State University, Columbus: *cis*-dominant and *trans*-recessive splicing-defective mutants in the *oxi3* gene of yeast mtDNA.

Haldi, M.L., Anziano, P.Q., Perlman, P.S., Dept. of Genetics, Ohio State University,Columbus: New insights to *cis*-acting domains in yeast mitochondrial introns.

Martin, N.C., Miller, D.L., Najarian, R., Underbrik-Lyon, K., Dept. of Biochemistry, University of Texas Health Science Center, Dallas: Yeast mitochondrial tRNA gene transcripts and tRNA biosynthesis.

SESSION 9 *Mechanisms of Cleavage and Ligation*

Chairperson: T. Cech, University of Colorado, Boulder, Colorado

Filipowicz, W.,[1,2] Shatkin, A.J.,[1] Konarska, M.,[2] Tyc, K.,[2,3] Kikuchi, Y.,[3] Gross, H.J.,[3] [1]Roche Institute of Molecular Biology, Nutley, New Jersey; [2]Institute of Biochemistry and Biophysics, Polish Academy of of Science, Warsaw; [3]Institute für Biochemie, University of Würzburg, Federal Republic of Germany: Distinct ligation pathways for tRNA splicing in plant and animal cell extracts.

Furneaux, H.M., Pick, L., Arenas, J., Reinberg, D., Hurwitz, J., Dept. of Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, New York: Isolation and characterization of an RNA ligase from wheat germ.

Pyle, V.S., Gumport, R.I., Dept. of Biochemistry, University of Illinois, Urbana: T4 RNA ligase forms cyclic-phosphate-terminated oligoribonucleotides.

David, M., Borasio, G.D., Teichman, A., Vekstein, R., Kaufmann, G., Dept. of Biochemistry, Weizmann Institute, Rehovot, Israel: Processing of mature host tRNA by T4 anticodon nuclease, polynucleotide kinase, and RNA ligase.

Greer, C.,[1] Javor, B.,[2] Abelson, J.,[1] [1]Division of Biology, California Institute of Technology, Pasadena; [2]Scripps Institute of Oceanography, La Jolla, California: Identification of an RNA ligase activity in bacterial extracts and formation of a novel 2′, 5′ linkage.

Engberg, J., Nielsen, H., Panum Institute, University of Copenhagen, Denmark: Model for secondary studies involved in the excision of the intervening sequence of the *Tetrahymena* tRNA precursor.

Bass, B., Cech, T.R., Dept. of Chemistry, University of Colorado, Boulder: Ribozymic catalysis.

Mount, S.M., Pettersson, I., Hinterberger, M.,Karmas, A., Steitz, J.A., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Isolated U1 snRNA-protein complexes can recognize a 5′ splice site in vitro.

Watson, N., Gurevitz, M., Ford, J., Apirion, D., Dept. of Microbiology and Immunology, Washington University School of Medicine, St. Louis, Missouri: Possible self-cleavage of a precursor RNA of a tRNA-like molecule from bacteriophage T4.

SESSION 10 *Functions of RNP Particles*

Chairperson: S. Berget, Rice University, Houston, Texas

Albrecht, G., Brawerman, G., Tufts University School of Medicine, Boston, Massachusetts: Specific RNA-protein interactions in rabbit β-globin mRNP particles – Relation to mRNA function.

Schmid, H.-P., Köhler, K., Setyono, B., Institute of Biology, University of Stuttgart, Federal Republic of Germany: Evidence that the interaction of a scRNP with globin-free mRNPs inhibits the messenger translation.

Sarkar, S., Mukherjee, A.K., Roy, R.K., Chandrika, S.R., Dasgupta, S., Dept. of Muscle Research, Boston Biomedical Research Institute, Massachusetts: RNP particles of chick embryonic muscle – Isolation, characterization, and possible role in regulation of myogenesis.

Walter, P., Blobel, G., Dept. of Cell Biology, Rockefeller University, New York, New York: Disassembly and reconstitution of signal recognition particle.

Wolin, S.L., Steitz, J.A., Dept. of Molecular Biophysics, Yale University, New Haven, Connecticut: The Ro small cytoplasmic RNAs–Gene structure and clues to snRNP function.

Gottesfeld, J.M., Hoch, S.O., Division of Cellular Biology, Research Institute of Scripps Clinic, La Jolla, California: Association of an RNA polymerase III transcription factor with the La RNP complex.

Stefano, J., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Purified Lupus antigen La recognizes uridylate resides at the 3′ terminus of RNA polymerase III transcripts.

Mathews, M.B.,[1] Bernstein, R.M.,[2] [1]Cold Spring Harbor Laboratory, New York; [2]Royal Postgraduate Medical School, London, England: Characterization of cytoplasmic RNP complexes recognized by myositis autoantibodies.

RNA TUMOR VIRUSES

May 25–May 29

SESSION 1 *Oncogene Proteins*

Chairperson: J. Brugge, State University of New York, Stony Brook, New York

Brugge, J., Darrow, D., Dept. of Microbiology, State University of New York, Stony Brook: Isolation of protease-resistant fragments from pp60^{src} and pp90^{src} that function as tyrosine-specific protein kinases.

Ralston, R.,[1] Bishop, J.M.,[1] Deuel, T.F.,[2] [1]Dept. of Microbiology, University of California, San Francisco; [2]Dept. of Medical Oncology, Washington University School of Medicine, St. Louis, Missouri: Novel phosphorylation of pp60$^{c\text{-}src}$ and enhancement of its kinase activity in response to PDGF.

Maness, P., Levy, B., Sorge, L., Perry, M., Dept. of Biochemistry, University of North Carolina, Chapel Hill: Differential sensitivity of viral and cellular pp60^{src} to inhibition by Ap4A.

Greenberg, M.E., Edelman, G.M., Rockefeller University, New York, New York: Characterization of the 34-kD pp60^{src} substrate using monoclonal antibodies.

Tamura, T.,[1] Bauer, H.,[1] Pipkorn, R.,[2] Birr, C.,[2] Friis, R.R.,[1] [1]Institut für Virologie, Universität Giessen; [2]Max-Planck-Institut für Medizinische Forschung, Heidelberg, Federal Republic of Germany: Structure and function of pp60^{src} as investigated by means of antibodies prepared against synthetic oligopeptides.

Yamamoto, T., Nishida, T., Miyajima, N., Kawai, S., Toyoshima, K., Institute of Medical Science, University of Tokyo, Japan: Amino acid sequence of a domain of the *erbB* protein is homologous to pp60^{src}.

Prywes, R.,[1] Foulkes, J.G.,[1] Rosenberg, N.,[2] Baltimore, D.,[1] [1]Whitehead Institute for Biomedical Research and Massachusetts Institute of Technology, Cambridge; [2]Tufts University School of Medicine, Boston, Massachusetts: Sequences of Ab-MLV required for transformation.

Wang, J.Y.J.,[1] Baltimore, D.,[2] [1]Whitehead Institute for Biomedical Research; [2]Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Structural and functional domains of the transforming gene of Ab-MLV.

Watanabe, S.M.,[1] Robertson, D.L.,[1] Rosenberg, N.,[2] Whitlock,C.,[1] Ponticelli, F.,[1] Witte, O.N.,[1] [1]Molecular Biology Institute, University of California, Los Angeles; [2]Cancer Research Center, Tufts University School of Medicine, Boston, Massachusetts: Different subcellular localization regulates *v-abl* protein phosphorylation and functional activity.

Reynolds, F.H., Jr., Stephenson, J.R., NCI, Federick Cancer Research Facility, Frederick, Maryland: Tyrosine phosphorylation of a cellular serine-specific protein kinase by virus (*v-fes* and *v-abl*)-encoded protein kinases.

Curran, T., Miller, A.D., Verma, I.M., Molecular Biology and Virology Laboratory, Salk Institute, San Diego, California: The FBJ-MSV oncogene product (p55) is located in the nucleus.

Alitalo, K.,[1] Colby, W.,[2] McGrath, J.,[2] Levinson, A.,[2] Bishop, J.M.,[1] [1]Dept. of Microbiology, University of California, San Francisco; [2]Genetech, Inc., South San Francisco, California: Search for *c-myc* protein product with antibodies against a *v-myc*-encoded polypeptide expressed in bacteria.

Hann, S., Eisenman, R., Fred Hutchinson Cancer Research Center, Seattle, Washington: Identification of *v-myc* and *c-myc* proteins by means of an antiserum against a carboxy-terminal peptide of *v-myc*.

SESSION 2 *Viral Genetics*

Chairperson: J.T. Parsons, University of Virginia, Charlottesville, Virginia

Duyk, G.,[1] Leis, J.,[1] Longiaru, M.,[2] Skalka, A.,[2] [1]Case Western Reserve University School of Medicine, Cleveland, Ohio; [2]Roche Institute of Molecular Biology, Nutley, New Jersey: Selective cleavage of the RAV-2 LTR sequence by the endonuclease associated with the $\alpha\beta$ form of reverse transcriptase.

Hippenmeyer, P.J., Grandgenett, D.P., Institute for Molecular Virology, St. Louis, Missouri: In vitro mutagenesis of the RSV pp32 region.

Ju, G., Cullen, B., Dept. of Molecular Genetics, Hoffmann-La Roche, Inc., Nutley, New Jersey: Site-directed mutagenesis of the avian retrovirus LTR.

Kawai, S., Nishizawa, M., Koyama, T., Yamamoto, T., Institute for Medical Science, University of Tokyo, Japan: Genome structure of a mutant of RSV that is defective in packaging of its own genome.

Mann, R., Baltimore, D., Whitehead Institute for Biomedical Research; Massachusetts Institute of Technology, Cambridge: A packaging deletion mutant of Mo-MLV reverts to wild type after transfected into NIH-3T3 cells.

Schwartzberg, P., Colicelli, J., Goff, S.P., Dept. of Biochemistry, Columbia University, College of Physicians and Surgeons, New York, New York: Biochemical and genetic analysis of mutants of Mo-MLV constructed by in vitro mutagenesis.

Hunter, E., Wills, J., Hardwick, M., Shaw, K., Dept. of Microbiology, University of Alabama, Birmingham: Site-directed mutations in the *env* gene of RSV.

Buetti, E., Diggelmann, H., Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland: Identification of a short segment of the MMTV LTR involved in the hormone stimulation of transcription.

Richter, A.,[1] Ozer, H.L.,[2] DesGroseillers, L.,[1] Jolicoeur, P.,[1] [1]Institut de Recherches Cliniques de Montréal, Université de Montréal, Canada; [2]Dept. of Biological Sciences, Hunter College, City University of New York, New York: An X-linked gene affecting mouse-cell DNA synthesis also affects production of competent unintegrated linear and supercoiled DNAs of MLV.

DesGroseillers, L., Jolicoeur, P., Institut de Recherches Cliniques de Montréal, Université de Montréal, Canada: Physical mapping and sequencing of the *Fv-1* tropism host-range determinant of BALB/c MLV.

Boone, L.R.,[1,2] Ou, C.-Y.,[2] Myer, F.E.,[2] Yang, D.-M.,[2] Tennant, R.W.,[1] Yang, W.K.,[2] [1]National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina; [2]Biology Division, Oak Ridge National Laboratory, Tennessee: Analysis of the *Fv-1* gene target in N-tropic and B-tropic MLVs by restriction fragment exchange and nucleotide sequence analysis.

Sorge, J.,[1] Hughes, S.H.,[2] [1]Research Institute of Scripps Clinic, La Jolla, California; [2]Cold Spring Harbor Laboratory, New York: Suppression of retroviral RNA splicing.

SESSION 3 *Poster Session*

Canaani, E.,[1] Gale, R.P.,[2] [1]Dept. of Chemical Immunology, Cell Biology, and Medicine, Weizmann Institute of Science, Rehovot, Israel; [2]University of California School of Medicine, Los Angeles: Oncogenes in human leukemia – Abnormal expression of *c-abl* in chronic myelogenous leukemia (CML).

Collins, S.,[1] Groudine, M.,[2] [1]Veterans Administration Hospital, Seattle; [2]Fred Hutchinson Cancer Research Center, Seattle, Washington: Rearrangement and amplification of endogenous *c-abl* and λ immunoglobulin sequences in the chronic myelogenous leukemia cell line K562.

Dale, B., Born, W., Mason, I., Ozanne, B., University of Texas Health Science Center, Dallas: Comparison of methylated status of the *c-abl* locus with transcriptional activity of *c-abl* in mouse tissues.

Leibowitz, D., Miller, C., Donovan-Peluso, M., Cubbon, R., Bank, A., Depts. of Medicine, Human Genetics, and Development, Columbia University, New York, New York: Organization of *c-abl* in K562 cells.

Groffen, J., Heisterkamp, N., Stephenson, J.R., NCI, Frederick Cancer Research Facility, Frederick, Maryland: The human *v-abl* cellular homolog.

Youngren, S.D., de Noronha, F., New York State College of Veterinary Medicine, Cornell University, Ithaca: A transmissible FeSV containing sequences related to the *ras*^{K} "onc" gene.

Ellis, R.W.,[1] Lowe, R.,[1] Scolnick, E.M.,[1] Brinster, R.L.,[2] Palmiter, R.D.,[3] [1]Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania; [2]School of Veterinary Medicine, University of Pennsylvania, Philadelphia; [3]Howard Hughes Medical Institute Laboratory, University of Washington, Seattle: Cloning and expression of p21 *ras*^{H} genes adjacent to the metallothionein promoter.

Swan, D., Gol, R., Tronick, S., McBride, W., Aaronson, S., NCI, National Institutes of Health, Bethesda, Maryland: Chromosomal localization of the human homologs of the *ras* gene family.

Rasheed, S.,[1] Norman, G.,[1] Heidecker, G.,[2] [1]University of Southern California, School of Medicine, Los Angeles; [2]University of California, School of Medicine, Davis: Two new mutations in the rat sarcoma virus oncogene are similar to those present in the human bladder carcinoma gene.

Teumer, J.,[1] Wigler, M.,[2] Friedman, E.,[1] Lipkin, M.,[1] Winawer, S.,[1] Stavnezer, E.,[1] [1]Memorial Sloan-Kettering Cancer Center, New York; [2]Cold Spring Harbor Laboratory, New York: Comparison of *c-ras*^{K} mRNA levels in normal, premalignant, and malignant colonic epithelia.

Cooper, C.S.,[1] Blair, D.G.,[2] Oskarsson, M.K.,[1] Vande Woude, G.F.,[1] [1]NCI, National Institutes of Health, Bethesda; [2]NCI, Frederick Cancer Research Facility, Frederick, Maryland: Transforming activity of DNA from chemically transformed human cells and cell lines derived from human tumors.

Lautenberger, J.A., Court, D., Papas, T.S., NCI, National Institutes of Health, Bethesda, Maryland: High-level expression in *E. coli* of the carboxyterminal sequences of the avian myelocytomatosis virus (MC29) *v-myc* protein.

Soe, L.H.,[1] Devi, B.G.,[1] Roy-Burman, P.,[1,2] [1]Dept. of Pathology, [2]Dept. of Biochemistry, University of Southern California, School of Medicine, Los Angeles: Allelic variation in the feline *c-myc* gene.

Dalla-Favera, R.,[1] Gelmann, E.P.,[2] [1]Dept. of Pathology, New York University School of Medicine, New York; [2]NCI, National Institutes of Health, Bethesda, Maryland: Cloning and molecular analysis of the t(8:14) chromosomal translocation involving the *c-myc* onc gene in human Burkitt lymphoma.

Keath, E.J., Piccoli, S.P., Cole, M.D., Dept. of Biochemistry, St. Louis University School of Medicine, Missouri: Alteration of the *c-myc* oncogene by chromosomal translocation in mouse plasmacytomas.

Krump-Konvalinkova, V., Vaessen, M.J., Radiobiological Institute TNO, Rijswijk, The Netherlands: Oncogenic capacity of normal vertebrate DNAs.

Milly, M.I.,[1] Haas, M.,[1,2] [1]Cancer Center, [2]Dept. of Biology, University of California, San Diego: Oncogene expression in factor-dependent T-lymphoblastoma cells and autonomous T-lymphoma cells.

Levy, L.S.,[1] Gardner, M.,[2] Casey, J.,[1] [1]Dept. of Biochemistry, Louisiana State University Medical Center, New Orleans; [2]Dept. of Pathology, University of California, Davis: Structural alterations in the feline *c-myc* locus in naturally occurring tumors.

Narayanan, R., Srinivasan, A., Dunn, C.Y., Reddy, E.P., Aaronson, S.A., NCI, National Institutes of Health, Bethesda, Maryland: Activation of the *mos* gene by 3' LTR–Analysis of *cis*-acting sequences.

Kloetzer, W., Maxwell, S., Gallick, G., Stanker, L., Arlinghaus, R., Dept. of Tumor Virology, University of Texas System Cancer Center, M.D. Anderson Hospital, Houston: P85$^{gag\text{-}mos}$ encoded by *ts*110 Mo-MSV has an associated protein kinase activity.

Somers, K.D., Murphey, M.M., Martin, D., Dept. of Microbiology and Immunology, Eastern Virginia Medical School, Norfolk: Detection of candidate *v-mos* gene products in a morphological revertant of Mo-MSV-transformed rat cells.

Gallick, G.E.,[1] Sparrow, J.,[2] Stanker, L.H.,[1] Arlinghaus, R.B.,[1] [1]University of Texas Systems Cancer Center, M.D. Anderson Hospital, Houston; [2]Dept.of Medicine, Baylor College of Medicine, Houston, Texas: Recognition of a normal cellular protein structurally related to the transforming protein of MSV 124 with antisera to synthetic peptides of the *v-mos*^{mo} gene product.

Kan, N., Papas, T., NCI, National Institutes of Health, Bethesda, Maryland: Molecular cloning of MH2 provirus.

Lipsich, L.A., Lewis, A., Brugge, J.S., Dept. of Microbiology, State University of New York, Stony Brook: Isolation of monoclonal antibodies that recognize the transforming proteins of ASVs.

Tanaka, T., Löwer, J., Kurth, R., Paul-Ehrlich-Institute, Frankfurt, Federal Republic of Germany: Monoclonal antibodies specific for RSV-coded structural and transforming proteins.

Parsons, S.J., McCarley, D.J., Ely, C.M., Benjamin, D.C., Parsons, J.T., University of Virginia, Charlottesville: Monoclonal antibodies to the *src* protein of RSV.

Ingman-Baker, J.,[1] Buchan, A.,[2] Ginter, G.,[1] Pawson, T.,[1] [1]Dept. of Microbiology; [2]Dept. of Physiology, University of British Columbia, Vancouver, Canada: Isolation of monoclonal antibodies to the FuSV-transforming protein.

Wang, L.-H., Edelstein, B., Rockefeller University, New York, New York: Molecularly cloned *src* deletion mutants of RSV are able to induce tumors and generate recovered sarcoma virus in chickens.

Weinmaster, G., Stone, J., Hinze, E., Pawson, T., Dept. of Microbiology, University of British Columbia, Vancouver, Canada: Analysis of structural and functional regions of the FuSV-transforming protein.

Neckameyer, W.S., Wang, L.-H., Rockefeller University, New York, New York: Molecular cloning and characterization of UR-2 ASV DNA and analysis of the cellular homolog of the UR-2 transforming gene.

Naharro, G.,[1] Tronick, S.R.,[1] Rasheed, S.,[2] Gardner, M.B.,[3] Aaronson, S.A.,[1] Robbins, K.C.,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Pathology, University of Southern California, School of Medicine, Los Angeles; [3]Dept.of Pathology, University of California, Davis: The *onc* sequence of Gardner-Rasheed FeSV is distinct from the transforming genes of other retroviruses with associated tyrosine kinase activity.

Huang, C.-C., Hammond, C., Bishop, J.M., Dept. of Microbiology and Immunology, University of California, San Francisco: Nucleotide sequence of the oncogene of PRCII ASV and its cellular homolog.

Parker, R.C., Mardon, G., Varmus, H.E., Bishop, J.M., Dept. of Microbiology and Immunology, University of California, San Francisco: Human cellular *src*–Isolation and characterization of two distinct loci.

Gibbs, C.,[1] Anderson, S.K.,[2] Tanaka, A.,[2] Ridgway, A.,[2] Radul, J.,[2] Kung, H.-J.,[1] Fujita, J.,[2] [1]Dept. of Biochemistry, Michigan State University, East Lansing; [2]Cancer Research Laboratory, University of Western Ontario, London, Canada: Human *c-src*–Structural analysis of cloned DNA reveals extensive homology with avian *v-src*.

Hill, K., Robertson, D.L., Dept. of Chemistry, Brigham Young University, Provo, Utah: Biochemistry of pp60^{src}-cAMP–dependent phosphorylation.

Jullien, M.,[1] Harel, L.,[1] Golde, A.,[2] Villaudy, J.,[2] Pugnet, P.,[1] [1]Institut de Recherches Scientifiques sur le Cancer, Villejuif; [2]Institut Curie, Paris, France: Inhibition by quercetin of the early effect of *src* gene expression on ATP turnover.

Schofield, M.A., Mardon, G., Varmus, H.E., Dept. of Microbiology and Immunology, University of California, San Francisco: Molecular and biological characterization of frame-shift mutants and back mutants of *src* arising in a single Rous sarcoma provirus.

Balachandran, R., Swan, D.C., Aaronson, S.A., Reddy, E.P., NCI, National Institutes of Health, Bethesda, Maryland: Ra-MLV-induced stage-specific transformation of B cells and its association with changes in the *c-myc* locus.

Barker, P.E.,[1] Verma, I.,[2] Ruddle, F.H.,[1] [1]Dept. of Biology, Yale University, New Haven, Connecticut; [2]Tumor Virology Laboratory, Salk Institute, La Jolla, California: Chromosomal mapping of the human *c-fos* oncogene.

MacConnell, W.P., Verma, I.M., Molecular Biology and Virology Laboratory, Salk Institute, San Diego, California: Expression of FBJ-MSV *fos* gene product in bacteria.

Moelling, K., Bunte, T., Donner, P., Greiser-Wilke, I., Owada, M.K., Max-Planck-Institut für Molekulare Genetik, Berlin-Dahlem, Federal Republic of Germany: Analysis of different transforming polyproteins of acute avian leukemia viruses.

Josephs, S.F., Wong-Staal, F., NCI, National Institutes of Health, Bethesda, Maryland: 3' viral and human cellular sequences corresponding to the transforming gene of SSV.

Thiel, H.-J., Hafenrichter, R., Greger, B., Federal Research Institute for Animal Virus Diseases, Tübingen, Federal Republic of Germany: Characterization of a transformation-specific glycopeptide in SSV-NP cells.

Robbins, K.C., Devare, S.G., Aaronson, S.A., NCI, National Institutes of Health, Bethesda, Maryland: Posttranslational modifications of the SSV transforming gene product.

Jansen, H.W., Patschinsky, T., Bister, K., Max-Planck-Institut für Molekulare Genetik, Berlin, Federal Republic of Germany: Genetic structure of avian leukemia and carcinoma virus MH2 and its relationship to other *myc*-containing viruses.

Schultz, A.M., Oroszlan, S., NCI, Federick Cancer Research Facility, Frederick, Maryland: Retroviral transforming proteins and *gag* polyproteins of type-B, -C, and -D viruses are myristylated.

Teich, N.M., Rowe, J., Harrison, M.A., Imperial Cancer Research Fund, London, England: Studies of BSB, a murine erythroleukemia virus complex distinct from Friend virus.

Wright, S.,[1,3] Harmon, S.,[2,3] Smith, D.,[3] Hayes, J.,[3] Robertson, P.,[3] Wayne, A.,[3] [1]Dept. of Medicine and Cellular, Viral and Molecular Biology, University of Utah School of Medicine; [2]Dept. of Biology, University of Utah; [3]Veterans Administration Medical Center, Salt Lake

City, Utah: Candidate products of the AMV oncogene region produced by in vitro translation of genomic RNA.

Bonner, T.I.,[1] Mark, G.E.,[1] Goldsborough, M.D.,[1] O'Brien, S.J.,[1] Nash, W.G.,[1] Minna, J.D.,[2] Rapp, U.R.,[1] [1]Laboratory of Viral Carcinogenesis, NCI, Federick, Maryland; [2]NCI-Navy Medical Oncology Branch, Bethesda, Maryland: The human cellular homologs of a new oncogene, *v-raf.*

Roussel, M.F.,[1] Baker, P.F.,[2] Fedele, L.A.,[1] Ruddle, F.H.,[2] Sherr, C.J.,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Biology, Yale University, New Haven, Connecticut: Molecular characterization and chromosomal localization of the human proto-oncogene *c-fms.*

Hampe, A.,[1] Gobet, M.,[1] Galibert, F.,[1] Sherr, C.J.,[2] [1]Laboratoire d'Hematologie Experimentale, Hôpital Saint-Louis, Paris, France; [2]NCI, National Institutes of Health, Bethesda, Maryland: Nucleotide sequence of the polyprotein-coding region of McDonough FeSV and its oncogene *v-fms.*

Heisterkamp, N., Groffen, J., Stephenson, J.R., NCI, Frederick Cancer Research Facility, Frederick, Maryland: Molecular cloning and chromosomal localization of the human *c-fms* oncogene.

Kollek, R.,[1] Stocking, C.,[1] Arbuthnott, C.,[2] Ostertag, W.,[1] [1]Heinrich-Pette-Institut für Experimentelle Virologie und Immunologie an der Universität Hamburg, Federal Republic of Germany; [2]Beatson Institute for Cancer Research, Glasgow, Scotland: Molecular and biological analysis of the MPSV and its temperature-sensitive mutants.

Leprince, D., Gegonne, A., Coll, J., Schneeberger, A., Lagrou, C., Stehelin, D., INSERM, Institut Pasteur, Lille, France: The AMV E26 contains a new specific nucleotide sequence (*v-ets)* in addition to the *v-myb* oncogene.

Brennan, L., Li, Y., Magarian, C., Brodeur, D., Barkas, A., Stavnezer, E., Dept. of Molecular Biology and Virology, Sloan-Kettering Institute, New York, New York: Novel features of *v-ski* and *c-ski* revealed by molecular cloning and DNA sequencing.

Brodeur, D., Barkas, A., Brennan, L., Teumer, J., Myers, D., Nguyen, H., Stavnezer, E., Dept. of Molecular Biology and Virology, Sloan-Kettering Institute, New York, New York: Transformation and tumor induction by biologically and molecularly cloned SK viruses.

Lederman, L., Singhal, M.C., Zuckerman, E.E., Hardy, W.D., Jr., Besmer, P., Signorelli, K.L., Snyder, H.W., Jr., Memorial Sloan-Kettering Cancer Center, New York, New York: Subcellular localization of the transforming proteins of FeSV.

Snyder, H.W., Jr., Singhal, M.C., Zuckerman, E.E., Jones, F.R., Hardy, W.D., Jr., Memorial Sloan-Kettering Cancer Center, New York, New York: Characterization of FOCMA from feline lymphosarcoma cells.

SESSION 4 *Poster Session*

Balduzzi, P., Christensen, J.R., Dept. of Microbiology, University of Rochester School of Medicine and Dentistry, New York: Initial characterization of a temperature-sensitive mutation in the transforming gene of ASV UR-2.

Bryant, D., Wilkerson, V., Coffman, A., Parsons, J.T., Dept. of Microbiology, University of Virginia Medical School, Charlottesville: Site-directed mutagenesis in the *src* gene of RSV.

Cross, F.R., Pellman, D., Hanafusa, H., Rockefeller University, New York, New York: Mapping regions in $p60^{src}$ of RSV that are required for transformation.

Foster, D.A., Hanafusa, H., Rockefeller University, New York, New York: A *gag*-deficient mutant of FuSV transforms fibroblasts in culture and induces tumors in chickens.

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Joyner, A., Bernstein, A., Ontario Cancer Institute, Toronto, Canada: Generation of infectious retroviruses expressing dominant and selectable genes is associated with in vivo recombination and deletion events.

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Norton, P., Coffin, J.M., Tufts University School of Medicine, Boston, Massachusetts: Expression and propagation of the bacterial gene for β-galactosidase in avian cells by a retrovirus.

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Muller, R.,[1] Tremblay, J.M.,[1] Adamson, E.D.,[2] Verma, I.M.,[1] [1]Molecular Biology and Virology Laboratory, Salk Institute, San Diego; [2]Cancer Research Center, La Jolla Cancer Research Foundation, California: Expression of cellular oncogenes during mouse development.

Pederson, N.C.,[1] Johnson, L.,[1] Theilen, G.H.,[2] [1]Dept. of Medicine, [2]Dept. of Surgery, School of Veterinary Medicine, University of California, Davis: Latent FeLV infection.

SESSION 5 *Mechanisms of Leukemogenesis*

Chairperson: W. Hayward, Memorial Sloan-Kettering Cancer Institute, New York, New York

van Ooyen, A.J.J.[1] Nusse, R.,[1] Varmus, H.E.,[2] [1]Dept. of Virology, The Netherlands Cancer Institute, Amsterdam; [2]Dept. of Microbiology, University of California, San Francisco: Integration of MMTV in a specific chromosomal region and activation of a cellular gene.

Peters, G., Dickson, C., Imperial Cancer Research Fund Laboratories, London, England: *int-2*—A second common integration region

for MMTV proviruses in mouse mammary tumors.

Raines, M.,[1] Lewis, W.,[1] Crittenden, L.B.,[2] Kung, H.J.,[1] [1]Dept. of Biochemistry, Michigan State University; [2]USDA Poultry Research Laboratory, East Lansing, Michigan: LTR insertional activation of *c-erb-B*—The molecular basis of ALV-induced erythroblastosis.

Miles, B., Robinson, H., Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: High-frequency occurrence of transduced *erb* sequences in ALV-induced erythroblastosis.

Swift, R.A., Schaller, E., Kung, H.-J., Dept. of Biochemistry, Michigan State University, East Lansing: Insertion-activation of *c-myc* by REV—Cloning and structure analysis of an activated *c-myc* gene.

Lemay, G., Jolicoeur, P., Institut de Recherches Cliniques de Montréal, Université de Montreal, Canada: Specific rearrangement of a unique cellular sequence in Mo-MLV-induced rat thymoma.

Tsichlis, P.N., Strauss, P.G., Hu, L.F., NCI, National Institutes of Health, Bethesda, Maryland: Mo-MLV integration in rat thymic lymphomas.

Steffen, D., Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Proviruses are integrated adjacent to *c-myc* in some MLV-induced lymphomas.

Herman, S.A., Coffin, J.M., Dept. of Microbiology and Molecular Biology, Tufts University School of Medicine, Boston, Massachusetts: Downstream transcription in viruses of differing oncogenic potential.

Cullen, B., Lomedico, P.T., Ju, G., Dept. of Molecular Genetics, Hoffmann-La Roche, Inc., Nutley, New Jersey: Interference between LTR promoters in the avian retrovirus transcriptional unit.

Lamph, W., Dudley, J., Arfsten, A., Green, P., Risser, R., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Phenotypic changes and DNA rearrangement in clonal Abelson lymphoma cells.

Casey, J.W.,[1] Levy, L.S.,[1] Derse, D.D.,[1] Caradonna, S.J.,[2] Deininger, P.L.,[1] [1]Dept. of Biochemistry, [2]Dept. of Pharmacology, Louisiana State University Medical Center, New Orleans: The nucleotide sequence of the BLV LTR and host-virus junctions suggests a dependence on host DNA for transcriptional activity.

Frykberg, L.,[1] Graf, T.,[2] Vennström, B.,[1] [1]Dept. of Medical Genetics, Uppsala University, Sweden; [2]German Cancer Research Center, Heidelberg, Federal Republic of Germany: Transformation capacities of avian retroviruses containing chicken *c-myc* sequences.

SESSION 6 *Human Retroviruses*

Chairperson: M. Martin, National Institutes of Health, Bethesda, Maryland

Seiki, M., Hattori, S., Hirayama, Y., Yoshida, M., Dept. of Viral Oncology, Cancer Institute, Kam-Ikebukuro, Tokyo, Japan: Human retrovirus (ATLV)—Complete nucleotide sequence of the provirus genome integrated in leukemic cell DNA.

Hahn, B., Wong-Staal, F., Manzari, V., Franchini, G., Gelmann, E.P., Gallo, R.C., NCI, National Institutes of Health, Bethesda, Maryland: A survey of human leukemias and lymphomas using cloned HTLV sequences and adjacent cellular sequences.

Yoshida, M., Watanabe, T., Seiki, M., Dept. of Viral Oncology, Cancer Institute, Tokyo, Japan: New proviral genomes with small human retroviral (ATLV) and integration sites in leukemic and nonleukemic cell DNAs.

Franchini, G.,[1] Wong-Staal, F.,[1] Manzari, V.,[1] Hahn, B.,[1] Croce, C.,[2] Gallo, R.C.,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Wistar Institute, Philadelphia, Pennsylvania: Characterization and chromosomal localization of a specific integration site of HTLV.

Merl, S.,[1] Kloster, B.,[1] Moore, J.,[1,3] Hubbell, C.,[1] Tomar, R.,[1] Davey, F.,[1] Gordon, L.,[1] Comis, R.,[1,3] Poiesz, B.,[1,2,3] [1]Upstate Medical Center, State University of New York, Syracuse; [2]Syracuse Veterans Administration Medical Center; [3]Barbara Rapp Research Center, Auburn, New York: A sensitive biologic assay for HTLV—Transformation of previously activated T lymphocytes.

Souyri, M., Fleissner, E., Memorial Sloan-Kettering Cancer Center, New York, New York: Analysis of transforming sequences in DNA of human T-cell leukemias.

Steele, P.E., Rabson, A.B., Bryan, T., Repaske, R., O'Neill, R.R., Martin, M.A., NIAID, National Institutes of Health, Bethesda, Maryland: Human DNA contains at least two families of endogenous retroviral sequences.

Rabson, A.B., Steele, P.E., Bryan, T., Martin, M.A., NIAID, National Institutes of Health, Bethesda, Maryland: Organization and expression of full-length human endogenous retroviral DNA.

Cohen, M., Bonner, T., O'Connell, C., NCI, Frederick Cancer Research Facility, Frederick, Maryland: A full-length endogenous human retroviral genome.

Gelmann, E., Popovic, M., Robert-Guroff, M., Blayney, D., Gallo, R.C., NCI, National Institutes of Health, Bethesda, Maryland: HTLV proviral sequences in peripheral lymphocytes of two patients with AIDS.

Mullins, J.I.,[1] McLane, M.F.,[1] Essex, M.,[1] Schooley, R.,[2] Gold, J.,[3] [1]Dept. of Cancer Biology, Harvard School of Public Health, Boston; [2]Dept. of Infectious Diseases, Massachusetts General Hospital, Boston; [3]Special Microbiology Laboratory, Memorial Sloan-Kettering Cancer Center, New York, New York: HTLV sequences in AIDS.

SESSION 7 *Poster Session*

Wolfe, J.H.,[1] Blankenhorn, E.P.,[2] Blank, K.J.,[1] [1]Dept. of Pathology, [2]Dept. of Microbiology, University of Pennsylvania School of Medicine, Philadelphia: *gag* precursor polyprotein alteration in Gross-virus-induced cell lines derived from H-2 congenic mice.

Etzerodt, M., Andersen, H.D., Jørgensen, P., Kjeldgaard, N.O., Pedersen, F.S., Dept. of Molecular Biology, University of Aarhus, Den-

mark: The nucleotide sequence of Akv MLV.

Elder, J.,[1] Mullins, J.,[2] [1]Research Institute of Scripps Clinic, La Jolla, California; [2]Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts: Nucleotide sequence of the envelope gene of GA-FeLV-B reveals unique sequence homologies with a murine MCF virus.

Buchhagen, D.L., Downstate Medical Center, State University of New York, Brooklyn, New York: Integration of recombinant and ecotropic proviruses in preleukemic and leukemic AKR/J thymus DNAs.

Famulari, N.G.,[1] Cieplensky, D.,[1] Koehne, C.,[1] Lenz, J.,[2] Haseltine, W.,[2] O'Donnell, P.V.,[1] [1]Memorial Sloan-Kettering Cancer Center, New York, New York; [2]Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Viral gene expression in thymocytes and primary leukemias of mice inoculated with leukemogenic ecotropic virus.

O'Donnell, P.V., Famulari, N.G., Chu, A., Memorial Sloan-Kettering Cancer Center, New York, New York: Stages in development of virus-accelerated leukemia in AKR mice.

Melief, C.J.M., Zijlstra, M., de Goede, R.E.Y., Schoenmakers, H.J., Central Laboratory of the Netherlands, Red Cross Blood Transfusion Services, and University of Amsterdam, The Netherlands: Influence of the H-2 complex on the phenotype of lymphomas induced by different host-range classes of MLV.

Bach, R.G., Meruelo, D., Irvington House Institute, New York University Medical Center, New York: Identification of a 36,000-molecular-weight *gag*-related phosphoprotein in lymphoma cells transformed by RadLV.

Bach, R.G., Meruelo, D., Irvington House Institute, New York University Medical Center, New York: Heterogeneity of p15(E)-related polypeptides expressed by MLV-infected cells—Phenotypic marker for oncogenic RadLV?

Pinter, A., Honnen, W.J., Memorial Sloan-Kettering Cancer Center, New York, New York: Comparison of structural and antigenic features of specific domains of ecotropic and MCF MLV gp70 molecules.

Trauger, R.J., Luftig, R.B., Dept. of Microbiology and Immunology, University of South Carolina School of Medicine, Columbia: Studies on Mo-MLV gp70 and a presumptive 45K limit-digest breakdown product (gp45).

Pepinsky, R.B., Vogt, V.M., Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca: Localization of lipid-protein and protein-protein interactions within the murine retrovirus *gag* precursor by a novel peptide-mapping technique.

Oroszlan, S.,[1] Copeland, T.D.,[1] Gerard, G.,[2] [1]NCI, Frederick Cancer Research Facility, Frederick; [2]Bethesda Research Laboratory, Gaithersburg, Maryland: Primary structure studies of Mo-MLV reverse transcriptase.

Murphy, E.C., Jr., Nash, M.A., Brown, N.V., Dept. of Tumor Virology, University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston: Nuclease-S1 mapping of viral RNA from MSV *ts*110—A possible temperature-sensitive splicing mutant of MSV.

Miller, A.D., Verma, I.M., Molecular Biology and Virology Laboratories, Salk Institute, San Diego, California: A single-base-pair change between infectious and non-infectious Mo-MLV.

Karpel, R.L.,[1,2] Henderson, L.E.,[1] Oroszlan, S.,[1] [1]NCI, Frederick Cancer Research Facility, Frederick; [2]Dept. of Chemistry, University of Maryland Baltimore County, Catonsville: Interactions of the basic nucleic-acid-binding protein of type-C viruses with RNA.

Yoshinaka, Y., Shames, R., Luftig, R.B., University of South Carolina School of Medicine, Columbia: Partial purification and characterization of the MLV-associated protein kinase.

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Katoh, I., Yoshinaka, Y., Luftig, R.B., Dept. of Microbiology and Immunology, University of South Carolina School of Medicine, Columbia: MLV p30 heterogeneity as revealed by 2-D electrophoresis and column chromatofocusing methods.

Fan, H., Chute, H., Chao, E., Feuerman, M., Dept. of Molecular Biology and Biochemistry, University of California, Irvine: Mutants of Mo-MLV unable to synthesize glycosylated *gag* polyprotein.

Stoltzfus, C.M., Dane, R.W., Dept. of Microbiology, University of Iowa, Iowa City: Localization of adenosine methylations in RSV genomic RNA.

Staskus, K.,[1] Resnick, R.,[1] Omer, C.,[2] Retzel, E.,[1] Faras, A.,[1] [1]Dept. of Microbiology, University of Minnesota, Minneapolis; [2]Dept. of Genetics, Stanford University, California: RNase-H-mediated initiation of strong-stop (+)DNA synthesis.

Stacey, D.W.,[1] Mulcahy, L.,[1] Pugatsch, T.,[2] [1]Roche Institute of Molecular Biology, Nutley, New Jersey; [2]International Genetics Institute, Jerusalem, Israel: Correlation of a sequence required for virion packaging with a 28-bp self-complementing sequence in SR-B.

Smith, J.K., Hsu, T.W., Cywinski, A., Taylor, J.M., Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania: Synthesis of DNA intermediates by ASV.

Wang, L.-H., Rockefeller University, New York, New York: Deletion in *pol* sequences affects the RNA expression of certain ASVs.

Leis, J., Jentoft, J., Case Western Reserve University School of Medicine, Cleveland, Ohio: RNA-binding properties of the avian retrovirus pp12 protein—Regulation by phosphorylation.

Edbauer, C.A., Naso, R.B., University of Texas System Cancer Center, M.D. Anderson Hospital, Houston: Cytoskeleton-associated $Pr65^{gag}$ and retrovirus assembly.

Crawford, S., Goff, S.P., Dept. of Biochemistry, Columbia University, College of Physicians and Surgeons, New York, New York: Deletion mutants in the P12 *gag* gene of Mo-MLV generated by site-specific mutagenesis.

Lerner, T., Hanafusa, H., Rockefeller University, New York, New York: The nature of the *pol* and *env* defects in Bryan RSV.

Hughes, S.H., Kosik, E., Cold Spring Harbor Laboratory, New York: Mutagenesis of the region between *env* and *src* in the SR-A strain of RSV.

Katz, R.A.,[1] Malavarca, R.,[1] Cullen, B.,[2] Ju, G.,[2] Skalka, A.M.,[1] [1]Roche Institute of Molecular Biology; [2]Dept. of Molecular Genetics, Hoffmann-La Roche, Inc., Nutley, New Jersey: Localization of a defect in the mRNA leader region of the endogenous avian retrovirus *ev*-1.

Ficht, T.A., Chang, L.-J., Stoltzfus, C.M., Dept. of Microbiology, University of Iowa, Iowa City: Amino-terminal analysis of the primary

translation products of the *gag* and *env* genes of avian RNA tumor viruses.

Bizub, D., Katz, R.A., Skalka, A., Roche Institute of Molecular Biology, Nutley, New Jersey: Comparison of the noncoding sequences of avian retroviruses.

Caballero, A., Luftig, R.B., Dept. of Microbiology and Immunology, School of Medicine, University of South Carolina, Columbia: Comparative effects of divalent cations (Mn^{++}, Mg^{++}) and membrane-disruptive agents (melittin, Sterox SL) on the reverse transcriptase activities of murine and avian retroviruses.

Notani, G.W., Sauerbier, W., Dept. of Genetics and Cell Biology, University of Minnesota, St. Paul: Variations in the LTR of REVs.

Woodland, E.C., Horowit, T.S., Shank, P.R., Division of Biology and Medicine, Brown University, Providence, Rhode Island: Highly defective viral RNA and DNA molecules in cells infected with transformation-defective RSV.

Brooks, B.R., Priester, E., Dept. of Neurology and Medical Microbiology, University of Wisconsin Medical School, and William S. Middleton Veterans Hospital, Madison: Virus-specific protein synthesis in murine neurotropic retrovirus-infected astrocytes in vitro.

Mamoun, R.Z., Astier, T., Guillemain, B., INSERM, Bordeaux, France: BLV protein expression in cultured cells.

Kashmiri, S.V.S., Mehdi, R., Altland, B., Ferrer, J.F., University of Pennsylvania, Kennett Square: Molecular cloning of covalently closed, circular DNA of BLV.

Wernicke, D.,[1] Trainin, Z.,[1] Ungar-Waron, H.,[2] Essex, M.,[1] [1]Dept. of Cancer Biology, Harvard University School of Public Health, Boston, Massachusetts; [2]Dept. of Immunology, Kimron Veterinary Institute, Bet-Dagan, Israel: Suppression of the humoral antibody response associated with infection by FeLV.

Reeves, R.H., O'Brien, S.J., NCI, Frederick Cancer Research Facility, Frederick, Maryland: Molecular characterization of endogenous feline RD114 viral sequences.

Haapala, D.K.,[1] Denniston, K.J.,[2] Robey, W.G.,[1] [1]NCI, Frederick Cancer Research Facility, Frederick, Maryland; [2]Dept. of Microbiology, Georgetown University, Washington, DC: Isolation and characterization of an RD114-like virus with a novel coat.

Firzlaff, J., Diggelmann, H., Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland: Glucocorticoids stimulate the rate of MMTV transcription in isolated nuclei of transfected cells.

Ponta, H.,[1] Groner, B.,[1] Kennedy, N.,[1] Herrlich, P.,[1] van Ooyen, A.,[2] Scheidereit, C.,[3] Beato, M.,[3] Hynes, N.E.,[1] [1]Kernforschungszentrum Karlsruhe, Federal Republic of Germany; [2]Netherlands Cancer Institute, Amsterdam; [3]Phillips University, Institute of Physiological Chemistry, Marburg, Federal Republic of Germany: Transcriptional control signals contained in the LTR sequences of MMTV.

Zavada, J.,[1] Huang, A.S.,[1] Svec, J.,[2] [1]Harvard Medical School and Children's Hospital Medical Center, Boston, Massachusetts; [2]Institute of Experimental Oncology, Bratislava, Czechoslovakia: Phenotypic mixing of VSV with human tumor cell proteins.

Wilson, M., Gautsch, J., Research Institute of Scripps Clinic, La Jolla, California: Extent of methylation of proviral and other genes in differentiated and undifferentiated teratocarinoma cells.

Brémont, M., d'Auriol, L., Cavalieri, F., Emanoil-Ravicovitch, R., Périès, J., Dépt. d'Oncologie Expérimentale, Institut de Recherches sur les Maladies du Sang, Hôpital Saint-Louis, Paris, France: Methylation and restriction of ecotropic MLV expression in cloned embryonal carcinoma cells.

Bandyopadhyay, A.K., Liang, L.T., Chang, K.S.S., NCI, National Institutes of Health, Bethesda, Maryland: DNA methylation affecting retrovirus expression in murine embryonal carcinoma and trophoblast cells.

SESSION 8 *Poster Session*

Robert-Lézénés, J., Moreau-Gachelin, F., INSERM Unit 248, Faculté de Mèdecine Lariboisière-Saint Louis, Paris, France: Expression of SFFV *env*-gene-related sequences in normal mice.

Tsichlis, P.N.,[1] Strauss, P.G.,[1] Kozak, C.,[2] [1]NCI; [2]NIAID, National Institutes of Health, Bethesda, Maryland: The *MLVI-2* locus maps in chromosome 15 in the mouse.

Strauss, P.G., Tsichlis, P.N., NCI, National Institutes of Health, Bethesda, Maryland: Nature of DNA rearrangements in the *MLVI-1* locus in Mo-MLV-induced rat thymic lymphomas.

Robey, W.G., Dekaban, G.A., Fischinger, P.J., NCI, Frederick Cancer Research Center, Frederick, Maryland: Isolation of newly generated recombinant MLVs after infection with ecotropic MLV.

Thomas, C.Y.,[1] Khiroya, R.,[2] Schwartz, R.S.,[2] Coffin, J.M.,[2] [1]University of Virginia School of Medicine, Charlottesville; [2]Tufts University School of Medicine, Boston, Massachusetts: Role of ecotropic viruses in the generation of recombinant leukemogenic retroviruses of HRS mice.

Ou, C.Y.,[1] Boone, L.R.,[2] Boone, G.,[1] Yang, W.K.,[1] [1]Biology Division, Oak Ridge National Laboratory, Tennessee; [2]National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: A novel, mouse-specific, interdispersed, short, repetitive sequence found in the LTR of most MLV-related proviral sequences of mouse genome.

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Halpern, W.S.,[1] England, J.M.,[1] Deery, D.T.,[1] Petcu, D.J.,[2] Mason, W.S.,[2] Molnar-Kimber, K.L.,[2] [1]Wistar Institute of Anatomy and Biology; [2]Institute for Cancer Research, Philadelphia, Pennsylvania: Synthesis of duck hepatitis-B virus nucleic acids and antigen accumulation in tissues of infected ducks.

Mark, G.E., Schultz, A., Goldsborough, M.D., Rapp, U.R., NCI, Frederick Cancer Research Facility, Frederick, Maryland: Nucleotide sequence and expression of *v-raf*, the oncogene of 3611-MSV.

SESSION 9 *Oncogenes*

Chairperson: J. Coffin, Tufts University School of Medicine, Boston, Massachusetts

Hoffmann, F.M.,[1] Fresco, L.D.,[1] Hoffman-Falk, H.,[2] Shilo, B.Z.,[2] [1]Cell and Developmental Biology, Harvard University, Cambridge, Massachusetts; [2]Dept. of Virology, Weizmann Institute, Rehovot, Israel: Nucleotide sequence comparison of *v-abl* and *v-src* to the *Drosophila* oncogenes *Dash* and *Dsrc*.

DeFeo, D., Papageorge, A., Stokes, P., Temeles, G., Scolnick, E.M., Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania: *ras*-related gene sequences and *ras*-related gene products are present in *S. cerevisiae*.

Hammond, C.I., Bishop, J.M., Dept. of Microbiology and Immunology, University of California, San Francisco: Analysis of DNA and RNA homologous to *fps* in *S. cerevisiae*.

Simon, M.A.,[1] Kornberg, T.B.,[1] Bishop, J.M.,[2] [1]Dept. of Biochemistry and Biophysics; [2]Dept. of Microbiology and Immunology, University of California, San Francisco: The cellular *src* gene of *D. melanogaster*.

Steele, R.E., Reeder, R.H., Fred Hutchinson Cancer Research Center, Seattle, Washington: Characterization and cloning of oncogenes of the frog *X. laevis*.

Ruta, M.,[1] Wolford, R.,[1] Dhar, R.,[2] Ellis, R.,[3] Scolnick, E.M.,[3] [1]Laboratory of Tumor Virus Genetics; [2]Laboratory of Molecular Virology, National Institutes of Health, Bethesda, Maryland; [3]Merck Sharp and Dohme, West Point, Pennsylvania: Comparison of the nucleotide sequences of *c-ras*^{H}-1, *c-ras*^{H}-2, and *v-ras*^{H}.

Keshet, E., Itin, A., Rotman, G., Asher, A., Dept. of Virology, Hebrew University-Hadassah Medical School, Jerusalem, Israel: Murine viruslike (VL30) DNA – Sequence analysis of the LTR and adjacent DNA, and apparent recombinants with MLV-related proviruses.

Goldsborough, M.D., Mark, G.E., Bonner, T.I., Rapp, U.R., NCI, Frederick Cancer Research Facility, Frederick, Maryland: Structure and biological activity of *v-raf* – A new oncogene transduced by retrovirus.

Noda, M.,[1] Selinger, Z.,[2] Bassin, R.,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Life Sciences Institute, Hebrew University, Jerusalem, Israel: Flat revertants from Ki-MSV-transformed NIH-3T3 cells – Fusion studies indicate possible functional relationships among retroviral oncogenes.

Samarut, J.,[1] Mathey-Prevot, B.,[2] Hanafusa, H.,[2] [1]Université Claude Bernard Lyon-I, Lyon, France; [2]Rockefeller University, New York, New York: Preferential expression of NCP98 in chicken cells of the granulocytic cell lineage.

Waneck, G., Rosenberg, N., Cancer Research Center, Tufts University School of Medicine, Boston, Massachusetts: Abelson- and Harvey-virus-induced modulation of erythropoiesis.

Ihle, J.N., Scott, A., Gilbert, D., Palaszynski, E., NCI, Frederick Cancer Research Facility, Frederick, Maryland: Mo-MLV-induced interleukin-3-dependent lymphomas in BALB/c mice.

Ralston R., Bishop, J.M., Dept. of Microbiology, University of California, San Francisco: Evolutionary relationships among oncogenes.

SESSION 10 *Oncogene Activation*

Chairperson: G. Vande Woude, National Institutes of Health, Bethesda, Maryland

Parada, L.F., Land, H., Dautry, F., Cunningham, J.M., Murray, M.J., Weinberg, R.A., Center for Cancer Research, Massachusetts Institute of Technology; Whitehead Institute for Biomedical Research, Cambridge: N-*ras* and 3MC-Ki-*ras* – Two cellular oncogenes that are activated in a variety of tissues.

Shimizu, K., Taparowsky, E., Fasano, O., Suard, Y., Birnbaum, D., Goldfarb, M., Wigler, M., Cold Spring Harbor Laboratory, New York: Structure and mutational activation of three human *ras* genes.

Parker, R.C., Varmus, H.E., Bishop, J.M., Dept. of Microbiology and Immunology, University of California, San Francisco: High levels of pp60$^{v\text{-}src}$ do not morphologically transform Rat-2 cells – Lower levels of *v-src* are adequate for transformation.

Wood, T., Blair, D., McGeady, M., Baroudy, B., Vande Woude, G., NCI, National Institutes of Health, Bethesda, Maryland: The normal mouse DNA sequences preceding *c-mos* can prevent activation of the transforming potential of *c-mos*.

Blair, D.,[1] Woodworth, A.,[2] Oskarsson, M.,[2] McGeady, M.,[2] Tainsky, M.,[2] Vande Woude, G.,[2] [1]Frederick Cancer Research Facility, Frederick; [2]NCI, National Institutes of Health, Bethesda, Maryland: Biological properties of the human DNA sequence homologous to the *v-mos* transforming gene of Mo-MSV.

Dreazen, O., Horowitz, M., Cohen, J., Rechavi, G., Givol, D., Canaani, E., Dept. of Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel: Viral *A*-particle-related sequences are associated with the activation of a cellular oncogene (*c-mos*) in a murine plasmacytoma.

Gol, R., Eva, A., Yuasa, Y., Kraus, M., Needleman, S., Tronick, S., Aaronson, S., NCI, National Institutes of Health, Bethesda, Maryland: Isolation and characterization of the normal human homolog of the neuroblastoma oncogene – Frequent activation of closely related oncogenes in diverse human tumors.

Marshall, C.J., Hall, A., Vousden, K., Institute of Cancer Research, Chester Beatty Laboratories, London, England: Activated *ras* genes in human and mouse tumors.

Yuasa, Y., Srivastava, S.K., Rhim, J.S., Reddy, E.P., Aaronson, S.A., NCI, National Institutes of Health, Bethesda, Maryland: Isolation from a human lung carcinoma-derived cell line of a *bas/has* oncogene whose site of activation differs from that of T24 and EJ bladder carcinoma oncogenes.

Moroni, C.,[1] Senn, H.-P.,[2] Gambke, C.,[1] [1]Friedrich Miescher-Institut; [2]Research Dept., Ciba-Geigy Ltd., Basel, Switzerland: Identification and characterization of an oncogene from fresh bone marrow from a patient with AML.

Sodroski, J., Haseltine, W., Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Activation of the human *c-fes* locus by recombination with FeSV sequences.

Shalloway, D.,[1] Coussens, P.M.,[1] Yaciuk, P.,[1] Zelenetz, A.D.,[2] [1]Dept. of Biochemistry, Molecular Biology, Molecular and Cell Biology, Penn-

sylvania State University, University Park; [2]Sidney Farber Cancer Institute, Boston, Massachusetts: *c-src* overexpression causes enhanced in vivo tyrosine phosphorylation but not neoplastic transformation.

Schwab, M., Alitalo, K., Varmus, H.E, Bishop, J.M., Dept. of Microbiology, University of California, San Francisco: Molecular rearrangements in the 5'-flanking region of an amplified *c-myc* oncogene in the human colon carcinoma cell line COLO 320 result in altered *c-myc* transcripts.

Schwab, M.,[1] Alitalo, K.,[1] Varmus, H.E.,[1] Bishop, J.M.,[1] George, D.,[2] [1]Dept. of Microbiology, University of California, San Francisco; [2]Dept. of Human Genetics, University of Pennsylvania, Philadelphia: Double minute chromosomes (DMs) and a homogeneously staining chromosomal region (HSR) contain an amplified and abundantly expressed cellular oncogene (c-Ki-*ras)* in mouse adrenocortical tumor cells.

Alitalo, K.,[1] Lin, C.C.,[1] George, D.,[2] Schwab, M.,[1] Varmus, H.E.,[1] Bishop, J.M.,[1] [1]Dept. of Microbiology and Immunology, University of California, San Francisco; [2]Dept. of Human Genetics, University of Pennsylvania School of Medicine, Philadelphia: Amplification of *c-myc* and *c-myb* oncogenes in human colon carcinoma cells—Relationship with chromosomal abnormalities.

Mushinski, J.F., Bauer, S.R., Potter, M., Reddy, E.P., NCI, National Institutes of Health, Bethesda, Maryland: Expression and rearrangement of *c-myb* locus in lymphosarcomas arising in pristane-treated mice infected with Abelson MLV.

SESSION 11 *Vectors/Transforming Viruses*

Chairperson: A. Skalka, Roche Institute, Nutley, New Jersey

Emerman, M., Bandyopadhyay, P., Panganiban, A., Tarpley, G., Watanabe, S., Temin, H.M., McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Infectious retroviral vectors that express foreign DNA.

Cone, R., Cepko, C., Mann, R., Baltimore, D., Mulligan, R., Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Construction of highly transmissible mammalian cloning vectors derived from murine retroviruses.

Cepko, C.L., Mulligan, R.C., Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Rescue of Mo-MLV-derived recombinant genomes as bacterial plasmids.

Joyner, A., Keller, G., Phillips, R., Bernstein, A., Ontario Cancer Institute, Toronto, Canada: Retrovirus-mediated transduction of normal bone-marrow progenitor cells to neomycin resistance.

Van Beveren, C., Curran, T., Enami, S., Verma, I.M., Molecular Biology and Virology Laboratory, Salk Institute, San Diego, California: Organization of the *fos* gene—FBR murine osteosarcoma virus encodes a *gag-fos* fusion protein.

Lipsick, J., Boyle, W., Lampert, M., Dvorak, M., Baluda, M., Dept. of Pathology, University of California School of Medicine, Los Angeles: The product of the AMV oncogene and its normal cellular homolog.

Klempnauer, K.-H.,[1] McGrath, J.P.,[2] Levinson, A.D.,[2] Bishop, J.M.,[1] [1]Dept. of Microbiology, University of California, San Francisco; [2]Genentech, Inc., South San Francisco, California: Identification of the proteins encoded by *v-myb* and *c-myb.*

Schiff-Maker, L.,[1] Ponticelli, A.S.,[2] Witte, O.N.,[2] Rosenberg, N.,[1] [1]Cancer Research Center, Tufts University School of Medicine, Boston, Massachusetts; [2]Molecular Biology Institute, University of California: Los Angeles: Expression of *v-abl* and *c-abl.*

Wolff, L.,[1] Scolnick, E.,[2] Ruscetti, S.,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania: Unique carboxyl terminus of SFFV gp52 is encoded by envelope sequences that contain both a large deletion and insertions.

Clark, S.P., Mak, T.W., Ontario Cancer Institute, Toronto, Canada: Complete nucleotide sequence of Friend SFFV—gp55 is an envelope fusion glycoprotein.

Benedict, S.H.,[1] Beug, H.,[2] Graf, T.,[2] Wallbank, A.M.,[3] Vogt, P.K.,[1] [1]Dept. of Microbiology, University of Southern California School of Medicine, Los Angeles; [2]Deutsches Krebsforschungszentrum, Institut für Virusforschung, Heidelberg, Federal Republic of Germany; [3]Dept. of Medical Microbiology, University of Manitoba, Winnepeg, Canada: The properties of S13 avian sarcoma and erythroleukosis virus.

SESSION 12 *Genetics of Retroviral Diseases*

Chairperson: H. Robinson, Worcester Institute for Experimental Biology, Shrewsbury, Massachusetts

Robinson, H.,[1] Eisenman, R.,[2] [1]Worcester Institute for Experimental Biology, Shrewsbury, Massachusetts; [2]Fred Hutchinson Cancer Center, Seattle, Washington: New findings on the congenital transmission of ALVs.

Robinson, H., Miles, B., Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Unintegrated proviral DNA is a characteristic of ALV-induced osteopetrosis.

Stuhlmann, H.,[1] Jähner, D.,[1] Mulligan, R.,[2] Jaenisch, R.,[1] [1]Heinrich-Pette-Institut für Experimentelle Virologie und Immunologie an der Universität Hamburg, Federal Republic of Germany; [2]Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Infection of mouse embryos with recombinant retrovirus containing the *Eco gpt* gene.

Schnieke, A., Harbers, K., Jaenisch, R., Heinrich-Pette-Institut für Experimentelle Virologie und Immunologie an der Universität Hamburg, Federal Republic of Germany: Retroviruses and insertion mutagenesis—Germ-line integration identifies the gene essential for mouse embryogenesis.

Robinson, D.R.,[1] Luciw, P.A.,[2] Crittenden, L.B.,[3] Kung, H.-J.,[1] [1]Dept. of Biochemistry, Michigan State University, East Lansing; [2]Chiron Corp., Emeryville, California; [3]USDA Regional Poultry Research Laboratory, East Lansing, Michigan: "Enhancer" sequences are required for in vivo tumor induction by injection of cloned *v-src* DNAs.

Rein, A.,[1] Oliff, A.,[2] [1]NCI, Frederick Cancer Research Facility, Frederick, Maryland; [2]Sloan-Kettering Research Institute, New York, New York: Studies on MLV receptor specificity in NIH-3T3 cells.

Chatis, P.A.,[1] Holland, C.A.,[1] Hartley, J.W.,[2] Rowe, W.P.,[2] Hopkins, N.,[1] [1]Massachusetts Institute of Technology, Cambridge; [2]National Institutes of Health, Bethesda, Maryland: A role for the 3′ end of the genome in determining disease specificity of Fr-MLV and Mo-MLV.

Silver, J.,[1] Fredrickson, T.,[2] Rowe, W.,[1] [1]Laboratory of Viral Diseases, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Pathology, University of Connecticut, Storrs: Mouse genes control the type of leukemia induced by Fr-MLV.

Holland, C.A.,[1] Hartley, J.W.,[2] Rowe, W.P.,[2] Hopkins, N.H.,[1] [1]Center for Cancer Research, Massachusetts Institute of Technology, Cambridge; [2]National Institutes of Health, Bethesda, Maryland: Leukemogenicity of in vitro recombinants between molecule clones of Akv and MCF 247 viruses.

Lenz, J., Celander, D., Crowther, R., Patarca, R., Perkins, D., Sheldon, A., Trus, M., Haseltine, W., Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusets: Localization of the leukemogenic determinants of SL3-3.

DesGroseillers, L., Rassart, E., Jolicoeur, P., Institut de Recherches Cliniques de Montréal, Université de Montréal, Canada: The thymotropism of MLV is conferred by its LTR.

Baba, T.W., Humphries, E.H., Dept. of Microbiology, University of Texas Health Science Center, Dallas: Quantitative difference of hyperplasia in chickens susceptible and resistant to lymphomas induced by ALV.

THE MOLECULAR BIOLOGY OF YEAST

August 16–August 21

SESSION 1 *Cell Cycle, Cell Structure, and Chromosome Stability*

Chairperson: J. Pringle, University of Michigan, Ann Arbor, Michigan

Adams, A.E.M., Jacobs, C.W., Pringle, J.R., Division of Biological Sciences, University of Michigan, Ann Arbor: Fluorescence localization of actin and tubulin in wild-type, mutant, and inhibitor-treated *Saccharomyces.*

Katz, M.E., Reed, S.I., Dept. of Biological Sciences, University of California, Santa Barbara: Isolation and characterization of suppressors of *ste* mutations.

Singer, R.A., Johnston, G.C., Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada: The G_1 cell-cycle interval is shortened by differentially slowing the DNA-division sequence or increasing growth capacity.

Uno, I.,[1] Matsumoto, K.,[2] Ishikawa, T.,[1] [1]Institute of Applied Microbiology, University of Tokyo, [2]Dept. of Industrial Chemistry, Tottori University, Japan: Roles of cAMP in mitosis and meiosis of *S. cerevisiae.*

Shuster, E.O., Byers, B., Dept. of Genetics, University of Washington, Seattle: Meiotic analysis of the "start" class of *cdc* mutations.

Novick, P.,[1] Shortle, D.,[2] Osmond, B.,[1] Grisafi, P.,[1] Botstein, D.,[1] [1]Dept. of Biology, Massachusetts Institute of Technology, Cambridge; [2]Dept. of Microbiology, State University of New York, Stony Brook: Genetic analysis of actin and interacting proteins.

Thomas, J.H., Botstein, D., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Phenotypic and genetic analysis of mutants in the gene encoding β-tubulin (*TUB2*).

Neff, N., Botstein, D., Solomon, F., Center for Cancer Research and Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Isolation of trifluoperazine-resistant yeast mutants.

Huberman, J.A., El Assouli, S., Potashkin, J.A. Saavedra, R.A., Spotila, L.D., Dept. of Cell and Tumor Biology, Roswell Park Memorial Institute, Buffalo, New York: Initiation of DNA replication in yeast cells.

Wagner, D.W., Wood, J.S., Hartwell, L.H., Dept. of Genetics, University of Washington, Seattle: Identifying genes involved in chromosomal structures important for replication and segregation.

Hieter, P., Mann, C., Snyder M., Davis, R.W., Dept. of Biochemistry, Stanford University School of Medicine, California: Functions involved in chromosome stability.

SESSION 2 *DNA Replication and Chromosome Structure*

Chairperson: C.S. Newlon, University of Iowa, Iowa City, Iowa

Sclafani, R.A., Wagner, D.W., Fangman, W.L., Dept. of Genetics, University of Washington, Seattle: Genetics of DNA replication in yeast *CDC7*- and *CDC8*-gene products.

Campbell, J.L., Kuo, C.-I., Celniker, S.E., Dept. of Chemistry, Caltech, Pasadena, California: Analysis of DNA sequences and proteins important for yeast replication using in vitro replication systems.

Makkuni, J., Wu, L., Li, Y.-Y., Broach, J.R., Dept. of Microbiology, State University of New York, Stony Brook: Control of 2u circle replication.

Maine, G., Sinha, P., Tye, B.-K., Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Mutants of *S. cerevisiae* defective in the maintenance of minichromosomes.

Newlon, C.S., Lipchitz, L.R., Dept. of Zoology, University of Iowa,

Iowa City: Cloning, mapping, and localization of ARSs on a circular derivative of yeast chromosome III.

Scott, J., Long, C., Woontner, M., Strich, R., Dept. of Microbiology, University of Illinois, Urbana: Structure of *ARS1* chromatin and functional domains of its DNA sequence.

Szostak, J.W., Claus, T.E., Murray, A.W., Dunn, B., Dana-Farber Cancer Institute and Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Structure and function of telomeres in yeast.

Clarke, L., Carbon, J., Dept. of Biological Sciences, University of California, Santa Barbara: Genomic substitutions of centromeres in *S. cerevisiae*.

Dani, G.M., Zakian, V.A., Fred Hutchinson Cancer Research Center, Seattle, Washington: Behavior of linear centromere plasmids in meiosis.

DiNardo, S., Voelkel, K.A., Sternglanz, R., Dept. of Bochemistry, State University of New York, Stony Brook: Mutation in the gene coding for *S. cerevisiae* DNA topoisomerase II affects termination of DNA replication.

Jazwinski, S.M., Edelman, G.M., Rockefeller University, New York, New York: Evidence for the involvement of a multiprotein complex in 2u and *ARS1* DNA replication.

SESSION 3 *Poster Session*

CDC and Cell Structure

Basson, M., Barnes, G., Rine, J., Dept. of Biochemistry, University of California, Berkeley: Studies of gene controlling the activity of HMG-CoA reductase in *S. cerevisiae*.

Casperson, G.F., Walker, N., Brasier, A.R., Bourne, H.R., Dept. of Pharmacology and CVRI, University of California, San Francisco: Adenylate cyclase in the yeast *S. cerevisiae*.

Matsumoto, K.,[1] Uno, I.,[2] Ishikawa, T.,[2] [1]Dept. of Industrial Chemistry, Tottori University; [2]Institute of Applied Microbiology, University of Tokyo, Japan: Identification of the structural gene and nonsense alleles for adenylate cyclase in *S. cerevisiae*.

Coleman, K.G., Lillie, S.H., Jacobs, C.W., Robinson, J.S., Haarer, B., Stapleton, A.E., Pringle, J.R., Division of Biological Sciences, University of Michigan, Ann Arbor: Formal and molecular genetic analysis of cellular morphogenesis in *Saccharomyces*.

Yochem, J., Byers, B., Dept. of Genetics, University of Washington, Seattle: Molecular analysis of two G_1-arrest *CDC* mutants.

Chlebowicz-Sledziewska, E., Brewer, B.J., Fangman, W.L., Dept. of Genetics, University of Washington, Seattle: Unequal mother/daughter cell cycle phase lengths in yeast.

Okamoto, S., Laboratory of Molecular Genetics, Institute of Medical Science, University of Tokyo, Japan: Isolation of methyl-benzimidazole-2-yl-carbamate-resistant mutants and cloning of a MBC^r gene in *S. cerevisiae*.

Peterson, T., Reed, S., Dept. of Biological Sciences, University of California, Santa Barbara: *CDC9* transcription is cell-cycle-regulated.

Lorincz, A.T., Cheetham, B.F., Richardson, S.M., Reed, S.I., Biochemistry and Molecular Biology Section, Dept. of Biological Sciences, University of California, Santa Barbara: Genetic and molecular analysis of the cell cycle control gene *CDC28*.

Hiraoka, Y., Yanagida, M., Dept. of Biophysics, Faculty of Science, Kyoto University, Japan: Movement of mitotic chromosomes in the fission yeast *S. pombe*.

Soll, D.R., Herman, M.A., Anderson, J.M., Dept. of Zoology, University of Iowa, Iowa City: Use of a perfusion chamber to analyze budding characteristics of *S. cerevisiae*.

Elder, R., Easton Esposito, R., Dept. of Biology, University of Chicago, Illinois: Cloning of the *SPO12* gene—A gene necessary for the meiosis-I division.

Giroux, C.N., National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Selective system for isolating meiosis-specific genes—Cloning of a *spo11-1* complementing function.

Ninfa, E.G., Kaback, D.B., Dept. of Microbiology, UMDNJ-New Jersey Medical School, Newark: Isolation of DNA sequences complementary to sporulation-specific transcripts.

Magee, P.T.,[1] Clancy, M.J.,[2] Primerano, D.,[1] [1]Dept. of Microbiology, Michigan State University, East Lansing; [2]Dept. of Microbiology, Notre Dame University, South Bend, Indiana: Transcriptional control of mutant phenotype of genes involved in sporulation in *S. cerevisiae*.

Clancy, M.,[1] Pugh, T.,[1] Lehman, D.,[2] Magee, P.T.,[2] [1]Dept. of Microbiology, Notre Dame University, South Bend, Indiana; [2]Dept. of Microbiology and Public Health, Michigan State University, East Lansing: Program of gene expression during sporulation in *S. cerevisiae*.

Percival-Smith, A., Segall, J., Dept. of Biochemistry, University of Toronto, Ontario, Canada: Identification of sporulation genes of *S. cerevisiae*.

DNA Replication and Chromosome Structure

Amin, A.A., Pearlman, R.E., Dept. of Biology, York University, Downsview, Ontario, Canada: ARS regions from rDNA of *T. thermophila*.

Bloom, K., Yeh, E., Dept. of Biology, University of North Carolina, Chapel Hill: Topology of yeast centromere DNA.

Yeh, E.,[1,2] Carbon, J.,[1] Bloom, K.,[2] [1]Dept. of Biological Sciences, University of California, Santa Barbara; [2]Dept. of Biology, University of North Carolina, Chapel Hill: RNA transcripts from the centromere region of chromosomes III and XI in yeast.

Karns, L.R., Smith, M.M., Dept. of Microbiology, University of Virginia School of Medicine, Charlottesville: Analysis of the regulation of expression of yeast histone–β-galactosidase gene fusions.

Bouton, A., Stirling, V., Smith, M.M., Dept. of Microbiology, University of Virginia, Charlottesville: Characterization of an ARS closely linked to one copy of the histone H3-H4 genes in *S. cerevisiae*.

Cross, S.L., Smith, M.M., Dept. of Microbiology, University of Virginia, Charlottesville: Transcription of the yeast genes for histones H3 and H4.

Mittman, B.A., Smith, M.M., Dept. of Microbiology, University of Virginia, Charlottesville: Expression of the histone H4 gene and gene mutants constructed in vitro.

Hsiao, C.L., Jonak, G.J., E.I. du Pont de Nemours and Company, Experi-

mental Station, Central Research and Development Dept., Wilmington, Delaware: Effect of yeast centromeric DNA on pSV2*neo* vector in monkey cells.

Thrash, C., Voelkel, K.A., DiNardo, S., Sternglanz, R., Dept. of Biochemistry, State University of New York, Stony Brook: Identification of *S. cerevisiae* DNA topoisomerase I mutants.

Williams, S.J., Shaw, W.V., Dept. of Biochemistry, University of Leicester, England: Replication of staphylococcal plasmid DNA in yeast.

Koshland, D., Hartwell, L., Dept. of Genetics, University of Washington, Seattle: Fidelity of minichromosome transmission.

Gaudet, A., Fitzgerald-Hayes, M., Dept. of Biochemistry, University of Massachusetts, Amherst: Investigation of the DNA sequence organization required for mitotic segregation of centromere plasmids.

Schmitt, E., Olson, M.V., Dept. of Genetics, Washington University School of Medicine, St. Louis, Missouri: Use of SUP4-CEN3 plasmids as genetic tools.

Helms, C., Graham, M.Y., Brodeur, G.M., Scheinman, R., Olson, M.V., Dept. of Genetics, Washington University School of Medicine, St. Louis, Missouri: Physical mapping of yeast chromosomal DNA.

Dunn, B., Szostak, J.W., Dana-Farber Cancer Institute and Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Evidence for interaction between linear plasmids and the ends of yeast chromosomes.

Murray, A.W.,[1,2] Szostak, J.W.,[1,3] [1]Dana-Farber Cancer Institute; [2]Program in Cell and Developmental Biology; [3]Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Construction and properties of artificial linear chromosomes.

Strich, R., Scott, J., Dept. of Microbiology, University of Illinois, Urbana: Insertion mutagenesis and subclones of *ARS1*.

Woontner, M., Scott, J., Dept. of Microbiology, University of Illinois, Urbana: Deletion analysis of *ARS1* in TRP1 RI Circle.

Conrad, M.N., Zakian, V.A., Fred Hutchinson Cancer Research Center, Seattle, Washington: Association of nascent DNA with the yeast nuclear matrix.

Dani, G.M., Hager, L.J., Zakian, V.A., Fred Hutchinson Cancer Research Center, Seattle, Washington: 2u DNA can provide ARS function for centromere and linear plasmids.

Pluta, A.F., Dani, G.M., Zakian, V.A., Fred Hutchinson Cancer Research Center, Seattle, Washington: Ends from *O. fallax* macronuclear DNA can provide telomere function in yeast.

McMahon, M.E., Petes, T.D., Dept. of Microbiology, University of Chicago, Illinois: A novel yeast 5S rRNA gene at the junction of rDNA with single-copy chromosomal DNA.

Goebl, M.G., Petes, T.D., Dept. of Microbiology, University of Chicago, Illinois: Isolation and characterization of poly(dA)-poly(dT) sequences in the yeast *S. cerevisiae*.

Walmsley, R.W.,[1] Chan, C.,[2] Tye, B.,[2] Peters, T.D.,[1] [1]Dept. of Microbiology, University of Chicago, Illinois; [2]Section of Biochemistry, Cornell University, Ithaca, New York: Poly(GT) tracts in the yeast genome.

Vincent, A., Petes, T.D., Dept. of Microbiology, University of Chicago, Illinois: Repeated sequences in yeast—A Ty element associated with an rDNA sequence.

Potashkin, J.A.,[1] Ziegel, R.,[2] Huberman, J.A.,[1] [1]Dept. of Cell and Tumor Biology; [2]Biophysics Research, Roswell Park Memorial Institute, Buffalo, New York: Isolation and characterization of yeast nuclear matrices.

Saavedra, R.A., Huberman, J.A., Dept. of Cell and Tumor Biology, Roswell Park Memorial Institute, Buffalo, New York: Yeast 2u plasmid DNA undergoes alterations in topology and in tightly bound proteins as cells enter S phase.

Spotila, L.D., Huberman, J.A., Dept. of Cell and Tumor Biology, Roswell Park Memorial Institute, Buffalo, New York: General method for mapping replication origins.

Walmsley, R.,[1] Johnston, L.H.,[2] Oliver, S.G.,[1] Fennell, D.J.,[2] McCready, S.,[3] Williamson, D.H.,[2] [1]University of Manchester Institute of Science and Technology; [2]National Institute for Medical Research, London; [3]Botany School, University of Oxford, England: Replicon organization of rDNA in *S. cerevisiae*.

Panzeri, L.,[1] Groth-Clausen, I.,[2] Altenburger, W.,[2] Philippsen, P.,[2] [1]Instituto di Genetica, Università di Milano, Italy; [2]Dept. of Microbiology, Biozentrum, University of Basel, Switzerland: DNA in the centromere of chromosome VI.

Bachmair, A., Fessl, F., Mattes E., Ruis, H., Institut für Allgemeine Biochemie der Universität Wien and Ludwig Boltzmann-Forschungsstelle für Biochemie, Vienna, Austria: Isolation of minichromatin from yeast cells transformed with plasmids derived from 2u DNA.

Fasullo, M.T., Davis, R.W., Dept. of Biochemistry, Stanford University School of Medicine, California: Method for choosing the type and location of chromosome rearrangements.

Grunstein, M., Rykowski, M., Wallis, J., Molecular Biology Institute, University of California, Los Angeles: Genetic evidence suggesting functional differences between the amino and carboxyl termini of yeast histone H2B.

Holm, C., Botstein, D., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Analysis of the functional region of *ars1* by in vitro mutagenesis.

Chan, C.S.M.,[1] Walmsley, R.M.,[2] Petes, T.D.,[2] Tye, B.-K.,[1] [1]Dept. of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York; [2]Dept. of Microbiology, University of Chicago, Illinois: Organization of DNA sequences and ARSs at yeast telomeres.

Surosky, T., Tye, B.-K., Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Construction of chromosomes in vivo.

Murakami, S.,[1] Chan, C.S.M.,[2] Tye, B.-K.,[2] Livingston, D.M.,[3] [1]Cancer Research Institute, Kanazawa University, Japan; [2]Dept. of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York; [3]Dept. of Biochemistry, University of Minnesota Medical School, Minneapolis: Nuclease-hypersensitive sites of chromatin structure around autonomous replicating segments of *S. cerevisiae*.

Fagrelius, T.J., Livingston, D.M., Dept. of Biochemistry, University of Minnesota, Minneapolis: Location of DNase-I-hypersensitive cleavage sites in 2u plasmid DNA chromosomes.

Kim, K.E., Newlon, C.S., Dept. of Zoology, University of Iowa, Iowa City: Telomeric sequences in a library constructed from alkaline-denatured DNA.

Montiel, F.J., Norbury, C.J., Tuite, M.F., Dobson, M.J., Mills, J.S., Cook, P.R., Kingsman, A.J., Kingsman, S.M., Dept. of Biochemistry, Oxford, England: Char-

acterization of human chromosomal DNA sequences that replicate in *S. cerevisiae*.

DiGate, R.J., Hinkle, D.C., Dept. of Biology, University of Rochester, New York: DNA primase activity associated with yeast DNA polymerase I.

Crowley, J.C.,[1] Coleman, K.G.,[2] Steensma, H.Y.,[1] Kramer, B.,[1] Pringle, J.R.,[2] Kaback, D.B.,[1] [1]Dept. of Microbiology, UMDNJ-New Jersey Medical School, Newark; [2]Dept. of Molecular and Cellular Biology, University of Michigan, Ann Arbor: Molecular organization of chromosome I.

Kuo, C.-I., Jong, A., Campbell, J.L., Dept. of Chemistry, California Institute of Technology, Pasadena: Analysis of the yeast *CDC8* gene and the *CDC8* protein.

Walmsley, R.M., Gardner, D.J., Oliver, S.G., Dept. of Biochemistry and Applied Molecular Biology, University of Manchester Institute of Science and Technology, England: Plasmid stability in yeast—Evidence for a phenotype conferred by 2u DNA.

Sidhu, R., Bollon, A.P., Dept. of Molecular Genetics, Wadley Institutes of Molecular Medicine, Dallas, Texas: Identification of proteins associated with unique yeast vectors isolated as minichromosomes.

Recombination and Repair

Cottrelle, P., Thiele, D., Micouin, J.Y., Memmet, S., Buhler, J.M. Sentenac, A., Dept. de Biologie, Service de Biochimie, CEN, Gif-sur Yvette Cédex, France: Molecular cloning of a gene for protein synthesis EFI from *S. cerevisiae*.

Friedberg, E.C., Naumovski, L., Pure, G.A., Robinson, G.W., Schultz, R.A., Weiss, W.A., Yang, E.W., Stanford University, California: Characterization of cloned genes required for the excision repair of UV-irradiated DNA in *S. cerevisiae*.

Keil, R.L., Roeder, G.S., Dept. of Biology, Yale University, New Haven, Connecticut: A mitotic recombination hot spot in the rDNA of *S. cerevisiae*.

Lambie, E.J., Roeder, G.S., Dept. of Biology, Yale University, New Haven, Connecticut: Transposition of *CEN3*—Effects of meiotic recombination and segregation of chromosome III.

Orr-Weaver, T.L.,[1] Rothstein, R.J.,[2] Szostak, J.W.,[1] [1]Dana-Farber Cancer Institute and Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts; [2]Dept. of Microbiology, New Jersey Medical School, Newark: Gene conversion in yeast by double-strand gap repair.

Haber, J., Thorburn, P., Liu, P.S., Klucznik, M., Rosenstiel Center, Brandeis University, Waltham, Massachusetts: Breaking and healing of yeast chromosomes.

Haber, J.,[1] McCusker, J.,[1] Soltis, A.,[1] Resnick, M.,[2] [1]Brandeis University, Waltham, Massachusetts; [2]NIEHS, Research Triangle Park, North Carolina: Mitotic recombination induced by double-strand breaks.

Edwards, C.W., III, Malone, R.E., Dept. of Microbiology, Loyola University Medical Center, Maywood, Illinois: Examination of the pleiotropic effects of *rad52* mutations in yeast.

Hoekstra, M.F., Malone, R.E., Dept. of Microbiology, Loyola University Medical Center, Maywood, Illinois: Error-prone repair is not required for *rem1*-produced hypermutability.

Jordan, K.B., Malone, R.E., Dept. of Microbiology, Loyola University Medical Center, Maywood, Illinois: Isolation and characterization of extragenic revertants of the *rad50-4* mutation.

Barker, D.G., Johnston, L.H., National Institute for Medical Research, London, England: *S. cerevisiae CDC9*—A structural gene for DNA ligase.

Barker, D.G., Johnston, L.H., National Institute for Medical Research, London, England: A new sensitive assay for DNA ligase.

Game, J., Mortimer, R., Donner Laboratory, Lawrence Berkeley Laboratory, Berkeley, California: Use of a cold-sensitive *rad50* allele to study meiotic recombination.

Schild, D.,[1] Johnston, J.,[2] Chang, C.,[1] Mortimer, R.,[1] [1]Lawrence Berkeley Laboratory and University of California, Berkeley; [2]University of Strathclyde, Glasgow, Scotland: Cloning of a yeast photoreactivation gene.

Sitney, K.C.,[1] Contopoulou, C.R.,[1] Calderon, I.L.,[2] Mortimer, R.K.,[1] [1]Dept. of Biophysics and Medical Physics, University of California, Berkeley; [2]Dept. de Genetica, Universidad de Sevilla, Spain: Two plasmids that suppress the *rad50* mutation in *S. cerevisiae*.

Rockmill, B., Fogel, S., University of California, Berkeley: Meiotic disjunction mutants.

Choi, T., Lusnak, K., Williamson, M., Fogel, S., Dept. of Genetics, University of California, Berkeley: Gene conversion of a centromeric *ade8-18*.

Williamson, M., Fogel, S., Dept. of Genetics, University of California, Berkeley: Meiotic correction-defective yeast mutants.

Picologlou, S., Dicig, M.E., Liebman, S.W., University of Illinois, Chicago: Inversions sometimes accompany Ty1-associated deletions in yeast.

Pacquin, C., Williamson, V., ARCO Plant Cell Research Institute, Dublin, California: Temperature affects the rate of transposition of yeast Ty elements.

Embretson, J.E., Livingston, D.M., Dept. of Biochemistry, University of Minnesota, Minneapolis: Initial characterization of a plasmid model to study genetic recombination in yeast.

Prakash, L., Polakowska, R., Reynolds, P., Weber, S., Dept. of Radiation Biology and Biophysics, University of Rochester School of Medicine, New York: Molecular cloning and preliminary characterization of the *RAD6* gene of yeast.

Nisson, P., Lawrence, C., University of Rochester, School of Medicine and Dentistry, New York: Yeast strains with altered nitrosoguanidine mutability.

Nitiss, J.,[1,2] Resnick, M.A.,[1] [1]National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina; [2]Illinois Institute of Technology, Chicago: DNA metabolism during meiosis in *rad50* and *rad52* mutants of *S. cerevisiae*.

Resnick, M.A.,[1] Sugino, A.,[2] Nitiss, J.,[1,3] Chow, T.,[1] [1]National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina; [2]Dept. of Molecular Genetics, University of Georgia, Athens; [3]Dept. of Biology, Illinois Institute of Technology, Chicago: DNA polymerases, deoxyribonucleases, and recombination during meiosis.

Bruschi, C.V., Bjornstad, K.A., Maleas, D.T., Esposito, M.S., Lawrence Berkeley Laboratory, University of California, Berkeley: Miotic recombination and 2u DNA plasmid profiles of *spo11-1/spo11-1* yeast hybrids.

Kunes, S., Fox, M., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Homology-dependent and -independent repair of plasmid double-strand breaks.

Hoshizaki, D.K., Dept. of Molecular Biology, University of Edinburgh, Scotland: Recombination of plasmids in yeast.

Braun, R.J., Roth, R.M., Dept. of Biology, Illinois Institute of Technology, Chicago: Exonuclease A from *S. cerevisiae* specific for 5'-P-terminated SS and DS DNAs.

Higgins, D., Prakash, S., Dept. of Biology, University of Rochester, New York: Isolation and characterization of *S. cerevisiae* genes that function in incision of UV-irradiated DNA.

Moore, C.W., Dept. of Radiation Biology and Biophysics, University of Rochester School of Medicine, New York: Genetic and molecular control of the sensitivity of *S. cerevisiae* to the "radiomimetic" bleomycin group antibiotics.

Mazzara, G.P., Greer, H., Harvard University, Cambridge, Massachusetts: Sequence analysis of *HIS4* chromosomal rearrangements implicate short regions of homology.

Ruby, S.W., Szostak, J.W., Dana-Farber Cancer Institute and Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Regulation of yeast genes induced by DNA-damaging agents.

Whiteway, M.S., Szostak, J.W., Dana-Farber Cancer Institute and Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusets: Yeast mutant defective in cell-cycle arrest.

Toda, T., Adachi, Y., Yanagida, M., Dept. of Biophysics, Faculty of Science, Kyoto University, Japan: Two α-tubulin genes of the fission yeast *S. pombe*—Isolation and sequence analysis.

Nunes, E.,[1] Brum, G.,[1] Candreva, E.C.,[1] Schenberg Frascino, A.C.,[2] [1]Facultad de Medicina, Montevidea, Uruguay; [2]Universidade de São Paulo, Brazil: Interacting repair pathways for UV and X-ray inactivation of *S. cerevisiae* diploid cells.

Osley, M.A., Gould, J., Hereford, L.M., Dana-Farber Cancer Institute and Dept. of Microbiology and Molecular Biology, Harvard Medical School, Boston, Massachusetts: Analysis of an *ars* region involved in histone gene regulation.

SESSION 4 *Recombination and Repair*

Chairperson: M.S. Esposito, University of California, Berkeley, California

Chow, T.Y.-K, Resnick, M.A., National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Purification of a deoxyribonuclease controlled by the *RAD52* gene of *S. cerevisiae.*

Esposito, M.S., Bruschi, C.V., Maleas, D.T., Bjornstad, K.A., Lawrence Berkeley Laboratory, University of California, Berkeley: Yeast *REC* genes—Genetic analysis and molecular isolation.

Wagstaff, J., Waddell, C., Klapholz, S., Cwirla, S., Easton Esposito, E., Dept. of Biology, University of Chicago, Illinois: Functional analysis of meiotic Rec⁻ mutations using the *spo13-1* haploid meiosis system.

Klein, H., Dept. of Biochemistry, New York University Medical Center, New York: Genetic interactions of tandem duplications in yeast.

Klar, A., Strathern, J., Hicks, J., Abraham, J., Ivy, J., Weisbrod, S., Kelly, M., Stephens, C., Cold Spring Harbor Laboratory, New York: Resolution of the recombination intermediate generated by the mating-type gene-transposition mechanism.

Lichten, M., Borts, R., Hearn, M., Haber, J., Rosenstiel Center, Brandeis University, Waltham, Massachusetts: Analysis of two cloned regions with high frequencies of recombination in yeast.

Eibel, H., Gafner, J., Stotz, A., Fleig, U., Schirmaier, F., Iten, E., Philippsen, P., Dept. of Microbiology, Biozentrum, University of Basel, Switzerland: Transposition and excision of Ty elements.

Rothstein, R., Helms, C., Chrebet, G., Rosenberg, N., Dept. of Microbiology, UMDNJ-New Jersey Medical School, Newark: Mechanism of recombination between short repeated sequences.

Andrews, B.J., Vetter, D., Beatty, L., Sadowski, P.D., Dept. of Medical Genetics, University of Toronto, Canada: FLP-mediated recombination of yeast 2u DNA in vitro.

McLeod, M., Makkuni, J., Li, Y.-Y., Broach, J.R., Dept. of Microbiology, State University of New York, Stony Brook: Site-specific recombination associated with the yeast plasmid 2u circle.

Symington, L., Kolodner, R., Dana-Farber Cancer Institute and Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Plasmid recombination catalyzed by cell-free extracts of yeast.

SESSION 5 *Regulation I*

Chairperson: J. Broach, State University of New York, Stony Brook, New York

Beier, D.J., Young, E.T., Biochemistry Dept., University of Washington, Seattle: Characterization of the regulatory sequences that control *ADH2* expression.

Losson, R., Fuchs, R.P.P., Lacroute, F., IBMC de CNRS, Strasbourg, France: Positive control of *URA1* and *URA3* transcription initiation.

West, R.W., Jr., Yocum, R.R., Ptashne, M., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: *GAL1-GAL10* UAS—Structure vs. function.

Laughon, A., Gesteland, R.F., Howard Hughes Medical Institute, University of Utah, Salt Lake City: Characterization of the *GAL4* gene of yeast.

Post-Beittenmiller, M.A., Hopper, J.E., Dept. of Biological Chemistry, M.S. Hershey Medical Center, Pennsylvania State University, Hershey: Transcriptional expression of the structural gene for α-galactosidase.

Johnston, M., Dept. of Genetics, Washington University School of Medicine, St. Louis, Missouri: Selection for *GAL* regulatory mutants using *GAL-HIS3* gene fusions.

Celenza, J., Neigeborn, L., Sarokin, L., Carlson, M., Dept. of Human Genetics and Development, Columbia University, College of Physi-

cians and Surgeons, New York, New York: Regulation of *SUC2*-gene expression.

Goldenthal, M.J., Chow, T., Cohen, J., Marmur, J., Dept. of Biochemistry, Albert Einstein College of Medicine, Bronx, New York: Physical and functional organization of the *MAL* loci of yeast *Saccharomyces.*

Michels, C.A.,[1] Needleman, R.B.,[2] [1]Dept. of Biology, Queens College of the City of New York, Flushing, New York; [2]Dept. of Biochemistry, Wayne State University School of Medicine, Detroit, Michigan: The *MAL* loci—A dispersed family of repeated genes controlling the fermentation of maltose.

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Stuhl, K., Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: *his3* chromatin structure and gene regulation.

SESSION 6 *Poster Session—Regulation I*

Chakraburtty, K., Medical College of Wisconsin, Milwaukee: Elongation factor 3 is the temperature-labile translational component in the yeast mutant *ts*275.

Werner, M.,[1] Feller, A.,[1] Messenguy, F.,[2] Crabeel, M.,[3] Piérard, A.,[1,2] [1]Laboratoire de Microbiologie, Université Libre de Bruxelles; [2]Institut de Recherches du CERIA; [3]Microbiologie, Vrije Universiteit Brussel, Belgium: Sequences of the regulatory regions of genes *CPA1* and *CPA2* encoding the arginine pathway carbamoylphosphate synthase of *S. cerevisiae.*

Hauge, B., Greer, H., Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: 5′ *HIS4* and *TRP5* transcripts implicated in general control of amino acid biosynthesis.

Penn, M.D., Thireos, G., Greer, H., Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: Cloning and molecular analysis of positive regulatory genes involved in general control of amino acid biosynthesis.

Greenberg, M., Barr, R., Ganem, M., Greer, H., Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: New regulatory mutations affecting histidine biosynthesis.

Thireos, G., Penn, M.D., Greer, H., Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: General control of amino acid biosynthesis involves the coordinate action of "initiation" and "maintenance" genes.

Alexander, P.,[1] Cooper, T.G.,[2] Adams, B.G.,[1] [1]University of Hawaii, Honolulu; [2]University of Pittsburgh, Pennsylvania: Role of metabolite transport in nitrogen catabolite repression.

Di Mauro, E.,[1] Caserta, M.,[1] Negri, R.,[1] Carnevali, F.,[2] [1]Dept. Genetica e Biologia Molecolare, Università di Roma; [2]Centro di Studio per gli Acidi Nucleici, CNR Roma, Italy: Transitions in topological organization of supercoiled DNA domains as potential regulatory mechanism.

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Fukasawa, T.,[1] Nogi, Y.,[1] Shimada, H.,[2] Hashimoto, H.,[1] Matsuzaki, Y.,[1] [1]Laboratory for Molecular Genetics; [2]Dept. of Biochemistry, Keio University School of Medicine, Tokyo, Japan: Isolation and characterization of the regulatory gene *GAL80* in the galactose metabolism in *S. cerevisiae.*

Bajwa, W., Meyhack, B., Rudolf, H., Hinnen, A., Friedrich Miescher-Institut, Basel, Switzerland: Structural analysis of two tandemly repeated acid phosphatase genes in yeast.

Rudolf, H., Hinnen, A., Friedrich Miescher-Institut, Basel, Switzerland: Deletion scanning of the yeast *PHO5* promoter.

Meyhack, B., Hinnen, A., Ciba-Geigy AG, Basel, Switzerland: High levels of expression of foreign genes under the control of the yeast *PHO5* promoter.

Allison, D.,[1] Goh, S.H.,[2] Hall, B.,[1] [1]Dept. of Genetics, University of Washington, Seattle; [2]Dept. of Biochemistry, University of British Columbia, Vancouver, Canada: Internal promoter sequence of the yeast *SUP4* $tRNA^{Tyr}$ gene.

Hurley, D.L.,[1] Hopper, J.E.,[2] [1]Molecular and Cell Biology Program, [2]Dept. of Biological Chemistry, M.S. Hershey Medical Center, Pennsylvania State University, Hershey: Nucleotide sequence of the *S. cerevisiae* α-galactosidase gene and deletion analysis of the controlling region.

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Ingles, C.J.,[1] Himmelfarb, H.J.,[2] Shales, M.,[1] Friesen, J.D.,[2] [1]Banting and Best Dept. of Medical Research; [2]Dept. of Medical Genetics, University of Toronto, Canada: A family of *S. cerevisiae* RNA polymerase genes—Identification, molecular cloning, and mutagenesis.

Klootwijk, J., Verbeet, M.P., van Heerikhuizen, H., Veldman, G.M., de Regt, V.C.H.F., Planta, R.J., Dept. of Biochemistry, Free University, Amsterdam. The Netherlands: Initiation of transcription of 37S pre-rRNA on *S. carlsbergensis* rDNA in vivo and in vitro.

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Panchal, C.J., Peacock, L., Whitney, G., Stewart, G.G., Production Research Dept., Labatt Brewing Company Limited, London, Ontario, Canada: Drug resistance phenomenon in amaylase-producing yeasts of the genus *Schwanniomyces*.

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Elliott, S.G., Carbon, J.A., Dept. of Biology, University of California, Santa Barbara: Fusion of *ARG4* (arginosuccinate lyase) with β-galactosidase—Control of *ARG4* expression.

Knowlton, R.G., Stearman, R.S., Dept. of Molecular Genetics, Collaborative Research, Inc., Lexington, Massachusetts: Regulated expression of interferon from the *SUC2* promoter.

Lawyer, F.C., Stoffel, S., Gelfand, D.H., Cetus Corporation, Berkeley, California: Derivation and use of a dominant selectable marker for yeast.

Gjermansen, C.,[1] Holmberg, S.,[1] Petersen, J.G.L.,[1] Nilsson-Tillgren, T.,[2] Kielland-Brandt, M.C.,[1] Sigsgaard, P.,[3] Pedersen, M.B.,[2] [1]Dept. of Physiology, Carlsberg Laboratory, Copenhagen Valby; [2]Institute of Genetics, University of Copenhagen; [3]Dept. of Brewing Chemistry, Carlsberg Research Laboratory, Copenhagen Valby, Denmark: Genetics of lager yeast.

Gritz, L., Davies, J., Biogen S.A., Geneva, Switzerland: Hygromycin-B resistance—A new selectable marker for yeast transformation.

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Hsu, Y.-P., Schimmel, P., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Yeast

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Mowatt, N.,[1] Johnston, J.,[1] Watson, D.,[2] [1]Dept. of Bioscience and Biotechnology, University of Strathclyde, Glasgow, Scotland; [2]Chivas Brothers, Ltd., Strathisla-Glenlivet Distillery, Keith, Scotland: Hybridization of distillery yeasts by spheroplast fusion.

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Keng, T., Guarente, L., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Mutants in heme biosynthesis.

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McAlister, L., Holland, M., Dept. of Biological Chemistry, Medical School, University of California, Davis: Expression of yeast genes encoding glyceraldehyde-3-phosphate dehydrogenase.

Kurtz, S., Petko, L., McGarry, T., Lindquist, S., Dept. of Biology, University of Chicago, Illinois: Expression of heat-shock genes in *S. cerevisiae*.

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Finkelstein, D.B., Farrelly, F.W., Davis, A.H., Division of Molecular Biology, Dept. of Biochemistry, University of Texas Health Science Center, Dallas: *HSP90* gene family.

Craig, E.A., Jacobsen, K., University of Wisconsin, Madison: Characterization and analysis of mutations of the 70K heat-shock and related genes of yeast.

Ellwood, M., Slater, M., Craig, E., Dept. of Physiological Chemistry, University of Wisconsin, Madison: Differential regulation of the 70K heat-shock and related genes in *S. cerevisiae*.

Keegan, L., Silver, P., Ptashne, M., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Characterization of the GAL4-gene product.

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Hill, D.E., Struhl, K., Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Mechanism of *HIS3* regulation—Cloning of the regulatory gene *TRA3*.

Letts, V.A., Moss, L., Henry, S.A., Depts. of Genetics and Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: A mutant of yeast altered in phosphatidylmonomethylethanolamine biosynthesis.

Cooperman, B.S., Letts V.A., Henry, S.A., Depts. of Genetics and Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Solubilization and characterization of the phospholipid *N*-methyltransferases from yeast.

Loewy, B.S., Klig, L.S., Henry, S.A., Depts. of Genetics and Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Regulatory mutants that uncouple the coordinate regulation between inositol metabolism and phosphatidylcholine synthesis.

Letts, V.A.,[1] Klig, L.S.,[1] Lee-Bae, M.S.,[2] Carman, G.M.,[2] Henry, S.A.,[1] [1]Depts. of Genetics and Molecular Biology, Albert Einstein College of Medicine, Bronx, New York; [2]Dept. of Food Science, Cook College, New Jersey Agricultural Experimental Station, Rutgers University: *CHO1* locus of yeast—Cloning and analysis of its gene product, phosphatidylserine synthase.

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Platt, T., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Toxicity of 2-deoxygalactose to cells induced for the galactose enzymes and analysis of survivors.

Niederberger, P., Aebi, M., Furter, R., Prantl, F., Hütter, R., Mikrobiologisches Institut ETH-Z, Zurich, Switzerland: Expression of an artificial yeast *TRP*-gene cluster in *S. cerevisiae* and *E. coli*.

Romero, D.P., Dahlberg, A.E., Division of Biology and Medicine, Brown University, Providence, Rhode Island: Phosphorylation of initiation factor 2 (eIF2) in the yeast *S. cerevisiae*.

Brandriss, M.C., Dept. of Microbiology, New Jersey Medical School, Newark: Proline utilization in yeast—Analysis of the cloned *PUT2* gene.

Winston, M., Bhattacharjee, J.K., Dept. of Microbiology, Miami University, Oxford, Ohio: Regulation of lysine biosynthesis in *S. cerevisiae*.

Wright, C.F., Weiss, J.L., Walthall, D.A., Zitomer, R.S., Dept. of Biological Sciences, State University of New York, Albany: Regulation of *CYC7* expression.

Rymond, B.C., Zitomer, R.S., Dept. of Biological Sciences, State University of New York, Albany: Isolation of *CYC1* regulatory mutations using a *CYC1/galK* gene fusion.

Breunig, K., Dahlems, U., Hollenberg, C.P., Institut für Mikrobiologie, Universität Düsseldorf, Federal Republic of Germany: Characterization of the regulatory region of the β-galactosidase gene in *K. lactis*.

Carlson, M., Taussig, R., Dept. of Human Genetics and Development, Columbia University, College of Physicians and Surgeons, New York, New York: Structure of the *SUC* gene family.

Bisson, L.J., Fraenkel, D.G., Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Yeast mutants deficient in hexose uptake.

Sedivy, J.M., Fraenkel, D.G., Dept. of Microbiology and Molecular Ge-

netics, Harvard Medical School, Boston, Massachusetts: Genetics of fructose-1,6-bisphosphatase in *S. cerevisiae.*

Mitchell, A.P., Magasanik, B., Massachusetts Institute of Technology, Cambridge: Multiple regulatory systems for glutamine synthetase.

Turner, K.J.,[1] Elliott, Q.,[2] Lemire, J.M.,[2] Thill, G.P.,[3] Rogers, D.T.,[1] Kramer, R.A.,[4] Bostian, K.A.,[2] [1]Genetics Institute, Boston, Massachusetts; [2]Division of Biology and Medicine, Brown University, Providence, Rhode Island; [3]SIBIA, La Jolla, California; [4]Dept. of Molecular Genetics, Hoffman-LaRoche, Inc., Nutley, New Jersey: Regulation and organization of two tandemly duplicated yeast acid phosphatase genes.

Kawasaki, G.H., Woodbury, R.G., Forstrom, J.F., Zymos Corporation, Seattle, Washington: Production of human AT in yeast.

Russell, P., Woodbury, R., Zymos Corporation, Seattle, Washington: Expression of human AT in fission yeast.

Eccleshall, T.R.,[1] Federoff, H.J.,[2] Marmur, J.,[3] [1]Sungene Technologies Corp., Palo Alto, California; [2]Massachusetts General Hospital, Boston; [3]Albert Einstein College of Medicine, Bronx, New York: Regulation of the inducible and repressible maltase in *S. carlsbergensis.*

Abovich, N., Tung, L., Rosbash, M., Dept. of Biology, Brandeis University, Waltham, Massachusetts: Characterization of the expression of two genes coding for ribosomal protein 51.

Overbye, K.M., Rose, M., Grisafi, P., Botstein, D., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Segment-directed mutagenesis in vitro of the promoter and regulatory regions of the yeast *URA3* gene.

Gordon, C., Fantes, P., Dept. of Zoology, University of Edinburgh, Scotland: Cloning of a gene required for DNA synthesis in *S. pombe.*

McKnight, G., McConaughy, B., Dept. of Genetics and Center for Inherited Diseases, University of Washington, Seattle: Cloning of cDNAs by functional complementation in yeast.

Baker, S.M., Jaehning, J.A., Dept. of Biochemistry, University of Illinois, Urbana: Transcription of the *GAL7* gene from autonomous plasmids.

SESSION 7 *Regulation II*

Chairperson: J. Hicks, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Greer, H., Hauge, B., Thireos, G., Penn, M., Greenberg, M., Mazzara, G., Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts: General control of amino acid biosynthesis.

Hinnebusch, A.G., Fink, G.R., Whitehead Institute for Biomedical Research and Massachusetts Institute of Technology, Cambridge: *AAS3* is a positive regulator of general amino acid control.

Winston, F., Fink, G.R., Whitehead Institute for Biomedical Research and Massachusetts Institute of Technology, Cambridge: Genes that affect Ty-mediated gene expression in yeast.

Farabaugh, P., Dept. of Microbiology, University of Connecticut Health Center, Farmington: Sequence and genetic analysis of the Ty912 transposable element.

Bowen, B.A., Fulton, A.M., Dobson, M.J., Tuite, M.F., Kingsman, S.M., Kingsman, A.J., Dept. of Biochemistry, Oxford, England: Expression of Ty-*lacZ* fusions in yeast.

Pearlman, R.E.,[1] Rose, A.,[1] Roeder, G.S.,[2] [1]Dept. of Biology, York University, Downsview, Ontario, Canada; [2]Dept. of Biology, Yale University, New Haven, Connecticut: Control of *HIS4* expression by Ty elements.

Baim, S.B., Sherman, F., Dept. of Radiation Biology and Biophysics, University of Rochester School of Medicine, New York: Mutations affecting the translation of *CYC1* mRNA.

Guarente, L., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Regulation of the *CYC1* gene of *S. cerevisiae.*

Lowry, C.V., Zitomer, R.S., Dept. of Biological Sciences, State University of New York, Albany: Regulatory mutations affecting oxygen-regulated expression of the *ANB1, CYC1,* and *tr-1* genes.

Cooper, T.G., Platoniotis, D., Yoo, H.-S., Rai, R., Chisholm, G., Genbauffe, F., Buckholz, R., Sumrada, R.A., University of Pittsburgh, Pennsylvania: Regulatory elements of nitrogen catabolic genes and their target sequences.

Klig, L.S., Henry, S.A., Dept. of Genetics and Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: *INO1* locus—Cloning and analysis of transcripts.

SESSION 8 *Regulation III*

Chairperson: J. Marmur, Albert Einstein College of Medicine, Bronx, New York

Welch, J.W.,[1] Fogel, S.,[1] Karin, M.,[2] [1]Dept. of Genetics, University of California, Berkeley; [2]Dept. of Microbiology, University of Southern California School of Medicine, Los Angeles: Molecular genetics of copper resistance in industrial yeast strains.

Woolford, J.L., Levy, A., Rotenberg, M.O., Larkin, J.C., Last, R., Dept. of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania: Structure and expression of yeast ribosomal protein genes.

Warner, J.R.,[1] Schwindinger, W.F.,[1] Mitra, G.,[1] Fried, H.M.,[2] Pearson, N.,[3] [1]Albert Einstein College of Medicine, Bronx, New York; [2]University of North Carolina, Chapel Hill; [3]University of Maryland, Catonsville: Varieties of regulatory experience.

Shaw, K.J.,[1] Olson, M.V.,[2] [1]Unigene Laboratories, Inc., Fairfield, New Jersey; [2]Dept. of Genetics, Washington University School of Medicine, St. Louis, Missouri: Role of 5′-flanking sequences in the in vivo expression of the *SUP4-o* gene.

Stillman, D.J., Sivertsen, A.L., Geiduschek, E.P., Dept. of Biology, University of California, San Diego, La Jolla: *S. cerevisiae* proteins binding to genes that are transcribed by RNA polymerase III.

Hitzeman, R., Chen, C., deBoer, H., Kastalein, R., Etcheverry, T., Oppermann, H., Dept. of Molecular

Biology, Genentech, Inc., South San Francisco, California: Factors affecting homologous and heterologous gene expression in yeast.

Valenzuela, P.,[1] Medina, M.A.,[1] Bishop, R.,[1] Najarian, R.C.,[1] Barr, P.J.,[1] Karin, M.,[2] [1]Chiron Research Laboratories, Chiron Corporation, Emeryville; [2]Dept. of Microbiology, University of Southern California School of Medicine, Los Angeles: Structure of the yeast *CUP1* locus and its use in the regulation of the expression of foreign proteins in yeast.

Miyajima, A., Miyajima, I., Arai, K., Arai, N., DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, California: Expression of plasmid-encoded R388 type-II dihydrofolate reductase gene as a dominant selection marker in *S. cerevisiae.*

Cohen, E.H., Kelly, J.D., Heinikoff, S., Fred Hutchinson Cancer Research Center, Seattle, Washington: Transcription termination control region in *S. cerevisiae.*

Saffer, L., Miller, O.L., Jr., Dept. of Biology, University of Virginia, Charlottesville: Electron microscope study of replication and its interaction with transcription in synchronized yeast cells.

Swanson, M., Holland, M., Dept. of Biological Chemistry, School of Medicine, University of California, Davis: Transcription of yeast ribosomal cistrons.

SESSION 9 *Poster Session*

Regulation

Potter, A.A.,[1] Nestmann, E.R.,[2] Iyer, V.N.,[1] [1]Dept. of Biology, Carleton University; [2]Mutagenesis Section, Dept. of National Health and Welfare, Ottawa, Ontario, Canada: Transfer of the pKM101-associated *muc* genes to *S. cerevisiae.*

Pall, M.L., Program in Genetics and Cell Biology, and Biochemistry/Biophysics, Washington State University, Pullman: cAMP stimulation of glycolysis and inhibition of gluconeogenesis—An evolutionarily conserved response.

Sutton, A., Broach, J.R., Dept. of Microbiology, State University of New York, Stony Brook: Precise mapping of 2u circle transcripts.

Willsky, G.R., Dosch, S.F., Dept. of Biochemistry, State University of New York, Buffalo: Vanadate-resistant mutants in *S. cerevisiae.*

Courchesne, W.E., Magasanik, B., Massachusetts Institute of Technology, Cambridge: Nitrogen regulation in *S. cerevisiae.*

Buckholz, R.G., Cooper, T.G., University of Pittsburgh, Pennsylvania: Oxalurate induction of multiple *URA3* transcripts in *S. cerevisiae.*

Rai, R., McKelvey, J., Cooper, T.G., University of Pittsburgh, Pennsylvania: Transport and metabolism of GABA, an alternative means of degrading glutamate to ammonia.

Genbauffe, F.S., Chisholm, G.E., Cooper, T.G., University of Pittsburgh, Pennsylvania: Isolation and characterization of two novel repeated DNA sequences in *S. cerevisiae.*

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Killer

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Kurjan, J., Dept. of Biological Sciences, Columbia University, New York, New York: Mutagenic analysis of two α-factor genes.

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Soidla, T.R., Dept. of Genetics, Leningrad University, USSR: One more way to make ends meet—A possible role of tRNA in splicing.

Mitochondria

Tabak, H.F., Osinga, K.A., Van der Bliek, A.M., De Vries, E., Groot Koerkamp, M.J.A., Section for Molecular Biology, Laboratory of Biochemistry, Amsterdam, The Netherlands: Initiation of transcription of yeast mtDNA.

Poutre, C.G., Fox, T.D., Section of Genetics and Development, Cornell University, Ithaca, New York: Characterization of a nuclear mutation, *petE11-1*, that affects mitochondrial gene expression and can be partially suppressed by a mitochondrial mutation.

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Nagley, P., Vaughan, P.R., Linnane, A.W., Dept. of Biochemistry, Monash University, Clayton, Victoria, Australia: Cotransformation of yeast with mtDNA and the plasmid YEp13.

Cumsky, M.G., Trueblood, C.E., McEwen, J.E., Ko, C., Poyton, R.O., Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: Molecular cloning of cytochrome *c* oxidase nuclear genes.

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Najarian, D.R., Martin, N.C., Dept. of Biochemistry, University of Texas Health Science Center, Dallas: Mitochondrial $tRNA^{Asp}$ biosynthesis in *syn*$^-$ and petite mutants of yeast.

Khan, N.A., Mendelsohn, A., Eisenberg, H., Gaskin, S., Dept. of Biology, Brooklyn College, New York: Concomitant reversion of the petitite mutants induced by manganese on glycerol and maltose plates in yeast.

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Secretion

Ramirez, R.M., Atkinson, K.D., Dept. of Biology, University of California, Riverside: Secretion proceeds uncoupled from plasma membrane growth in sucrose-grown inositol-starved yeast.

Cuoto, J.R., Huffaker, T.C., Robbins, P.W., Dept. of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Cloning and expression of a mannosyl transferase in the N-linked protein glycosylation pathway.

Runge, K.W., Robbins, P.W., Dept. of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Two yeast mutants in asparagine-linked glycosylation.

Call, P., Schekman, R., Dept. of Biochemistry, University of California, Berkeley: Golgi isolation from a yeast secretory mutant—Presence of secreted and vacuolar enzymes.

Julius, D.J.,[1,2] Blair, L.C.,[1] Brake, A.J.,[3] Thorner, J.,[1] Depts. of [1]Microbiology and Immunology, [2]Biochemistry, University of California, Berkeley; [3]Chiron Corporation, Emeryville, California: Yeast pheromone biosynthesis—Overglycosylation of prepro-α-factor in *kex2* mutants.

Brown, P.A., Perlman, D., Halvorson, H.O., Rosenstiel Center, Brandeis University, Waltham, Massachusetts: Investigation of the role of the signal sequence in the secretion of yeast invertase.

Bitter, G.A., Chen, K.K., Banks, A.R., Lai, P.-H., Amgen, Thousand Oaks, California: Secretion of foreign peptides from *S. cerevisiae* directed by α-factor gene fusions.

Singh, A.,[1] Lugovoy, J.M.,[1] Chen, E.Y.,[1] Perry, J.,[2] Kohr, W.J.,[2] Hitzeman, R.A.,[1] Depts. of [1]Molecular Biology; [2]Protein Biochemistry, Genentech, Inc., South San Francisco, California: α-Factor genes and the synthesis and secretion of mammalian proteins in yeast.

Burn, V.,[1] Buckholz, R.,[2] Adams, B.,[3] Bostian, K.,[1] [1]Brown University, Providence, Rhode Island; [2]University of Pittsburgh, Pennsylvania; [3]University of Hawaii, Honolulu: Analysis of a putative processing mutant of yeast α-galactosidase.

Wise, J.A., Guthrie, C., University of California, San Francisco: Genetic analysis of the role of 7S RNA in protein secretion.

Heyer, W.-D.,[1] Amstutz, H.,[1] Szankasi, P.,[1] Kohli, J.,[1] Aebi, R.,[1] Gysler, C.,[1] Munz, P.,[1] Gamulin, V.,[2] Söll, D.,[2] Leupold, U.,[1] [1]Institute of General Microbiology, University of Bern, Switzerland; [2]Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Intergenic conversion in a family of serine tRNA genes in *S. pombe*.

Killer Workshop

SESSION 10 *Mating Type*

Chairperson: A. Klar, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Siliciano, P., Tatchell, K., Dept. of Biology, University of Pennsylvania, Philadelphia: Deletion mapping in the *MATα1-MATα2* intergenic region.

Brake, A.J.,[1] Najarian, R.C.,[1] Laybourn, P.J.,[1] Thorner, J.W., [2]Merryweather, J.P.,[1] [1]Chiron Research Laboratories, Chiron Corporation, Emeryville, California; [2]Dept. of Microbiology and Immunology, University of California, Berkeley: Identification and characterization of a structural gene for the yeast peptide mating pheromone α-factor.

Ammerer, G.,[1] Tatchell, K.,[2] Sprague, G.,[3] [1]Zymos Corp., Seattle, Washington; [2]Dept. of Biology, University of Pennsylvania, Pittsburgh; [3]Institute of Molecular Biology, University of Oregon, Eugene: Regulation of α-specific genes.

Hagen, D., McCaffrey, G., Sprague, G., Institute of Molecular Biology, University of Oregon, Eugene: Regulation of α-specific *STE3* gene and a possible role for the *STE3* product in mating.

Stetler, G.L., Thorner, J., Dept. of Microbiology and Immunology, University of California, Berkeley: Yeast pheromone action—Isolation and characterization of genes under transcriptional control by α-factor.

Beach, D., Klar, A., Cold Spring Harbor Laboratory, New York: Mating-type switching in fission yeast.

Stern, M., Jensen, R., Herskowitz, I., Dept. of Biochemistry and Biophysics, University of California, San Francisco: Five *SWI* genes are required for *HO* expression.

Haber, J., Stewart, S., Weiffenbach, B., Brandeis University, Waltham, Massachusetts: Effect of various mutations on homothallic switching of yeast mating-type genes.

Schnell, R., Kimmerly, W., Rine, J., Dept. of Biochemistry, University of California, Berkeley: Genetic interaction between the genes controlling expression of the silent mating-type genes in *S. cerevisiae*.

Hicks, J., Strathern, J., Klar, A., Abraham, J., Ivy, J., Kelly, M., Stephens, C., Cold Spring Harbor Laboratory, New York: Mating-type switching and control of gene expression.

Wilson, K.L., Herskowitz, I., Dept. of Biochemistry and Biophysics, University of California, San Francisco: Analysis of the *STE6* gene and its expression.

SESSION 11 *RNA Processing and tRNA Suppressors*

Chairperson: J. Abelson, University of California, San Diego, La Jolla, California

Gallwitz, D., Langford, C.J., Klinz, F.-J., Donath, C., Physiologisch-Chemisches Institut I, Universität Marburg, Lahnberge, Federal Republic of Germany: Splicing of RNA polymerase II transcripts in *S. cerevisiae* requires an intron-contained conserved sequence element.

Kaufer, N.F., Warner, J.R., Albert Einstein College of Medicine, Bronx, New York: Effect of intron deletions on the *cyh2* gene.

Teem, J.,[1] Pikielny, C.,[2] Rosbash, M.,[1] Depts. of [1]Biology, [2]Biochemistry, Brandeis University, Waltham, Massachusetts: A conserved sequence within mRNA introns is required for splicing and may be a counterpart of U1 snRNA of metazoans.

Parker, R., Guthrie, C., Dept. of Biochemistry and Biophysics, University of California, San Francisco: Genetic analysis of mRNA splicing in *S. cerevisiae*.

Hopper, A.K.,[1] Hurt, D.J.,[1] Morales, M.,[2] Martin, N.C.,[2] [1]Dept. of Biological Chemistry, M.S. Hershey Medical Center, Pennsylvania; [2]Dept. of Biochemistry, University of Texas Health Science Center, Dallas: Modification of both cytoplasmic and mitochondrial tRNAs is affected by single nuclear mutations.

Thompson, J.R.,[1] Last, R.,[2] Larkin, J.Z.,[2] Levy, A.,[2] Woolford, J.L.,[2] Fournier, M.J.,[1] [1]Dept. of Biochemistry, University of Massachusetts, Amherst; [2]Dept. of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania: A yeast snRNA is tightly linked to a ribosomal protein gene.

Tollervey, D., Wise, J.A., Dunn, E.J., Maloney, D., Guthrie, C., Dept. of Biochemistry and Biophysics, University of California, San Francisco: Yeast snRNAs—Characterization and genetic analyses of U2 and U4 analogs.

Cummins, C.M., Culbertson, M.R., Laboratories of Genetics and Molecular Biology, University of Wisconsin, Madison: Frameshift suppressor mutations outside the anticodon in proline tRNAs containing an intervening sequence.

Wakem, P.,[1] Consaul, S.,[1] Wilhelm, J.,[2] Sherman, F.,[1] Depts. of [1]Radiation Biology and Biophysics, [2]Microbiology, University of Rochester School of Medicine, New York: Isolation and characterization of omnipotent suppressors.

Willis, I., Chisholm, V., Pearson, D., Schaack, J., Frendewey, D., Söll, D., Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Second-site mutations in the *sup3-e* and *sup9-e* loci of *S. pombe*—New insights on transcription and processing of eukaryotic tRNAs.

SESSION 12 *Mitochondria*

Chairperson: F. Sherman, University of Rochester Medical Center, Rochester, New York

Haldi, M.L., Anziano, P.Q., Perlman, P.S., Genetics Dept. and MCD Program, Ohio State University, Columbus: New insights into *cis*-acting domains in yeast mitochondrial introns.

Novitski, C.E., John, U.P., Ooi, B.-G., McMullen, G.L., Macreadie, I.G., Maxwell, R.J., Choo, W.M., Watkins, L.C., Marzuki, S., Lukins, H.B., Linnane, A.W., Nagley, P., Dept. of Biochemistry, Monash University, Clayton, Victoria, Australia: Mitochondrial *aap1* gene coding for mitochondrial ATPase subunit 8—Nature of mutants and mitochondrial revertants.

Szekely, E., Montgomery, D., Dept. of Biochemistry, University of Texas Health Science Center, San Antonio: Glucose represses transcription of nuclear genes coding for mitochondrial components.

Prezant, T., Guarente, L., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Regulation of mitochondrial genes in *S. cerevisiae*.

Bonitz, S.,[1] Tzagoloff, A.,[2] [1]Cold Spring Harbor Laboratory, New York; [2]Dept. of Biological Sciences, Columbia University, New York, New York: Yeast nuclear gene required for the correct expression of a mitochondrial cytochrome oxidase gene.

Underbrink-Lyon, K., Miller, D., Ross, N., Martin, N., Dept. of Biochemistry, Division of Molecular Biology, University of Texas Health Science Center, Dallas: Deletion and restriction mapping of a mitochondrial gene required for yeast mitochondrial tRNA biosynthesis.

Mueller, P., Reif, M.K., Fox, T.D., Section of Genetics and Development, Cornell University, Ithaca, New York: The *pet494-1* mutation blocks a posttranscriptional step leading to accumulation of cytochrome oxidase subunit III, but it can be suppressed by a mitochondrial gene rearrangement.

McEwen, J.E., Ko., C., Cumsky, M.G., Forrester, H., Kloeckener-Gruissem, B., Poyton, R.O., Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: Genetics of mitochondrial membrane biogenesis in *S. cerevisiae*—Identification of mutants altered in nuclear structural genes for cytochrome oxidase subunits.

Grivell, L.A., Van Loon, A.P.G.M., De Haan, M., Maarse, A.C., Section for Molecular Biology of the Laboratory of Biochemistry, University of Amsterdam, The Netherlands: Protein import by mitochondria. Characterization and expression of genes coding for imported subunits of the ubiquinol-cytochrome *c* reductase.

McCammon, M.,[1] Geller, B.,[1] Emr, S.,[2] Douglas, M.,[1] [1]Dept. of Biochemistry, University of Texas Health Science Center, San Antonio; [2]Dept. of Biology, California Institute of Technology, Pasadena: Targeting and mitochondrial import of *ATP2-lacZ* fusion protein.

Sor, F.,[1] Bolotin-Fukuhara, M.,[2] Fukuhara, H.,[1] [1]Institut Curie, Section de Biologie; [2]Laboratoire de Biologie Générale, Faculté des Sciences, Orsay, France: Mutations of the mitochondrial gene coding for the large rRNA—Identification and localization on the secondary structure model of the RNA.

SESSION 13 *Secretion and Protein Localization*

Chairperson: E.W. Jones, Carnegie-Mellon University, Pittsburgh, Pennsylvania

Emr, S.,[1] Schekman, R.,[1] Flessel, M.C.,[2] Thorner, J.,[2] Depts. of [1]Biochemistry, [2]Microbiology and Immunology, University of California, Berkeley: Yeast pheromone biosynthesis—Use of an α-factor-invertase (*MFα1-SUC2*) gene fusion to study protein localization and cell-type-specific gene expression.

Jones, E.W.,[1] Hospodar, M.,[1] Stevens, T.,[2] [1]Dept. of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania; [2]Dept. of Biochemistry, University of Califor-

nia, Berkeley: Mutations of yeast that affect processing of vacuolar enzyme precursors.

Stevens, T.H.,[1] Emr, S.D.,[2] [1]Institute of Molecular Biology, University of Oregon, Eugene; [2]Division of Biology, California Institute of Technology, Pasadena: Intracellular localization of carboxypeptidase Y-*LacZ* fusion proteins in yeast.

Schauer, I., Emr, S., Schekman, R., Dept. of Biochemistry, University of California, Berkeley: *SUC2* mutants defective for secretion of active invertase.

Chang, C.N., Matteucci, M., Perry, J., Chen, C.Y., Hitzeman, R.A., Genentech, Inc., South San Francisco, California: Recognition and cleavage of hybrid invertase signals and mature forms of human interferon (IFN-α2) in yeast.

Moreland, R.B.,[1] Rozdzial, M.,[2] Hereford, L.,[1] [1]Dana-Farber Cancer Institute and Dept. of Microbiology and Genetics, Harvard Medical School, Boston, Massachusetts; [2]Dept. of Biology, University of California, Riverside: Nuclear localization of yeast histones.

Hall, M.N.,[1] Hereford, L.,[2] Herskowitz, I.,[1] [1]Dept. of Biochemistry and Biophysics, University of California, San Francisco; [2]Sidney Farber Cancer Institute, Boston, Massachusetts: Targeting of proteins to the nucleus.

Barnes, G., Hansen, W., Holcomb, C., Rine, J., Dept. of Biochemistry, University of California, Berkeley: Identification of genes and mutations affecting an enzyme of the endoplasmic reticulum.

Huffaker, T., Robbins, P.W., Dept. of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Mutants deficient in protein glycosylation.

Ogrydziak, D.M., Fukayam, J.W., Institute of Marine Resources, University of California, Davis: Precursors and processing of the alkaline extracellular protease of *S. lipolytica*.

PHAGE AND BACTERIAL REGULATORY MECHANISMS

August 23 – August 28

SESSION 1 *Regulatory Mechanisms I*

Danchin, A.,[1] Guiso, N.,[2] Roy, A.,[1] Ullmann, A.,[2] [1]Institut de Biologie Physico-Chimique, Paris; [2]Institut Pasteur, Paris, France: Structure and regulation of adenylate cyclase in *E. coli*.

Aiba, H., Radioisotope Laboratory, Faculty of Medicine, Kyoto University, Japan: CRP-cAMP regulates negatively the *cya* gene transcription in vitro.

Nieuwkoop, A.J., Boylan, S.A., Bender, R.A., Division of Biological Sciences, University of Michigan, Ann Arbor: Regulation of *hutUH* operon expression by the CAP-cAMP complex in *K. aerogenes* – "Double-negative" control.

Menzel, R., Gellert, M., NIADDK, National Institutes of Health, Bethesda, Maryland: Analysis of the control of *gyr*B gene expression.

Braun, R., O'Day, K., Wright, A., Dept. of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts: Regulation and autoregulation of the *dnaA* gene in *E. coli* K12.

Maloy, S., Menzel, R., Roth, J., Dept. of Biology, University of Utah, Salt Lake City: Genetic regulation by a membrane-bound protein – Cloning and characterization of the regulatory sites of the *put* operon.

Hu, J.C., Gross, C.A., Dept. of Bacteriology, University of Wisconsin, Madison: Mutations of the σ subunit of *E. coli* RNA polymerase that affect expression of the *ara* operon.

Mizusawa, S., Court, D., Gottesman, S., NCI, National Institutes of Health, Bethesda, Maryland: Cell division regulation – Transcription of the *sulA* gene and repression by LexA.

Nguyen, T., Postle, K., Isackson, D., Bertrand, K., Dept. of Microbiology, University of California, Irvine: Structure and regulation of the Tn*10* and pSC101 tetracycline-resistance determinants.

Wolf, R.E., Jr., Baker, H.V. II, Dept. of Biological Sciences, University of Maryland, Baltimore County, Catonsville: A site within the 6-phosphogluconate dehydrogenase-coding sequence for maximal growth-rate-dependent expression of *gnd-lac* gene fusions.

Garrett, S., Taylor, R.K., Sodergren, E.J., Silhavy, T.J., NCI, Frederick Cancer Research Facility, Frederick, Maryland: Regulation of porin expression in *E. coli* K12.

French, S.,[1] Martin, K.,[2] Patterson, T.,[2] Bauerle, R.,[1] Miller, O.L., Jr.,[1] [1]Dept. of Biology, University of Virginia, Charlottesville; [2]Cold Spring Harbor Laboratory, New York: Electron microscopic visualization of *trp* operon expression in *S. typhimurium*.

SESSION 2 *Regulatory Mechanisms II*

Magasanik, B., Hunt, T., Ueno-Nishio, S., Reitzer, L., Bueno, R., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Transcriptional and translational regulation of the complex *glnALG* operon.

Garges, S., Adhya, S., NCI, National Institutes of Health, Bethesda, Maryland: Regulation of expression of the *rho* gene of *E. coli*.

Barik, S., Bhattacharya, P., Das, A., University of Connecticut School of Medicine, Farmington: Regulation of *rho* – Alterations of the protein and depression of the gene activity.

Shigesada, K., Matsumoto, Y., Tsurushita, N., Hirano, M., Imai, M., Institute for Virus Research, Kyoto University, Japan: Attenuation control of the gene for transcription termination factor *rho* in *E. coli*.

Lupski, J., Ruiz, A.A., Godson, G.N., Biochemistry Dept., New York University Medical Center, New York: Regulation of the *rpsU-dnaG-rpoD* macromolecular synthesis operon of *E. coli* K12.

Grossman, A.D., Gross, C.A., Dept. of Bacteriology, University of Wisconsin, Madison: Regulation of the σ operon of *E. coli*.

Burton, Z.,[1] Watanabe, K.,[1] Gross, C.,[2] Burgess, R.,[1] [1]McArdle Laboratory for Cancer Research; [2]Dept. of Bacteriology, University of Wisconsin, Madison: Structure of the operon that encodes the sigma subunit of RNA polymerase from *S. typhimurium*.

Guidi-Rontani, C.,[1] Danchin, A.,[2] Ullmann, A.,[1] [1]Institut Pasteur; [2]Institut de Biologie Physico-Chimique, Paris, France: Transcriptional control of polarity in lactose and galactose operons in *E. coli* by cAMP.

Zengel, J.M., Archer, R.H., Lindahl, L., Dept. of Biology, University of Rochester, New York: Attenuation control of the S10 ribosomal protein operon.

Mackie, G.A., Parsons, G.D., Dept. of Biochemistry, University of Western Ontario, London, Canada: Structure and expression of the gene for ribosomal protein S20.

Cerretti, D.P., Nomura, M., Institute for Enzyme Research, University of Wisconsin, Madison: The *spc* operon of *E. coli*—Localization of the S8 repressor target site in the S8 translational control unit.

SESSION 3 *Regulatory Mechanisms III*

Sambucetti, L., Silverman, P., Albert Einstein College of Medicine, Bronx, New York: Regulation of conjugative plasmid transfer gene expression in *E. coli*.

Krylov, V.N., Yanenko, A.S., Gorbunova, S.A., Institute for Genetics of Microorganisms, Moscow, USSR: RP4-promoted expression of a silent genetic material and SOS effects in *E. coli* and *P. aeruginosa*.

Brooks, J.E.,[1] Theriault, G.,[2] Gingeras, T.R.,[1] [1]Cold Spring Harbor Laboratory, New York; [2]Dept. of Biochemistry, Université Laval, Quebec, Canada: Control of expression of a type-II restriction-modification system from *P. aeruginosa*.

Bhagwat, A., Brooks, J., Starr, P., Gingeras, T., Cold Spring Harbor Laboratory, New York: Cloning and analysis of *Eco*RII restriction-modification genes.

Torres-Cabassa, A., Gottesman, S., NCI, National Institutes of Health, Bethesda, Maryland: *Ion* regulation of capsular polysaccharide synthesis in *E. coli* K12.

Strauch, K., Miller, C., Dept. of Molecular Biology and Microbiology, Case Western Reserve University, Cleveland, Ohio: *S. typhimurium* genes regulated by growth phase.

Young, F.S.,[1] Furano, A.V.,[2] Cashel, C.M., [1]Dept. of Molecular Biology, Genentech, Inc., South San Francisco, California; [2]NIADDK, [3]NICHHD, National Institutes of Health, Bethesda, Maryland: The *tufB* gene is essential for the regulation of growth by *E. coli*.

Sarmientos, P., Cashel, M., NICHHD, National Institutes of Health, Bethesda, Maryland: The *rrnA* downstream P2 promoter behaves as a maintenance promoter during growth rate control and carbon starvation.

Shinagawa, H., Makino, K., Amemura, M., Nakata, A., Research Institute for Microbial Diseases, Osaka University, Japan: Regulation of the phosphate regulon in *E. coli*.

Lau, E.T.,[1] Landick, R.,[2] Van-Bogelen, R.A.,[1] Neidhardt, F.C.,[1] [1]Dept. of Microbiology and Immunology; [2]Dept. of Biological Chemistry, University of Michigan, Ann Arbor: Regulatory gene for the heat-shock response in *E. coli*.

Woolford, C.,[1] Hendrix, R.,[1] Tilly, K.,[2] Georgopoulos, C.,[3] [1]Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania; [2]Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts; [3]Dept. of Cellular, Viral, and Molecular Biology, University of Utah Medical Center, Salt Lake City: Studies on the *E. coli* heat-shock genes, *groEL* and *groES*.

Freundlich, M., Friden, P., Tsui, P., Dept. of Biochemistry, State University of New York, Stony Brook: IHF—A global regulator in *E. coli*?

SESSION 4 Nus*ness*

Franklin, N.C., Biology Dept., University of Utah, Salt Lake City: *N* antitermination proteins of λ, ϕ21, and P22—Initial probings by comparison and by mutation.

Schauer, A.T., Friedman, D.I., Dept. of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor: λ and P22 antiterminator interactions with phage sites and host factors.

Olson, E.R.,[1] Tomich, C.-S.C.,[2] Friedman, D.I.,[1] [1]Dept. of Microbiology and Immunology, University of Michigan, Ann Arbor; [2]Molecular Biology Research, Upjohn Co., Kalamazoo, Michigan: Evidence that a unique nucleotide sequence, "boxA," is involved in the action of the *nusA*-gene product.

Haber, R.,[1] Levin, J.,[1] Carver, D.,[2] Friedman, D.,[2] Adhya, S.,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]University of Michigan, Ann Arbor: Characterization of the *nusA* gene of *E. coli*.

Shoemaker, K., Wolska, K., Das, A., University of Connecticut School of Medicine, Farmington: Modulation of transcription termination by *nus*-gene products.

Warren, F., Shoemaker, K., Das, A., University of Connecticut School of Medicine, Farmington: Formation of termination-resistant transcription complex at *nut* locus—Evidence bearing on a tentative model.

Greenblatt, J., Li, J., Dulhanty, A., Banting and Best Dept. of Medical Research, University of Toronto, Canada: Interactions among the *nusA* and RNA polymerase of *E. coli* and the *N*-gene protein of bacteriophage λ.

Lau, L.F., Roberts, J.W., Wu, R., Dept. of Biochemistry, Cell and Molecular Biology, Cornell University, Ithaca, New York: Transcription termination at λtR1.

Zuber, M., Court, D., NCI, National Institutes of Health, Bethesda, Maryland: Deletion analysis of *nut*R and *t*R1 sites of phage λ.

Leason, K.R., Baumann, M., Friedman, D.I., Dept. of Microbiology and Immunology, University of Michigan, Ann Arbor: Analysis of the *nin* region.

Kröger, M., Decker, T., Hobom, G., Universität Frieburg, Institut für

Biologie, Federal Republic of Germany: Control mechanism for the expression of bacteriophage λ late control gene *Q*.

Grayhack, E.J., Lau, L., Hart, C., Goliger, J., Verner, K., Yang, X., Roberts, J.W., Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Antitermination by purified λ *Q*-gene protein in vitro.

Chinali, G., Sarmientos, P., Glaser, G., Mozer, B.A., Cashel, M, NICHHD, National Institutes of Health, Bethesda, Maryland: Ribosomal RNA operon transcription termination and antitermination.

Gottesman, M.E.,[1] Chinali, G.,[2] Sarmientos, P.,[2] Mozer, B.,[2] Cashel, M.,[2] [1]NCI; [2]NICHHD, National Institutes of Health, Bethesda, Maryland: A plasmid *rrn*A clone that restricts phage λ growth.

Malik, S., Goldfarb, A., Dept. of Microbiology, Columbia University College of Physicians and Surgeons, New York, New York: Effect of bacteriophage-T4-induced modification of RNA polymerase on *rho*-dependent and *rho*-independent termination.

SESSION 5 *Poster Session*

Adley, C.C., Bukhari, A.I., Cold Spring Harbor Laboratory, New York: Methylation-dependent expression of the *mom* gene of bacteriophage Mu—The promoter is located about 200 bp downstream from the methylation sites.

Bear, S.E.,[1] Court, D.L.,[2] Friedman, D.,[2] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Microbiology, University of Michigan Medical School, Ann Arbor: Characterization of a λ mutant that grows with reduced efficiency on integration host factor (IHF) mutants of *E. coli*.

Brown, A.,[1] Drahos, D.,[2] Szybalski, W.,[1] [1]McArdle Laboratory for Cancer Research, University of Wisconsin, Madison; [2]Corporate Research Laboratory, Monsanto Co., St. Louis, Missouri: Synthesis and effect of transcription antitermination of the postulated NusA-protein-binding sequence of *E. coli* phage λ.

Bussman, K., Pelzer-Reith, B., Rasched, I., Fakultät für Biologie, Universität Konstanz, Federal Republic of Germany: Bacteriophage fd—Amplifying the expression of gene III (adsorption protein).

Chattoraj, D.K.,[1] Das, A.,[2] Cordes, K.,[1] [1]NCI, Frederick Cancer Research Facility, Frederick, Maryland; [2]University of Connecticut Health Center, Farmington: Mutagenesis and mutation transfer induced by UV in plasmid-cloned DNA.

Craig, N.L., Nash, H.A., NIMH, National Institutes of Health, Bethesda, Maryland: *att* sites juxtaposition during phage λ site-specific recombination—Collison versus sliding.

Craigie, R.A., Mizuuchi, K., NIADDK, National Institutes of Health, Bethesda, Maryland: Cloning of bacteriophage Mu A and B proteins.

Daniels, D., Nguyen, T., Moyed, H., Bertrand, K., Dept. of Microbiology, University of California, Irvine: Mutations in the divergent overlapping promoters that control Tn*10 tetA* and *tetR*.

Eliason, J.L., Ptashne, M., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: The bases in the phage λ operators recognized by repressor and Cro—A mutational study.

Franklin, N.C., Biology Dept., University of Utah, Salt Lake City: Conservation of structure but not sequence in the DNA coding for transcription of the *N* region in bacteriophages λ, φ21, and P22.

Freedman, L.P., Zengel, J.M., Lindahl, L., Dept. of Biology, University of Rochester, New York: Study of autogenous regulation of the S10 operon of *E. coli* using gene fusion plasmids.

Goldfarb, A., Malik, S., Dept. of Microbiology, Columbia University College of Physicians and Surgeons, New York, New York: New promoter specificity resulting from bacteriophage-T4-induced modification of RNA polymerase.

Grundy, F.J., Howe, M.M., Dept. of Bacteriology, University of Wisconsin, Madison: Phage particle structures found in lysates of Mu amber mutants defective in essential genes.

Guzmán, P., Guarneros, G., Dept. of Genetics and Molecular Biology, Centro de Investigación y de Estudios Avanzaos del IPN, Mexico City, Mexico: Four regions in λ phage genome are involved in *rap* exclusion by *E. coli*.

Hodgson, D.A., Shapiro, L., Amemiya, K., Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Induction of a protein kinase after infection of *C. crescentus* by bacteriophage φCd1.

Hyman, D., Malone, C., Rothman-Denes, L.B., Dept. of Biophysics and Theoretical Biology, University of Chicago, Illinois: Characterization of bacteriophage N4 deletion mutants.

Koop, A.H., Bourgeois, S., Regulatory Biology Laboratory, Salk Institute, San Diego, California: The isolation and characterization of cAMP receptor protein mutants in *E. coli*.

Kutsukake, K., Dept. of Biology, University of Tokyo, Japan: Invertible DNA in *E. coli*.

Lifson, E.,[1] Zengel, J.,[2] Lindahl, L.,[2] Depts. of [1]Microbiology, [2]Biology, University of Rochester, New York: An altered form of EFTu from *E. coli* is associated with the outer membrane.

Lin, C.-S.,[1] Six, E.,[1] Christie, G.E.,[2] Dale, E.,[2] Calendar, R.,[2] [1]Microbiology Dept., University of Iowa, Iowa City; [2]Molecular Biology Dept., University of California, Berkeley: Organization of the late gene cluster of bacteriophage P4.

Lopez, J., Webster, R.E., Dept. of Biochemistry, Duke University Medical Center, Durham, North Carolina: Filamentous phage f1 extrusion occurs at adhesion zones between the inner and outer membranes of the host cell.

Lynn, S.P.,[1] Burton, W.,[2] Gould, R.,[2] Gumport, R.I.,[2] Gardner, J.F.,[1] Depts. of [1]Microbiology, [2]Biochemistry, University of Illinois, Urbana: Oligodeoxyribonucleotide site-directed mutagenesis of the threonine operon regulatory region.

Patterson, T.A.,[1] Bauerle, R.H.,[2] [1]Cold Spring Harbor Laboratory, New York; [2]University of Virginia, Charlottesville: A system for in vivo cloning of mutant and wild-type genes of the *S. typhimurium trp* operon.

Peterson, K.R.,[1] Mount, D.W.,[1] [1]Dept. of Molecular and Medical Microbiology, University of Arizona, Tucson: Characterization of noncleavable and hypocleavable mutants of the *E. coli lexA* gene.

Shultz, J., Berman, M., NCI, Frederick Cancer Research Facility, Frederick, Maryland: Construction of an *E. coli* tRNA suppressor gene regulated by the *lac* operon promoter/operator.

Spanier, J.G., Cleary, P.P., Dept. of Microbiology, University of Minnesota, Minneapolis: Terminal redundancy and cycle permutation in the group A streptococcal bacteriophage SP24.

Sung, W.,[1] Brousseau, R.,[1] Narang, S.,[1] Patterson, T.,[2] Bukhari, A.I.,[2] [1]National Research Council of Canada, Ottawa; [2]Cold Spring Harbor Laboratory, New York: Transposition of IS*5*.

Whalen, W.A., Berg, C.M., Biological Sciences Group, University of Connecticut, Storrs: *avtA* is under repressor control in *E. coli* K12.

SESSION 6 *Regulatory Mechanisms IV*

Basu, S., Sarkar, P., Adhya, S., Sengupta, D., Maitra, U., Albert Einstein College of Medicine, Bronx, New York: Locations and nucleotide sequences of promoters for bacteriophage T3 RNA polymerase on T3 DNA.

Markiewicz, P., Haynes, L.,[1] Chase, J.,[2] Rothman-Denes, L.B.,[1] [1]Dept. of Biophysics and Theoretical Biology, University of Chicago, Illinois; [2]Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Promoters for the bacteriophage N4 virion-encapsulated RNA polymerase.

Reyes, O., Section de Radiobiologie Cellulaire, Laboratoire d'Enzymologie du CNRS, Gif-sur-Yvette, France: Toward a physiological understanding of bacteriophage ϕ80 induction—A preliminary description of some regulatory loci.

Christie, G.E., Calendar, R., Dept. of Molecular Biology, University of California, Berkeley: Common features of bacteriophage P2 late gene promoters.

Lagos, R., Godstein, R., Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Functions of satellite phage P4 affecting plasmid copy number.

Abeles, A., Austin, S., Chattoraj, D., Laboratory of Molecular Biology, NCI, Frederick Cancer Research Facility, Frederick, Maryland: P1 plasmid maintenance.

Blumer, K.J., Steege, D.A., Dept. of Biochemistry, Duke University Medical Center, Durham, North Carolina: Host cell enzymatic activity processes bacteriophage f1 mRNAs.

Guarneros, G.,[1] Galindo, J.M.,[1] Bear, S.,[2] Court, D.,[2] [1]Dept. of Genetics and Molecular Biology, CINVESTAV, Mexico City, Mexico; [2]NCI, National Institutes of Health, Bethesda, Maryland: Retrostimulation—Positive regulation of gene expression by a transcription terminator.

Oppenheim, A.B., Altuvia, S., Dept. of Molecular Genetics, Hebrew University-Hadassah Medical School, Jerusalem, Israel: Regulation of phage λ lysogenic response by the *E. coli rnc* gene.

Baker, T.A., Grossman, A.D., Gross, C.A., Dept. of Bacteriology, University of Wisconsin, Madison: Proteolysis defect in *htpR* mutants of *E. coli*.

Baker, T.A., Gross, C.A., Dept. of Bacteriology, University of Wisconsin, Madison: Identification of genetic loci involved in nonessential proteolysis in *E. coli* K12.

Burbee, D., Roberts, J.W., Field of Biochemistry, Cornell University, Ithaca, New York: Kinetics of LexA cleavage by RecA protease.

Hoyt, M.A.,[1] Banuett, F.,[2] Herskowitz, I.,[2] Echols, H.,[1] [1]Dept. of Molecular Biology, University of California, Berkeley; [2]Dept. of Biochemistry and Biophysics, University of California, San Francisco: Control of phage λ development by stability of cII protein—Characterization of the host *hflA* degradative function.

SESSION 7 *Protein Processing and Phage Morphogenesis*

Molineux, I.J., Schmitt, M., Condreay, J.P., Dept. of Microbiology, University of Texas, Austin: Mutants of bacteriophage T3 that are subject to F-mediated restriction.

Karam, J., Trojanowska, M., Dawson, M., Alford, C., Dept. of Biochemistry, Medical University of South Carolina, Charleston: Molecular cloning, nucleotide sequence, and expression of the *regA* gene of T4 phage.

Macdonald, P.,[1] Kutter, E.,[2] Mosig, G.,[1] [1]Dept. of Molecular Biology, Vanderbilt University, Nashville, Tennessee; [2]Evergreen State College, Olympia, Washington: Regulation of a late bacteriophage T4 gene, *soc*, which maps in an early region.

McGraw, T., MacKenzie, G., Mindich, L., Public Health Research Institute of the City of New York, Inc., New York: cDNA cloning and sequencing of the ϕ6 genome.

Putterman, D.G.,[1] Boyle, P.D.,[1] Casadevall, A.,[1] Yang, H.-L.,[1] Frangione, B.,[2] Marzec, C.J.,[1] Day, L.A.,[1] [1]Public Health Research Institute of the City of New York; [2]Dept. of Pathology, New York University School of Medicine, New York: Genomic sequence of the filamentous phage Pf3.

Krikos, A.,[1] Mutoh, N.,[1] Boyd, A.,[2] Simon, M.,[1] [1]Division of Biology, California Institute of Technology, Pasadena; [2]Leicester Biocentre, Medical Sciences Building, England: Sensory transducers of *E. coli*—Structural and functional domains.

Normark, S.,[1] Norgren, M.,[1] Lark, D.,[2] O'Hanley, P.,[2] Schoolnik, G.,[2] Uhlin, B.E.,[1] Svanborg-Eden, C.,[3] Falkow, S.,[2] Goldstein, R.,[4] [1]University of Umea, Sweden; [2]Stanford University School of Medicine, California; [3]University of Goteborg, Sweden; [4]Harvard Medical School, Boston, Massachusetts: Adhesion mediated by the Pap pili operon can be genetically separated from the presence of Pap pili fibers.

Berget, P.B., Schwarz, J., Chidambaram, M., Dept. of Biochemistry and Molecular Biology, University of Texas Medical School, Houston: Assembly-defective phage P22 tail protein mutants.

Casjens, S., Hayden, M., Dept. of Cellular, Viral and Molecular Biology, University of Utah Medical Center, Salt Lake City: Bacteriophage P22 headful DNA packaging.

Sternberg, N., Hoess, R., NCI, Frederick Cancer Research Facility, Frederick, Maryland: The P1 *pac*-cutting system—Possible involvement in phage replication.

Mindich, L.,[1] Bamford, D.,[2] McGraw, T.,[1] [1]Public Health Research

Institute of the City of New York, Inc., New York; [2] University of Helsinki, Finland: Bacteriophage PRD1 has terminal proteins on its DNA.

Rosenberg, S.M.,[1] Stahl, M.M.,[1] Kobayashi, I.,[1] Leach, D.R.F.,[2] Stahl, F.W.,[1] [1]Institute of Molecular Biology, University of Oregon, Eugene; [2]Dept. of Molecular Biology, University of Edinburgh, Scotland: In vitro packaging of λ DNA—A one-component *cos*-less packaging system free from endogenous phages.

Frackman, S., Siegele, D.A., Momany, T., Feiss, M., Dept. of Microbiology, University of Iowa, Iowa City: Structure of λ terminase.

Murialdo, H., Fife, W., Becker, A., Dept. of Medical Genetics, University of Toronto, Canada: λ*cos*-cutting in vivo in the absence of proheads.

SESSION 8 *Tricks*

Gottesman, M.E., Court, D., NCI, National Institutes of Health, Bethesda, Maryland: A λ vector for the cloning of prokaryotic promoters.

Wang, A., Roth, J., Dept. of Biology, University of Utah, Salt Lake City: Activation of silent genes by transposons Tn*5* and Tn*10*.

Bremer, E., Silhavy, T.J., Weisemann, J.M., Weinstock, G.M., NCI, Fredrick Cancer Research Facility, Frederick, Maryland: A λ-Mu-*lac* hybrid phage for creating *lacZ* gene fusions in a single step.

Hughes, K.T., Roth, J.R., Dept. of Biology, University of Utah, Salt Lake City: Mu-*d*8, a conditionally transposition-defective derivative of Mu-*d*1(Ap, *lac*).

Berg, C.M., Simpson, D.A., Biological Science Group, University of Connecticut, Storrs: Bacteriophage P1 as a vector for the Tn*5* mutagenesis in the Enterobacteriaceae.

Reynolds, A.E.,[1] Wright, A.,[2] [1]Dana-Farber Cancer Research Institute, Boston; [2]Tufts University, Boston, Massachusetts: Characterization of *bglR* mutations that activate the cryptic *bgl* operon of *E. coli* K12.

Wertman, K.F, Mount, D.W., Dept. of Molecular and Medical Microbiology, University of Arizona, Tucson: Gene fusions on bacteriophage M13 allow for rapid mutational analysis of regulatory loci in *E. coli* K12.

SESSION 9 *DNA-Protein Interactions*

Mandecki, W.,[1] Caruthers, M.H.,[2] [1]Dept. of Molecular Biology, Abbott Laboratories, North Chicago, Illinois; [2]Dept. of Chemistry, University of Colorado, Boulder: Large insertions and deletions between the CAP site and the −35 region of the *lac* promoter.

Cossart, P., Gicquel-Sanzey, B., Dépt. de Biochimie et Génétique Moléculaire, Institut Pasteur, Paris, France: A genetic and structural study of the cAMP receptor protein of *E. coli*.

Ebright, R.H.,[1] Beckwith, J.,[1] Cossart, P.,[2] Gicquel-Sanzey, B.,[2] [1]Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusets; [2]Unite de Biochimie Cellulaire, Institut Pasteur, Paris, France: Mutations that alter the DNA-sequence specificity of the catabolite gene activator protein (CAP) of *E. coli*.

Adhya, S., Irani, M., Orosz, L., Majumdar, A., NCI, National Institutes of Health, Bethesda, Maryland: Use of repressor titration to isolate operator mutations—An operator within a *gal* structural gene.

Majumdar, A., Adhya, S., NCI, National Institutes of Health, Bethesda, Maryland: Repressor operator interaction in the *gal* operon of *E. coli*.

Kelley, R., Yanofsky, C., Dept. of Biological Sciences, Stanford University, California: Mapping functional domains of the *E. coli* tryptophan repressor by in vitro mutagenesis.

Hahn, S., Dunn, T.M., Schleif, R., Dept. of Biochemistry, Brandeis University, Waltham, Massachusetts: A site required for repression of the *E. coli* L-arabinose operon, which lies 270 bp upstream from the start of transcription.

Hendrickson, W., Schleif, R., Dept. of Biochemistry, Brandeis University, Waltham, Massachusetts: *E. coli araC* protein binding to the *araBAD* promoter sites assayed by the altered migration of DNA-protein complexes in polyacrylamide gels.

Youderian, P.,[1] Bouvier, S.,[1] Susskind, M.,[1] Vershon, D.,[2] Sauer, R.,[2] [1]Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical School; [2]Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Altered specificity repressor mutations.

Vershon, A.,[1] Youderian, P.,[2] Susskind, M.,[2] Sauer, R.,[1] [1]Dept. of Biology, Massachusetts Institute of Technology, Cambridge; [2]Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester: The Mnt and Arc proteins of bacteriophage P22.

Pakula, A.A., Sauer, R.T., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Lambda *cro* mutants.

Nelson, H.C.M., Hecht, M.H., Sauer, R.T., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Interaction of mutant λ repressors with operator DNA.

Kang, I., Fien, K., Wulff, D.L., Dept. of Biological Sciences, State University of New York, Albany: The *c*II proteins of phages λ and 21 are highly specific for their respective p_{RE} promoters.

Shih, M.-C., Gussin, G.N., Genetics Ph.D. Program and Zoology Dept., University of Iowa, Iowa City: Analysis of mutations affecting cII activation of the λ p_{RE} promoter.

Hoopes, B.C., McClure, W.R., Dept. of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania: Activation of the λ promoters p_E and p_I by λ *c*II protein.

SESSION 10 *DNA Replication and Recombination*

Maurer, R.,[1,2] Osmond, B.C.,[1] Botstein, D.,[1] [1]Dept. of Biology, Massachusetts Institute of Technology, Cambridge; [2]Dept. of Molecular Biology and Microbiology, Case Western Reserve University School of Medicine, Cleveland, Ohio: Interaction of the *dnaQ* and *dnaE* proteins of *Salmonella*.

Engstrom, J., Maurer, R., Dept. of Molecular Biology and Microbiology, Case Western Reserve University School of Medicine, Cleveland, Ohio: Genetics of the *dnaX* and *dnaZ* genes of *S. typhimurium*.

Zylicz, M., Georgopoulos, C., Dept. of Cellular, Viral and Molecular Biology, University of Utah Medical Center, Salt Lake City: Properties of the *dnaK* protein of *E. coli*.

Johnston, S.A., Ray, D.S., Molecular Biology Institute, University of California, Los Angeles: Identification of a DNA replication enhancer sequence in the genome of bacteriophage M13.

Dotto, G.P., Horiuchi, K., Zinder, N.D., Rockefeller University, New York, New York: The functional origin of bacteriophage f1 DNA replication—Its signals and domains.

Roth, M., Brown, D., Schmidt-Glenewinkel, T., Hu, M., Belgado, N., Goetz, G., Hurwitz, J., Albert Einstein College of Medicine, Bronx, New York: Replication of ϕX174 DNA—Characterization of the ϕX A protein-DNA complex.

Mosig, G., Macdonald, P., Lin, G., Levin, M., Seaby, R., Dept. of Molecular Biology, Vanderbilt University, Nashville, Tennessee: Gene expression and initiation of DNA replication of bacteriophage T4 in phage and host topoisomerase mutants.

Kreuzer, K.N., Alberts, B.M., Dept. of Biochemistry and Biophysics, University of California, San Francisco: Specific recognition of bacteriophage T4 DNA by the T4-induced type-II DNA topoisomerase.

Zahn, K., Blattner, F.R., Dept. of Genetics, University of Wisconsin, Madison: DNA binding properties of phage ϕO protein.

Poteete, A.,[1] Sauer, R.,[2] Hendrix, R.,[3] [1]University of Massachusetts Medical School, Worcester; [2]Massachusetts Institute of Technology, Cambridge; [3]University of Pittsburgh, Pennsylvania: Structure of the P22 Erf protein.

Fassler, J.S., Tessman, I., Tessman, E.S, Purdue University, West Lafayette, Indiana: Inviability of *rho-rep* and *rho-ssb* double mutants—A role for *rho* in DNA replication.

Fassler, J.S., Tessman, E.S., Tessman, E., Purdue University, West Lafayette, Indiana: *rep-rec* double mutants—A role for *recA* in DNA synthesis.

Chaudhury, A.M., Smith, G.R., Fred Hutchinson Cancer Research Center, Seattle, Washington: Novel recombination-proficient *recB* mutants of *E. coli* lacking ATP-dependent exonuclease activity.

Thaler, D.S., Stahl, M.M., Stahl, F.W., Institute of Molecular Biology, University of Oregon, Eugene: The *red* recombination pathway of λ has double-strand break repair activity; the *recA*, *recBC* pathway of *E. coli* does not.

SESSION 11 *Transposition and Site-specific Recombination*

Biel, S.W., Berg, D.E., Dept. of Microbiology, and Immunology, Washington University Medical School, St. Louis, Missouri: Two modes of IS*1*-mediated gene transposition—Conservative and replicative.

Sasakawa, C., Carle, G.F., Berg, D.E., Dept. of Microbiology, Washington University School of Medicine, St. Louis, Missouri: The termini of IS*50*—Sequences essential for transposition.

Bukhari, A.I.,[1] Godson, G.N.,[2] [1]Cold Spring Harbor Laboratory, New York: [2]Dept. of Biochemistry, New York University Medical School, New York: Transposase recognizes a site that is located 53 bp from the left of bacteriophage Mu.

Mizuuchi, M., Mizuuchi, K., NIADDK, National Institutes of Health, Bethesda, Maryland: Substrate requirement of bacteriophage Mu transposition.

Mizuuchi, K., NIADDK, National Institutes of Health, Bethesda, Maryland: In vitro transposition of bacteriophage Mu.

Pato, M.,[1] Waggoner, B.,[1] Reich, C.,[1] Howe, M.,[2] [1]National Jewish Hospital and Research Center, Denver, Colorado; [2]Dept. of Bacteriology, University of Wisconsin, Madison: Requirements for maximal levels of replication of bacteriophage Mu DNA.

Miller, J.L., Lynn, D.L., Obukowicz, M.G., Glasgow, A.C., Howe, M.M., Dept. of Bacteriology, University of Wisconsin, Madison: Mutants of λ::mini-Mu altered in Mu-specific integration, replication, and growth inhibition properties.

Roberts, D., Kleckner, N., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Host mutants of *E. coli* affected for Tn*10* transposition.

Plasterk, R.H.A., van de Putte, P., Laboratory of Molecular Genetics, State University of Leiden, The Netherlands: A DNA invertase and methylation-regulated DNA-modifying protein coded for by the Mu β region.

Flamm, E., Weisberg, R., NICHHD, National Institutes of Health, Bethesda, Maryland: Primary structure of the *hip* gene and its protein.

Craig, N.L., Nash, H.A., NIMH, National Institutes of Health, Bethesda, Maryland: Sequence-specific interaction of *E. coli* integration host factor with DNA.

Bear, S.E., Court, D.L., NCI, National Institutes of Health, Bethesda, Maryland: Characterization of λ*int* mutants—A negative complementation test.

Redfield, R.J., Campbell, A.M., Dept. of Biological Sciences, Stanford University, California: IS2 and the generation of cryptic λ prophages.

Summers, D., Sherratt, D., Institute of Genetics, Glasgow University, Scotland: Role of site-specific recombination in the stable maintenance of the natural plasmid ColE1.

SESSION 12 *Open Discussion*

MODERN APPROACHES TO VACCINES

August 31–September 4

SESSION 1 *Viral Structure and Function*

Chairperson: W.K. Joklik, Duke University Medical Center, Durham, North Carolina

Wilson, I.A.,[1] Skehel, J.J.,[2] Wiley, D.C.,[3] [1]Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California; [2]National Institute for Medical Research, London, England; [3]Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Three-dimensional structure of a viral antigen–Haemagglutinin glycoprotein of influenza virus.

Hogle, J.M., Dept. of Molecular Biology, Scripps Clinic and Research Foundation, La Jolla, California: High-resolution structural studies of poliovirus.

Joklik, W.K., Antzcak, J.B., Cashdollar, L.W., Chmelo, R., Gaillard, R.K., Lee, P.W.K., Li, J.K.K., Dept. of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina: Comparison of the genes of the three serotypes of reovirus and of the proteins that they encode.

Norrby, E.,[1] Varsanyi, T.,[1] Utter, G.,[1] Appel, M.,[2] [1]Dept. of Virology, Karolinska Institute School of Medicine, Stockholm, Sweden; [2]Dept. of Microbiology, New York State College of Veterinary Medicine, Cornell University, Ithaca, New York: Morphology and immunogenicity of purified measles virus envelope components.

Schild, G.C., Minor, P., Evans, P., Almond, J.W., National Institute for Biological Standards and Control, London, England: Molecular basis of antigenicity and virulence of poliovirus type 3.

Venkatesan, S., Elango, N., Satake, M., Camargo, E., NIAID, National Institutes of Health, Bethesda, Maryland: Organization and expression of human respiratory syncytial virus.

Both, G.W.,[1] Siegman, L.J.,[1] Atkinson, P.H.,[2] Poruchynsky, M.S.,[2] Kabcenell, A.K.,[2] Street, J.E.,[3] Gunn, P.R.,[3] Sato, F.,[3] Powell, K.F.H.,[3] Bellamy, A.R.,[3] [1]CSIRO, Molecular and Cellular Biology Unit, North Ryde, Australia; [2]Dept. of Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, New York; [3]Dept. of Cell Biology, University of Auckland, New Zealand: Analysis of cloned genes corresponding to the rotavirus group- and type-specific antigenic determinants.

Purcell, R.H., Feinstone, S.M., Daemer, R.J., Ticehurst, J.R., Baroudy, B.M., NIAID, National Institutes of Health, Bethesda, Maryland: Approaches to hepatitis A vaccine–The old and the new.

SESSION 2 *The Chemistry of Virus Neutralization*

Chairperson: E. Wimmer, State University of New York, Stony Brook, New York

Wimmer, E.,[1] Emini, E.A.,[2] Jameson, B.A.,[1] [1]Dept. of Microbiology, State University of New York, Stony Brook; [2]Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania: Poliovirus-specific synthetic peptides–Priming and induction of a neutralizing antibody response and identification of viral neutralizing antigenic sites.

Lerner, R.A., Wilson, I., Niman, H.L., Dept. of Molecular Biology, Scripps Clinic and Research Foundation, La Jolla, California: Learning the chemistry of antigen-antibody union.

Rowlands, D.J.,[1] Clarke, B.E.,[1] Carroll, A.R.,[1] Brown, F.,[1] Nicholson, B.H.,[2] Bittle, J.L.,[3] Houghten, R.A.,[3] Lerner, R.A.,[3] [1]Animal Virus Research Institute, Pirbright; [2]Biochemistry Dept., The University, Whiteknights Park, England; [3]Research Institute of Scripps Clinic, La Jolla, California: Chemical basis for variation in the major antigenic site eliciting neutralizing antibodies in FMDV.

Arnon, R., Shapira, M., Dept. of Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel: Anti-influenza synthetic vaccine.

Dreesman, G.R.,[1] Sparrow, J.T.,[2] Kennedy, R.C.,[1] Melnick, J.L.,[1] [1]Dept. of Virology and Epidemiology; [2]Dept. of Medicine, Baylor College of Medicine, Houston, Texas: Immunogenic and antigenic activity of a cyclic synthetic HBsAg peptide.

Gerin, J.,[1] Purcell, R.,[2] Lerner, R.,[3] [1]Georgetown University, Rockville, Maryland; [2]NIAID, National Institutes of Health, Bethesda, Maryland; [3]Research Institute of the Scripps Clinic, La Jolla, California: Chemically synthesized peptides of hepatitis B surface antigens–Immunogenicity and protective efficacy in chimpanzees.

Shih, J.W.-K.,[1] Gerety, R.L.,[1] Liu, D.T.,[1] Yajima, H.,[2] Fujii, N.,[2] Nomizu, M.,[2] Katakura, S.,[2] [1]NCDB, FDA, National Institutes of Health, Bethesda, Maryland; [2]Kyoto University, Japan: Immunogenicity of the unconjugated synthetic polypeptides of HBsAg.

Parker, J.M.R., Bhargave, S., Hodges, R.S., Dept. of Biochemistry and Medical Research Council of Canada, University of Alberta, Edmonton: A general method to prepare peptide conjugates.

SESSION 3 *Poster Session*

Arora, D.J.S., Justewicz, D.M., Mandeville, R., Institut Armand-Frappier, Université du Québec, Canada: In vitro stimulation of NK cell activity with purified influenza virus surface antigens.

Bittle, J.,[1] Worrell, P.,[1] Houghten, R.,[1] Brown, F.,[2] Lerner, R.,[1] [1]Dept. of Molecular Biology, Scripps Clinic and Research Foundation, La Jolla, California; [2]Animal Virus Research Institute, Pirbright, England: Immunization with a chemically synthesized peptide derived from FMDV polypeptide VP1.

Casali, P., Rice, P.A., Oldstone, M.B.A., Scripps Clinic and Research Foundation, La Jolla, California: Viruses perturb functions of human lymphocytes–Effects of measles virus and influenza virus on lymphocyte-mediated killing and antibody production.

Fox, G.M., Langley, D., Hu, S., Amgen, Inc., Thousand Oaks, California: Development of PPV subunit

vaccine by recombinant DNA methods.

Gallick, G.E., Brown, D.B., Murphy, E.C., Arlinghaus, R.B., University of Texas Science Center, M.D. Anderson Hospital, Houston: Inhibition of transformation in a cell line infected with a temperature-sensitive mutant of MSV by cytoplasmic microinjection of purified IgG from an antisera generated against a synthetic *v-mos* peptide.

Klemm, P., Gaastra, W., Josephsen, J., Petersen, J.K., Dept. of Microbiology, Technical University of Denmark, Lyngby: *E. coli* fimbriae as basis for vaccines.

McCahon, D., King, A.M.Q., Slade, W.R., Saunders, K., Newman, J.W.L., Animal Virus Research Institute, Pirbright, England: Study of the nature and extent of RNA recombination in a picornavirus (FMDV).

Morgan, A.J., Smith, A.R., Barker, R.N., Epstein, M.A., Dept. of Pathology, University of Bristol Medical School, England: Progress in the development of an EBV subunit vaccine.

Moore, D.,[1] Morgan, D.,[1] Robertson, B.,[1] McKercher, P.,[1] Patzer, E.,[2] Shire, S.,[2] Kleid, D.,[2] [1]Plum Island Animal Disease Center, Greenport, New York; [2]Genentech, Inc., South San Francisco, California: A highly antigenic portion of FMDV O_1 VP_1 elicits bovine antibodies that protect mice but not cattle from FMDV infection.

Murphy, B.,[1] Green, N.,[2] Alexander, S.,[2] Lerner, R.,[2] Chanock, R.,[1] [1]NIAID, National Institutes of Health, Bethesda, Maryland; [2]Research Institute of Scripps Clinic, La Jolla, California: Evaluation in hamsters of synthetic peptides representing different antigenic sites of the influenza A virus hemagglutinin.

Petteway, S.R., Ray, J., Ivanoff, L.A., Korant, B.D., E.I. Du Pont de Nemours and Co., Wilmington, Delaware: Expression of viral antigens in *E. coli*.

Rowlands, D.J.,[1] Clarke, B.E.,[1] Carroll, A.R.,[1] Brown, F.,[1] Nicholson, B.H.,[2] Bittle, J.L.,[3] Houghten, R.A.,[3] Lerner, R.A.,[3] [1]Animal Virus Research Institute, Pirbright; [2]Biochemistry Dept., The University, Whiteknights Park, England; [3]Research Institute of Scripps Clinic, La Jolla, California: Comparative structural studies of the antigenic sites of FMDV.

Sela, M., Jacob, C.O., Arnon, R., Dept. of Chemical Immunology, Weizman Institute of Science, Rehovot, Israel: Synthetic approaches to vaccination against bacterial toxins.

van der Marel, P.,[1] Osterhaus, A.D.M.E.,[1] van Steenis, G.,[1] van Wezel, A.L.,[1] Sundquist, B.,[2] Morein, B.,[2] [1]Rijsinstiuut voor de Volksgezondheid, Bilthoven, The Netherlands; [2]Dept. of Virology, National Veterinary Institute, Uppsala, Sweden: Toward a measles virus subunit vaccine.

SESSION 4 *Cloning and Expression of Viral Genes I*

Chairperson: F. Brown, Animal Virus Research Institute, Pirbright, England

Flores, J., Sereno, M., Kalica, A., Keith, J., Kapikian, A., Chanock, R., NIAID, National Institutes of Health, Bethesda, Maryland: Molecular cloning of rotavirus genes for use in immunoprophylaxis.

Nayak, D.P., Dept. of Microbiology and Immunology, University of California School of Medicine, Los Angeles: Immunological response to human influenza virus hemagglutinin expressed in *E. coli*.

De Wilde, M., Cabezon, T., Harford, N., Simoen, E., Rutgers, T., Van Wijnendaele, F., Genetic Research Dept., Smith Kline-RIT, Rixensart, Belgium: Hepatitis-B vaccine produced in yeast by R-DNA.

van der Marel, P.,[1] Hazendonk, A.G.,[1] Henneke, M.A.C.,[1] de Vries, F.A.J.,[1] Wieringa, B.,[1] van Wezel, A.L.,[1] Jore, J.,[2] Pouwels, P.H.,[2] Enger-Valk, B.E.,[2] [1]National Institute of Public Health, Bilthoven; [2]Medical Biological Laboratory, TNO, Rijswijk, The Netherlands: Expression in *E. coli* of capsid-protein VP1 of poliovirus type 1.

Leung, W.C., Jing, G.Z., Hasnain, S.E., Manavathu, E.K., Leung, M., Zwaagstra, J., Dept. of Medicine, University of Alberta, Edmunton, Canada: Expression of cDNA clones for HSV-1 and HSV-2 neutralization antigen glycoprotein gB.

Amann, E., Bröker, M., Wurm, F., Research Laboratories, Marburg, Federal Republic of Germany: High-level production in *E. coli* of hybrid proteins expressing parts of the coding sequences of HSV-1 glycoproteins gC and gD.

Carlson, J., Maxwell, I., Maxwell, F., McNab, A., Rushlow, K., Midbrand, M., Teramoto, Y., Winston, S., Syngene Products and Research, Fort Collins, Colorado: Expression of FPV antigens in *E. coli*.

Malek, L., Soostmeyer, G., James, E., Garvin, R.T., Genetic Engineering Group, Connaught Research Institute, Willowdale, Ontario: Expression of rabies glycoprotein gene in *E. coli*.

SESSION 5 *Cloning and Expression of Viral Genes II*

Chairperson: R. Purcell, National Institutes of Health, Bethesda, Maryland

Cavanagh, D., Binns, M., Boursnell, M.E.G., Brown, T.D.K., Dept. of Microbiology, Houghton Poultry Research Station, England: Genetically engineered vaccine to avian infectious bronchitis virus (IBV) with "live" and "killed" vaccine characteristics.

Hu, S., Bruszewski, J., Boone, T., Souza, L., Amgen, Inc., Thousand Oaks, California: Cloning and expression of the surface glycoprotein gp195 of porcine transmissible gastroenteritis virus (TGEV).

Bouges-Bocquet, B., Guesdon, J.L., Hofnung, M., INSERM, Institut Pasteur, Paris, France: In vitro genetic construction devised to express given antigenic determinants at the surface of bacteria.

Tiollais, P., INSERM, Institut Pasteur, Paris, France: Synthesis and excretion of HBsAg in animal cells.

Stratowa, C., Wang, Y., Hughes, J., Doehmer, J., Hofschneider, P.H., Max-Planck-Institut für Biochemie, Martinsried, Federal Republic of Germany: Construction of cell lines producing HBsAg using eukaryotic viral vectors.

Burnette, W.N., Samal, B., Bitter, G.A., Browne, J.K., Fenton, D., Amgen, Inc., Thousand Oaks, California: Production of hepatitis-B recombinant vaccines.

Wampler, D.E., Buynak, E., Harder, B.J., Herman, A.C., Hilleman, M.R., McAleer, W., Scolnick, E., Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania: HBsAg purification by immune affinity chromatography.

Chow, M., Baltimore, D., Whitehead Institute, Massachusetts Institute of Technology, Cambridge: Expression of poliovirus capsid proteins and antigenic activity of synthetic peptides.

SESSION 6 *Attenuation of Virulence I*

Chairperson: E. Norrby, Karolinska Institute of Virology, Stockholm, Sweden

Kilbourne, E.D., Mount Sinai School of Medicine of the City University of New York, New York: Immunization strategy—Infection-permissive vaccines for the modulation of infection.

Roizman, B., Marjorie B. Klover Oncology Laboratory, University of Chicago, Illinois: Genetic engineering of the herpes simplex virus genome for the purpose of immunoprophylaxis.

Fields, B.N.,[1] Sharpe, A.H.,[2] Spriggs, D.,[2] Greene, M.,[3] [1]Dept. of Medicine, Brigham and Women's Hospital, Boston; [2]Dept. of Microbiology; [3]Dept. of Pathology, Harvard Medical School, Boston, Massachusetts: Reovirus hemagglutinin—Specific attenuation and antigenic mimicry as approaches for immunization.

Flamand, A.,[1] Coulon, P.,[1] Pepin, M.,[2] Blancou, J.,[2] Rollin, P.,[3] [1]Université Paris-Sud; [2]Centre National d'Etude sur la Rage; [3]Institut Pasteur, Paris, France: Immunogenic and protective power of avirulent mutants of rabies virus selected with neutralizing monoclonal antibodies.

Paoletti, E., Panicali, D., Center for Laboratories and Research, New York State Department of Health, Albany: Genetically engineered poxviruses as live recombinant vaccines.

Mackett, M., Smith, G.L., Moss, B., NIAID, National Institutes of Health, Bethesda, Maryland: General method for the production and selection of infectious vaccinia virus recombinants expressing foreign genes.

Plotkin, S.A., Children's Hospital of Philadelphia, and Wistar Institute, Pennsylvania: Vaccination against human cytomegalovirus.

SESSION 7 *Attenuation of Virulence II*

Chairperson: I.A. Wilson, Scripps Research Foundation, La Jolla, California

Smith, G., Mackett, M., Murphy, B., Moss, B., NIAID, National Institutes of Health, Bethesda, Maryland: Vaccinia virus recombinants expressing genes from pathogenic agents have potential as live vaccines.

Greenberg, H., Midthun, K., Wyatt, R., Flores, J., Hoshino, Y., Chanock, R., Kapikian, A., NIAID, National Institutes of Health, Bethesda, Maryland: Use of reassortant rotaviruses and monoclonal antibodies to make gene-coding assignments and construct rotavirus vaccine candidates.

Murphy, B.,[1] Clements, M.,[2] Buckler-White, A.,[1] Tian, S.F.,[1] Maassab, H.F.,[3] London, W.,[4] Chanock, R.,[1] [1]NIAID, National Institutes of Health, Bethesda, Maryland; [2]Center for Vaccine Development, University of Maryland School of Medicine, Baltimore; [3]School of Public Health, University of Michigan, Ann Arbor; [4]NINCDS, National Institutes of Health, Bethesda: Attenuation of human influenza A viruses by genetic reassortment with attenuated donor viruses.

Lai, C.-J., Markoff, L., Sveda, M., Lin, B.-C., Chanock, R.M., NIAID, National Institutes of Health, Bethesda, Maryland: Engineering the genome of influenza virus for immunoprophylaxis—Progress and obstacles.

Rott, R., Scholtissek, C., Institut für Virologie, Justus-Liebig-Universität, Giessen, Federal Republic of Germany: Alterations in pathogenicity of influenza virus through reassortment.

Kew, O.M., Nottay, B.K., Division of Viral Diseases, Centers for Disease Control, Atlanta, Georgia: Evolution of the oral polio vaccine strains in humans occurs by both mutation and intramolecular recombination.

SESSION 8 *Enhancement of Antigenicity and Immunogenicity I*

Chairperson: E.D. Kilbourne, Mt. Sinai Medical Center, New York, New York

Morein, B.,[1] Sundquist, B.,[2] Dalsgaard, K.,[3] Osterhaus, A.,[4] [1]Institute of Microbiology, Swedish University of Agricultural Sciences, Uppsala, Sweden; [2]National Veterinary Institute for Virus Research, Kalvehave, Denmark; [4]National Institute of Health, Bilthoven, The Netherlands: Iscom, a cagelike immunostimulating complex of membrane proteins.

Hopp, T.P., Immunex Corporation, Seattle, Washington: Use of palmitic acid as a carrier for chemically synthesized vaccines.

Thibodeau, L.,[1] Perrin, P.,[2] Sureau, P.,[2] [1]Institut Armand-Frappier; [2]Université du Québec, Laval, Canada: Rabies immunosome—A new candidate for an RNA-free vaccine.

Sanderson, A.R., MRC Immunology Team, Guy's Hospital, London, England: MHCI molecules—Widely applicable purification technology and evidence for superior carrier function.

Howard, C.R., Young, P., Tsiquaye, K., Zuckerman, A.J., London School of Hygiene and Tropical Medicine, England: Development of hepatitis-B polypeptide micelle vaccines.

Gordon, L.K., Connaught Research Institute, Toronto, Canada; Connaught Laboratories, Swiftwater, Pennsylvania: Characterization of a hapten-carrier conjugate vaccine. *H. influenzae*-diphtheria conjugate vaccine, H-flu-VAX™.

Audibert, F., Chedid, L., Immunothérapie Expérimentale, Institut Pasteur, Paris, France: Of antigens, adjuvants, and carriers in synthetic vaccines.

SESSION 9 *Cloning and Expression of Viral Genes III*

Chairperson: J. Gerin, Georgetown University School of Medicine and Dentistry, Rockville, Maryland

Koprowski, H., Dietzschold, B., Macfarlan, R., Sutcliffe, J.G., Lerner, R.A., Wistar Institute, Philadelphia, Pennsylvania: Localization of the immunodominant domains of rabies virus glycoprotein.

Dietzschold, B., Cohen, G.H., Wister Institute, Philadelphia, Pennsylvania: Synthesis of an antigenic determinant of HSV glycoprotein D that stimulates production of virus-neutralizing antibody and confers protection against a lethal challange infection of HSV.

Valenzuela, P.,[1] Coit, D.,[1] Heberlein, U.,[1] Masiarz, F.R.,[1] Medina, M.A.,[1] Rosenberg, S.,[1] Tekamp-Olson, P.,[1] Burlingame, A.,[2] Rutter, W.J.,[2] [1]Chiron Research Laboratories, Chiron Corporation, Emeryville, California; [2]Dept. of Biochemistry, University of California, San Francisco: Characterization of HBsAg particles produced in yeast and in mammalian cells.

Kendal, A.,[1] Nakajima, S.,[2] Nakajima, K.,[2] Raymond, L.,[3] Caton, A.,[3] Brownlee, G.,[3] Webster, R.,[4] [1]Centers for Disease Control, Atlanta, Georgia; [2]Institute of Public Health, Tokyo, Japan; [3]University of Oxford, England; [4]St. Jude's Hospital, Memphis, Tennessee: Structures in influenza A/USSR/90/77 hemagglutinin associated with epidemiologic and antigenic change.

Gething, M.J.,[1] Braciale, T.J.,[2] Sambrook, J.F.,[1] [1]Cold Spring Harbor Laboratory, New York; [2]Washington University School of Medicine, St. Louis, Missouri: Comparison of different eukaryotic vector systems for the high-level expression of a cloned copy of the hemagglutinin glycoprotein of influenza virus.

SESSION 10 *Enhancement of Antigenicity and Immunogenicity II*

Chairperson: M. Oldstone, Scripps Clinic and Research Foundation, La Jolla, California

Mandeville, R., Justewicz, D.M., Lecomte, J., Institut Armand-Frappier, Université du Québec, Laval, Canada: Specific inhibition of influenza-virus-induced boosting of natural killer-cell activity by an anti-HA-specific monoclonal antibody.

Gallick, G.E.,[1] Sparrow, J.,[2] Stanker, L.H.,[1] Nash, M.A.,[1] Arlinghaus, R.B.,[1] [1]University of Texas Science Center, M.D. Anderson Hospital, Houston; [2]Dept. of Medicine, Baylor College of Medicine, Houston, Texas: Recognition of native proteins with antisera to synthetic peptides may depend on the size and conformation of the peptide.

Parker, J.M.R., Taneja, A.K., Worobec, E.A., Paranchych, W., Hodges, R.S., Dept. of Biochemistry and Medical Research Council of Canada, University of Alberta, Edmonton: Immunodominant region of EDP208 pili—A model system for the design of pilus synthetic vaccines.

Welling, G.W., Groen, G., Boer, T., Nijmeijer, J., van der Zee, R., Wilterdink, J.B., Welling-Wester, S., Laboratorium voor Medische Microbiologie, Rijksuniversiteit Groningen, The Netherlands: HPLC of viral proteins as a tool in the design of synthetic vaccines.

Kennedy, R.C.,[1] Sparrow, J.T.,[2] Sanchez, Y.,[1] Melnick, J.L.,[1] Dreesman, G.R.,[1] [1]Dept. of Virology and Epidemiology, [2]Dept. of Medicine, Baylor College of Medicine, Houston, Texas: Enhancement of the immune response to a cyclic synthetic HBsAg peptide by prior injection of anti-idiotype antibodies.

Trudel, M., Payment, P., Boudreault, A., Institut Armand-Frappier, Université du Québec, Laval, Canada: Efficiency of subunit vaccines—Case illustration with influenza immunosomes and rubella virosomes.

Summary: R. Chanock, National Institutes of Health, Bethesda, Maryland

THE CANCER CELL

September 8–September 13

Welcoming Remarks: J.D. Watson

SESSION 1 *src I*

Chairperson: G. Vande Woude, NCI, National Institutes of Health, Bethesda, Maryland

Hanafusa, H.,[1] Iba, H.,[1] Takeya, T.,[2] Cross, F.,[1] [1]Rockefeller University, New York, New York; [2]Institute for Chemical Research, Kyoto University, Japan: Transforming activity of the *c-src* gene.

Shalloway, D., Coussens, P.M., Yaciuk, P., Molecular and Cell Biology Program, Pennsylvania State University, University Park: Effect of *c-src* overexpression in mouse cells.

Parker, R.C., Swanstrom, R., Varmus, H.E., Bishop, J.M., Dept. of Microbiology, University of California, San Francisco: Cloning and expression of chicken *c-src* reveals structural and biological differences from *v-src*.

Graziani, Y.,[1] Maller, J.,[2] Sugimoto, Y.,[1] Erikson, R.L.,[1] [1]Dept. of Pathology, [2]Dept. of Pharmacology, University of Colorado School of Medicine, Denver: Modification of the activity of catalytic subunit of cAMP-dependent protein kinase by $pp60^{v\text{-}src}$.

Parsons, J.T., Bryant, D., Parsons, S.J., Wilkerson, V., Dept. of Microbiology, University of Virginia, Charlottesville: Site-directed mutagenesis of RSV $pp60^{src}$—Identification of functional domains required for transformation.

Brugge, J.S., Jarvis-Morar, M., Cotton, P., Lipsich, L., Yonemoto, W.,

Darrow, D., Dept. of Microbiology, State University of New York, Stony Brook: Structural and functional studies on the tyrosine kinase transforming proteins.

Kamps, M., Sefton, B., Molecular Biology and Virology Laboratory, Salk Institute, San Diego, California: Lysine 295 comprises part of the active site of pp60^{src}.

SESSION 2 *Growth Factors*

Chairperson: G. Todaro, Oncogen, Inc., Seattle, Washington

Sporn, M.B., Anziano, M.A., Assoian, R.K., De Larco, J.E., Frolik, C.A., Roberts, A.B., NCI, National Institutes of Health, Bethesda, Maryland: Isolation and characterization of transforming growth factors from human, bovine, and murine sources.

Cowley, J.,[1] Gustason, B.,[1] Smith, J.,[1] Hendler, F.,[2] Ozanne, B.,[1] [1]Ludwig Institute of Cancer Research, London, England; [2]University of Texas Health Science Center, Dallas: EGF receptor is expressed at high levels on squamous carcinomas.

Kamata, T., Feramisco, J.R., Cold Spring Harbor Laboratory, New York: Nucleotide effects on EGF receptors in normal and transformed cells—A relationship to Ha-*ras* oncogenes?

McClure, D.B.,[1] Dermody-Weisbrod, M.,[2] Topp, W.C.,[2] [1]Cancer Center, University of California, San Diego, La Jolla; [2]Cold Spring Harbor Laboratory, New York: In vitro correlates of tumorigenicity of REF52 cells transformed by SV40.

Waterfield, M.D.,[1] Scarce, G.T.,[1] Whittle, N.,[1] Stockwell, P.,[1] Stroobant, P.,[1] Johnsson, A.,[2] Wasteson, A.,[2] Westermark, B.,[2] Heldin, C.-H.,[2] Huang, J.S.,[3] Deuel, T.F.,[3] [1]Imperial Cancer Research Fund, London, England; [2]University of Uppsala, Sweden; [3]Washington University School of Medicine, Jewish Hospital of St. Louis, Missouri: Relationship between PDGF and the transforming protein of simian sarcoma virus.

Robbins, K.C.,[1] Antoniades, H.N.,[2] Devare, S.G.,[1] Hunkapiller, M.W.,[3] Aaronson, S.A.,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Center for Blood Research, Harvard School of Public Health, Boston, Massachusetts; [3]Division of Biology, California Institute of Technology, Pasadena: Structural and functional relationships between the transforming gene product(s) of a primate sarcoma virus and human PDGF.

Cochran, B.H., Reffel, A.C., Stiles, C.D., Dana-Farber Cancer Institute and Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Molecular cloning of intermittently firing gene sequences whose expression is regulated by platelet-derived growth factor.

SESSION 3 *Cytoskeleton*

Chairperson: K. Weber, Max-Planck Institute, Goettingen, Federal Republic of Germany

Matsumura, F., Lin, J.J.-C., Yamashiro-Matsumura, S., Cold Spring Harbor Laboratory, New York: Differential expression of tropomyosin forms in the microfilaments isolated from normal and transformed cultured cells.

Kakunaga, T.,[1] Taniguchi, S.,[1] Leavitt, J.,[2] Hamada, H.,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Linus Pauling Institute, Palo Alto, California: Point mutation and other changes in cytoplasmic actins associated with the expression of transformed phenotype.

Chen, L.B., Summerhayes, I.C., Weiss, M.J., Davis, S., Bernal, S.D., McIsaac, R.M., Lampidis, T.J., Nadakavukaren, K.K., Shepherd, E.L., Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Effect of altered cytoskeleton on mitochondrial distribution in tumor cells.

Albrecht-Buehler, G., Northwestern University Medical School, Chicago, Illinois: Movement of nucleus and centrosphere in animal tissue cells.

SESSION 4a *src II*

Chairperson: J. Brugge, State University of New York, Stony Brook, New York

Hunter, T., Cooper, J.A., Gould, K., Saris, C.J., Molecular Biology and Virology Laboratory, Salk Institute, San Diego, California: Tyrosine protein kinases and the control of cell proliferation.

Weber, M.J., Bishop, R.W., Crabb, G., Martinez, R., Monteagudo, C., Nakamura, K.D., Tondravi, M., Williams, D., Dept. of Microbiology, University of Illinois, Urbana: Phosphotyrosine-containing proteins in normal and transformed cells.

Goldberg, A.R., Krueger, J.G., Garber, E.A., Wong, T.W., Rockefeller University, New York, New York: Subcellular localization of viral and cellular tyrosyl protein kinases.

Martin, G.S., Radke, K., Carter, V.C., Moss, P., Dehazya, P., Gilmore, T., Young, J., Dept. of Zoology, University of California, Berkeley: Role of tyrosine phosphorylation in transformation and mitogenesis.

Greenberg, M.E., Brackenbury, R., Edelman, G.M., Rockefeller University, New York, New York: Changes in the tissue distribution of the 34-kD pp60^{src} substrate during differentiation and maturation.

SESSION 4b *Polyoma*

Chairperson: J. Sambrook, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Cuzin, F.,[1] Rassoulzadegan, M.,[1] Lemieux, L.,[1] Rouget, P.,[2] Renaud, J.F.,[1] [1]Centre de Biochimie du CNRS, Université de Nice; [2]Institut Jacques Monod, Paris, France: Functions of the three polyoma virus oncogenes in the transformation and immortalization of cells in culture.

Benjamin, T., Dept. of Pathology, Harvard Medical School, Boston, Massachusetts: The polyoma HR-T gene—An "oncogene" with a purpose.

Smith, A.E.,[1] Oostra, B.A.,[1] Kalderon, D.D.,[1] Belsham, G.J.,[1] Ely, B.K.,[1] Harvey, R.W.,[1] Markland, W.,[1] Markham, A.F.,[2] Paucha, E.,[1] Courtneidge, S.A.,[1] [1]Biochemistry Division, National Institute for Medical Research, London; [1]ICI Pharmaceuticals Division, Macclesfield, England: Biochemical basis of transformation by polyoma virus middle T and SV40 large T.

Ito, Y., National Institutes of Health, Bethesda, Maryland: Evaluation of the importance of the sequence of middle T antigen of polyoma virus. GLU-GLU-GLU-GLU-TYR-MET-PRO-MET-GLU.

SESSION 5 *myb, myc*

Chairperson: D. Baltimore, Massachusetts Institute of Technology, Cambridge, Massachusetts

Baluda, M.A., Lipsick, J.S., Boyle, W.J., Dvorak, M., Lampert, M.A., University of California School of Medicine, Jonsson Cancer Center, Los Angeles: Oncogene of AMV is an altered proto-oncogene.

Papas, T.S., Watson, D., Kan, N., Psallidopoulos, M., Flordellis, C., Samuel, K., Lautenberger, J., NCI, National Institutes of Health, Bethesda, Maryland: Oncogenes of avian acute leukemia viruses MC29 (*myc*) and MH2 (*mht-myc*) and their normal cellular avian and human homologs (*c-myc*).

Bishop, J.M., University of California, San Francisco: Tumorigenesis by *myb* and *myc*.

Moelling, K., Bunte, T., Donner, P., Greiser-Wilke, I., Max-Planck-Institut für Molekulare Genetik, Berlin, Federal Republic of Germany: Analysis of transformation-specific polyproteins of acute avian leukemia and sarcoma viruses.

Eisenman, R., Hann, S., Abrams, H., Tachibana, C., Rohrschneider, L., Fred Hutchinson Cancer Research Center, Seattle, Washington: *myc* oncogene encodes a class of nuclear proteins—Possible association with the nuclear matrix.

Hayward, W.S., Goodenow, M., Simon, M.C., Shih, C.-K., Wiman, K., Memorial Sloan-Kettering Cancer Center, New York, New York: Activation of host *c-onc* genes.

Cole, M.D., Keath, E.J., Piccoli, S.P., Caimi, P., Kelekar, A., Dept. of Biochemistry, St. Louis University School of Medicine, Missouri: Activation of the *c-myc* oncogene by chromosomal translocation.

Croce, C., Wistar Institute, Philadelphia, Pennsylvania: Differential expression of the normal and of the translocated human *c-myc* oncogenes in B cells.

Diamond, A., Devine, J., Cooper, G.M., Ritz, J., Lane, M.-A., Dana-Farber Cancer Institute, Boston, Massachusetts: Transforming genes in human B-cell lymphomas.

SESSION 6 *Chromosomal Rearrangements*

Chairperson: J. Rowley, University of Chicago, Chicago, Illinois

Marcu, K.B.,[1] Stanton, L.W.,[1] Harris, L.J.,[1] Yang, J.Q.,[1] Remmers, E.F.,[1] Fahrlander, P.,[1] Watt, R.,[2] Eckhardt, L.A.,[3] Birshtein, B.K.,[3] [1]Biochemistry Dept., State University of New York, Stony Brook; [2]Wistar Institute, Philadelphia, Pennsylvania; [3]Cell Biology Dept., Albert Einstein College of Medicine, Bronx, New York: Activation of *c-myc* oncogene by chromosomal translocation in murine plasmacytomas.

Leder, P.,[1] Battey, J.,[1] Lenoir, G.,[2] Murphy, W.,[1] Stewart, T.,[1] Taub, R.,[1] [1]Dept. of Genetics, Harvard Medical School, Boston, Massachusetts; [2]International Agency for Research on Cancer, Lyon, France: Translocations involving the *c-myc* oncogene in Burkitt lymphoma.

Hayday, A.,[1] Saito, H.,[1] Wood, C.,[1] Tonegawa, S.,[1] Wiman, K.,[2] Hayward, W.,[2] [1]Center for Cancer Research and Dept. of Biology, Massachusetts Institute of Technology, Cambridge; [2]Memorial Sloan-Kettering Cancer Center, New York, New York: Structure and expression of the human *c-myc* gene from germ line and from rearranged loci.

Wirschubsky, Z., Dept. of Tumor Biology, University of Stockholm, Sweden: Specific chromosomal translocations and the genesis of B-cell-derived tumors in mice, rat, and man.

Groffen, J.,[1] Heisterkamp, N.,[1] Grosveld, G.,[2] Stephenson, J.,[1] [1]NCI, Frederick Cancer Research Facility, Frederick, Maryland; [2]Dept. of Cell Biology and Genetics, Erasmus University, Rotterdam, The Netherlands: Chromosomal translocations involving human cellular oncogenes.

Hawley, R.G.,[1,2] Shulman, M.J.,[2,3] Hozumi, N.,[1,2] [1]Ontario Cancer Institute; [2]Dept. of Medical Biophysics, University of Toronto; [3]Wellesley Hospital, Toronto, Canada: Intracisternal A-particle genes are mammalian transposable elements.

Dickson, C., Peters, G., Smith, R., Brookes, S., Laboratory of Viral Carcinogenesis, Imperial Cancer Research Fund Laboratories, London, England: Tumorigenesis by MMTV may involve provirus integration in a specific region and activation of a cellular gene.

Varmus, H.,[1] Westaway, D.,[1] Fung, Y.K.,[1] van Ooyen, A.A.,[2] Nusse, R.,[2] [1]Dept. of Microbiology and Immunology, University of California, San Francisco; [2]Dept. of Virology, Antoni van Leeuwenhoekhuis, Amsterdam, The Netherlands: Retroviral insertion mutations during oncogenesis by retroviruses without viral oncogenes.

SESSION 7 *mos, fos, erb, fes, rel, abl*

Chairperson: H. Hanafusa, Rockefeller University, New York, New York

Blair, D.G., Wood, T.G., Propst, F., Seth, A., McGeady, M.L., Oskarsson, M.K., Woodworth, A.M., Vande Woude, G.F., NCI, Frederick Cancer Research Facility, Frederick, Maryland: Properties of the mouse and human *mos* oncogene locus.

Danos, O., Yaniv, M., Molecular Biology Dept., Pasteur Institut, Paris, France: An homologous domain between the human *c-mos* gene

product and a papillomavirus polypeptide with putative role in cellular transformation.

Cohen, J.B., Dreazen, O., Horowitz, M., Klar, A., Unger, T., Rechavi, G., Givol, D., Canaani, E., Dept. of Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel: Insertion of endogenous intracisternal A-particle genomes into the *c-mos* oncogene in mouse plasmacytoma.

Wilhelmsen, K.C., Tarpley, W.G., Temin, H.M., McArdle Laboratory, University of Wisconsin, Madison: Identification of some of the parameters governing transformation by oncogenes in retroviruses.

Verma, I.M., Curran, T., Miller, A.D., Müller, R., Van Beveren, C., Molecular Biology and Virology Laboratory, Salk Institute, San Diego, California: Viral and cellular *fos* gene – Structure and expression.

Witte, O.N., Whitlock, C., Ziegler, S., Swanson, S., Robertson, D., Stafford, J., Dept. of Microbiology and Molecular Biology Institute, University of California, Los Angeles: Transformation and differentiation of defined stages of murine B lymphocytes by the *abl* oncogene.

Baltimore, D., Prywes, R., Wang, J., Foulkes, J.G., Whitehead Institute for Biomedical Research, Massachusetts Institute of Technology, Cambridge: Transformation by Ab-MLV.

Anderson, S.J., Sherr, C.J., Division of Human Tumor Cell Biology, St. Jude Children's Research Hospital, Memphis, Tennessee: Transforming glycoproteins encoded by the oncogene *v-fms*.

Hayman, M.J.,[1] Beug, H.,[2] [1]Imperial Cancer Research Fund, London, England; [2]European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: Analysis of the avian erythroblastosis virus *erbB* gene product.

SESSION 8 *Gene Expression*

Chairperson: A. Levine, State University of New York, Stony Brook, New York

Rigby, P.W.J.,[1] Brickell, P.M.,[1] Latchman, D.S.,[1] Murphy, D.,[1] Westphal, K.-H.,[1] Willison, K.,[2] Scott, M.R.D.,[1] [1]Dept. of Biochemistry, Imperial College of Science and Technology; [2]Chester Beatty Research Laboratories, Institute of Cancer Research, London, England: Cellular genes activated in transformed cells and their relationship to normal development.

Linzer, D.I.H., Nathans, D., Dept. of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, Maryland: Growth-related changes in specific mRNAs of cultured mouse cells.

Celis, J.E., Fey, S.J., Larsen, P.M., Celis, A., Dept. of Chemistry, Aarhus University, Denmark: Expression of cellular proteins in normal and tumor cells of human origin.

Garrels, J.I., Franza, R., Cold Spring Harbor Laboratory, New York: Transformation-sensitive proteins detected by computer-analyzed two-dimensional gel electrophoresis.

Bravo, R., European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: Epidermal growth-factor-induced changes in the polypeptide synthesis of A431 cells.

SESSION 9 *Keratins*

Chairperson: H. Green, Harvard Medical School, Boston, Massachusetts

Weber, K., Geisler, N., Max-Planck Institute for Biophysical Chemistry, Goettingen, Federal Republic of Germany: Structure of nonepithelial and epithelial intermediate filament proteins – A molecular basis of their diversity.

Fuchs, E., Dept. of Biochemistry, University of Chicago, Illinois: Two distinct classes of keratins from the intermediate filaments of vertebrate epithelial cells.

Sun, T.-T.,[1,2] Eichner, R.,[2] Weiss, R.A.,[2] Nelson, W.G.,[2] Cooper, D.,[1] [1]Dept. of Dermatology and Pharmacology, New York University Medical School, New York; [2]Depts. of Dermatology, Opthamology, and Cell Biology and Anatomy, Johns Hopkins University Medical School, Baltimore, Maryland: Immunological characterization and functional significance of mammalian epithelial keratins.

Franke, W.W.,[1] Schiller, D.L.,[1] Hatzfeld, M.,[1] Magin, T.,[1] Jorcano, J.,[2] Mittnacht, S.,[2] Achtstaetter, T.,[2] Cohlberg, J.,[1] Quinlan, R.,[1] Schmid, E.,[1] Moll, R.,[1] Franz, J.K.,[1] Kartenbeck, J.,[1] Venetianer, A.,[3] [1]Institute of Cell and Tumor Biology, German Cancer Research Center; [2]Center of Molecular Biology, University of Heidelberg, Federal Republic of Germany; [3]Institute of Genetics, Hungarian Academy of Sciences, Szeged, Hungary: Cytokeratins – Biosynthesis, complex formation, and interactions with desmosomes.

Osborn, M.,[1] Debus, E.,[1] Altmannsberger, M.,[2] Weber, K.,[1] [1]Max-Planck Institute for Biophysical Chemistry; [2]Dept. of Pathology, University of Goettingen, Federal Republic of Germany: Use of conventional and monoclonal antibodies to intermediate filament proteins to type human tumor material.

Geiger, B.,[1] Gigi, O.,[1] Kreis, T.E.,[1] Schmid, E.,[2] Jorcano, J.L.,[2] Mittnacht, S.,[3] Franke, W.W.,[2] [1]Dept. of Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel; [2]Institute of Cell and Tumor Biology, German Cancer Research Center; [3]Center of Molecular Biology, University of Heidelberg, Federal Republic of Germany: Dynamic rearrangements of cytokeratin polypeptides in cells.

Rheinwald, J.G., Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Expression of subsets of the keratin family and vimentin in differentiated epithelial cell types as a function of growth and malignant transformation.

SESSION 10 *T, t, P53 EBNA*

Chairperson: D. Nathans, Johns Hopkins University, Baltimore, Maryland

Kriegler, M., Botchan, M., Dept. of Molecular Biology, University of California, Berkeley: Enhanced morphological transformation by DNA tumor viruses mediated by retroviral expression systems.

Pipas, J.M., Chiang, L.-C., Barnes, D.W., Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: Effect of cell type, hormonal conditions, and viral genetic background on transforming infections of SV40.

Tijan, R., Dynan, W., Rio, D., Jones, K., Dept. of Biochemistry, University of California, Berkeley: Positive and negative regulation of transcription by binding of Sp1 and T antigen to the SV40 early promoter region.

Livingston, D.M., Murphy, C.I., Ellman, M.S., Bikel, I., Figge, J., Schlossman, R., Dana-Farber Cancer Institute and Harvard Medical School, Boston, Massachusetts: Immunofluorescent localization and antigenic modulation of SV40 small T antigen in cells producing T but not large T antigen.

Mercer, W.E., Avignolo, C., Baserga, R., Dept. of Pathology, Temple University Medical School, Philadelphia, Pennsylvania: p53 protein in cell proliferation.

Harlow, E.,[1] Benchimol, S.,[2] Crawford, L.,[2] Lamb, P.,[2] Leppard, K.,[2] Jenkins, J.,[3] [1]Cold Spring Harbor Laboratory, New York; [2]Imperial Cancer Research Fund Laboratories, London; [3]Marie Curie Memorial Foundation, Oxted, England: Molecular analysis of the gene for the p53 cellular tumor antigen.

Oren, M., Zakut, R., Bienz, B., Givol, D., Dept. of Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel: Structure and expression of the genes coding for murine p53.

Hearing, J., Levine, A.J., Dept. of Microbiology, State University of New York, Stony Brook: Expression and genetic mapping of an EBV nuclear antigen.

SESSION 11 *Promoters*

Chairperson: B. Weinstein, Columbia University, New York, New York

Nishizuka, Y., Dept. of Biochemistry, Kobe University School of Medicine; Dept. of Cell Biology, Institute for Basic Biology, Okazaki, Japan: Protein kinase C and mechanism of action of tumor promoters.

Blumberg, P.M., Jaken, S., Jeng, A.Y., Konig, B., Leach, K.L., Sharkey, N.A., Yeh, E., NCI, National Institutes of Health, Bethesda, Maryland: Membrane and cytosolic receptors for the phorbol ester tumor promoters.

Shoyab, M., Boaze, B., Jr., NCI, Frederick Cancer Research Facility, Frederick, Maryland: Isolation and characterization of specific receptor for biologically active phorbol and ingenol esters—Homogeneous receptor is a calcium-independent protein kinase.

SESSION 12 *ras*

Chairperson: P. Duesberg, University of California, Berkeley, California

Wigler, M., Shimizu, K., Taparowsky, E., Fasano, O., Birnbaum, D., Ruley, M.A., Goldfarb, M., Cold Spring Harbor Laboratory, New York: Structures of three *ras* genes activated in human tumor cell lines.

McGrath, J., Capon, D., Seeburg, P., Goeddel, D., Levinson, A., Dept. of Molecular Biology, Genentech Inc., South San Francisco, California: Structure, organization, and activation of the human Kirsten-*ras* gene family.

Barbacid, M., Santos, E., Sukumar, S., Notario, V., Pulciani, S., Reddy, E.P., NCI, National Institutes of Health, Bethesda, Maryland: *ras*-transforming genes in human and experimentally induced tumors.

DeFeo-Jones, D., Scolnick, E.M., Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania: *ras*-related gene sequence identified and isolated from *S. cerevisiae*.

Yuasa, Y., Eva, A., Needleman, S., Kraus, M., Pierce, J., Rhim, J., Srivastava, S., Gazit, A., Srinivasan, A., Gol, T., Tronick, S., Reddy, P., Aaronson, S., NCI, National Institutes of Health, Bethesda, Maryland: *ras*-related oncogenes of human tumors.

Marshall, C.J., Hall, D., Newbold, R., Malcolm, S., Weiss, R., Institute for Cancer Research, Chester Beatty Laboratories, London, England: *ras* genes and cell transformation.

Nakano, H., Yamamoto, H., Neville, C., Evans, D., Perucho, M., Dept. of Biochemistry, State University of New York, Stony Brook: Structure and mechanisms of activation of the human *c-ras*^{K1} oncogene in lung tumors.

Guerrero, I., Mayer, A., Pellicer, A., Dept. of Pathology and Kaplan Cancer Center, New York University Medical Center, New York: Carcinogen- and radiation-induced mouse lymphomas contain an activated *c-ras* oncogene.

Bassin, R.H.,[1] Noda, M.,[1] Scolnick, E.M.,[2] Selinger, Z.,[3] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Merck Sharp and Dohme Laboratories, West Point, Pennsylvania; [3]Hebrew University, Jerusalem, Israel: Study of possible relationships among *onc* genes using flat revertants isolated from Ki-MSV-transformed cells.

SESSION 13 *E1A, E1B*

Chairperson: J. Darnell, Rockefeller University, New York, New York

Land, H., Parada, L.F., Cunningham, J., Murray, M., Weinberg, R.A., Center for Cancer Research, Massachusetts Institute of Technology; Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: Two cooperating oncogenes are required for tumorigenic conversion of primary rat embryo fibroblasts.

Ruley, E., Cold Spring Harbor Laboratory, New York: Separate establishment and transforming functions are required for transformation of primary cells by viral and cellular genes.

van der Eb, A.J.,[1] Bernards, R.,[1] Schrier, P.I.,[1] Bos, J.L.,[1] Vaessen, R.T.M.J.,[1] Jochemsen, A.G.,[1] Melief, C.,[2] [1]Dept. of Medical Biochemistry, State University, Leiden; [2]Dept. of Tumor Immunology, Amsterdam, The Netherlands: Al-

tered expression of cellular genes in adenovirus-transformed cells.

Chinnadurai, G., Institute for Molecular Virology, St. Louis University Medical Center, Missouri: Ad2 E1B-coded 19-kD tumor antigen plays an essential role in cell transformation.

Gallimore, P.H.,[1] Byrd, P.J.,[1] Grand, R.,[1] Whittaker, J.,[1] Breiding, D.,[2] Williams, J.F.,[2] [1]Cancer Research Campaign Laboratories, University of Birmingham, England; [2]Dept. of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania: Examination of the transforming and tumor-inducing capacity of a number of Ad12 E1 host-range mutants and cells transformed by subgenomic fragments of Ad12 E1 region.

Logan, J., Winberg, G., Pilder, S., Hearing, P., Shenk, T., Dept. of Microbiology, State University of New York, Stony Brook: Functional analysis of the Ad5 E1A and E1B transcription units.

Jat, P.S., Kingston, R.E., Sharp, P.A., Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Regulation of gene expression by the E1A region of Ad5.

Treisman, R., Green, M., Maniatis, T., Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Activation of globin gene transcription by viral immediate early gene products.

Nevins, J.R., Imperiale, M.J., Feldman, L.T., Kao, H.-T., Rockefeller University, New York, New York: Function of the adenoviral E1A gene product.

SESSION 14 *Surface Antigens*

Chairperson: S. Weissman, Yale University, New Haven, Connecticut

Gooding, L.,[1] Geib, R.,[1] O'Connell, K.,[1] Harlow, E.,[2] [1]Dept. of Microbiology and Immunology, Emory University School of Medicine, Atlanta, Georgia; [2]Cold Spring Harbor Laboratory, New York: Antibody and cellular detection of SV40 T antigenic determinants on the surfaces of transformed cells.

Tevethia, S., Lewis, A., Tevethia, M., Dept. of Microbiology, Pennsylvania State University College of Medicine, Hershey: Localization of antigenic sites reactive with cytotoxic lymphocytes on the proximal half of SV40 T antigen.

Schlom, J., Colcher, D., Hand, P.H., Greiner, J., Wunderlich, D., NCI, National Institutes of Health, Bethesda, Maryland: Human breast carcinoma and colon-carcinoma-associated antigens defined by monoclonal antibodies.

Koprowski, H., Dept. of Anatomy and Biology, Wistar Institute, Philadelphia, Pennsylvania: Human tumor antigens.

HUMAN T-CELL LEUKEMIA VIRUSES

September 14–September 15

Welcoming Address: M. Essex, Harvard School of Public Health

Introduction: R. Gallo, National Cancer Institute

SESSION 1 *Animal Models and Related Viruses*

Chairpersons: Y. Ito, Kyoto University, Japan, and P. Vogt, School of Medicine, University of California, Los Angeles

Vogt, P.K., Dept. of Microbiology, University of Southern California School of Medicine, Los Angeles: Mechanisms of viral leukemogenesis.

Bruck, C.,[1] Ghysdael, J.,[1] Portetelle, D.,[1,2] Burny, A.,[1,2] [1]University of Brussels; [2]Faculty of Agronomy, Gembloux, Belgium: Antigenicity of the envelope glycoprotein (gp51) of BLV.

Hardy, W.D., Jr., Zuckerman, E.E., Laboratory of Veterinary Oncology, Memorial Sloan-Kettering Cancer Center, New York, New York: T-cell lymphoid malignancies and feline acquired immune deficiency syndrome (FAIDS) of pet cats induced by FLV.

de Thé, G., Faculty of Medicine, CNRS, Lyon, France: Pathobiology and epidemiology of Epstein-Barr virus.

Shafritz, D.A., Rogler, C.E., Liver Research Center, Albert Einstein College of Medicine, Bronx, New York: Review of properties of hepatitis B virus related to DNA and RNA tumor viruses.

Short Talks

Ito, Y., Dept. of Microbiology, Faculty of Medicine, Kyoto University, Japan: Tumor-promoting diterpene esters as possible environmental cofactor(s) for ATL.

Goodenow, M.M., Shih, C.-K., Hayward, W.S., Memorial Sloan-Kettering Cancer Center, New York, New York: ALV and *c-myc* activation.

Rowley, J., University of Chicago Medical School, Illinois: Chromosome abnormalities in human lymphoid disease.

SESSION 2 *HTLV: Proteins and Nucleic Acids*

Chairpersons: F. Wong-Staal, National Cancer Institute, Bethesda, Maryland, and M.M. Yoshida, Cancer Institute, Tokyo, Japan

Sarngadharan, M.G., Dept. of Cell Biology, Litton Bionetics, Inc., Kensington, Maryland: Introduction.

Oroszlan, S.,[1] Copeland, T.D.,[1] Kalyanaraman, V.S.,[2] Sarngadharan,

M.G.,[2] Schultz, A.M.,[1] Gallo, R.C.,[3] [1]NCI, Frederick Cancer Research Facility, Frederick; [2]Dept. of Cell Biology, Litton Bionetics, Inc., Kensington; [3]NCI, National Institutes of Health, Bethesda, Maryland: Chemical analysis of HTLV structural proteins.

Lee, T.H., Howe, C.W.S., McLane, M.F., Tachibana, N., Essex, M., Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts: HTLV-associated antigens recognized by human serum antibodies.

Weiss, R., Nagy, K., Clapham, P., Cheingsong-Popov, R., Institute of Cancer Research, Chester Beatty Laboratories, London, England: Biological properties of HTLV envelope antigens.

Wong-Staal, F., Franchini, G., Hahn, B., Gelmann, E.P., Manzari, V., Gallo, R.C., NCI, National Institutes of Health, Bethesda, Maryland: Integration and expression of HTLV genomes in fresh and cultured T cells of adult T-cell leukemia patients.

Yoshida, M., Seiki, M., Hattori, S., Watanabe, T., Dept. of Viral Oncology, Cancer Institute, Tokyo, Japan: Genome structure of ATLV (HTLV) and its involvement in leukemogenesis of ATL.

Haseltine, W.A., Sodroski, J.G., Patarca, R., Trus, M., Perkins, D., Crowther, R., Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Structure and function of HTLV.

Mullins, J.I.,[1] Brody, D.,[1] Kim, H.J.,[1] McLane, M.F.,[1] Essex, M.,[1] Rubsamen, H.,[2] Gold, J.W.,[3] Feorino, P.,[4] Cabradilla, C.,[4] Schooley, R.,[5] Hirsch, M.,[5] Wong-Staal, F.,[6] Franchini, B.,[6] Gallo, R.C.,[6] [1]Harvard School of Public Health, Boston, Massachusetts; [2]Paul Ehrlich Institut, Frankfurt, Federal Republic of Germany; [3]Memorial Sloan-Kettering Cancer Center, New York, New York; [4]Centers for Disease Control, Atlanta, Georgia; [5]Massachusetts General Hospital, Boston; [6]NCI, National Institutes of Health, Bethesda, Maryland: HTLV sequences in leukemias and in AIDS.

Seigel, L.J.,[1] Nash, W.G.,[2] Manzari, V.,[1] Wong-Staal, F.,[1] Gallo, R.C.,[1] O'Brien, S.J.,[2] [1]Laboratory of Tumor Cell Biology, [2]Laboratory of Viral Carcinogenesis, NCI, National Institutes of Health, Bethesda, Maryland: On the dynamic nature of proviral integration in HTLV-infected malignancies—A genetic analysis.

Reitz, M.S., Jr., Clarke, M.F., Trainor, C.D., Mann, D.L., Gallo, R.C., NCI, National Institutes of Health, Bethesda, Maryland: HTLV and HLA antigen expression.

Short Talks

Gelmann, E., Popovic, M., Franchini, G., Wong-Staal, F., Gallo, R.C., NCI, National Institutes of Health, Bethesda, Maryland: A new human retrovirus associated with a T-cell hairy cell leukemia.

SESSION 3 *T-cell Biology and the Effects of HTLV*

Chairpersons: K. Lennert, Christian-Albrechts University, Kiel, Federal Republic of Germany, and H. Kaplan, Stanford University, Stanford, California

Greaves, M.F., Imperial Cancer Research Fund Laboratories, London, England: T-cell differentiation and malignancy.

Haynes, B.F.,[1] Palker, T.J.,[1] Scearce, R.M.,[1] Robert-Guroff, M.,[2] Kalyanaraman, V.S.,[2] Bolognesi, D.P.,[1] Gallo, R.C.,[2] [1]Depts. of Medicine and Surgery, Duke University, Durham, North Carolina; [2]NCI, National Institutes of Health, Bethesda, Maryland: Monoclonal antibodies against human T-cell leukemia virus (HTLV) p24 internal core protein and HTLV-associated p19 protein identify antigens in normal human tissue.

Weissman, I., McGrath, M., Tidmarsh, G., Marian, J., Dept. of Pathology, Stanford University School of Medicine, California: Retrovirus receptors on T- and B-cell lymphocytes.

Popovic, M.,[1] Markham, P.,[1] Sarin, P.,[1] Salahuddin, Z.,[1] Robert-Guroff, M.,[1] Mann, D.,[1] Allavena, P.,[2] Minowada, J.,[3] Gallo, R.C.,[1] [1]NCI, National Institutes of Health, Bethesda; [2]NCI, Frederick Cancer Research Facility, Frederick, Maryland; [3]Veterans Administration Hospital, Hines, Illinois: Infection and transformation of human T cells by HTLV.

Michalski, M., Popovic, M., NCI, National Institutes of Health, Bethesda, Maryland: Induction of syncytia of HTLV isolates.

Miyoshi, I.,[1] Taguchi, H.,[1] Fujishita, M.,[1] Yoshimoto, S.,[1] Ohtsuki, Y.,[2] [1]Dept. of Medicine, [2]Dept. of Pathology, Kochi Medical School, Japan: Natural infection in monkeys with adult T-cell leukemia virus or a closely related agent.

Mituya, H., Gallo, R., Broder, S., NCI, National Institutes of Health, Bethesda, Maryland: Cytotoxic T cells specific for HTLV.

Short Talks

Mann, D.L.,[1] Clark, J.,[1] Clarke, M.F.,[1] Strong, D.M.,[2] Franchini, G.,[1] Trainor, C.D.,[1] Gallo, R.C.,[1] Reitz, M.,[1] [1]NCI, National Institutes of Health; [2]Uniformed Services University of the Health Sciences, Bethesda, Maryland: HTLV-infected B-cell lines established from patients with adult T-cell leukemias.

Poiesz, B.,[1,2,3] Moore, J.,[1,3] Merl, S.,[1,2,3] Tomar, R.,[1] Zamkoff, K.,[1,2] Davey, F.,[1] Planas, A.,[1,2] Gottlieb, A.,[1] Runge, L.,[1] Cowan, B.,[1,2,3] Reeves, W.,[1,2] Ruscetti, F.,[5] Han, T.,[6] Cabradilla, C.,[7] Ehrlach, G.,[4] R. Comis,[1,3] [1]Upstate Medical Center, [2]Veterans Administration Medical Center, Syracuse; [3]Barbara Kopp Research Center, Auburn; [4]Syracuse University, New York; [5]NCI, Frederick Cancer Research Facility, Frederick, Maryland; [6]Roswell Park Medical Institute, Buffalo, New York; [7]Centers for Disease Control, Atlanta, Georgia: Biology and epidemiology of HTLV.

SESSION 4 *Epidemiology and Natural History of HTLV-related Diseases*

Chairpersons: D.P. Bolognesi, Duke University, Durham, North Carolina, and K. Takatsuki, Kumamoto University, Japan

Takatsuki, K., Yamaguchi, K., Kawano, F., Nishimura, H., Tsuda, H., Sanada, I., Second Dept. of Internal Medicine, Kumamoto University Medical School, Japan: Adult T-cell leukemia/lymphoma

(ATL)—Clinical feature, epidemiology, and cytogenetic, phenotypic, and functional studies of leukemia cells.

Blattner, W., Saxinger, C., Blayney, D., Clark, J., Robert-Guroff, M., Gallo, R., NCI, National Institutes of Health, Bethesda, Maryland: HTLV—Epidemiology and relationship to human malignancy.

Catovsky, D., Matutes, E., Brito-Babapulle, V., MRC Leukaemia Unit, Royal Postgraduate Medical School, London, England: Laboratory studies in Caribbean patients with ATL.

Robert-Guroff, M.,[1] Saxinger, W.C.,[1] Schupbach, J.,[1] Blayney, D.W.,[1] Popovic, M.,[1] Kalyanaraman, V.S.,[2] Sarngadharan, M.G.,[2] Gallo, R.C.,[1] [1]NCI, National Institutes of Health, Bethesda; [2]Litton Bionetics, Inc., Kensington, Maryland: Seroepidemiologic studies on HTLV.

Essex, M.,[1] McLane, M.,[1] Lee, T.H.,[1] Tachibana, N.,[1] Homma, T.,[1] Mullins, J.,[1] Falk, L.,[1] de Thé, G.,[1] Gold, J.,[2] Schooley, R.,[3] Hirsch, M.,[3] Evatt, B.,[4] Cabradilla, C.,[4] Francis, D.,[4] [1]Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts; [2]Memorial Sloan-Kettering Cancer Center, New York, New York; [3]Massachusetts General Hospital, Boston; [4]Centers for Disease Control, Atlanta, Georgia: Distribution of antibodies to HTLV-MA in patients with AIDS and related control groups.

Greaves, M.F.,[1] Verbi, W.,[1] Blattner, W.,[2] Robert-Guroff, M.,[2] Hong-Guong, G.,[2] Reitz, M.,[2] Gallo, R.C.,[2] [1]Imperial Cancer Research Fund, London, England; [2]NCI, National Institutes of Health, Bethesda, Maryland: HTLV in immigrants to the United Kingdom.

Aoki, T., Hamada, C., Ohno, S., Miyakoshi, H., Robert-Guroff, M., Ting, R.C., Gallo, R.C., Research Division, Shinrakuen Hospital; Dept. of Virology, Niigata University School of Medicine, Niigata, Japan; Dept. of Cell Biology, Biotech Research Laboratory, Rockville; NCI, National Institutes of Health, Bethesda, Maryland: Location of HTLV p19 antigen on HTLV-producing cells and a role of natural antibody to HTLV-related antigens in antibody-dependent cell-mediated cytotoxicity against HTLV-producing cells.

Miyoshi, I.,[1] Taguchi, H.,[1] Fujishita, M.,[1] Yoshimoto, S.,[1] Ohtsuki, Y.,[2] [1]Dept. of Medicine, [2]Dept. of Pathology, Kochi Medical School, Japan: Natural infection in monkeys with ATLV or a closely related agent.

Saxinger, C.,[1] Blattner, W.,[1] Lange-Wantzin, G.,[2] Lapin, B.,[3] Yakoleva, L.,[3] Guo, C.,[1] Robert-Guroff, M.,[1] Gallo, R.,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Dermatology, Finsen Institute, Denmark; [3]USSR Academy of Medical Science, Sukhami: HTL—Seroepidemiological studies in diverse populations.

SESSION 5 *Possible Role of HTLV in AIDS*

Chairpersons: J. Gutterman, University of Texas, Houston, and D. Francis, Centers for Disease Control, Phoenix, Arizona

Fauci, A.S., NIAID, National Institutes of Health, Bethesda, Maryland: The immunobiology of AIDS.

Safai, B., Pollack, M., Dupont, B., Memorial Sloan-Kettering Cancer Center, New York, New York: HLA in cutaneous T-cell lymphoma and Kaposi's sarcoma—A possible genetic susceptibility to HTLV.

Letvin, N.L.,[1,2] Hunt, R.D.,[1] [1]Dana-Farber Cancer Institute, Boston; [2]New England Regional Primate Research Center, Southborough, Massachusetts: Transmissible immunodeficiency syndrome and lymphomas in Macaque monkeys.

Essex, M.,[1] McLane, M.,[1] Lee, T.H.,[1] Tachibana, N.,[1] Homma, T.,[1] Mullins, J.,[1] Falk, L.,[1] de Thé, G.,[1] Gold, J.,[2] Schooley, R.,[3] Hirsch, M.,[3] Evatt, B.,[4] Cabradilla, C.,[4] Francis, D.,[4] [1]Dept. of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts; [2]Memorial Sloan-Kettering Cancer Center, New York, New York; [3]Massachusetts General Hospital, Boston; [4]Centers for Disease Control, Atlanta, Georgia: Distribution of antibodies to HTLV-MA in patients with AIDS and related control groups.

Yoshida, M.,[1] Seiki, M.,[1] Watanabe, T.,[1] Sugano, H.,[1] Kinoshita, T.,[2] Kazawa, M.,[2] Abe, T.,[2] [1]Dept. of Viral Oncology, Cancer Institute; [2]Dept. of Internal Medicine, School of Medicine, Teikyo University, Tokyo, Japan: Proviral DNA of ATLV (HTLV) in a hemophilia patient with AIDS-like symptoms in Japan.

Gallo, R.C., Sarin, P., Popovic, M., NCI, National Institutes of Health, Bethesda, Maryland: The family of human retroviruses called human T-cell leukemia/lymphoma virus (HTLV)—Their role in lymphoid disorders (AIDS).

Montagnier, L.,[1] Barré-Sinoussi, F.,[1] Dauguet, C.,[1] Rozenbaum, W.,[2] Brun-Vézinet, F.,[3] Rouzioux, C.,[3] Klatzmann, D.,[2] Gluckman, J.C.,[2] Chermann, J.C.,[2] [1]Dept. of Virology, Institut Pasteur; [2]Hôpital la Pitié Salpetriere; [3]Hôpital Claude Bernard, Paris, France: A human T-lymphotropic retrovirus—Characterization and possible role in AIDS.

Scott, G., Parks, W., Kanner, S., Fischl, M., Dickenson, G., de Medina, M., Schiff, E., Depts. of Pediatrics, Microbiology, and Immunology and Medicine, University of Miami School of Medicine, Florida: AIDS and HTLV.

BANBURY MEETINGS PROGRAMS

ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS) AND KAPOSI'S SARCOMA

February 6–February 8

OVERVIEW

B. Safai, Memorial Sloan-Kettering Cancer Center, New York, New York: Clinical aspects.

J.W. Curran, Centers for Disease Control, Atlanta, Georgia: Epidemiology.

J. Shuster, McGill University, Montreal, Canada: AIDS and the hemophiliac population.

SESSION 1 *Immunological Aspects I*

Chairperson: R.A. Good, Oklahoma Medical Research Foundation, Oklahoma City

B. Dupont, Memorial Sloan-Kettering Cancer Center, New York, New York.
P. Rubinstein, The New York Blood Center, New York, New York.

H.C. Lane, National Institute of Allergy and Infectious Disease, Bethesda, Maryland.
A. Rubinstein, Albert Einstein College of Medicine, Bronx, New York.

A.J. Ammann, University of California, San Francisco.

Discussants: J. Reuben, M.D. Anderson Hospital and Tumor Institute, Houston, Texas; J.L. Fahey, University of California, Los Angeles; F.P. Siegal, Mt. Sinai School of Medicine, New York, New York.

SESSION 2 *Immunological Aspects II*

Chairperson: K.W. Sell, National Institute of Allergy and Infectious Disease, Bethesda, Maryland

S. Cunningham-Rundles, Memorial Sloan-Kettering Cancer Center, New York, New York.
J. Laurence, Rockefeller University, New York, New York.

G. Quinnan, National Institutes of Health, Bethesda, Maryland.
S. Zolla-Pazner, New York University School of Medicine, New York, New York.

G.M. Shearer, National Institutes of Health, Bethesda, Maryland.
E.M. Shevach, National Institute of Allergy and Infectious Disease, Bethesda, Maryland.

SESSION 3 *Pathology and Cell Biology*

Chairperson: R.C. Gallo, National Cancer Institute, Bethesda, Maryland

D. Gospodarowicz, University of California, San Francisco.

L.C.M. Reid, Albert Einstein College of Medicine, Bronx, New York.

SESSION 4 *Virology I*

D. Armstrong, Memorial Sloan-Kettering Cancer Center, New York, New York.
M.S. Hirsch, Massachusetts General Hospital, Boston.

G. Noble, Centers for Disease Control, Atlanta, Georgia.
W.L. Drew, University of California, San Francisco.

D. Francis, Centers for Disease Control, Phoenix, Arizona.

SESSION 5 *Virology II*

K.K. Takemoto, National Institute of Allergy and Infectious Disease, Bethesda, Maryland.
R.C. Gallo, National Cancer Institute, Bethesda, Maryland.

M.S. Horwitz, Albert Einstein College of Medicine, Bronx, New York.
G.S. Hayward, Johns Hopkins University, Baltimore, Maryland.

D.H. Spector, University of California, San Diego, La Jolla, California.
I.M. Arias, Albert Einstein College of Medicine, Bronx, New York.

PLANT VIRUSES AND VIROIDS

February 27–March 2

SESSION 1

S.H. Howell, University of California, San Diego, La Jolla: Introduction to DNA viruses.

K.E. Richards, Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France: Structure and expression of CaMV DNA.

R. Hull, John Innes Institute, Norwich, England: Unencapsidated nucleic acids of CaMV and their significance in virus replication.

T. Hohn, Friedrich Miescher-Institut, Basel, Switzerland: Reverse transcription involved in CaMV replication.

T.J. Guilfoyle, University of Minnesota, St. Paul: CaMV minichromosome.

S.H. Howell, University of California, San Diego, La Jolla, California: Recombination of CaMV genomes.

L. Hirth, Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France: CaMV DNA in the elaboration of gene vectors.

SESSION 2

R.M. Goodman, Calgene, Inc., Davis, California: Genome structure and relationships among whitefly-borne geminiviruses.

K.W. Buck, Imperial College of Science, London, England: Intracellular forms of TGMV DNA.

R.H. Symons, University of Adelaide, Australia: Characterizations of the DNA genomes of the Australian geminiviruses.

M. Zaitlin, Cornell University, Ithaca, New York: Introduction to RNA viruses.

P. Goelet, MRC Laboratory of Molecular Biology, Cambridge, England: Nucleotide sequence of TMV RNA.

M. Zaitlin, Cornell University, Ithaca, New York: Characterization of single- and double-stranded subgenomic RNAs from TMV-infected plants.

A. Siegel, Wayne State University, Detroit, Michigan: Characterization of the subgenomic and host RNA species encapsidated in vivo by TMV capsid protein.

T.J. Morris, University of California, Berkeley: Virus-specific dsRNA—Functional role in RNA virus infection.

SESSION 3

W.O. Dawson, University of California, Riverside: Examination of the mRNA component of TMV RNA synthesis.

D.L.D. Caspar, Brandeis University, Waltham, Massachusetts: Electrostatic interactions in the structure and assembly of TMV.

A.O. Jackson, Purdue University, West Lafayette, Indiana: Characterization of the RNAs of four strains of BSMV.

G. Bruening, University of California, Davis: RNA and nucleocapsid accumulation in protoplasts resistant to CPMV.

R.W. Goldbach, Agricultural University, Wageningen, The Netherlands: Structure and genetic organization of CPMV RNAs.

D.L. Nuss, New York State Department of Health, Albany: Molecular biology of WTV—Characterization of subgenomic RNAs associated with extravectorial isolates.

SESSION 4

T.C. Hall, University of Wisconsin, Madison: Template and product specificity of plant virus replicases.

A. van Kammen, Agricultural University, Wageningen, The Netherlands: Isolation and characterization of the CPMV RNA replication complex.

J. Mottinger, University of Rhode Island, Kingston: Genetic and molecular examination of virally associated mutations in maize.

S. Dellaporta, Cold Spring Harbor Laboratory, New York: Molecular and genetic examination of virally associated mutations in maize.

H.D. Robertson, Rockefeller University, New York, New York: Introduction to viroids, virusoids, and satellites.

H. Sänger, Max-Planck-Institut für Biochemie, Munich, Federal Republic of Germany: Studies on viroid replication.

P. Palukaitis, Cornell University, Ithaca, New York: Reexamination of the nature and biological significance of linear viroid molecules—Sequence of the 5′ end of linear PSTV molecules.

A.D. Branch, Rockefeller University, New York, New York: Structure of the viroid replication complex.

J. Semancik, University of California, Riverside: Nuclear replication and cellular pathology in CEV infection.

SESSION 5

D. Riesner, Universität Dusseldorf, Federal Republic of Germany: Structure and cellular organization of viroids.

R.A. Owens, Plant Protection Institute, USDA, Beltsville, Maryland: Biological activity of cloned PSTV cDNAs.

G. Bruening, University of California, Davis: Properties and biological effects of satellite RNA of TobRV.

C.W. Collmer, Plant Protection Institute, USDA, Beltsville, Maryland: Structural analyses of the cucumovirus satellite RNAs differing in biological function.

P. Palukaitis, Cornell University, Ithaca, New York: Comparison of four satellite RNAs of CMV.

R.I.B. Francki, University of Adelaide, Glen Osmond, South Australia: Satellite nature of some viroidlike RNAs.

R.H. Symons, University of Adelaide, Australia: Comparative structure and properties of virusoids.

ENHANCERS AND CONTROLLING ELEMENTS

April 3–April 6

SESSION 1 *Papova Enhancers*

P. Chambon, Faculté de Médecine de Strasbourg, France: Upstream elements (72-bp and 21-bp repeats) of the SV40 early promoter—In vivo and in vitro studies.

P. Gruss, University of Heidelberg, Germany: Competition for cellular factors required for the transcriptional activation of enhancers.

T. Kadesch, Stanford University School of Medicine, California: Transcriptional position effects and enhancement of the SV40 early promoter.

A. Nordheim, Massachusetts Institute of Technology, Cambridge: Potential Z-DNA in the SV40 enhancer region.

W. Schaffner, University of Zurich, Switzerland: Transcriptional enhancers of viral and cellular origin.

M. Botchan, University of California, Berkeley: Anatomy of the BPV activator and the relationship between enhancers of transformation and activators of transcription.

G. Khoury, National Cancer Institute, Bethesda, Maryland: Specificity associated with viral enhancers.

M.R. Capecchi, University of Utah, Salt Lake City: Location and function of retroviral and SV40 sequences that enhance biochemical transformation after microinjection of DNA.

SESSION 2 *Promoters*

G.L. Hager, National Cancer Institute, Bethesda, Maryland: Glucocorticoid regulatory sequence from MMTV—A negative element?

K.R. Yamamoto, University of California, San Francisco: Fusions of a glucocorticoid regulatory element to the *tk* promoter region—Hormone-regulated enhancement of promoter activity.

D.H. Hamer, National Institutes of Health, Bethesda, Maryland: Distinct promoter and regulatory sequences of an inducible metallothionein gene.

B. Roizman, University of Chicago, Illinois: Identification of the regulatory regions of alpha genes of HSV by construction of a chimeric gene.

J. Nevins, Rockefeller University, New York, New York: Regulatory signals in adenovirus-inducible promoter.

W. Dynan, University of California, Berkeley: A promoter-specific transcription factor that allows recognition of upstream sequence in early SV40 promoter.

SESSION 3 *Enhancers II*

R. Kamen, Genetics Institute, Boston, Massachusetts: Relationship between the polyoma virus enhancer and the *cis*-acting element required for viral DNA replication—Studies using SV40/polyoma recombinants.

E. Linney, La Jolla Cancer Research Foundation, California: Virus enhancing sequences for teratocarcinoma cells.

M. Yaniv, Institut Pasteur, Paris, France: Function of polyoma virus enhancer in embryonal and differentiated cells of the mouse.

J.A. Hassell, McGill University, Montreal, Canada: Deletion mapping of the polyoma virus early promoter-enhancer region.

I.M. Verma, The Salk Institute, San Diego, California: Enhancer elements in murine LTR.

T.G. Wood, National Cancer Institute, Bethesda, Maryland: Sequences required for the activation of the transforming potential of a normal cellular gene, *c-mos*.

B.H. Howard, National Institutes of Health, Bethesda, Maryland: Comparison of enhancer functions in SV40 and RSV.

T.E. Shenk, State University of New York, Stony Brook: The Ad5 E1A transcription unit contains an enhancerlike element.

P. Sassone-Corsi, Faculté de Medecine de Strasbourg, France: An activator element upstream from the Ad2 E1A promoter.

SESSION 4 *Promoters II*

S. Beckendorf, University of California, Berkeley: Chromatin structure and expression of a *Drosophila glu* protein gene.

P.M. Bingham, State University of New York, Stony Brook: Properties of putative enhancerlike elements at the *white* locus of *Drosophila*.

L.P. Guarente, Massachusetts Institute of Technology, Cambridge: Regulation of the yeast iso-l-cytochrome *c* gene by heme via an upstream activation site.

A.G. Hinnebusch, Massachusetts Institute of Technology, Cambridge: Repeated DNA sequences and regulatory genes that control amino acid biosynthetic genes in yeast.

S. Mitrani-Rosenbaum, National Cancer Institute, Bethesda, Maryland: Regulation of the human interferon gene expression.

K. Zinn, Harvard University, Cambridge, Massachusetts: DNA sequences controlling expression of the human β-interferon gene.

H.R.B. Pelham, MRC Laboratory of Molecular Biology, Cambridge, England: Anatomy of stress-inducible promoters.

SESSION 5 *Special Systems*

M. Fried, Imperial Cancer Research Fund Laboratories, London, England: Host sequences that enhance the expression of adjacent DNA.

K. Calame, University of California, Los Angeles: Regions in the mouse immunoglobulin heavy-chain locus that enhance transcription from the SV40 early promoter.

V.T. Oi, Stanford University School of Medicine, California: Control of immunoglobulin gene expression in transfected lymphoid cells.

S. Tonegawa, Massachusetts Institute of Technology, Cambridge: Tissue-specific enhancer element in the major intron of an immunoglobulin heavy-chain gene.

A. Rich, Massachusetts Institute of Technology, Cambridge: Nucleotide sequences, Z-DNA formation, and protein interactions.

M. Green, Harvard University, Cambridge, Massachusetts: Activation of human globin gene transcription by *cis*- and *trans*-acting factors.

MECHANISMS OF PERCEPTION: MARR MEMORIAL CONFERENCE

April 24–April 29

Participants

Allman, John, California Institute of Technology, Pasadena
Bajcsy, Ruzena, Moore School of Electrical Engineering, Philadelphia, Pennsylvania
Baker, Curtis, Dalhousie University, Halifax, Nova Scotia
Binford, Thomas, Stanford University, California
Bobick, Aaron, Massachusetts Institute of Technology, Cambridge
Braddick, Oliver J., University of Cambridge, England
Brady, Michael, Massachusetts Institute of Technology, Cambridge
Brown, Christopher M., University of Rochester, New York
Bulthof, Heinrich, Max-Planck-Institut für Biologische Kybernetik, Tubingen, Federal Republic of Germany
Chien, Y.T., National Science Foundation, Washington, D.C.
Crick, Francis H.C., Salk Institute, San Diego, California
Daugman, John, Harvard University, Cambridge, Massachusetts
Dawson, Benjamin, Massachusetts Institute of Technology, Cambridge
Fahle, Manfred, Eberhard-Karls-Universität, Tubingen, Federal Republic of Germany
Frisby, John, University of Sheffield, England
Glaser, Donald A., University of California, Berkeley
Grimson, Eric, Massachusetts Institute of Technology, Cambridge
Hildreth, Ellen, Massachusetts Institute of Technology, Cambridge
Hoffman, Donald, Massachusetts Institute of Technology, Cambridge
Kass, Michael, Massachusetts Institute of Technology, Cambridge
Koch, Cristof, Massachusetts Institute of Technology, Cambridge
Koenderink, J.J., Rijksuniversiteit Utrecht, The Netherlands
Longuet-Higgins, Christopher H., Sussex University, Brighton, England
Mayhew, John, University of Sheffield, England
Mandelbrot, Benoit B., Thomas J. Watson Research Center, IBM, Yorktown Heights, New York
McGill, Michael, National Science Foundation, Washington, D.C.
Mitchison, Graeme J., University of Cambridge, England
Morgan, Michael J., University College London, England
Nielsen, Kenneth R.K., Massachusetts Institute of Technology, Cambridge
Nishihara, Keith H., Massachusetts Institute of Technology, Cambridge
Poggio, Tomaso, Massachusetts Institute of Technology, Cambridge
Reichardt, Werner, Max-Planck-Institut für Biologische Kybernetik, Tubingen, Federal Republic of Germany
Richards, Whitman, Massachusetts Institute of Technology, Cambridge
Richter, Jacobi, Weizmann Institute of Science, Rehovot, Israel
Rosenfeld, Azriel, University of Maryland, College Park
Rubin, John, Massachusetts Institute of Technology, Cambridge
Scheuhammer, Joseph, Massachusetts Institute of Technology, Cambridge
Segev, Idan, National Institutes of Health, Bethesda, Maryland
Sejnowski, Terrence, Johns Hopkins University, Baltimore, Maryland
Stevens, Kent, University of Oregon, Eugene
Sutherland, Stuart N., Sussex University, Brighton, England
Terzopoulos, Demetri, Massachusetts Institute of Technology, Cambridge
Thompson, William, University of Minnesota, Minneapolis
Torre, Vincent, Universita di Genova, Italy
Treisman, Anne, University of British Columbia, Vancouver, Canada
Ullman, Shimon, Massachusetts Institute of Technology, Cambridge
Vaina, Lucia, Boston University, Massachusetts
Van Essen, David, California Institute of Technology, Pasadena
Watt, R.J., University College London, England
Westheimer, Gerald, University of California, Berkeley
Winston, Patrick, Massachusetts Institute of Technology, Cambridge
Witkin, Andrew, Fairchild Corporation, Palo Alto, California
Woodham, Robert J., University of British Columbia, Vancouver, Canada
Yuille, A., Massachusetts Institute of Technology, Cambridge
Zipser, David, University of California, San Diego, La Jolla

SLOAN JOURNALISTS' WORKSHOP: NEW CONCEPTS IN MUTATION

August 5–August 7

SESSION 1 *Genetic Origins of Human Disease*

J. Cairns, Harvard School of Public Health, Boston, Massachusetts.
J.F. Crow, University of Wisconsin, Madison.

SESSION 2 *Origins of Genetic Change*

J.H. Miller, University of California, Los Angeles.
M. Botchan, University of California, Berkeley.
R.T. Schimke, Stanford University, California.

SESSION 3 *Engineering the Mutation Process*

D. Shortle, State University of New York, Stony Brook.
M. Smith, University of British Columbia, Vancouver, Canada.

SESSION 4 *Mutation in Evolution*

A.C. Wilson, University of California, Berkeley.

SESSION 5 *Approaches to Human Genetic Disease*

C.T. Caskey, Baylor College of Medicine, Houston, Texas.

MECHANISMS OF MUTAGENESIS

August 7–August 10

SESSION 1 *Genomic Rearrangements*

J. Cairns, Harvard School of Public Health, Boston, Massachusetts.
M. Botchan, University of California, Berkeley.
W.R. Engels, University of Wisconsin, Madision.
G. Fink, Massachusetts Institute of Technology, Cambridge.
T.D. Tlsty, Stanford University, California.
R.T. Schimke, Stanford University, California.
M. Wigler, Cold Spring Harbor Laboratory, New York.

SESSION 2 *Mutagenesis in Prokaryotes*

J.H. Miller, University of California, Los Angeles.
F. Hutchinson, Yale University, New Haven, Connecticut.
J.E. LeClerc, University of Rochester, New York.
G.C. Walker, Massachusetts Institute of Technology, Cambridge.
E. Eisenstadt, Harvard School of Public Health, Boston, Massachusetts.
P.L. Foster, Harvard School of Public Health, Boston, Massachusetts.
L.A. Loeb, University of Washington, Seattle.

SESSION 3 *Origins of Eukaryotic Mutation I*

C.T. Caskey, Baylor College of Medicine, Houston, Texas.
M. Calos, Stanford University, California.
D.F. Barker, University of Utah, Salt Lake City.
R.J. Albertini, University of Vermont, Burlington.
D. Martin, Jr., Genentech, Inc., South San Francisco, California.
D. Patterson, Eleanor Roosevelt Institute for Cancer Research, Inc., Denver, Colorado.
F. Sherman, University of Rochester, New York.
L. Prakash, University of Rochester, New York.

SESSION 4 *Origins of Eukaryotic Mutation II*

J.F. Crow, University of Wisconsin, Madison.
S.M. Weissman, Yale University, New Haven, Connecticut.
F. Vogel, Ruprecht-Karls-Universität, Heidelberg, Federal Republic of Germany.
L.S. Lerman, State University of New York, Albany.
A.C. Wilson, University of California, Berkeley.
T. Maniatis, Harvard University, Cambridge, Massachusetts.
R.A. Flavell, Biogen Research Corporation, Cambridge, Massachusetts.

SESSION 5 *Directed Mutagenesis*

M. Smith, University of British Columbia, Vancouver, Canada.
D. Shortle, State University of New York, Stony Brook.
R.B. Wallace, City of Hope Research Institute, Duarte, California.
M. Inouye, State University of New York, Stony Brook.
M. Grunstein, University of California, Los Angeles.

THE ROLE OF GENETIC PREDISPOSITION IN RESPONSES TO CHEMICAL EXPOSURE

October 2–October 5

INTRODUCTION

G.S. Omenn, University of Washington, Seattle: From pharmacogenetics to ecogenetics.
W. Kalow, University of Toronto, Canada: A pharmacologist looks at pharmacogenetics and ecogenetics.

SESSION 1 *P-450 Systems*

Chairperson: G.S. Omenn, University of Washington, Seattle

R.N. Hines, University of Nebraska, Omaha: Regulation of expression of cytochrome P-450.
M.J. Coon, University of Michigan, Ann Arbor: P-450–Multiplicity of inducers, isozymes, and substrates.
H. Gelboin, National Institutes of Health, Bethesda, Maryland: Phenotyping cytochrome P-450 by monoclonal-antibody-directed enzyme inhibition and radioimmunoassay.
F.P. Guengerich, Vanderbilt University, Nashville, Tennessee: Purification and characterization of a rat liver microsomal cytochrome P-450 involved in debrisoquine 4-hydroxylation, a prototype for genetic polymorphism in drug metabolism.

SESSION 2 *Drug and Carcinogen Metabolism*

Chairperson: W.F. Bodmer, Imperial Cancer Research Fund Laboratories, London, England

D.S. Davies, University of London, England: Studies of the substrate specificity of human cytochrome P-450.

J.R. Idle, University of London, England: Lung cancer—Consequence of habit and inheritance?

C. Von Bahr, Huddinge University Hospital, Sweden: In vitro metabolism by human liver in relation to polymorphic drug oxidation.

E.S. Vesell, Pennsylvania State University, Hershey: Impact of multiple dynamically interacting genetic and environmental factors on methods to detect new polymorphisms of hepatic drug oxidation.

A.H. Conney, Hoffmann-La Roche, Inc., Nutley, New Jersey: Variability in chemical biotransformations in human beings.

R.E. Kouri, Microbiological Associates, Bethesda, Maryland: Variations in aryl hydrocarbon hydroxylase levels in mitogen-activated human lymphocytes.

W.F. Bodmer, Imperial Cancer Research Fund Laboratories, London, England: DNA polymorphisms in population and family studies—Examples from the HLA system.

SESSION 3 *Polymorphisms of Metabolizing Enzyme Systems*

Chairperson: A.H. Conney, Hoffmann-La Roche, Inc., Nutley, New Jersey

S.P. Spielberg, Hospital for Sick Children, Toronto, Canada: Pharmacogenetic susceptibility to toxic drug metabolites in man.

B.N. La Du, Jr., University of Michigan, Ann Arbor: Could the human paraoxonase polymorphism account for different responses to certain environmental chemicals?

A.G. Motulsky, University of Washington, Seattle: Biochemical genetics of paraoxonase polymorphism.

F. Oesch, University of Mainz, Federal Republic of Germany: Variations in epoxide hydrolase activities in human liver and blood.

E. Beutler, Scripps Clinic and Research Foundation, La Jolla, California: Sensitivity to drug-induced hemolytic anemia in glucose-6-dehydrogenase deficiency.

E.J. Calabrese, University of Massachusetts, Amherst: Animal model for blood hereditary disorders—Environmental applications.

SESSION 4 *Oncogene Activation and DNA and Chromosomal Markers*

Chairperson: J.E. Cleaver, University of California, San Francisco

R.A. Weinberg, Massachusetts Institute of Technology, Cambridge: Oncogenes and human carcinogenesis.

A. Pellicer, New York University, New York: A chemical carcinogen activates in vivo a mouse *c-ras* oncogene.

A. Balmain, Beatson Institute for Cancer Research, Glasgow, Scotland: Activation of oncogenes at stages of chemical carcinogenesis in different mouse strains.

C.C. Harris, National Cancer Institute, Bethesda, Maryland: Carcinogenesis studies using cultured human tissues and cells.

T. Kakunaga, National Cancer Institute, Bethesda, Maryland: Mutations associated with neoplastic transformation by chemical carcinogens.

J. Garrels, Cold Spring Harbor Laboratory, New York: Use of two-dimensional gel electrophoresis for detection of proteins indicating genetic risk.

SESSION 5 *Immunological and Molecular Genetic Approaches*

Chairperson: H. Gelboin, National Institutes of Health, Bethesda, Maryland

B. Dupont, Memorial Sloan-Kettering Cancer Center, New York, New York: Disease susceptibility genes in the HLA complex.

P.C. White, Memorial-Sloan Kettering Cancer Center, New York, New York: Cloning and expression of cDNA encoding an adrenal cytochrome P-450 specific for steroid-21 hydroxylation.

H. Erlich, Cetus Corporation, Emeryville, California: HLA DNA polymorphisms—Use as genetic markers in control and disease populations.

S. Wolff, University of California, San Francisco: Use of sister chromatid exchanges to determine possible genetic predisposition in response to chemical exposure.

M.-C. King, University of California, Berkeley: Genetic and epidemiologic approaches for detecting susceptibility in populations.

SESSION 6 *Population Correlations*

Chairperson: C.C. Harris, National Cancer Institute, Bethesda, Maryland

F. Kueppers, Temple University, Philadelphia: Effect of smoking on the development of emphysema in alpha-1 antitrypsin deficiency.

R.A. Cartwright, Yorkshire Regional Cancer Organisation, Leeds, England: Epidemiological studies on *N*-acetylation and C-center ring oxidation in neoplasia.

P.G. Archer, University of Colorado, Denver: Some statistical and methodological issues in cytogenetic testing.

COFFEE AND HEALTH
October 30–November 2

SESSION 1 *The Coffee Product*

Chairperson: R.G. Bost, General Foods Corporation, White Plains, New York

G.E. Boecklin, National Coffee Association of USA, Inc., New York, New York: Coffee—A social history.

A.F. Beltrao, International Coffee Organisation, London, England: Coffee in the world economy.

W.P. Clinton, General Foods Corporation, White Plains, New York: Chemistry of coffee.

S.N. Katz, General Foods Corporation, Hoboken, New Jersey: Decaffeination of coffee.

R.G.K. Strobel, Procter & Gamble Company, Ohio: Chemistry of instant coffee.

A. Leviton, Harvard Medical School, Boston, Massachusetts: Correlates of coffee consumption.

SESSION 2 *Coffee Mutagenesis—Experimental Approaches*

Chairperson: L.W. Wattenberg, University of Minnesota, Minneapolis

T. Sugimura, National Cancer Center Research Institute, Tokyo, Japan: Mutagens in coffee—Background and present knowledge of mutagens and carcinogens produced by pyrolysis.

M. Nagao, National Cancer Center Research Institute, Tokyo, Japan: Mutagens in coffee.

H.P. Wurzner, Nestle Products Technical Assistance Co. Ltd., Orbe, Switzerland: Preliminary findings of a carcinogen bioassay of coffee in mice.

H.-U. Aeschbacher, Nestle Products Technical Assistance Co. Ltd., Orbe, Switzerland: Risk evaluation of coffee based on in vitro and in vivo mutagenicity testing.

S. Takayama, Japanese Foundation for Cancer Research, Tokyo: Long-term carcinogenicity studies on caffeine, instant coffee, and methylglyoxal in rats.

SESSION 3 *Coffee and Human Carcinogenesis I*

Chairperson: T. Sugimura, National Cancer Center Research Institute, Tokyo, Japan

Pancreatic Cancer

B. MacMahon, Harvard School of Public Health, Boston, Massachusetts: Coffee and cancer of the pancreas—A review.

Bladder and GI Tract Cancer

L. Gordis, Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland: Coffee and pancreatic cancer.

A.S. Morrison, Harvard School of Public Health, Boston, Massachusetts: Control of cigarette smoking in evaluating the association of coffee drinking and bladder cancer.

L.W. Wattenberg, University of Minnesota, Minneapolis: Protective effects of coffee constituents on carcinogenesis in experimental animals.

SESSION 4 *Coffee and Human Carcinogenesis II*

Chairperson: R. Saracci, International Agency for Research on Cancer, Lyon, France

Ovarian Cancer

D. Trichopoulos, University of Athens School of Medicine, Greece: A case-control investigation of a possible association between coffee consumption and ovarian cancer in Greece.

S. Shapiro, Boston University School of Medicine, Cambridge, Massachusetts: Ovarian cancer and coffee drinking.

Breast Cancer and Fibrocystic Disease

V.L. Ernster, University of California, San Francisco: Epidemiological studies of coffee and breast disease.

F. Lubin, Chaim Sheba Medical Center, Tel-Hashomer, Israel: Coffee and methylxanthine in benign and malignant breast disease.

L. Rosenberg, Boston University School of Medicine, Cambridge, Massachusetts: Breast cancer and coffee drinking.

E.M. Grossman, General Foods Corporation, Tarrytown, New York: Caffeine and benign breast disease—A proposed clinical trial.

SESSION 5 *Physiological and Behavioral Effects*

Chairperson: S.R. Tannenbaum, Massachusetts Institute of Technology, Cambridge

G.D. Friedman, The Permanente Medical Group, Inc., Oakland, California: Coffee and coronary heart disease—Are there grounds for concern?

L. Welin, University of Gothenburg, Sweden: Coffee, traditional risk factors, coronary heart disease, and mortality.

A. Sivak, Arthur D. Little, Inc., Cambridge, Massachusetts: Chronic experimental animal studies with coffee.

BIOLOGICAL IMAGING AND NUCLEAR MAGNETIC RESONANCE (CONGRESSIONAL WORKSHOP)

December 2–December 4

INTRODUCTION

B. Chance, University of Pennsylvania, Philadelphia

OVERVIEW

P.C. Lauterbur, State University of New York, Stony Brook

SESSION 1

T. Brady, Massachusetts General Hospital, Boston, Massachusetts: Molecules in magnetic fields.

M. Ter-Pogossian, Washington University Medical School, St. Louis, Missouri: Positron emission tomography.

SESSION 2

B. Chance, University of Pennsylvania, Philadelphia: In vivo nuclear magnetic resonance.

C. Higgins, University of California, San Francisco, Medical School: Proton imaging and computed tomography.

SESSION 3

T. Budinger, University of California, Berkeley: Comparative and safety aspects.

P.C. Lauterbur, State University of New York, Stony Brook, and B. Chance, University of Pennsylvania, Philadelphia: Summary discussion.

Congressional redirect.

POSTGRADUATE COURSES
Participants and Seminars

THE CELLULAR AND MOLECULAR BIOLOGY OF BEHAVIOR

Participants

Blum, Andrew S., B.A., *Rockefeller University, New York, New York*
Bodnar, Deana A., B.S.E., *University of Iowa, Iowa City*
Earnest, Thomas N., M.A., *Boston University, Massachusetts*
Floeter, Mary Kay, B.S., *Washington University, St. Louis, Missouri*
Friedman, Alan M., A.B., *Yale Medical School, New Haven, Connecticut*
Goode, Marian I., B.A., *Georgia Institute of Technology, Atlanta*
Hakim, Vincent, *Attache de Recherche of the CNRS, Paris, France*
Indik, Jonathan H., M.D., *University of Pennsylvania, Philadelphia*
Jones, Bradley R., B.S., *Hopkins Marine Station, Pacific Grove, Pennsylvania*
LaMantia, Anthony-Samuel, B.A., *Yale Medical School, New Haven, Connecticut*
McFarland, Jenny Lee, S.B., *University of Washington, Seattle*
Mistler, Lisa A., S.B., *University of California, San Francisco*
Morielli, Anthony D., B.S., *University of California, Santa Cruz*
Reye, David N., B.S., *University of Alberta, Edmonton, Canada*
Sakurai, Masaki, M.D., *University of Tokyo, Japan*
Schachter, Leah A., B.A., *Yale University, New Haven, Connecticut*
Tamsky, Carolyn A., B.S., *Arizona State University, Tempe*
Wagner, Michael A., B.S., *Institute for Cancer Research, Philadelphia, Pennsylvania*
Walsh, John, *University of Texas, Houston*
Williams, Heather, B.A., *Rockefeller University, New York, New York*

Seminars

Axel, R., Columbia University. Introduction to the study of gene regulation.
Goldberg, D., Columbia University. Regulation of transmitter synthesis.
Scheller, R., Stanford University. Genes, peptides, and behavior.
Truman, J., University of Washington. Hormones and behavior.
Kupfermann, I., Columbia University. Motivation.
Wine, J., Stanford University. Nerve circuitry for simple behavioral acts and their control.
Getting, P., University of Iowa. Rhythm generation in invertebrate motor systems.
Thach, W.T., Jr., Washington University. Cerebellar content of posture and movement.
Fuchs, A., University of Washington. Adaptive regulation in the oculomotor system.
Ghez, C., Columbia University. Voluntary movements in mammals.
Suga, N., Washington University. Brain pathways for sound processing in bats.
Heiligenberg, W., University of California, San Diego. Electrolocation and electric communication in fish.
Ross, E., Southwestern Medical School. Language functions in the human brain and their location.
Black, I., Cornell University. The role of neurotransmitters in behavioral abnormalities.

MOLECULAR BIOLOGY OF PLANTS

Participants

Barnes, Wayne M., Ph.D., *Washington University, St. Louis, Missouri*
Choi, Kyung-Hee, Ph.D., *Albert Einstein College of Medicine, Bronx, New York*
Curtis, Stephanie E., Ph.D., *University of Chicago, Illinois*
De Lorenzo, Giulia, Ph.D., *Universita di Roma, Italy*
Fox, Thomas D., Ph.D., *Cornell University, Ithaca, New York*

Gesteland, Raymond F., Ph.D., *University of Utah, Salt Lake City*
Green, Pamela J., B.S., *State University of New York, Stony Brook*
Haugli, Finn B., Ph.D., *University of Tromsø, Norway*
Hombrecher, Gerd G., Ph.D., *John Innes Institute, Norwich, England*
Kinlaw, Claire S., Ph.D., *Rice University, Houston, Texas*
Kuhlemeier, Cris, M.S., *State University of Utrecht, The Netherlands*
Lemaux, Peggy G., Ph.D., *Stanford University, California*
Mackey, Catherine J., Ph.D., *Pfizer, Inc., Groton, Connecticut*
Murphy, Caroline, Ph.D., *University of Connecticut, Storrs*
Tenning, Paul P., *Max-Planck-Institut, Koln, Federal Republic of Germany*
Van Den Elzen, Peter J., Ph.D., *Advanced Genetic Sciences, Inc., Berkeley, California*

Seminars

Bidney, D., Advanced Genetic Sciences. The potato protoplast system and plant breeding.
Kemble, R., Kansas State University. Mitochondrial DNA and male sterility.
Maliga, P., Washington University. Cell culture mutant selection and protoplast fusion.
Long, S., Stanford University. Nitrogen fixation–Symbiosis.
Hanson, M., University of Virginia. Plant cell culture and organelle genetics.
Van Montagu, M., Rijksuniversiteit Gent. Molecular biology of crown gall disease.
Ausubel, F., Massachusetts General Hospital. Nitrogen fixation–Enzymology and genetics.
Harada, J., University of California, Los Angeles. Regulation of abundant genes during soybean embryogeny.
Dooner, H., Advanced Genetic Sciences. Maize controlling elements.
Ellingboe, A., University of Wisconsin. Genetics of plant-pathogen interactions.
Bogorad, L., Harvard University. Chloroplast molecular biology.
Zaitlin, M., Cornell University. Plant viruses, viroids, virusoids, and satellites.
Miles, D., University of Missouri. Mutants in the photosynthesis pathway.
Steinback, K., Advanced Genetic Sciences. Analysis of thylakoid polypeptides and electron flow in photosynthesis.
Chau, N.H., Rockefeller University. Nuclear chloroplast interactions and gene regulation.
Walbot, V., Stanford University. Developmental genetics of maize.
Ho, D., University of Illinois. Hormone response mutants of barley.
Dunsmuir, P., CSIRO. Chlorophyll ab binding protein.

NERVOUS SYSTEM OF THE LEECH

Participants

Davis, Robin L., B.S., M.A., *Stanford University, California*
Dietzel, Irmgard D., Ph.D., *Max-Planck-Institut, Heidelberg, Federal Republic of Germany*
Frederiksen, Kristen, M.S., *Cold Spring Harbor Laboratory, New York*
Friedman, Beth, Ph.D., *Montreal General Hospital Research Institute, Canada*
Giraldez, Fernando, M.D., Ph.D., *Physiological Laboratory, Cambridge, England*
Kotak, Vibhakar C., Ph.D., *Sardar Patel University, Kaira, India*
Loer, Curtis M., B.S., *University of California, San Diego*
Magill, Catherine, B.A., *University of California, Berkeley*
Sundin, Olof, Ph.D., *Cold Spring Harbor Laboratory, New York*
Thompson, Stephen W.N., B.S., *University of Glasgow, Scotland*

Seminars

Stent, G., University of California, Berkeley. Development of leech nervous system.
Weisblat, D., University of California, Berkeley. Development of leech nervous systems.
Friesen, O., University of Virginia. Swimming.
Kristan, W., University of California, San Diego. Swimming.
Hockfield, S., Cold Spring Harbor Laboratory. Monoclonal antibodies for leech CNS.

MOLECULAR CLONING OF EUKARYOTIC GENES

Participants

Alter, Barbara J., M.S., *University of Minnesota, Duluth*
Buxser, Stephen E., Ph.D., *University of Massachusetts Medical School, Worcester*
Carter, Richard, Ph.D., *National Institutes of Health, Bethesda, Maryland*
Chen, Jeff W., M.S., *Johns Hopkins University Medical School, Baltimore, Maryland*
Griep, Anne E., B.A., *University of Wisconsin, Madison*
Gurney, Mark E., Ph.D., *University of Chicago, Illinois*
Kimura, Shoji, Ph.D., *Memorial Sloan-Kettering Cancer Center, New York, New York*
Leonard, Warren J., M.D., *NCI, National Institutes of Health, Bethesda, Maryland*
Ponzetto-Zimmerman, Carola, Ph.D., *Columbia University College of Physicians & Surgeons, New York, New York*
Riddle, Donald L., Ph.D., *University of Missouri, Columbia*

Roberts, Anita B., Ph.D., *National Institutes of Health, Bethesda, Maryland*
Roitman, Isaac, Ph.D., *Universidad de Brasilia, Brazil*
Samson, Leona, Ph.D., *University of California, Berkeley*
Sinangil, Faruk, Ph.D., *University of Nebraska Medical Center, Omaha*
Tominaga, Akira, Ph.D., *Harvard Medical School, Boston, Massachusetts*
Van Pel, Aline, *Ludwig Institute, Brussels, Belgium*

Seminars

Croce, C., Wistar Institute. Genetics of Burkitt's lymphoma.
Lacy, E., Columbia University. Gene transfer into mice.
Seed, B., Harvard University. Recovery and manipulation of recombinant DNA in vivo.
Vande Woude, G., National Institutes of Health. Properties of the *mos* oncogene locus in human and mouse genomes.
Scheller, R., Stanford University. The molecular genetic basis of simple behaviors.
Bender, W., Harvard University. Molecular genetics of the bithorax complex in *Drosophila.*
Shortle, D., State University of New York, Stony Brook. Directed mutagenesis.
Davis, M., Stanford University. Lymphocyte-specific gene expression and cloning.
Sutcliff, J.G., Scripps Institute. Localizing the products of brain-specific genes.
Fischer, S., State University of New York, Albany. DNA sequence-specific separation in denaturing gradient gel electrophoresis.
Fink, G., Massachusetts Institute of Technology. The control of recombination between repeated DNA elements.
Irwin, N., Harvard University. Expression of foreign genes in *E. coli.*
Hirsch, J., Harvard University. Reintroduction and expression of genes into *Drosophila.*
St. John, T., Stanford University. cDNA cloning in phage and manipulation of insert material.
McKnight, S., Fred Hutchinson Cancer Research Center. Regulation of cellular and viral TK enzyme expression.
Wigler, M., Cold Spring Harbor Laboratory. Human transforming genes.
Ruley, E., Cold Spring Harbor Laboratory. Ela, *c-myc.*
Ward, D., Yale University. Biotin-labeled polynucleotides as hybridization probes and affinity selection reagents.
Mulligan, R., Massachusetts Institute of Technology. New vectors for gene transfer in the mammalian cell and the intact animal.
Maniatis, T., Harvard University. Regulated expression of eukaryotic genes.

GENETICS AND MOLECULAR BIOLOGY OF *CHLAMYDOMONAS*

Participants

Adams, G.M.W., *Louisiana State University, Baton Rouge*
Aldrich, Jane, Ph.D., *Standard Oil of Ohio, Cleveland*
Ausich, Rodney L., Ph.D., *Amoco Research Center, Naperville, Illinois*
Bartlett, Sue G., *Louisiana State University, Baton Rouge*
Bean, Barry, Ph.D., *Lehigh University, Bethlehem, Pennsylvania*
Benedick, Michael J., Ph.D., *DNAX Research Institute, Palo Alto, California*
Blair, Lindley C., Ph.D., *University of California, Berkeley*
Brunke, Karen, Ph.D., *Institute for Cancer Research, Philadelphia, Pennsylvania*
Cox, John C., Ph.D., *Martin Marietta Laboratories, Baltimore, Maryland*
Detmers, Patricia A., Ph.D., *Albert Einstein College of Medicine, Bronx, New York*
Dutcher, Susan K., Ph.D., *Rockefeller University, New York, New York*
Forest, Charlene L., Ph.D., *Brooklyn College, New York*
Foster, Ken, *Mt. Sinai School of Medicine, New York, New York*
Galloway, Ruth E., *Washington University, St. Louis, Missouri*
Greenbaum, Elias, Ph.D., *Oak Ridge National Laboratory, Tennessee*
Hicks, James, Ph.D., *Cold Spring Harbor Laboratory, New York*
Hodson, Robert, Ph.D., *University of Delaware, Newark*
Homan, Wieger L., Ph.D., *University of Amsterdam, The Netherlands*
Hourcade, Dennis, *Washington University, St. Louis, Missouri*
Howell, Stephen H., Ph.D., *University of California, San Diego, La Jolla*
Huang, Bessie, *Baylor College of Medicine, Houston, Texas*
Jarvik, Jonathan, *Carnegie Mellon University, Pittsburgh, Pennsylvania*
Lee, Robert W., *Dalhousie University, Halifax, Nova Scotia*
Lemieux, Claude, Ph.D., *National Research Council, Ottawa, Canada*
Lewin, Ralph, *Scripps Institute of Oceanography, La Jolla, California*
Matagne, Rene F., *Laboratory of Molecular Genetics, Liege, Belgium*
Merlin, Ellis, *Standard Oil of Ohio, Cleveland*
Mets, Laurens, *University of Chicago, Illinois*
Morel, Nicole, Ph.D., *Tufts University, Medford, Massachusetts*
Mosig, Gisela, Ph.D., *Vanderbilt University, Nashville, Tennessee*
Muskavitch, Karen, Ph.D., *Harvard University, Cambridge, Massachusetts*
Polley, David L., Ph.D., *Wabash College, Crawfordsville, Indiana*

Rochaix, Jean-David, *University of Geneva, Switzerland*
Sager, Ruth, *Sidney Farber Cancer Center, Boston, Massachusetts*
Schloss, Jeffrey, *Yale University, New Haven, Connecticut*
Segal, Rosalind, *Rockefeller University, New York, New York*
Sklar, Robert, *Dana Farber Cancer Institute, Boston, Massachusetts*
Small, Gary D., Ph.D., *University of South Dakota, Vermillion*
Surzycki, Stefan, *University of Geneva, Switzerland*
Togasaki, Robert, *Indiana University, Bloomington, Indiana*
Van Winkle-Swift, Karen, Ph.D., *University of California, San Diego*
Wang, Wei-Yeh, *University of Iowa, Iowa City*
Weeks, Donald P., Ph.D., *Zoecon Corporation, Palo Alto, California*
Weiss, Richard L., Ph.D., *San Diego State University, California*
White, Frank F., Ph.D., *University of Washington, Seattle*
Wydrzyndki, Thomas, Ph.D., *Amoco Research Center, Naperville, Illinois*

Seminars

Sager, R., Sidney Farber Cancer Institute. Control of maternal inheritance of chloroplast DNA by methylation.
Mets, L., University of Chicago. Identification of the physical sites of genetic markers in the chloroplast genome.
Bartlett, S., Louisiana State University. Genetics of photosynthetic membranes.
Togasaki, R., Indiana University. Genetics of the dark reactions and ribulose bisphosphate carboxylase.
Wang, W.-Y., University of Iowa. Genetic dissection of porphyrin biosynthesis.
Adams, G.M.W., Louisiana State University. Selection of motility mutants.
Lee, R., Dalhousie University. Induction and selection of chloroplast mutants/synchronized cultures and density transfer experiments.
Lewin, R., Scripps Institute of Oceanography. The genus *Chlamydomonas*.
———.The mating reaction.
Huang, B., Baylor College of Medicine. Genetic dissection of flagellar axonemes.
Schloss, J., Yale University. Molecular genetics of tubulin.
Foster, K., Mt. Sinai School of Medicine. Phototaxis.
Howell, S., University of California, San Diego. Genetics and molecular biology of the cell cycle.
Van Winkle-Swift, K., University of California, San Diego. Mating type in the genus *Chlamydomonas*.
Matagne, R., University of Liege. Cell fusion.
Surzycki, S., Indiana University. In vitro transcription systems from *Chlamydomonas*.
Brunke, K., Institute for Cancer Research. Shared homologies in the 5′ promoter regions of the coordinately regulated tubulin gene.
Rochaix, J.-D., University of Geneva. Transformation.
Hourcade, D., Washington University, St. Louis. Gene transfer from bleomycin-treated *Chlamydomonas*.
Ausich, R., Amoco Research Center. Transformation of *Chlamydomonas* using *Agrobacterium tumefaciens* containing the T_i plasmid.

MOLECULAR EMBRYOLOGY OF THE MOUSE

Participants

Chung, Su-yun, Ph.D., *Princeton University, New Jersey*
Crenshaw, E. Bryan III, S.B. *University of California, San Diego*
Hanahan, Doug, Ph.D., *Cold Spring Harbor Laboratory, New York*
Krangel, Michael, Ph.D., *Cold Spring Harbor Laboratory, New York*
Lehrach, Hans, Ph.D., *European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany*
Rassoulzadegan, Minoo, Ph.D., *Centre de Biochimie, Nice, France*
Rigby, Peter, Ph.D., *Imperial College, London, England*
Schmidt, Azriel, Ph.D., *National Institutes of Health, Bethesda, Maryland*
Silver, Lee, Ph.D., *Cold Spring Harbor Laboratory, New York*
Stubbs, Lisa, *University of California, San Diego*
Taniguchi, Tadatsugu, Ph.D., *Cancer Institute, Tokyo, Japan*
Yisraeli, Joel K., *Hebrew University, Jerusalem, Israel*

Seminars

McLaren, A., MRC Mammalian Development Unit. Origin, growth, and differentiation of germ cells.
———. X-inactivation.
Pratt, H., University of Cambridge. Gene expression in preimplantation embryos.
Chapman, V., Roswell Park Memorial Institute. X-inactivation.
Stewart, T., Harvard Medical School. Chimeras with teratocarcinoma cells.
McGrath, J., Wistar Institute. Nuclear transplantation with mouse embryos.
Axelrod, H., Wistar Institute. Isolation of pluripotent cell lines from mouse embryos.
Spradling, A., Carnegie Institution of Washington. Gene insertion in *Drosophila*.
Angerer, R., University of Rochester. In situ hybridization with sea urchin embryos.
Lo, C., University of Pennsylvania. Intercellular communication in early mouse embryos.
———. Iontophoresis of DNA into mouse eggs.
Verma, I., Salk Institute. Oncogene expression in mouse embryos.
Mulligan, R., Massachusetts Institute of Technology. Viral vectors.
Jaenisch, R., Heinrich-Pette Institut. Introduction of retroviruses into the germ line.
Rigby, P., Imperial College, London. Transformation activators in embryonic genes.
Silver, L., Cold Spring Harbor Laboratory. The T complex in mice.

SINGLE-CHANNEL RECORDING

Participants

Ashcroft, Frances M., Ph.D., *University of Oxford, England*
Assmann, Sarah M., B.A., *Stanford University, California*
Carrow, Grant M., Ph.D., *Harvard University, Cambridge, Massachusetts*
Dawson, David C., Ph.D., *University of Michigan Medical School, Ann Arbor*
Ebihara, Lisa, Ph.D., *University of Colorado, Boulder*
Jahr, Craig E., Ph.D., *Harvard Medical School, Boston, Massachusetts*
Jalonen, Tuula O., M.S., *University of Turku, Finland*
Matthews, Hugh R., B.A., *University of Cambridge, England*
McArdle, Joseph J., Ph.D., *New Jersey Medical School, West Orange*
Robinson, Hugh P.C., B.A., *Rockefeller University, New York, New York*

Seminars

Tank, D.W., Bell Laboratories. Patch-clamp procedures for liposomes.
Steinbach, J.H., Salk Institute. Properties of acetylcholine receptors in BC_3H1 cells.

ADVANCED NEUROANATOMICAL METHODS

Participants

Catsicas, Stefano, B.S., *Institute of Anatomy, Bugnon, Switzerland*
Chuong, Cheng-Ming, M.D., *Rockefeller University, New York, New York*
Davidson, Robert M., M.S., *State University of New York, Buffalo*
Hemmings, Hugh Carroll, Jr., B.S., *Yale School of Medicine, New Haven, Connecticut*
Kennedy, Mary B., Ph.D., *California Institute of Technology, Pasadena*
Leonard, Debra S., B.S., *Purdue University, West Lafayette, Indiana*
LeTaillanter, Michel, Ph.D., *University of Minnesota, Minneapolis*
Ronchi, Elettra, M.S., *University of Rome, Italy*
Vallury, Visalakshi, M.S., *University of Illinois, Urbana*
Wald, Steven L., M.D., *University of Vermont, Burlington*

Seminars

Raviola, E., Harvard University. Freeze fracture and related techniques in the study of the retina.
McKay, R., Cold Spring Harbor Laboratory. Neuroimmunology and hybridoma technology.

ADVANCED BACTERIAL GENETICS

Participants

Armengod, Maria-Eugenia, Ph.D., *Instituto de Investigaciones Citologicas, Valencia, Spain*
Berberich, Mary Anne, Ph.D., *National Institutes of Health, Bethesda, Maryland*
Boxer, David H., Ph.D., *Dundee University, Scotland*
Brownstein, Bernard H., Ph.D., *Abbott Laboratories, North Chicago, Illinois*
Coulton, James W., Ph.D., *McGill University, Montreal, Canada*
Eichinger, Daniel J., *New York University Medical Center, New York*
Fekete, Thomas, M.D., *University of Chicago, Illinois*
Hom, Sherman, S.M., Ph.D., *Johns Hopkins University, Baltimore, Maryland*
Levesque, Roger C., Ph.D., *Massachusetts General Hospital, Boston*
Marcotte, William R., Jr., B.S., *University of Virginia, Charlottesville*
Ruether, James E., B.A., *Sloan-Kettering Institute, New York, New York*
Saunders, Roger, Ph.D., *Chappaqua, New York*
Shin, Hee-Sup, Ph.D., *Sloan-Kettering Institute, New York, New York*
Warren, Fred D., B.S., *University of Connecticut Health Center, Farmington*
Wolfe, Paul B., Ph.D., *University of California, Los Angeles*
Yakobson, Emanuel A., Ph.D., *University of California, San Diego*

Seminars

Adhya, S., NCI, National Institutes of Health. Control of the *gal* operon of *E. coli*.
Susskind, M.M., University of Massachusetts Medical School. Changing the binding specificity of the repressor.
Schwartz, M., Institut Pasteur. Positive control in the maltose regulation of *E. coli*.
Shuman, H., Columbia University. Use of gene fusions to study membrane transport proteins.

Oliver, D., Genetics Institute. Mechanism of protein localization in *E. coli*.

Grindley, N., Yale University Medical School. Rearrangements mediated by Tn*903* and what they tell us about transposition mechanisms.

Roth, J., University of Utah. Novel genetic approaches to interesting biological problems.

Miller, C., Case Western Reserve University. *Salmonella* genes regulated by growth phase.

MOLECULAR AND CELLULAR NEUROBIOLOGY

Participants

Akerblom, Ingrid E., B.S., *University of California, San Diego*

Baetscher, Manfred W., *University of Basel, Switzerland*

Bhakoo, Kishore K., B.S., *Institute of Neurology, London, England*

Bonini, Nancy M., A.B., *University of Wisconsin, Madison*

Chamberlain, Susan, B.S., *University of Walk, Bristol, England*

Chun, Jerold J.M., B.A., *Stanford Medical School, California*

DeVries, Steven H., B.S., *University of Chicago, Illinois*

Erondu, Ngozi E., B.S., *California Institute of Technology, Pasadena*

Ge, Bang-lun, Ph.D., *University of California, San Francisco*

Giovanni, Levi, *Weizmann Institute, Rehovot, Israel*

Grumet, Martin, Ph.D., *Rockefeller University, New York, New York*

Holton, Thomas, Ph.D., *California Institute of Technology, Pasadena*

Kuffer, Pierre, *Institut de Physiologie, Lausanne, Switzerland*

Lindgren, Clark A., M.S., *University of Wisconsin, Madison*

Nef, Patrick, *University of Geneva, Switzerland*

Perkel, David J., *Harvard University, Cambridge, Massachusetts*

Raghavan, K. Vijay, Ph.D., *Tata Institute of Fundamental Research, Bombay, India*

Ring, George D., M.S., *Hebrew University, Jerusalem, Israel*

Stiemer, Rainer Helmut, *Institut for Neurobiologie, Federal Republic of Germany*

Tuttle, Rebecca, B.S., *Purdue University, West Lafayette, Indiana*

Waddell, Pamela J., B.S., *University of Bristol, Scotland*

Williams, Brenda P., *University College London, England*

Yaffa, Mizrachi, M.Sc., *Weizmann Institute of Science, Rehovot, Israel*

Zelingher, Julian, B.S., *Hebrew University, Jerusalem, Israel*

Seminars

Lindstrom, J., Salk Institute. Acetylcholine receptor: Purification, structure of subunits, and reconstitution.

Merlie, J., Washington University, St. Louis. Regulation of the acetylcholine receptor.

Lindstrom, J., Salk Institute. Myosthenia gravis.

Jan, Y.-N., University of California, San Francisco. Do peptides act as neurotransmitters?

———. Differences between peptidergic transmissions and classical transmission.

McKay, R., Cold Spring Harbor Laboratory. How genes make brains.

Hockfield, S., Cold Spring Harbor Laboratory. Immunological probes of CNS structure.

Jan, Y.-N., University of California, San Francisco. P/M hybrid dysgenesis and the cloning of the K^+ channel in the *shaker*.

Basbaum, A., University of California, San Francisco. Principles of immunocytochemistry.

———. Substance P and pain.

———. Endorphins.

Corey, D., Yale University. Voltage-dependent Ca conductance in photoreceptors.

Roberts, J., Columbia University. Use of recombinant DNA technology to isolate neuroactive gene sequences.

Hume, R., Washington University, St. Louis. Neuroterminals release acetylcholine before synapse formation.

Lewis, R., University of California, San Francisco. Patch electrode recording from hair cells.

Scheller, R., Stanford University. Molecular basis of a "simple" behavior in *Aplysia*.

———. Neuroendocrine baf cells, cloning the ELH gene family, in situ hybridization, immunofluorescence.

Heinemann, S., Salk Institute, Molecular cloning of the acetylcholine receptors.

———. Structure and function of the acetylcholine receptors.

Frank, E., Introduction to sensory-motor synapses in the spinal cord.

———. Mechanism of synaptic transmission at sensory-motor synapses in the spinal cord.

———. Development and plasticity of sensory-motor synapses in the spinal cord.

Dionne, V., University of California, San Diego. From acetylcholine receptors to olefaction.

Steinbach, J., Salk Institute. Developmental changes in junctional acetylcholine receptors.

Van Essen, D., California Institute of Technology. Synapse elimination at the neuromuscular junction.

Kehoe, J., École Normale Superiéure. Synaptic elimination of synaptic conductance.

Erulkar, S., University of Pennsylvania. Single-channel properties of the synaptically activated chloride channel in *Mollusca*.

Bennett, M., Albert Einstein College of Medicine. Electrical synapses and gap junctions: Morphology.

Brocks, J., California Institute of Technology. Glial growth factor.

Jones, S., State University of New York, Stony Brook. M currents of potassium in bullfrog autonomic ganglia.

Blaustein, M., University of Maryland. Hooked on angel dust: K channels phencyclidine and behavior.
Black, I., Cornell University Medical College. Calecholaminergic synapse.
———. Growth and development of calecholaminergic systems.
———. Peptide-calecholamin interactions.
———. Neurotransmitter diseases in humans.

NEUROBIOLOGY OF HUMAN DISEASE

Participants

Allen, Donald, B.S., *Rockefeller University, New York, New York*
Boone, Timothy, M.S., *University of Texas, Houston*
Boyle, Mary B., B.A., *Yale University, New Haven, Connecticut*
Bredesen, Dale E., M.D., *University of California, San Francisco*
Fitzpatrick, Susan, B.S., *Cornell University, Ithaca, New York*
Greenamyre, Timothy, B.S., *University of Michigan, Ann Arbor*
Halpain, Shelley, B.S., *Rockefeller University, New York, New York*
Johnson, Eric, B.A., *University of Pennsylvania, Philadelphia*
Kato, Ann, Ph.D., *University of Geneva, Switzerland*
Krause, James, Ph.D., *State University of New York, Stony Brook*
Levi, Giovanni, M.S., *Weizmann Institute, Rehovot, Israel*
Levin, Leonard, B.A., *Harvard Medical School, Boston, Massachusetts*
Lillien, Laura, B.A., *University of Wisconsin, Madison*
Lipkin, Walter, M.D., *University of California, San Francisco*
Orloff, Gregory, B.A., *Yale University, New Haven, Connecticut*
Patel, Urmi, Ph.D., *National Institutes of Health, Bethesda, Maryland*
Ruggieri, Michael, B.A., *University of Pennsylvania, Philadelphia*
Rutecki, Paul, M.D., *Baylor College of Medicine, Waco, Texas*
Sapolsky, Robert, B.A., *Rockefeller University, New York, New York*
Stewart, Kim, B.A., *State University of New York, Stony Brook*
Wexler, Nancy, Ph.D., *Hereditary Disease Foundation, Beverly Hills, California*
White, Jeffrey, Ph.D., *State University of New York, Stony Brook*
Zaczek, Robert, B.S., *Johns Hopkins University, Baltimore, Maryland*

Seminars

Coyle, J., Johns Hopkins University School of Medicine. Degenerative diseases: Alzheimer's, Huntington's, and experimental models.
Plum, F., Cornell University Medical College. Consciousness, stupor, and coma.
Brady, R., National Institutes of Health. The lipidoses.
Barker, J., National Institutes of Health. Epilepsy.
Fields, H., University of California Medical School. Pain syndromes and neuropeptides.
Jessell, T., Harvard Medical School. Pain syndromes and neuropeptides.
Lindstrom, J., Salk Institute. Autoimmune disease—Myasthenia gravis.
Gajdusek, C., National Institutes of Health. Slow viruses and the central nervous system.
Aguayo, A., McGill University, Montreal General Hospital. Neural regeneration in the central and peripheral nervous systems.
Gazzaniga, M., Cornell University Medical College. Cognitive disorders and split brain function.

ADVANCED TECHNIQUES IN MOLECULAR CLONING OF EUKARYOTIC GENES

Participants

Barber, Leslie, B.A., *University of California, Los Angeles*
Craik, Charles, *University of California, San Francisco*
Jacob, William, M.S., *Brown University, Providence, Rhode Island*
Kaczorek, Michel, Ph.D., *Institut Pasteur, Paris, France*
Kehry, Marilyn, Ph.D., *University of Oregon, Eugene*
Lenardo, Michael, M.D., *University of Iowa, Iowa City*
Mason, Philip, Ph.D., *ICRF, London, England*
Osborne, Barbara, Ph.D., *Amherst College, Massachusetts*
Rice, Nancy, Ph.D., *Frederick Cancer Research Facility, Frederick, Maryland*
Savage, Nancy, Ph.D., *Bethesda Research Laboratories, Maryland*
Searls, David, Ph.D., *Wistar Institute, Philadelphia, Pennsylvania*
Vincent, Karen, B.A., *University of California, Berkeley*

Seminars

Woods, D., Harvard Medical School. Cloning cDNA of complement.

Seeburg, P., Genentech. Site-directed mutagenesis of the human growth hormone gene.

Gallupi, G., Monsanto. Synthesis and use of oligonucleotides.

Messing, J., University of Minnesota. M13 cloning and gene structure.

Wallace, B., City of Hope. Hybridization with synthetic DNA.

Tatchell, K., University of Pennsylvania. Use of oligonucleotide linkers as mutagens.

ELECTROPHYSIOLOGICAL METHODS USED IN ANALYZING THE MODE OF ACTION OF TRANSMITTERS

Participants

Acevedo, Larisa, B.A., *University of California, Davis*

Agmon, Ariel, M.S., *Stanford University, California*

Block, Melodye, M.S., *University of California, Los Angeles*

Johansen, Jorgen, M.S., *Cold Spring Harbor Laboratory, New York*

Lipscombe, Diane, B.S., *University College London, England*

Mitchell, Clifford L., Ph.D., *National Institute of Environmental Health Sciences, Research Triangle Park, Maryland*

Numman, Randy, B.S., *University of Texas, Galveston*

Rogawski, Michael, Ph.D., *National Institutes of Health, Bethesda, Maryland*

Stansfeld, Catherine, M.S., *University College, Cardiff, Wales*

Swope, Sheridan, B.S., *Harvard School of Public Health, Cambridge, Massachusetts*

Seminar

Adams, P., State University of New York, Stony Brook. Slow and very slow postsynaptic currents.

YEAST GENETICS

Participants

Boyd, Alan, Ph.D., *The Leicester Biocentre, England*

Driscoll, Robert, B.A., *University of Utah, Salt Lake City*

Dowhan, William, Ph.D., *University of Texas, Houston*

Ellwood, Marian Sue, Ph.D., *University of Wisconsin, Madison*

Ford, Clark F., Ph.D., *University of Virginia, Charlottesville*

Hamer, Dean H., Ph.D., *National Institutes of Health, Bethesda, Maryland*

Howell, William M., Ph.D., *University of Oregon, Eugene*

Krisch, Henry M., Ph.D., *University of Geneva, Switzerland*

Lauquin, Guy J.-M., Ph.D., *Centre d'Etudes Nucleaires, Grenoble, France*

Lindberg, Martin J., Ph.D., *Biomedical Center, Uppsala, Sweden*

Meacock, Peter A., Ph.D., *University of Leicester, England*

Roulland-Dussoix, Daisy M., Ph.D., *Institut Pasteur, Paris, France*

Seale, Ronald L., Ph.D., *Scripps Clinic and Research Foundation, La Jolla, California*

Simons, Robert W., Ph.D., *Harvard University, Cambridge, Massachusetts*

West, Robert W., Jr., Ph.D., *Harvard University, Cambridge, Massachusetts*

Zonneveld, B.J.M., Ph.D., *State University of Leiden, The Netherlands*

Seminars

Bloom, K., University of North Carolina. Topology of yeast centromere DNA.

Warner, J., Albert Einstein College of Medicine. Ribosomal protein genes and their regulation.

Sternglanz, R., State University of New York, Stony Brook. DNA topoisomerase yeast mutants.

Broach, J., State University of New York, Stony Brook. Yeast plasmid 2u circle.

Szostak, J., Dana Farber Cancer Institute. Properties of telomeres and artificial chromosomes.

Petes, T., University of Chicago. Recombination of repeated yeast genes.

Hereford, L., Dana Farber Cancer Institute. Regulation of yeast histone gene expression.

Rosbash, M., Brandeis University. Splicing of mRNA introns in yeast.

Botstein, D., Massachusetts Institute of Technology. Genetics of actin and tubulin in yeast.

Smith, M., University of British Columbia. Site-directed mutagenesis.

Grunstein, M., University of California, Los Angeles. Deletion analysis of histone H2B genes.

Prakash, L., University of Rochester. RAD genes of yeast.

Klar, A. and Strathern, J., Cold Spring Harbor Laboratory. Mechanism of recombination in mating-type switching.

Beach, D., Cold Spring Harbor Laboratory. Genetics in *Schizosaccharomyces pombe*.

Fox, T., Cornell University. Interaction of mitochondrial and nuclear genes.

IMMUNOGLOBULINS: MOLECULAR PROBES OF THE NERVOUS SYSTEM

Participants

Bixby, John L., Ph.D., *University of California, San Francisco*

Borroni, Edilio, *Max-Planck-Institut, Gottingen, Federal Republic of Germany*

Caudy, Michael A., B.S., *University of California, Berkeley*

Chin, Gilbert, A.B., *Harvard University, Cambridge, Massachusetts*

Duguid, John R., Ph.D., *University of California, San Francisco*

Hishinuma, Akira, M.D., *Columbia University, New York, New York*

Hockfield, Susan, Ph.D., *Cold Spring Harbor Laboratory, New York*

Pirchio, Mario, M.D., *Istituto di Neurofisiologia del CNR, Rome, Italy*

Sarthy, P. Vijay, Ph.D., *University of Washington, St. Louis, Missouri*

Wagner, Michael A., B.S., *Institute for Cancer Research, Philadelphia, Pennsylvania*

Seminars

Poljak, R., Institut Pasteur. The three-dimensional structure of immunoglobulin.

Gefter, M., Massachusetts Institute of Technology. Idiotype regulation and the origin of antibody diversity.

Wilson, D., University of Pennsylvania. Cell interaction in the immune system.

Sprent, J., University of Pennsylvania. MHC restriction.

Anderson, D., Columbia University College of Physicians & Surgeons. Acetylcholine receptor biosynthesis.

Weber, E., Stanford University. Immunological analysis of opioid peptides.

Lindstrom, J., Salk Institute. The immunobiology of the acetylcholine receptor.

Feramisco, J., Cold Spring Harbor Laboratory. The organization of the cytoskeleton.

Matthew, W., Harvard Medical School. Immunological studies of neurite outgrowth.

Kelly, R., University of California, San Francisco. Synaptic vesicle structure and secretion mechanisms.

Hockfield, S., Cold Spring Harbor Laboratory. Cellular organization in the mammalian CNS.

Willard, M., Washington University. The structure and function of the axon.

Harlow, E., Cold Spring Harbor Laboratory. Immunological analysis of SV40 T antigen.

Karten, H., State University of New York, Stony Brook. Antibodies as probes of defined neural circuits.

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, 249 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 J.M. Foundation, Burroughs Wellcome Fund, and Bayer Aspirin (Glen Brook Labs). They are listed below with their Laboratory sponsors and topics of research.

Marvin Appel, Harvard University
Research Advisor: F. Daldal
Growth of anaerobes under normal atmospheric conditions in medium reduced by *E. coli* membrane extracts.

Michael Cahn, Dartmouth College
Research Advisor: P. Thomas
Sequence analysis of human stress protein genes.

Brad Cookson, University of Utah
Research Advisor: M. Wigler
Analysis of mutations altering expression of H-*ras*-1 genes.

Lillie Hsu, University of Michigan
Research Advisor: A.I. Bukhari
Vectors for shotgun cloning bacterial genes without restriction enzymes.

Kyu-Ho Lee, Massachusetts Institute of Technology
Research Advisor: F. Tamanoi
Use of M13 to express H-*ras*-1 T24 bladder carcinoma p21 protein in *E. coli*.

Ramona Morfeld, Wheaton College
Research Advisors: J. Hicks, J. Ivy
Anti-Mar−A disrupter of the negative regulation of the silent mating-type cassettes in *Saccharomyces cerevisiae.*

Andrew Nathanson, University of Pennsylvania
Research Advisor: R. McKay
The molecular diversity of the embryonic nervous system of the rat.

Michael Schor, Cornell University
Research Advisor: A.J.S. Klar
Search for a site-specific endonuclease gene in *Schizosaccharomyces pombe.*

Thomas Smart, Cornell University
Research Advisor: R. Malmberg
Cloning of the *Nicotiana tabacum* gene nitrate reductase through insertional mutagenesis of a modified T-DNA fragment of *Agrobacterium tumefaciens.*

Laurie Smith, Princeton University
Research Advisor: A.I. Bukhari
Tn*5* mutagenesis of the *gin* and *mom* genes of Mu.

Olney Fellow
Robert Dudley, Duke University
Research Advisor: R. McKay
The generation of antibodies to neural gene products by means of cDNA clones.

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.

1982-1983

September

Michael Smith, University of British Columbia, Vancouver, Canada: Applications of synthetic oligonucleotides in molecular biology in DNA sequence determination, as probes, and site-directed mutagenesis.

Victoria Bautch, University of Illinois, Urbana: Organization and expression of *Drosophila* tropomyosin genes.

Mary Jane Madonna, University of Indiana, Bloomington: Genetic and regulatory studies of the *glt* in *Salmonella typhimurium.*

Terrence Andreasen, University of Washington, St. Louis, Missouri: Studies of calcium-regulated systems using photoaffinity calmodulin probes.

October

Lindley Blair, University of California, Berkeley: Processing and secretion of yeast sex factors.

Micholas Robakis, Roche Institute, Nutley, New Jersey: DNA-directed in vitro synthesis of Dl- and tripeptides—A novel system to study the regulation of the expression of prokaryotic genes.

Robert Gerard, University of Miami, Florida: Genetic analysis of nuclease-sensitive sites in SV40 chromatin.

Howard Fox, University of San Francisco Medical Center, California: Molecular clones of the *t* complex.

Janet Tobian, Virginia Commonwealth University, Richmond: Molecular cloning in the streptococci.

Töran Wadell, Karolinska Institut, Stockholm, Sweden: Molecular epidemiology of adenoviruses.

Jeremy Thorner, University of California, Berkeley: Polypeptide pheromone processing and secretion in yeast.

November

Nick Hastie, Roswell Park Memorial Institute, Buffalo, New York: The complexities of gene expression in the mouse.

Amikam Cohen, Hebrew University, Jerusalem, Israel: General plasmidic recombination—Role of bacterial functions.

Mark Zoller, University of British Columbia, Vancouver, Canada: Assays of gene function using site-directed mutagenesis.

Michael Rabson, Yale University School of Medicine, New Haven, Connecticut: Genome structures of nontransforming Epstein-Barr virus variants.

George Cross, Rockefeller University, New York, New York: Variant surface glycoproteins of *Trypanosoma brucei*—Structure and expression.

December

Susan Watts, University of Minnesota, Duluth: Transformation by rabbit and human papilloma viruses.

Timothy Donohue, University of Illinois at Urbana-Champaign: Regulation of phospholipid biosynthesis in *Rhodopseudomonas sphaeroides.*

Markku Kurkinen, Imperial Cancer Research Fund, London, England: Basement membrane glycoproteins—Laminin and type IV collagen.

John Northup, University of Texas at Dallas: Guanine nucleotide regulatory component of adenylate cyclase.

Patrick Hallenbeck, University of California, Davis: Nitrogenase from the photosynthetic bacterium *Rhodopseudomonas capsulata*—Purification and regulation of activity.

Mike Freeling, University of California, Berkeley: Insertion elements in maize—Structure and function of Robertson's mutator.

Lou Cantley, Harvard University, Cambridge, Massachusetts: The Na/K ATPase and its role in cellular differentiation.

Ravi Dhar, National Institutes of Health, Bethesda, Maryland: Activation of the human bladder transforming gene.

January

Robert Weiss, University of Washington, St. Louis, Missouri: Ribosome frameshifting and mistranslation of the genetic code.

Barbara Thalenfeld and Lenore Gardner, Enzo Biochemicals, New York, New York: Nonradioactive labeling of DNA with biotin.

Richard Ogden, University of California, San Diego: In vitro mutagenesis of yeast tRNA genes.

George Pavlakis, NCI, National Institutes of Health, Bethesda, Maryland: The regulation of genes in bovine papilloma virus vectors.

Obaid Siddiqui, Tata Institute for Fundamental Research, Bombay, India: Neurogenetics of olfaction in *Drosophila.*

Larry Klobutcher, University of Colorado, Boulder: Genome rearrangement during macronuclear development in ciliated protozoa.

Peter Walter, Rockefeller University, New York, New York: Translocation of proteins across the endoplasmic reticulum.

Douglas Youvan, University of California, Berkeley: "R" site-directed transposon mutagenesis and isolation of enhanced fluorescence mutants of *Rhodopseudomonas capsulata.*

February

Diane Stassi, University of Syracuse, New York: The cloning and control of expression of the maltosaccharomyces utilization gene of *Streptococcus pneumoniae.*

Janet Lee, Imperial Cancer Research Fund, London, England: Molecular analysis of HLA-D region genes.

Alex Rich, Massachusetts Institute of Technology, Cambridge: Z-DNA and the replication of transcription.

Tim Baba, University of Texas, Dallas: Analysis of early events in avian leukosis virus-induced lymphomas.

Jerry Lingrel, University of Cincinnati, Ohio: Different evolutionary origins of the developmentally regulated globin genes.

March

Michael Cole, St. Louis University, Missouri: Activation of *myc* oncogene by chromosome translocation.

Dan Lundell, University of California, Berkeley: Structure of the phycombilisome.

Pat Higgins, University of Wyoming, Laramie: Bacteriophage Mu transposition in vitro.

Pablo Scolnick, University of Chicago, Illinois: Molecular genetics of nitrogen fixation of *Rhodopseudomonas capsulata.*

Michael Been, University of Washington, St. Louis, Missouri: Specificity of eukaryotic type-1 topoisomerase on native and single-stranded DNAs.

Jesse Summers, Institute for Cancer Research, Philadelphia, Pennsylvania: Replication of hepatitis-B-like viruses.

Mark Furth, Memorial Sloan-Kettering Cancer Center, New York, New York: Expression of p32 *ras* in *E. coli* and animal cells.

Bob Steinberg, University of Connecticut, Storrs: Fine-structure analysis of mutations in the regulatory subunit of cyclic AMP-dependent protein kinase.

Stephen Lam, Montana State University, Bozeman: The use of transposon mutagenesis in genetic studies of *Pseudomonas syringae.*

Graeme Laver, John Curtin School of Medical Research, Birmingham, and Gillian Air, University of Alabama, Birmingham: The structure and variation of antigenic sites of influenza virus proteins.

Arnold Berk, University of California, Los Angeles: Transcriptional control of adenovirus.

April

Mike Mackett, National Institutes of Health, Bethesda, Maryland: Infectious vaccinia virus recombinants that express foreign genes.

Clay Armstrong, University of Pennsylvania, Philadelphia: Gating currents—Or understanding sodium channels from its charge movements in the membrane.

Rolf Sternglanz, State University of New York, Stony Brook: Identification and characterization of yeast DNA topoisomerase mutants.

Joyce Hamlin, University of Virginia, Charlottesville: Characterization of a mammalian chromosomal replicon.

Richard Egel, Institute of Genetics, Copenhagen, Denmark: Pattern of mating-type switching in fission yeast.

May

Marilyn Kehry, University of Oregon, Eugene: Sensory adaptation in bacterial chemotaxis.

Clyde Slaughter, University of Texas Health Sciences Center, Dallas: Antibody diversity and idiotypes—Studies of murine anti-arsonate antibodies.

Chuck Lent, Brown University, Providence, Rhode Island: Serotonin and feeding behavior in the leech.

Michael Merchlinsky, Yale University School of Medicine, New Haven, Connecticut: Studies with an infectious molecular clone of the autonomous parvovirus MVM.

NATURE STUDY PROGRAM

The Nature Study Program gives elementary and high 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, Nature Photography, and the Geology of Long Island.

During the summer of 1983 a new high of 475 students participated in the Nature Study Program. Most classes were held outdoors, when weather permitted, or at the Uplands Farm Nature Preserve of the Long Island Chapter of the Nature Conservancy. The Laboratory has equipped and maintains a darkroom and classroom/laboratories 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, Montauk Point State Park, the Long Island Pine Barrens, Caumsett Park, and in other area parks.

In addition to the four-week courses, a series of one-day marine biology workshops was offered to students. Studies on the marine ecology of L.I. Sound were conducted aboard the 66-foot schooner J.N. Carter chartered from Schooner, Inc., of New Haven, Connecticut. Students were able to study the Sound chemically, physically, and biologically using the ship's instrumentation. The three-day Adventure Education class took children on an 18-mile bike hike to Caumsett State Park, a six-mile canoe trip on the Nissequogue River, and a day of sailing on the J.N. Carter.

PROGRAM DIRECTOR
Edward Tronolone, M.S., P.D., Science Curriculum Associate, East Williston Public Schools

INSTRUCTORS
Kathryn Bott, M.S., science teacher, Friends Academy
Ruth Burgess, B.A., naturalist, Nassau County BOCES
Michael Fricano, B.S., naturalist, Nassau County BOCES
Robert Jaeger, M.S., science teacher, Mineola High School
Eric Knuffke, M.S., science teacher, Mineola High School
Fred Maasch, M.S., science teacher, Islip High School
Bill Payoski, M.S., science instructor, Nassau Community College
Linda Payoski, B.A., naturalist, Nassau County BOCES
James Romanski, M.S., science teacher, Bay Shore High School

COURSES
Nature Bugs
Nature Detectives
Advanced Nature Study
Introduction to Ecology
Frogs, Flippers, and Fins
Pebble Pups
Bird Study
Fresh Water Life
Seashore Life
They Swim, Walk, and Crawl
Geology of Long Island
Marine Biology
Nature Photography
Adventure Education
Marine Biology Workshop